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Strong Protective Effect of The Aldehyde Dehydrogenase Gene (*ALDH2*) 504lys (*2) Allele Against Alcoholism And Alcohol-Induced Medical Diseases in Asians

Dawei Li¹, Hongyu Zhao^{2,3}, and Joel Gelernter^{1,3,4}

¹Department of Psychiatry, School of Medicine, Yale University, New Haven, Connecticut 06511, USA

²Department of Epidemiology and Public Health, School of Medicine, Yale University, New Haven, Connecticut 06511, USA

³Department of Genetics, School of Medicine, Yale University, New Haven, Connecticut 06511, USA

⁴VA Connecticut Healthcare Center, West Haven, CT, USA

Abstract

Alcohol is oxidized to acetaldehyde, which in turn is oxidized to acetate. The aldehyde dehydrogenase 2 gene (*ALDH2*) is the most important gene responsible for acetaldehyde metabolism. Individuals heterozygous or homozygous for the lys (A or *2) allele at the single nucleotide polymorphism (SNP) glu504lys (rs671) of *ALDH2* have greatly reduced ability to metabolize acetaldehyde, which greatly decreases their risk for alcohol dependence (AD). Case-control studies have shown association between this SNP and alcohol dependence as well as alcohol-induced liver disease. However, some studies have produced insignificant results. Using cumulative data from the past 20 years predominately from Asian populations (from both English and Chinese publications), this meta-analysis sought to examine and update whether the aggregate data provide new evidence of statistical significance for the proposed association. Our results (9,678 cases and 7,331 controls from 53 studies) support a strong association of alcohol abuse and dependence, with allelic *P* value of 3×10^{-56} and OR of 0.23 (0.2, 0.28) under the random effects model. The dominant model (lys-lys + lys-glu vs. glu-glu) also showed strong association with *P* value of 1×10^{-44} and OR of 0.22 (0.18, 0.27). When stricter criteria and various sub-group analyses were applied, the association remained strong (for example, OR = 0.23 (0.18, 0.3) and $P = 2 \times 10^{-28}$ for the alcoholic patients with alcoholic liver disease, cirrhosis, or pancreatitis). These findings provide confirmation of the involvement of the human *ALDH2* gene in the pathogenesis of AD as well as alcohol-induced medical illnesses in East-Asians.

*To whom correspondence should be addressed: Dawei Li, *Ph.D.*, Department of Psychiatry, School of Medicine, Yale University, New Haven, Connecticut 06511, USA. or 300 George Street, Suite 503, New Haven, Connecticut 06511, USA. dawei.li@yale.edu.

Conflict of Interest

None

Electronic-database information

Accession Numbers and URLs for data in this article are as follows:

GenBank, <http://www.ncbi.nlm.nih.gov/Genbank/> for genomic structure of *ALDH2*;

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim> for *ALDH2*;

Genotype data, <http://www.hapmap.org/> for *ALDH2*;

Genome data, <http://genome.ucsc.edu/> for *ALDH2*.

Keywords

Meta-analysis; Association; Linkage Disequilibrium; Ethanol Metabolism

Introduction

Alcohol dependence, which constitutes major public health problems, is a chronic relapsing disorder characterized by compulsive seeking, abuse, tolerance, and physical dependence on alcohol. It is a multifactorial disorder caused by complex interaction of genetic and environmental factors (Gelernter and Kranzler 2009; Kendler et al. 2007). The alcohol dehydrogenases and aldehyde dehydrogenases metabolize alcohol into acetaldehyde and then into acetate. There are two major aldehyde dehydrogenase (ALDH) liver isoforms, cytosolic and mitochondrial, encoded by the aldehyde dehydrogenase 1 gene (*ALDH1*) and aldehyde dehydrogenase 2 gene (*ALDH2*), respectively. The *ALDH2* gene was hypothesized to alter genetic susceptibility to alcohol dependence (AD) and alcohol-induced liver diseases.

The *ALDH2* gene maps to chromosome 12q24.2. A single nucleotide polymorphism (SNP) at exon 12 predicts lysine at residue 504 instead of glutamic acid. The common form of the SNP (rs671) (504glu) encodes the glu (G) allele and was previously referred to as the *ALDH2**1 allele; the 504lys (A, formerly *ALDH2**2 and 487lys) allele produces a catalytically inactive isozyme (Yoshida et al. 1991), which has greatly reduced or no ability to metabolize acetaldehyde (Yoshida et al. 1984). Individuals homozygous for the lys allele (lower frequency in alcoholic subjects) therefore have greatly reduced capacity to clear acetaldehyde and typically show facial flushing and nausea after alcohol consumption, whereas heterozygotes exhibit similar but less severe reactions (Mizoi et al. 1994). This variant can partially determine blood acetaldehyde concentrations after drinking: heterozygotes or homozygotes for the 504lys allele showed peak blood acetaldehyde concentrations after alcohol consumption 6- and 19-fold higher than homozygous common-allele individuals, respectively (Mizoi et al. 1994). Due to delayed oxidation in individuals with the 504lys allele, these individuals have high blood acetaldehyde concentrations, which can cause adverse reactions sufficient to deter drinking. Some individuals with a high daily intake of alcohol develop alcohol-induced medical diseases, e.g., alcoholic cirrhosis, which occurs in around 10% and hepatitis in 10–35% (Grant et al. 1988) of AD individuals. It was hypothesized that the 504lys allele could decrease the risk of AD and consequently influence risk for alcohol-induced medical illnesses, including alcohol liver disease, cirrhosis, and pancreatitis (Thomasson et al. 1991; Yoshida et al. 1991). In addition, genome-wide association studies showed that rs671 was associated with mean corpuscular hemoglobin concentration (Kamatani et al. 2010) and esophageal squamous cell carcinoma (Cui et al. 2009). Other *ALDH2* variants were found to be associated with blood pressure (Kato et al. 2011) or upper aerodigestive tract cancers (McKay et al. 2011).

Several issues prompted us to carry out the present meta-analysis. First, it is difficult to determine the effect of the minor allele without using a large sample, which can most readily be obtained by meta-analysis. Second, rates of AD differ across population subgroups, even within Asian populations, and this is further complicated because of the consequences of differing sampling strategies. Third, many new studies have been reported from various populations in recent years since the previous meta-analyses (Luczak et al. 2006; Zintzaras et al. 2006). Therefore, based on these factors together with the well known critical role of the gene product in alcohol and acetaldehyde metabolism and the fact that the SNP is known to alter protein activity, we performed a comprehensive meta-analysis of the *ALDH2* gene with AD as well as alcohol-induced diseases including alcohol liver disease, cirrhosis, and

pancreatitis, based on all identifiable published studies in both English and Chinese publications, to update the association and to compare the results with those previously described.

Methods

Literature Search

The publications included in the analysis were selected from both PubMed and the database of Chinese Academic Journals with keywords 'aldehyde dehydrogenase', 'ALDH', 'association', 'associated', 'drug', 'substance', 'alcoholism', 'alcohol', 'alcoholics', 'heroin', 'opiate', and 'opioid'. Both English and Chinese language keywords were used in searching the Chinese academic journals (the first author, who performed the search, is proficient in Chinese). All references cited in these studies and in published reviews were examined in order to identify additional works not indexed by the databases. The analyzed data cover all identified English and Chinese publications up to August 2010.

Inclusion Criteria

Eligible studies had to meet the following criteria: they (i) were published in peer-reviewed journals and contained original data; (ii) presented sufficient data to calculate the odds ratio (OR) with confidence interval (CI) and *P* value; (iii) were association studies investigating the specific SNP discussed here (rs671); (iv) described or referenced appropriate genotyping methods or protocols; (v) investigated alcohol or drug dependence (or abuse) diagnosed by valid and published criteria. For the studies investigating alcohol-induced liver disease, cirrhosis, or chronic pancreatitis, the cases were considered as alcoholics with the diseases attributable to alcoholism. Cirrhosis was diagnosed by histological, clinical, radiological, and (or) endoscopic findings; (vi) had no description of other major psychiatric disorders for the patients except anxiety or depressive disorder (patients of AD are often comorbid with anxiety disorder or depressive disorder, or the status was unknown in some studies); and (vii) used unrelated individuals in case-control studies and healthy individuals (or random population) as controls. Authors were contacted in cases where we determined it would be useful to have additional information regarding their studies.

Statistical Analyses

Studies were classified according to inclusion criteria, as "alcoholics with alcoholic liver disease"; "alcoholics with cirrhosis or pancreatitis"; "alcoholics not known to have alcoholic liver disease, cirrhosis, or pancreatitis (or without medical comorbidity specified)"; and "other groups", e.g., heroin dependence (abuse). A study that contained data from more than one population groups (e.g., Asian and Mexican American) was considered effectively as multiple independent studies. Data from the included studies were summarized by two-by-two tables. From each table a log-odds ratio and its sampling variance were calculated. The Cochran's χ^2 -based Q statistic test was performed in order to assess heterogeneity (Zintzaras and Ioannidis 2005) to ensure that each group of studies was suitable for meta-analysis. Where heterogeneity was found, the random effects model, which yields a wider CI, was adopted; otherwise, both the fixed and random effects models were used. Heterogeneity Q tests were also performed for differences in OR between studies using the World Health Organization's International Statistical Classification of Diseases and Related Health Problems (ICD) (World Health Organization) or the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders (DSM) (American Psychiatric Association) system and others; between the English-language publications and Chinese-language publications; between studies originating in mainland China and others (identified by institutions and addresses of the authors); and between studies originating in the United States and others.

A test for funnel plot asymmetry, described by Egger et al. (Egger et al. 1997), was applied to assess evidence for publication bias. The test used a linear regression approach to measure funnel plot asymmetry on the natural logarithm of the OR. Degree of funnel plot asymmetry was measured by the intercept from regression of standard normal deviates against precision. The larger the deviation of each study from the funnel curve, the more pronounced the asymmetry. Results from small studies are expected to scatter widely at the bottom of the graph, with the spread narrowing among larger studies. The significance of the intercept was evaluated using the T test. The classic fail-safe analysis (Rosenthal 1979) was also applied to address potential publication bias. If publication bias is found, the “Duval and Tweedie's Trim and Fill” procedure (Duval and Tweedie 2000) is used to impute the number of potentially-missing studies. The Trim and Fill procedure imputes these missing studies, adds them to the analysis, and then re-computes the adjusted overall effect size.

ORs were pooled using the method of DerSimonian and Laird (DerSimonian and Laird 1986), and 95% CIs were constructed using Woolf's method (Woolf 1955). The significance of the overall OR was determined using the Z test. To measure sensitivity of our analysis results, each study was removed in turn from the total, and the remainder then reanalyzed. This procedure was used to ensure that no individual study was entirely responsible for the combined results. Both allelic and genotypic analyses (e.g., dominant model) were applied, and different combinations of the alcohol-related medical conditions (e.g., alcohol liver disease, cirrhosis, and pancreatitis) were also analyzed. Retrospective analysis was performed to better understand the potential effect of year of publication upon the results. The type I error rate was set at 0.05. The tests were two-tailed.

In order to evaluate whether there are other polymorphisms in strong linkage disequilibrium (LD) with this SNP, haplotype construction, counting, and LD block defining over a broader genomic region that includes *ALDH2* were performed separately based on the published genotype data of 90 Asian (Chinese and Japanese) samples and 30 CEPH trios (Utah residents) from HapMap release 23a (http://ftp.hapmap.org/genotypes/2008-03/rs_strand/non-redundant). The multiallelic D' was computed by performing a series of pairwise D' calculations using each haplotype in turn as an allele, with all other haplotypes at the locus serving as the other allele. This was then repeated for each haplotype at each locus and averaged by haplotype frequency. Maximum likelihood haplotype blocks were calculated using the EM algorithm (Excoffier and Slatkin 1995). In addition, the genotype data from the 1000 Genomes Project were also used to calculate and build the LD block (Johnson et al. 2008).

Results

The search yielded 470 references. After discarding overlapping references and those which clearly did not meet the criteria, 70 studies remained. These studies were then filtered to ensure conformity with our inclusion criteria. One study (Higuchi et al. 1995) was excluded because subjects in this study were included in another study (Higuchi et al. 1996b); three studies (Choi et al. 2006; Harada et al. 1999; Yang et al. 2007) were excluded because they were investigating other polymorphisms, such as 1951G/A, rather than the SNP considered here; two (Shimosegawa et al. 2008; Tsuchihashi-Makaya et al. 2009) because the criteria to define “alcoholic” patients were not eligible (i.e., drinking and smoking behavior was assessed on the basis of the lifestyle questionnaire self-administered by the subjects (Tsuchihashi-Makaya et al. 2009); and patients with alcohol consumption of more than 80 g/day for at least two years were classified as alcoholic chronic pancreatitis (Shimosegawa et al. 2008)); two (Hendershot et al. 2009; Segado Soriano et al. 2005) because the patients were described nonspecifically as “drinkers”, rather than “dependent” or “abusers”; one (Nanakorn et al. 1999) because the patients had comorbid psychiatric disorders; one (Wei et

al. 1999) because the cases and controls were related; one (Macgregor et al. 2009) because it was investigating twin pairs; four (Higuchi et al. 1996a; Kimura et al. 2009; Muramatsu et al. 1996; Vaswani et al. 2009) because no control subjects were recruited or control data were not available; one (Kuo et al. 2008) due to very low observed minor allele frequency (0.001) in their samples; and one (Shafe et al. 2009) because there was no genotype data available. In the end, 53 case-control studies met our criteria for inclusion. The studies included 52 studies (Chai et al. 2005; Chao et al. 1994; Chao et al. 2000; Chao et al. 2003; Chao et al. 1997; Chen et al. 1999; Chen et al. 1996; Chen et al. 1997; Fan et al. 1998; Guo et al. 2010; Hasegawa et al. 2002; Higuchi et al. 1996b; Huang et al. 2004; Iwahashi et al. 1995; Jing et al. 2009; Kim et al. 2008; Kim et al. 2004; Lee et al. 2001; Lee et al. 1999; Lee et al. 2009; Lee et al. 2010; Leng et al. 2008; Maezawa et al. 1995; Matsushita et al. 2001; Miyatake et al. 1995; Muramatsu et al. 1995; Nakamura et al. 1996; Nakamura et al. 1999; Nishizawa et al. 2006; Paik et al. 2000; Park et al. 2001; Sasabe et al. 2007; Shen et al. 1997a; Shen et al. 1997b; Shin et al. 2010; Tan et al. 2010; Tanaka et al. 1996; Thomasson et al. 1994; Thomasson et al. 1991; Xu et al. 2002; Yamauchi et al. 1995; Yan et al. 2003; Yu et al. 2002) of Asian populations (including Chinese aboriginal populations) and one study (Konishi et al. 2004) of Mexican Americans. One study (Xu et al. 2002) investigated heroin dependence and abuse and 52 studies investigated AD and alcohol abuse or AD only. Among these studies of alcoholics 48 studies (Chai et al. 2005; Chen et al. 1999; Chen et al. 1996; Chen et al. 1997; Fan et al. 1998; Guo et al. 2010; Hasegawa et al. 2002; Higuchi et al. 1996b; Huang et al. 2004; Iwahashi et al. 1995; Jing et al. 2009; Kim et al. 2008; Kim et al. 2004; Konishi et al. 2004; Lee et al. 2001; Lee et al. 1999; Lee et al. 2009; Lee et al. 2010; Leng et al. 2008; Maezawa et al. 1995; Matsushita et al. 2001; Miyatake et al. 1995; Muramatsu et al. 1995; Nakamura et al. 1996; Nakamura et al. 1999; Nishizawa et al. 2006; Paik et al. 2000; Park et al. 2001; Sasabe et al. 2007; Shen et al. 1997a; Shen et al. 1997b; Shin et al. 2010; Tan et al. 2010; Tanaka et al. 1996; Thomasson et al. 1994; Thomasson et al. 1991; Yamauchi et al. 1995; Yan et al. 2003; Yu et al. 2002) investigated alcoholics without (or without specifying) alcoholic liver disease, cirrhosis, or pancreatitis; seven studies (Chao et al. 1994; Chao et al. 2000; Chao et al. 2003; Chao et al. 1997; Lee et al. 2001; Tanaka et al. 1996; Yamauchi et al. 1995) investigated the alcoholics with alcoholic liver disease, cirrhosis, or pancreatitis; and three studies (Guo et al. 2010; Shin et al. 2010; Thomasson et al. 1994) included the mixed patients of AD and alcohol abuse. For the study by Lee et al. (Lee et al. 2009), some additional data, unavailable in the published paper, were kindly provided by the authors. These studies included 9,678 cases and 7,331 controls (supplementary Table 1). The results are detailed below.

Based on all these samples, the frequency of the protective 504lys allele was 14% (overall population estimate) but varied widely across different populations and disease statuses: high in the Asian normal populations 23% (1% – 53%), lower in affected subjects, 7% (0 – 23%); very low in Mexican Americans (1%); and undetected in the European-ancestry populations. Of the 53 studies included, 52 studies showed lower 504lys allele frequency in cases than in controls. Drug dependence as a primary diagnosis and non-Asian samples were considered as extra information for comparison (i.e., allele frequency calculation), and thus excluded from the following meta-analysis.

For the allelic analysis, the combined studies of alcohol abuse and dependence produced strong evidence of association with an overall P value of 3×10^{-56} , the $\ln$ (OR) being -1.45 ($-1.63, -1.27$) with an OR of 0.23 (0.2, 0.28) under the random effects model (due to evidence of significant heterogeneity between studies ($PQ = 3 \times 10^{-14}$)). The subgroup of Asian populations also showed strong association with P value of 7×10^{-56} (Table 1).

For medical conditions related to alcoholism, the combined studies of alcoholics with alcoholic liver disease, cirrhosis (specifically), and (or) pancreatitis produced a strong

association with P value of 2×10^{-28} (OR = 0.23 (0.18, 0.3)) with no evidence of significant heterogeneity ($P = 0.5$). The studies of alcoholic cirrhosis also showed strong association with P value of 6×10^{-19} .

Strong association was also detected when the alcoholics with known (and reported) alcoholic liver disease, cirrhosis, or pancreatitis were excluded ($P = 7 \times 10^{-46}$ and OR = 0.23 (0.19, 0.28) under the random effects model). Furthermore, the association was still strong after all the three studies containing alcohol abuse were excluded ($P = 2 \times 10^{-43}$) or when the Mexican American subjects were excluded ($P = 1 \times 10^{-45}$); or both ($P = 5 \times 10^{-43}$). Additionally, when the study of heroin dependence and abuse was included, the between-studies heterogeneity was more significant but the association was still strong ($P = 6 \times 10^{-43}$).

For the dominant model (lys-lys + lys-glu vs. glu-glu) in genotypic analyses, overall the studies of alcohol abuse and dependence showed an overall P value of 1×10^{-44} with the ln (OR) of -1.51 ($-1.72, -1.3$) and OR of 0.22 (0.18, 0.27) under the random effects model (Table 1). The strong association did not change after the Mexican American subjects were excluded from the analysis ($P = 2 \times 10^{-44}$). Strong association was also found with alcoholic liver disease and alcoholics with cirrhosis and (or) pancreatitis with P value of 8×10^{-26} and OR of 0.21 (0.16, 0.29). No evidence of significant heterogeneity was found between studies. The combined studies of alcoholics without alcoholic liver disease, cirrhosis, or pancreatitis also produced strong association with P value of 1×10^{-37} and OR of 0.22 (0.17, 0.27). Furthermore, the consistently strong association was identified after the subjects of alcohol abuse or after the subjects of Mexican American were excluded with the P values of 5×10^{-35} and 2×10^{-37} , respectively (Table 1).

The demography of the combined studies is shown in supplementary Table 1. The results of overall and subgroup meta-analyses are shown for both allelic and genotypic analyses in Table 1. The forest plots of the allelic and genotypic analyses are shown in Figures 1 and 2, respectively. The funnel plots of allelic and genotypic analyses are shown in Figure 3 and supplementary Figure 1.

Publication Bias and Fail-safe Analyses

In the present study, no evidence of significant publication bias was found in either allelic or genotypic analysis (P values > 0.05 in both Egger's regression and Begg's rank correlation (Kendall's tau) (Begg and Mazumdar 1994) tests).

The classic fail-safe analysis showed that for the allelic analysis about 10,386 assumed non-significant studies would be required to bring the $P(Z)$ value to > 0.05 ; and for the dominant model about 10,120 non-significant studies would be required to bring the $P(Z)$ value to > 0.05 . These results further supported the strong associations detected in the meta-analysis.

Other Heterogeneity Analyses

There was no evidence of significant heterogeneity regarding different diagnosis criteria, publication languages, or study countries (or regions) using either allelic or genotypic analysis. The results of AD and alcohol abuse are shown in supplementary Table 2 ($P(Q) > 0.1$).

Sensitivity and Retrospective Analyses

Sensitivity analysis showed that no individual group biased the findings to the extent that it could account for the overall strong association. For instance, the results of AD and alcohol abuse were strongly consistent, regardless of the data set removed, with the P values never $> 6 \times 10^{-51}$ among the 50 studies in the allelic analysis; under the dominant model the results

also showed strong consistency, regardless of the data set removed, with the P value never $> 3 \times 10^{-41}$ among the 52 studies. The results are shown for the allelic and genotypic analyses in supplementary Tables 3 and 4, respectively.

The asymptote lines of the analyses in retrospect based on the 20 publication years showed that the cumulative synthesis of the SNP has tended to be stable, in line with the overall results of our meta-analysis. The results of the allelic and genotypic analyses are shown in Figure 4 and supplementary Figure 2 and the P values are shown in supplementary Tables 5 and 6, respectively.

LD and Haplotype Structure Analyses

In the Asian populations, the *ALDH2* gene was located within a large region spanning 13 haplotype blocks with strong LD, and more than ten genes were found within this LD structure (Figure 5). Compared with the Asian populations, the European-ancestry populations showed stronger LD in this large structure, which was composed of three big haplotype blocks (supplementary Figure 3). Two other genes (*BRAP* and *ACAD10*) share the same haplotype block with *ALDH2*. Although it is unlikely that the association of this SNP was due to linkage disequilibrium with a different closely-mapped polymorphism at *ALDH2* or another gene considering both the strength of the association and the compelling biological rationale, it is still desirable to investigate other variants within the large structure in subsequent studies. For example, one SNP rs4646776 was in strong LD with rs671 based on the 1000 Genomes Project (11,747 base pair; $r^2 = 0.81$; and recombination rate = 0.99) (supplementary Figure 4).

Discussion

These findings support that the *ALDH2* 504lys allele can greatly lower the risk for AD as well as alcohol-induced diseases in the Asian populations. The reduction in *ALDH2* activity in the 504lys/504glu heterozygotes is more than 100-fold compared with the *ALDH2* activity of the 504glu/504glu homozygotes (Brooks et al. 2009; Crabb et al. 2004). Studies on liver extracts demonstrated that the *ALDH2* 504lys allele was nearly dominant, and the heterozygous and 504lys/504lys homozygous has almost no detectable *ALDH2* activity in the liver (Edenberg 2007). The inactive *ALDH2* 504lys allele occurred mainly in some Asian populations (with the nearly complete absence of homozygous lys504lys alcoholics), but its prevalence varied across these samples. For instance, the 504lys allele was less commonly found in some aboriginal Chinese populations (e.g., Ami, Atayal, Bunun, Elunchan, Mongolian, and Paiwan) compared with Han Chinese. Figure 6 and supplementary Table 7 show the 504lys allele frequencies of by location. Most alcoholics with inactive *ALDH2* alleles were heterozygous; homozygous lys-lys was rarely found in the alcoholic subjects, although it was often observed in controls. As expected, the recessive model based on the lys-lys genotype could not be applied in the present analysis due to insufficient power. Regarding the discrepant results in the individual association studies included in this meta-analysis, there are some possible explanations besides ethnic heterogeneity (e.g., one study (Chen et al. 1997) reported different direction of odd ratio in Ami): first, in most studies, subjects with AD or alcohol abuse were diagnosed according to the ICD (World Health Organization) or DSM (American Psychiatric Association) system. ICD-10 criteria for alcohol and drug dependence are more stringent than DSM-IV criteria, which in turn are more stringent than DSM-III-R (Schuckit et al. 1994). Two studies (Yan et al. 2003; Yu et al. 2002) applied local criteria and six studies had no specified criteria. Since these classification criteria differ, certain subjects would be eligible for inclusion as “affected” in some studies but not in others. Less stringent diagnostic criteria could differentiate the patient and control groups to a smaller extent, potentially altering observed effect sizes for *ALDH2* 504lys. Second, studies using mixed-sex samples may yield

different results than studies using only male or female subjects, especially if the sexes are mixed in only patient or control group. Third, in terms of recruitment strategy, the allele differences between patients and controls may be greater in the samples recruited from clinical treatment than in the samples from general population due to well-recognized ascertainment bias. Fourth, the genetic effects of *ALDH2* 504lys on AD may change longitudinally over the course of illness. Fifth, the samples were not in Hardy-Weinberg equilibrium (HWE) (Zintzaras 2008) in several studies. Of the control samples that were not in HWE, either the samples were small or the disequilibrium was not highly significant. Disequilibrium in patients supports that the gene may be related to AD because the genotype distributions observed differ from those expected by chance; however, disequilibrium in controls is more likely to reflect genotyping error and thus distort the analysis in some degree (Luo et al. 2008). The sixth reason is different statistical power and type II error due to sample size as well as different genotyping methods. In addition, cultural differences may interact with the genetic components and result in differentially significance levels of protection of *ALDH2* 504lys against AD across different ethnic groups.

The present comprehensive meta-analysis identified stronger association ($P = 3 \times 10^{-56}$) than the previous studies (Luczak et al. 2006; Zintzaras et al. 2006), which can be explained by several differences besides different research purposes and statistical methods (Minelli et al. 2005; Trikalinos et al. 2008; Zintzaras 2008; Zintzaras and Lau 2008; Zintzaras et al. 2009; Zintzaras et al. 2008) from ours (Li and He 2007a, b, c, 2008): the meta-analysis by Zintzaras et al. (Zintzaras et al. 2006) a) included 23 studies published in or before 2004 (compared to 53 studies in our meta-analysis); b) only included English literature; c) provided OR without specific *P* values; and d) found no association with liver disease using allelic analysis (the result was only positive under the dominant model). Another meta-analysis by Luczak et al. (Luczak et al. 2006), in which the latest dataset was also published in 2004, a) included 22 Asian studies including 1,980 cases and 2,550 controls (we included 9,678 cases and 7,331 controls); b) performed genotypic analysis but not allelic analysis; c) provided no specific *P* values; d) reported negative results (under the model of lys-lys vs. lys-glu); and e) found a difference between possessing one and two *ALDH2* 504lys alleles that approached but did not reach statistical significance. Compared with each published study, our meta-analysis a) included the largest sample size from 45 English and seven Chinese publications (it was important to include Chinese-language publications). Our analysis showed that there was no publication bias, thus, the Chinese literature did not introduce overestimates of effect sizes in our meta-analysis (supplementary Table 2); b) performed both allelic and genotypic analyses using the random effects model; c) used both strict and extended criteria as well as carrying out subgroup analyses to detect the effects; d) applied comprehensive analysis methods, as shown in the results, to study additional questions not answered in those previous meta-analyses; and e) found strong evidence of association with both alcohol dependence and the alcohol-induced medical diseases. In addition, the procedure of 'extended-quality score' suggested in our previous study (Li et al. 2006) was also applied to assist the assessment of quality of the association studies. However, the limitations were that the majority of studies included were predominately from Asian populations, because of the protective 504lys allele was barely detected in the European-ancestry populations. The studies of both English and Chinese languages were included in this meta-analysis, and those of other Asian languages were not considered. In addition, it is possible that some negative results tend to be not published, which is hard to measure.

Findings of association studies depend on inclusion criteria and quality of the case and control subjects. Approximately 10% of Japanese alcoholics (Higuchi et al. 1994) develop their disease despite having the inactive (protective) *ALDH2* encoded by either heterozygous lys-glu or homozygous lys-lys. Compared with the entire alcoholic population,

the heterogeneity of alcoholism is considered to be relatively low in this genetically defined subgroup. Such alcoholics are considered to be advantageous for the identification of variations that produce minor effects; otherwise, the potential association may be masked by genetic variants with high effect.

In future studies, more precise analyses could be performed if information including sex, age, and age at onset was provided in most individual studies (sex differences and haplotypes (Liu et al. 2011) were not evaluated in this meta-analysis because the information was not available). The genetic risk effects of other factors can also be measured (see Dr. Zintzaras et al.'s papers (Trikalinos et al. 2008; Zintzaras ; Zintzaras and Lau 2008; Zintzaras et al. 2009; Zintzaras et al. 2008)), and other genetic models can be explored, as suggested in Minelli et al. study (Minelli et al. 2005). Additionally, full haplotypes as well as potential gene-gene and gene-environment interactions should be considered because these interactions have been found to alter the protective effect size. For example, Higuchi et al. (Higuchi et al. 1994) reported that rates of Japanese *ALDH2* lys-glu individuals seeking treatment for AD increased from 2.5% in 1979 to 8% in 1986 then to 13% in 1992 in Japan while the rates of alcohol consumption also increased. Thus, the secular trend of increased cultural acceptance of alcohol consumption might have reduced the protective effect of the 504lys allele. Third, a previous longitudinal cohort study confirmed the significant roles of anxiety disorders and of the *ADH1B* Arg allele as antecedents of alcoholism among aboriginal Taiwanese (Cheng et al. 2004). Such studies will allow for determination of how the effects change over the course of lifetime alcohol use. Fourth, future studies should also try to use control samples that include older subjects which are more conservative regarding statistical analysis (as they will have passed through more of the age of risk for AD). Fifth, it is necessary to investigate the potential treatments for alcoholism based on the findings, for example, the alcohol aversion caused by this polymorphism compared to the effects of disulfiram.

In conclusion, using the cumulative data of the past 20 years from both English and Chinese publications, predominately from East-Asian populations (but barely detected in the European populations), this meta-analysis found highly strong evidence of association using both allelic ($P = 3 \times 10^{-56}$) and genotypic ($P = 1 \times 10^{-44}$) analyses under the random effects model. When strict criteria and sub-group analyses were applied, the strong associations remained. Our meta-analysis provides strong evidence for the involvement of the human *ALDH2* gene in the pathogenesis of AD and alcohol abuse as well as alcohol-induced medical diseases in East-Asians.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Forest plots of $\ln(OR)$ with 95% CI for the allelic analysis. Black squares indicate the $\ln(OR)$ ($\ln(OR)$ can be better fitted than OR), with the size of the square inversely proportional to its variance, and horizontal lines represent the 95% CIs. The pooled results are indicated by the unshaded black diamond. Six datasets including Chen 1997 (Atayal) (Chen et al. 1997), Chen 1997 (Bunun) (Chen et al. 1997), Chen 1997 (Paiwan) (Chen et al. 1997), Shen 1997 (b) (Elunchun) (Shen et al. 1997b), Fan 1998 (Elunchun) (Fan et al. 1998), and Konishi 2004 (Konishi et al. 2004), are not shown on the forest plots because the wide CIs can not fit into the current version of the plots.

Figure 2.

Forest plots of $\ln(OR)$ with 95% CI for the dominant model of (lys-lys + lys-glu) vs. glu-glu. Six datasets are not shown on the forest plots as described above.

Figure 3.

Egger's funnel plots of publication bias analysis for the allelic analysis. A larger deviation from the funnel curve of each study means more pronounced asymmetry. Results from small studies will scatter widely at the bottom of the graph, with the spread narrowing among larger studies.

ALDH2 Allelic Analysis

Figure 4. Retrospective analysis of the alleles. Analysis in retrospect was based on publication year since 1991.

Figure 5.

Graphical representation of the LD structure of the *ALDH2* gene for the Asian populations. The LD structure, spanning 902kb, was constructed using the Asian genotype data of 351 SNPs. Vertical tick marks above the name indicate the relative genomic position of each SNP. The LD structure represents the pairwise calculation of D' for each possible combination of SNPs. $D' < 0.5$ is shown in white, $D' = 1.0$ in dark red, with increasing shades of red between representing increasing D' between the SNPs. The genes from left to right are: *ATXN2*, *BRAP*, *ACAD10*, *ALDH2*, *MAPKAPK5*, *TMEM116*, *ERP29*, *C12orf30*, *TRAFD1*, *C12orf51*. The *ALDH2* gene and the SNP rs671 are shown in red; and rs1062136 (Val/Glu), which is a nonsynonymous SNP, is shown in blue. The other genes are in black.

Figure 6.

504lys allele frequencies among different populations. Blue and red represent 504lys and 504glu, respectively. Upper graphs are based on the cases, lower graphs on controls. The geographical borders (Miyazaki et al. 1993) of Taiwan aborigines were from a previous study.

Table 1

Results of the overall and sub-grouped studies

Groups	N*	LnOR (95% CI)	OR (95% CI)	P(Z)	P(Q)
Allelic Analysis (lys vs. glu)					
Alcoholics (alcohol dependence and abuse)	50	-1.45 (-1.63,-1.27)	0.23 (0.2,0.28)	3×10⁻⁵⁶	3×10 ⁻¹⁴
Alcoholics (Asian)	49	-1.45 (-1.63,-1.27)	0.23 (0.2,0.28)	7×10⁻⁵⁶	2×10 ⁻¹⁴
Alcohol-induced diseases ^a	7	-1.45 (-1.71,-1.19)	0.23 (0.18,0.3)	2×10⁻²⁸	0.48
Alcoholic cirrhosis	5	-1.37 (-1.68,-1.07)	0.25 (0.19,0.34)	6×10⁻¹⁹	0.24
Alcoholics without induced diseases ^b	46	-1.46 (-1.67,-1.26)	0.23 (0.19,0.28)	7×10⁻⁴⁶	7×10 ⁻¹⁵
Alcoholics without induced diseases ^b (Asian)	45	-1.46 (-1.67,-1.26)	0.23 (0.19,0.28)	1×10⁻⁴⁵	4×10 ⁻¹⁵
AD without induced diseases ^c	43	-1.48 (-1.69,-1.27)	0.23 (0.18,0.28)	2×10⁻⁴³	1×10 ⁻¹⁴
AD without induced diseases ^c (Asian)	42	-1.48 (-1.69,-1.27)	0.23 (0.19,0.28)	5×10⁻⁴³	8×10 ⁻¹⁵
Alcoholics and heroin addiction	51	-1.43 (-1.63,-1.22)	0.24 (0.2,0.29)	6×10⁻⁴³	1×10 ⁻²⁶
Alcoholics and heroin addiction (Asian)	50	-1.43 (-1.63,-1.22)	0.24 (0.2,0.29)	1×10⁻⁴²	6×10 ⁻²⁷
Dominant Model (lys-lys + lys-glu) vs. glu-glu					
Alcoholics	52	-1.51 (-1.72,-1.3)	0.22 (0.18,0.27)	1×10⁻⁴⁴	2×10 ⁻²¹
Alcoholics (Asian)	51	-1.51 (-1.72,-1.3)	0.22 (0.18,0.27)	2×10⁻⁴⁴	8×10 ⁻²²
Alcohol-induced diseases ^a	7	-1.54 (-1.83,-1.25)	0.21 (0.16,0.29)	8×10⁻²⁶	0.27
Alcoholic cirrhosis	5	-1.44 (-1.78,-1.1)	0.24 (0.17,0.33)	9×10⁻¹⁷	0.17
Alcoholics without induced diseases ^b	48	-1.53 (-1.76,-1.29)	0.22 (0.17,0.27)	1×10⁻³⁷	3×10 ⁻²²
Alcoholics without induced diseases ^b (Asian)	47	-1.52 (-1.76,-1.29)	0.22 (0.17,0.28)	2×10⁻³⁷	2×10 ⁻²²
AD without induced diseases ^c	45	-1.54 (-1.78,-1.3)	0.21 (0.17,0.27)	5×10⁻³⁵	3×10 ⁻²²
AD without induced diseases ^c (Asian)	44	-1.54 (-1.79,-1.3)	0.21 (0.17,0.27)	4×10⁻³⁵	1×10 ⁻²²

* number of studies included in the analyses.

^a alcoholic patients with alcoholic liver disease, cirrhosis, or pancreatitis.

^b alcoholic patients without alcoholic liver disease, cirrhosis, or pancreatitis.

^c alcohol dependent patients without alcoholic liver disease, cirrhosis, or pancreatitis and those without liver disease status described.

P(Z): Z test used to determine significance of the overall OR. The *P* values < 0.05 are indicated in boldfaces.

P(Q): Cochran's X^2 -based Q statistic test used to assess heterogeneity.
P(T): T test used to evaluate significance of publication bias (not shown). $P(1\text{-tailed}) > 0.05$.