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Further Clarification of The Contribution of The *ADH1C* Gene to The Vulnerability of Alcoholism And Selected Liver Diseases

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Abstract

The alcohol dehydrogenase 1C (*ADH1C*) subunit is an important member of the alcohol dehydrogenase family, a set of genes that plays a major role in the catabolism of ethanol. Numerous association studies have provided compelling evidence that *ADH1C* gene variation (formerly *ADH3*) is associated with altered genetic susceptibility to alcoholism and alcohol-related liver disease, cirrhosis, or pancreatitis. However, the results have been inconsistent, partially because each study involved a limited number of subjects, and some were underpowered. Using cumulative data over the past two decades, this meta-analysis (6,796 cases and 6,938 controls) considered samples of Asian, European, African, and Native American origins to examine whether the aggregate genotype provide statistically significant evidence of association. The results showed strong evidence of association between *ADH1C* Ile350Val (rs698, formerly *ADH1C* *1/*2) and alcohol dependence (AD) and abuse in the combined studies. The overall allelic (Val vs. Ile or *2 vs. *1) *P* value was 1×10^{-8} and Odds Ratio (OR) was 1.51 (1.31, 1.73). The Asian populations produced stronger evidence of association with an allelic *P* value of 4×10^{-33} (OR = 2.14 (1.89, 2.43)) with no evidence of heterogeneity, and the dominant and recessive models revealed even stronger effect sizes. The strong evidence remained when stricter criteria and sub-group analyses were applied, while Asians always showed stronger associations than other populations. Our findings support that *ADH1C* Ile may lower the risk of AD and alcohol abuse as well as alcohol-related cirrhosis in pooled populations, with the strongest and most consistent effects in Asians.

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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 *ADH1C*; Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim> for *ADH1C*;

Genotype data, <http://www.hapmap.org/> for *ADH1C*;

Genome data, <http://genome.ucsc.edu/> for *ADH1C*.

Keywords

Meta-analysis; Association; Ethanol Oxidation; Addiction; *ADH1C*

Introduction

Substance abuse, which constitutes a major public health problem, is genetically influenced, but characterized by incomplete penetrance, phenocopies, heterogeneity, and polygenic inheritance. The alcohol dehydrogenase 1B and 1C genes (*ADH1B* and *ADH1C*) encode class I alcohol dehydrogenase beta and gamma subunits, respectively. These isoenzymes metabolize alcohol into acetaldehyde (among other physiological actions); acetaldehyde is then metabolized into acetate through the aldehyde dehydrogenase 2 gene (*ALDH2*). The gamma subunit encoded by *ADH1C* plays a key role in the oxidation catabolism of a wide variety of substrates, including ethanol, retinol, other aliphatic alcohols, hydroxysteroids, and lipid peroxidation products. *ADH1B* appears to play the greatest role in modulating alcohol dependence risk among the ADH loci (review, Li et al., 2011). The *ADH1C* gene (formerly called *ADH3*), located on chromosome 4q21-q23, is adjacent to *ADH1B* and in the region of a gene cluster of the alcohol dehydrogenase subunits 6, 1A, 1B, 1C, and 7. The common form of a single nucleotide polymorphism (SNP: rs698, Ile350Val in exon 8, formerly known as *ADH1C**1/*2) at the *ADH1C* gene locus is 350Val (G or *2). The other allele 350Ile (A or *1) encodes for a highly active allozyme. This allozyme is capable of altering ethanol metabolism (Yoshida et al. 1991) and reducing genetic susceptibility to alcohol dependence (AD) (Higuchi et al. 1995; Thomasson et al. 1991). A well-known and generally accepted hypothetical mechanism is that the highly active *ADH1C* 350Ile can increase the level of acetaldehyde, and then result in enhanced negative reactions to alcohol, which in turn reduces the likelihood of AD.

Over the last few years, an increasing number of association studies have provided compelling evidence regarding the role of the *ADH1C* gene in alcohol and drug dependence as well as in alcohol-related liver disease, cirrhosis, and pancreatitis. However, the results have been inconsistent, partially because each study obviously involved a limited number of subjects, and some were underpowered to the extent that there was not enough information to demonstrate a significant association. Second, the findings are complicated by the use of different ethnicities, sampling strategies, or genotyping procedures (e.g., the rates of alcohol abuse and alcohol-related medical diseases differ across various populations). Third, the low prevalence of Val350Val individuals in some Asian populations makes it particularly difficult to determine the effect of homozygous individuals without evaluating large samples.

Although two early meta-analyses (Whitfield 1997; Zintzaras et al. 2006) evaluated findings relevant to the gene, the limited data were not sufficient to provide a systematic explanation of the role of the SNP. However, the availability of genotype data from various populations has increased greatly in recent years. Considering the critical role of the gene in alcohol and acetaldehyde metabolism as well as this nonsynonymous SNP's function in modulating in protein activity, we performed a comprehensive meta-analysis of *ADH1C* Ile350Val with AD and alcohol abuse, as well as with alcohol-related medical diseases, based on both English and Chinese-language publications. The aim of this meta-analysis was to clarify and confirm the characteristics of this association; compare the results with those from previous studies; and if possible, to provide further evidence for the proposed mechanism of *ADH1C* Ile350Val.

Methods

Literature Search

The studies included in the meta-analysis were selected from PubMed and from the database of Chinese Academic Journals with keywords 'alcohol dehydrogenase', '*ADH3*', '*ADH1C*', 'association', 'associated', 'drug', 'substance', 'alcoholism', 'alcohol', 'alcoholics', 'heroin', 'cocaine', 'opiate', 'opioid', and 'methamphetamine'. 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 studies cover all identified English and Chinese publications up to August 2010.

Inclusion Criteria

Eligible studies had to meet the following criteria: they (1) were published in peer-reviewed journals; (2) contained original data; (3) presented sufficient data to calculate the odds ratio (OR) with confidence interval (CI) and *P* value; (4) were association studies investigating the specific SNP considered here; (5) described or referenced appropriate genotyping methods; (6) investigated alcohol, heroin, cocaine, or methamphetamine dependence (or abuse) diagnosed by valid published criteria. For the studies investigating alcohol-related liver disease, cirrhosis, or chronic pancreatitis, the cases were considered as alcoholics with the alcohol-related diseases. Cirrhosis was diagnosed by histological, clinical, radiological, and (or) endoscopic findings; (7) had no description of known comorbidity with major psychiatric disorders for the participants (this information was not available in all the studies); and (8) used unrelated individuals in case-control studies. Authors were contacted in cases where we determined it would be useful to have additional information regarding their studies.

Statistical Analyses

Studies were divided among those dealing with samples with European ancestries, those with Asian ancestries, those with African ancestries, and those with Mexican (or Native American) ancestries. For studies that contained data from multiple populations, each was considered as effectively an independent study. Data from the studies were summarized by two-by-two tables. From each table a log-odds ratio and its sampling variance were calculated (Li et al. 2006). The Cochran's χ^2 -based *Q* statistic test was computed in order to assess heterogeneity to ascertain whether 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 adopted. A test for funnel plot asymmetry (Egger et al. 1997) was used 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. The larger the deviation of each study from the funnel curve, the more pronounced the asymmetry. Results from small studies will 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 (Egger et al. 1997; Fan and Sklar 2005). For datasets with evidence for publication bias, the "Duval and Tweedie's Trim and Fill" procedure (Duval and Tweedie 2000) was used to impute the number of potentially-missing studies. In the absence of bias the funnel plot would be symmetric with respect to the summary effect. If there are more small studies on the right than on the left, some studies may be missing from the left. 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. For sensitivity analysis, 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. In addition, genotypic analyses were carried out under the dominant ((ValVal + ValIle) vs. IleIle) and recessive (ValVal vs. (ValIle + IleIle)) models. Different combinations of the ethnic populations 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 understand better 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 know whether there are other polymorphisms in strong linkage disequilibrium (LD) with this SNP in Asians and Europeans, haplotype construction, counting, and LD block defining over a broader genomic region of *ADH1C* were performed separately using the genotypes of 30 European and 90 Asian samples from HapMap (release 23a and ncbi_b36). The analysis procedure and additional LD information on the gene cluster (*ADH7*, *ADH1C*, *ADH1B* and *ADH1A*) and other statistical approaches (e.g., fail-safe analysis) were described previously (Li et al. 2011).

Results

The combined search yielded 820 references. After discarding overlapping references and those which clearly did not meet the inclusion criteria, 58 studies remained. These studies were then filtered to ensure conformity with the inclusion criteria. One study (Wei et al. 1999) was excluded because the cases and controls were related; one study (Poupon et al. 1992) because the controls were moderate alcohol drinkers; one study (Macgregor et al. 2009) because it was investigating twin pairs; one study (Khan et al. 2010) because the definition for “alcoholics” was not consistent with our inclusion criteria; and one study (Shafe et al. 2009) because no genotype data were available. In the end, 53 case-control studies (supplementary Table 1) met our criteria for inclusion. These studies included 30 studies (Chai et al. 2005; Chao et al. 1994; Chao et al. 2000; Chao et al. 1997; Chen et al. 1999; Chen et al. 1996; Chen et al. 1997; Choi et al. 2005; Fan et al. 1998; Higuchi et al. 1996; Kim et al. 2004; Lee et al. 2001; Lee et al. 1999; Montane-Jaime et al. 2006; Nakamura et al. 1996; Osier et al. 1999; Park et al. 2001; Shen et al. 1997a; Shen et al. 1997b; Thomasson et al. 1994; Thomasson et al. 1991; Yu et al. 2002) of Asian populations; 18 studies (Borras et al. 2000; Chambers et al. 2002; Cichoz-Lach et al. 2008; Couzigou et al. 1990; Day et al. 1991; Espinos et al. 1997; Foley et al. 2004; Frenzer et al. 2002; Gilder et al. 1993; Grove et al. 1998; Kuo et al. 2008; Luo et al. 2007; Neumark et al. 1998; Pares et al. 1994; Sherman et al. 1994; Sherva et al. 2009; Vidal et al. 2004) of European populations; three studies (Konishi et al. 2003; Konishi et al. 2004; Wall et al. 2003) of Mexican Americans, and two studies (Luo et al. 2007; Montane-Jaime et al. 2006) of African Americans. Among them one study (Neumark et al. 1998) investigated heroin dependence and abuse; two studies (Luo et al. 2007) investigated multi-drug dependence; and among the 50 studies of AD (or AD and abuse) 14 studies (Borras et al. 2000; Chao et al. 1994; Chao et al. 2000; Chao et al. 1997; Cichoz-Lach et al. 2008; Couzigou et al. 1990; Day et al. 1991; Frenzer et al. 2002; Grove et al. 1998; Lee et al. 2001; Pares et al. 1994; Sherman et al. 1994; Vidal et al. 2004) investigated alcohol-related liver disease, cirrhosis, and (or) pancreatitis (five of them also included data for alcoholics without any of these diseases). The 53 studies included 6,796 cases and 6,938 controls. The results are described below.

The frequency of the risk *ADH1C* 350Val allele varied widely across different populations, based on all the samples: low in the Asian control populations 8% (0% – 20%) and patients 14% (0% – 32%); higher in the European controls 45% (24% – 59%) and patients 45% (30% – 62%); between Asian and European frequencies in the Mexican controls 35% (34% – 38%) and patients 35% (32% – 39%); and less frequent in African controls 13% (12% –

13%) and patients 16% (13% – 17%). Among the 30 Asian studies, 26 studies showed higher 350Val frequency in cases than in controls and one showed no significant difference; among the 17 European studies 10 studies showed higher frequency and two showed equal frequencies; two of three studies of Mexican Americans showed higher frequency; and all the studies of African Americans showed higher frequency in cases than in controls (Table 2).

The combined studies of AD and alcohol abuse showed that there was strong evidence of association, in particular, in the Asian populations, using both the allelic (350Val vs. 350Ile) and genotypic analyses (Table 1). The strict random effects model was applied when evidence of heterogeneity was found throughout this meta-analysis. The *P* value was 1×10^{-8} with OR of 1.51 (1.31, 1.73). Strong evidence of association was also found under the dominant (ValVal + ValIle vs. IleIle: OR = 1.65 (1.38, 1.96)) and recessive models (Table 1). Furthermore, the Asian populations showed a highly significant association, with an allelic *P* value of 4×10^{-33} (OR = 2.14 (1.89, 2.43)) with no evidence of heterogeneity between studies ($P > 0.05$). It was interesting that the dominant and recessive models produced stronger effect sizes (ORs = 2.2 (1.92, 2.53) and 3.83 (2.26, 6.49), respectively) although the underlying biological mechanism was not yet established. Strong evidence of association was also detected in the combined Asian and European populations ($P = 3 \times 10^{-8}$ and 2×10^{-7} for the allelic analysis and dominant model, respectively). The Mexican samples revealed evidence of significant association with *P* value of 0.001 (OR = 1.52 (1.18, 1.97)) under the dominant model. No evidence of significant association was found in the European populations. When the alcoholic subjects with alcoholic liver disease, cirrhosis, or pancreatitis (designated as “alcohol-related diseases” in Table 1) were combined for the analysis, strong evidence of association was found in the Asian populations. The *P* values were 0.0002 (OR = 2.12 (1.42, 3.17)) and 0.0006 for the allelic analysis and dominant model, respectively.

In order to understand whether the strong association was driven primarily by the patients with alcoholic liver disease, cirrhosis, or pancreatitis, we also analyzed the alcoholic subjects without any alcohol-related diseases. The results showed consistently strong evidence of association in the combined populations, particularly in the Asian populations, in both allelic and genotypic analyses (Table 1). For instance, the allelic *P* values for the combined populations and Asian populations were 8×10^{-8} (OR = 1.53 (1.31, 1.78)) and 6×10^{-30} (OR = 2.14 (1.87, 2.44)), respectively; and the allelic *P* value for the combined Asians and Europeans was 2×10^{-7} . They were also significant under the dominant and recessive models.

In most of the studies, the patients were explicitly described as “alcohol dependent” (these “definite” alcohol dependent patients are designated as “AD” in Table 1) while six studies had no such clear description and one study indicated that the patients included both AD and alcohol abuse subjects. Therefore, these “definite” alcohol dependent patients without any specification regarding alcohol-related diseases were also analyzed independently. The results showed no major change and the association was still significant in the combined populations, in particular, in Asians and combined Asians and Europeans, for both allelic and genotypic analyses (Table 1). For example, the allelic *P* values were 4×10^{-8} (OR = 1.66 (1.39, 1.99)) and 7×10^{-30} (OR = 2.14 (1.87, 2.44)) in all the populations and Asian populations, respectively. Evidence of significant association was also found in the studies of non-Asians (Table 1).

In terms of different phenotypes, when the samples of subjects diagnosed with heroin and other drug dependence (only three studies, and they were investigating the European and African populations) were combined with those of AD and alcohol abuse as an independent

meta-analysis, based on the hypothesis that different types of addictions may share some common genetic risks (Fu et al. 2002; True et al. 1999; Xian et al. 2008), no significant change was found in the associations. Strong evidence of association was still identified using allelic and genotypic analyses in all the combined populations, combined Asians and Europeans, and combined Mexicans and Africans, but not in Europeans. The results of overall and sub-grouped analyses are shown for both allelic and genotypic analyses in Table 1. The forest plots of the allelic analysis and dominant model are shown in Figures 1 and 2; and the plots of the recessive model are shown in supplementary Figure 1.

Other Heterogeneity Analyses

Heterogeneity Q tests were also performed to evaluate possible differences in OR between the 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 criteria (allowing the inclusion of the studies with no description of specific criteria); between the studies with specified criteria and the studies with no description of criteria; between the English-language publications and Chinese-language publications; between the studies originating in mainland China and others (identified by information provided, e.g., institutions and addresses of the authors); and between the studies originating in the United States and others. The results showed that there was no evidence of significant heterogeneity in the Asian studies regarding different diagnosis criteria, publication languages, or research sites using either allelic or genotypic analysis. The results are shown in supplementary Table 2 ($P(Q) > 0.1$).

Publication Bias Analyses

In the present study, no evidence of publication bias was found in either Asian or European populations ($P(T) > 0.05$) for either allelic or genotypic analyses. However, evidence of publication bias was found when all the populations were combined (Egger's regression $P = 0.001$ (1-tailed) for the allelic analysis and $P = 0.002$ (1-tailed) for the recessive model but not significant using Kendall's tau (Begg and Mazumdar rank correlation) (Begg and Mazumdar 1994) with $P > 0.05$). The Duval and Tweedie's trim and fill analysis showed that for the allelic analysis there might potentially be 10 missing studies, and the adjusted overall effect size was 1.28 (1.11, 1.48), which was still significant (it was not significant for the recessive model).

On the other hand, the classic fail-safe analysis suggested the association was still strong. For instance, for the allelic analysis at least 1,033 assumed non-significant studies would be required to bring the significant $P(Z)$ value to > 0.05 (814 required for the Asian studies); for the dominant model at least 949 non-significant studies would be required to bring the P value to > 0.05 (716 required for the Asian studies); and for the recessive model at least 235 non-significant studies would bring the P value to > 0.05 (138 required for the Asian studies). These findings, therefore, support that there are strong associations between the SNP and alcoholism as well as alcohol-related diseases. The funnel plots of the Asian studies of AD and alcohol abuse are shown for the allelic analysis, dominant model, and recessive model in Figure 3, and supplementary Figures 2 and 3, respectively. The plots of all the studies of AD and alcohol abuse are shown in supplementary Figures 4–6, indicating the possible changes between the observed and adjusted values of effect size.

Sensitivity and Retrospective Analyses

According to the sensitivity analyses, no individual study biased the findings to the extent that it could account for the strong observed association. For example, in the Asian populations, the results of AD and alcohol abuse were consistent, regardless of the data set

removed, with the allelic P values always between 6×10^{-34} and 3×10^{-28} . For the dominant model, the results also were consistent, regardless of the data set removed, with the P values always between 8×10^{-30} and 1×10^{-24} . Supplementary Tables 3 and 4 show the results for the allelic analysis and dominant model of the Asian populations, respectively. The results of all the combined populations are not shown, but are available on request.

The asymptote lines of the analyses in retrospect based on 20 publication years showed that the association signal of the polymorphism has tended to be stable in the Asian populations. However, the results revealed a slight decrease on the effect size in all the combined studies in recent years, which implied that more non-Asian replication studies may be still necessary. The results of the allelic analysis, dominant model, and recessive model are shown for all the studies in Figures 4, and supplementary Figures 7 and 8, respectively. Those of the Asian populations are shown in supplementary Figures 9–11, respectively. The P values of the allelic analysis, dominant model, and recessive model are shown for all the studies in supplementary Table 5–7 and for the Asian populations in supplementary Table 8–10, respectively.

Discussion

The alcohol dehydrogenases (ADH) are a group of alcohol metabolizing enzymes that occur in many organisms. Aldehyde dehydrogenase (the most important in this context is ALDH2) is the next enzyme in this metabolic pathway. The first ADH was purified in 1937 from *Saccharomyces cerevisiae* (Negelein and Wulff 1937). In humans, the ADH isozymes are encoded by at least seven genes, and genetic association studies of genes in the ADH gene cluster with alcoholism and drug dependence have a longstanding history of decades with the most studied genes being *ADH1B* and *ADH1C*. Similarly, *ALDH2* has long been studied with respect to alcohol dependence. Our recent meta-analyses confirmed strong associations of the *ADH1B* (Li et al. 2011) and *ALDH2* (Li et al. 2012) genes with alcoholism and alcohol-related medical diseases ($P = 1 \times 10^{-36}$ and $OR = 2.06$; $P = 3 \times 10^{-56}$ and $OR = 0.23$, respectively). Because the three genes are biologically related in terms of the functions of their encoded enzymes or pathways, genotype data of *ADH1C* was also analyzed. In this study, our findings strongly support that *ADH1C* 350Ile may lower the risk for AD and alcohol abuse as well as some alcohol-related diseases, particularly in Asian populations. Regarding the studies included in this meta-analysis, the prevalence of 350Ile allele varied greatly across these populations: from 40% in the Polish population to 100% in some Chinese aboriginal populations (e.g., Ami). Table 2 and Figure 5 show allele frequencies by geographic location.

Significant LD was found in the region of the gene cluster composed of the *ADH6*, *ADH1A*, *ADH1B*, *ADH1C*, and *ADH7* genes (Figure 6). The first four genes were in a strong LD structure and two of them (the *ADH1A* and *ADH1B* genes) were in the same haplotype block, which implied that the contribution of the genes that make up the cluster to the associated effect on substance use may not be independent. One study (Osier et al. 1999) claimed the *ADH1C* association might be attributable to the *ADH1C* gene being in close proximity to the *ADH1B* gene on chromosome 4q so that their genotypes are correlated. Our results showed that *ADH1C* is in another haplotype block (multiallelic $D' = 0.99$) although it is physically close to *ADH1B* (multiallelic $D' = 0.6$). It is still necessary to investigate further the polymorphisms that are in the same haplotype block with *ADH1C* (e.g., the nonsynonymous SNPs shown on the LD plots and supplementary Table 11) or the polymorphisms on close genes within this strong LD structure. The LD plots are shown for the Asian and European populations in Figure 6 and supplementary Figure 12, respectively.

Previous studies were somewhat inconsistent regarding associations with alcoholism and related traits. The discrepancies may be due to a number of reasons. Most obviously, these include type II error and low power due to sample size limitations for some studies. Second, most subjects were diagnosed according to the ICD(World Health Organization) or DSM(American Psychiatric Association) system. However, ICD-10 criteria for AD have been shown to be more stringent than DSM-IV criteria, which in turn are more stringent than DSM-III-R(Schuckit et al. 1994). Therefore, different studies differ in their diagnostic criteria, and selection of more severely affected subjects could potentially increase the observed effect sizes. Third, different recruiting strategies could result in differing results (e.g., recruitment based on clinical treatment samples vs. that based on general population samples). Fourth, studies using only males or females may yield different results than studies using mixed sex samples, especially when this affects sex matching between cases or controls. Fifth, the genetic effects of *ADH1C* 350Ile may change over the course of lifetime alcohol use. For example, it may be a protective factor at one stage, but become a neutral or even a risk factor at another stage(Lenroot and Giedd 2008). Sixth, potential cultural differences in patterns of alcohol consumption (and potentially in reporting or diagnosing) constitute one easily identifiable set of environmental influences on this trait, and can also contribute to discrepancies by interacting with the effect of variation at the gene.

The present meta-analysis identified much stronger evidence for association than previous meta-analyses, as shown below. One study(Whitfield 1997) only included five studies of alcoholism, and the latest dataset was published in 1995. Another study(Zintzaras et al. 2006), in which the latest dataset was published in 2004, (i) included 24 studies, compared to 53 studies in the present meta-analysis; (ii) only included English-language literature; and (iii) did not consider association with alcoholic liver diseases. Compared with the previous meta-analyses, our study included the largest sample size up to 2010 from 49 English and four Chinese publications (it was important to include Chinese-language publications as well); investigated both AD and alcohol abuse as well as drug dependence; applied both strict and extended criteria; performed both allelic and genotypic analyses under the strict random effects model; and applied a comprehensive and systematic analysis procedure, as shown in the results, to study additional questions not answered in those previous meta-analyses. Our meta-analysis found highly significant evidence of associations with AD and alcohol abuse as well as alcohol-related diseases, in particular, in Asians. 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.

The Val350Val homozygote could not be observed or showed extremely low frequency in Asian and African American samples (but not in Europeans or Native Americans), thus, the analysis under the recessive model (ValVal vs. ValIle + IleIle) tended to produce a wider CI compared to that under the dominant model. On the other hand, despite the stringent criteria that were applied in the selection of studies, the samples in some studies might not be entirely random because the participants might have been screened for certain alcohol-related medical conditions. In addition, Hardy-Weinberg disequilibrium in patients or controls may support that the gene is related to AD, however, disequilibrium in controls may also reflect genotyping error and thus potentially reduce the power of the analysis (however, of the control samples that were not in equilibrium, either the samples were small or the disequilibrium was not highly significant considering our strong findings).

Future studies should, first, strive to examine joint and interactive effects of the genetic markers because interaction effects could account for some of the inconsistent findings. Second, future investigations should also ideally consider longitudinal or prospective studies. Such studies can improve the understanding of the genetic mechanism and how the effects will change over the course of lifetime substance use. Third, most inherited AD

involves the interaction of multiple genes that have minor effects and sociocultural factors. For example, increased cultural acceptance of alcohol consumption has been shown to reduce the protective effect of the *ALDH2* 350Val allele (Higuchi et al. 1994); thus, gene-environment interactions should also be considered in the future studies. Fourth, to identify genes of minor effect, alcohol dependent subjects with either heterozygous Ile350Val or homozygous Val350Val, could be selected based on their reduced heterogeneity. Fifth, quantitative tests may be of value. In addition, future studies should use older control samples that can be more accurately categorized, as younger individuals may not have fully transited the age of risk for alcohol dependence.

In conclusion, this meta-analysis combined the cumulative data of *ADH1C* Ile350Val with AD and alcohol abuse as well as alcohol-related medical diseases based on all 53 identified English and Chinese-language studies within the past 20 years. Strong evidence of association was found in the combined populations, in particular, in the Asian populations, using both allelic and genotypic analyses. The association remained strong using stricter and extended criteria as well as in various sub-group analyses. The findings strongly support that *ADH1C* 350Ile lowers the risk for AD and alcohol abuse as well as some alcohol-related diseases; and provide further evidence for the involvement of the human *ADH1C* gene in the pathogenesis of AD and alcohol-related diseases.

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. Three studies, including Chen 1997 (Atayal), Chen 1997 (Ami), and Chen 1997 (Paiwan), are not shown on the forest plots because the scale of the wide CIs can not fit into the current version of the plot.

*, alcoholic patients without alcoholic liver disease, cirrhosis or pancreatitis.

Figure 2.

Forest plots of $\ln(\text{OR})$ with 95% CI for the dominant model ((ValVal + ValIle) vs. IleIle). Three studies, including Chen 1997 (Atayal), Chen 1997 (Ami), and Chen 1997 (Paiwan), are not shown on the forest plots because the scale of the wide CIs can not fit into the current version of the plot.

*, the alcoholic patients without alcoholic liver disease, cirrhosis or pancreatitis.

Figure 3.

Egger's funnel plots of publication bias analysis for the allelic analysis of AD and alcohol abuse in Asians. 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.

ADH1C Allelic Analysis

Figure 4. Retrospective analysis for the allelic analysis. Analysis in retrospect was based on publication year since 1990.

Figure 5.

Allele frequencies among different populations. Blue and red represent Ile350 and 350Val, respectively. Upper graphs are based on the patients and , lower graphs on controls. The geographical borders(Miyazaki et al. 1993) of Taiwan aboriginals were from a previous study.

Figure 6.

Graphical representation of the LD structure of the *ADH1C* gene for the Asian populations. The LD structure, spanning 233 kb, was constructed using the Asian genotype data of 232 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 representing increasing D' between the SNPs. The genes from left to right are *ADH6*, *ADH1A*, *ADH1B*, *ADH1C* and *ADH7*. The *ADH1C* gene, and *ADH1C*Ile350Val are shown in red; the selected *ADH1C* nonsynonymous SNPs are shown in blue; and the other genes are in black.

Table 1

Results of the overall and sub-grouped studies

Groups	N*	OR (95% CI)	P(Z)	P(Q)	OR (95% CI)	P(Z)	P(Q)	OR (95% CI)	P(Z)	P(Q)
		Allelic Analysis Val vs. Ile			Dominant Model (ValVal + ValIle) vs. IleIle			Recessive Model ValVal vs. (ValIle + IleIle)		
Alcoholics ^a	50	1.51 (1.31,1.73)	1×10⁻⁸	6×10 ⁻¹⁷	1.65 (1.38,1.96)	2×10 ⁻⁸	7×10 ⁻¹¹	1.43 (1.11,1.86)	0.0067	0.0007
Alcoholics ^a (Asian)	30	2.14 (1.89,2.43)	4×10⁻³³	0.2145	2.2 (1.92,2.53)	7×10⁻²⁹	0.1597	3.83 (2.26,6.49)	6×10⁻⁷	0.9481
Alcoholics ^a (European)	16	1.04 (0.89,1.22)	0.6358	3×10 ⁻⁵	1.04 (0.79,1.36)	0.7968	1×10 ⁻⁴	1.21 (0.87,1.69)	0.2579	0.0001
Alcoholics ^a (Mexican)	3	1.22 (1.02,1.47)	0.0328	0.9613	1.52 (1.18,1.97)	0.0012	0.6814	0.92 (0.64,1.31)	0.6440	0.3034
Alcoholics ^a (Asian & European)	46	1.56 (1.33,1.83)	3×10⁻⁸	4×10 ⁻¹⁸	1.69 (1.39,2.05)	2×10⁻⁷	1×10 ⁻¹¹	1.6 (1.19,2.16)	0.0020	0.0007
Alcohol-related diseases ^b	14	1.2 (0.91,1.57)	0.1966	7×10 ⁻⁶	1.08 (0.78,1.5)	0.6381	0.0015	1.52 (0.91,2.54)	0.1104	0.0009
Alcohol-related diseases ^b (Asian)	4	2.12 (1.42,3.17)	0.0002	0.3004	2.15 (1.39,3.31)	0.0006	0.3041	2.82 (0.59,13.38)	0.1921	0.7982
Alcohol-related diseases ^b (European)	10	1.02 (0.77,1.35)	0.8956	1×10 ⁻⁴	0.84 (0.68,1.04)	0.1125	0.0822	1.42 (0.8,2.49)	0.2281	0.0001
Cirrhosis	8	1.48 (1.03,2.12)	0.0320	0.0031	1.34 (0.84,2.15)	0.2202	0.0103	2.1 (1.03,4.27)	0.0407	0.0140
Cirrhosis (Asian)	3	2.15 (1.31,3.53)	0.0025	0.1609	2.09 (1.22,3.58)	0.0075	0.1652	4.15 (0.54,31.87)	0.1709	0.7121
Cirrhosis (European)	5	1.27 (0.87,1.85)	0.2191	0.0136	0.92 (0.68,1.24)	0.5895	0.090	1.95 (0.87,4.34)	0.1041	0.0027
Alcoholic liver disease (European)	3	1.16 (0.91,1.47)	0.2298	0.6717	0.93 (0.65,1.33)	0.6766	0.2863	1.95 (1.21,3.15)	0.0062	0.8942
Alcoholics ^c	42	1.53 (1.31,1.78)	8×10⁻⁸	4×10 ⁻¹⁴	1.69 (1.4,2.05)	7×10⁻⁸	4×10 ⁻⁹	1.39 (1.08,1.8)	0.0119	0.0302
Alcoholics ^c (Asian)	27	2.14 (1.87,2.44)	6×10⁻³⁰	0.1525	2.2 (1.9,2.54)	5×10⁻²⁶	0.1097	4.01 (2.29,7.01)	1×10⁻⁶	0.8841
Alcoholics ^c (European)	11	1.02 (0.86,1.21)	0.8075	0.0039	1.03 (0.72,1.47)	0.8616	0.0002	1.11 (0.93,1.33)	0.2627	0.0978
Alcoholics ^c (Asian& European)	38	1.6 (1.34,1.91)	2×10⁻⁷	3×10 ⁻¹⁵	1.74 (1.4,2.17)	8×10⁻⁷	8×10 ⁻¹⁰	1.61 (1.19,2.2)	0.0023	0.0316
AD ^d	36	1.66 (1.39,1.99)	4×10⁻⁸	1×10 ⁻¹³	1.86 (1.54,2.26)	2×10⁻¹⁰	2×10 ⁻⁵	1.47 (1.04,2.09)	0.0312	0.0350
AD ^d (Asian)	26	2.14 (1.87,2.44)	7×10⁻³⁰	0.1229	2.2 (1.9,2.54)	6×10⁻²⁶	0.0866	4.01 (2.29,7.01)	1×10⁻⁶	0.8841
AD ^d (European)	6	0.98 (0.77,1.23)	0.8396	0.0170	1.1 (0.91,1.33)	0.3255	0.0662	0.99 (0.78,1.26)	0.9595	0.1665
AD ^d (Asian & European)	32	1.78 (1.44,2.2)	1×10⁻⁷	1×10 ⁻¹⁴	1.97 (1.57,2.47)	4×10⁻⁹	7×10 ⁻⁶	2.05 (1.25,3.34)	0.0042	0.0324
AD ^d (non-Asian)	10	1.06 (0.9,1.24)	0.4776	0.0299	1.23 (1.06,1.43)	0.0073	0.0858	0.97 (0.8,1.18)	0.7826	0.3029
All the studies	53	1.47 (1.29,1.68)	1×10⁻⁸	2×10 ⁻¹⁶	1.61 (1.37,1.9)	9×10⁻⁹	2×10 ⁻¹⁰	1.36 (1.07,1.73)	0.0129	0.0008
European	18	1.04 (0.9,1.2)	0.5759	1×10 ⁻⁴	1.05 (0.83,1.33)	0.6707	0.0003	1.16 (0.87,1.56)	0.3064	0.0003

Groups	N*	OR (95% CI) Allelic Analysis Val vs. Ile	P(Z)	P(Q)	OR (95% CI) Dominant Model (ValVal + ValIle) vs. IleIle	P(Z)	P(Q)	OR (95% CI) Recessive Model ValVal vs. (ValIle + IleIle)	P(Z)	P(Q)
Asian & European	48	1.52 (1.31,1.76)	3×10⁻⁸	6×10 ⁻¹⁸	1.65 (1.37,1.98)	1×10⁻⁷	2×10 ⁻¹¹	1.49 (1.13,1.96)	0.0042	0.0007
Mexican & African	5	1.23 (1.03,1.46)	0.0235	0.9963	1.49 (1.18,1.89)	0.0008	0.8306	0.92 (0.64,1.31)	0.633	0.4236

* number of studies included in the analyses.

^a alcoholic patients with and without alcohol liver disease, cirrhosis or pancreatitis (only one study described the patients included both alcohol dependent and abuse subjects).

^b alcoholics with alcoholic liver disease, cirrhosis or pancreatitis.

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

^d “definite” alcohol dependence patients without alcoholic liver disease, cirrhosis or pancreatitis (i.e., including only patients clearly described as alcohol dependent).

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.

Table 2

350Val allele frequencies in different populations

Populations	Cases	Controls
Asians		
Ami	2.2%	0.0%
Paiwan	0.0%	1.4%
Atayal	1.3%	1.7%
Japanese	12.0%	5.8%
Bunun	5.9%	6.0%
Taiwanese Chinese	12.6%	6.6%
Chinese	15.2%	7.5%
Korean	13.0%	7.9%
Han Chinese	16.1%	9.1%
Mongolian	19.4%	10.0%
Elunchun	32.3%	13.5%
East Indian Trinidadian	30.5%	19.8%
Europeans		
New Zealand Maori	40.0%	23.9%
Jewish	34.9%	31.5%
Spanish	40.8%	40.0%
European, European American, or White	41.5%	40.7%
French	43.5%	42.3%
Australian	53.4%	43.7%
UK or Irish	48.2%	49.6%
Polish	36.2%	59.3%
Native Americans		
Native or Mexican American	37.6%	33.2%
Africans		
African American	15.7%	12.6%

Allele frequencies were based on all the 53 studies. For the categories of European, European American, "White", Chinese, and Taiwanese: no specific geographic origins were described or the subjects were mixed.