# Functional polymorphism of *CYP2E1* gene and alcohol use disorders in a Tibetan population

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**Abstract**: **Objective** To investigate the relationship between the *CYP2E1* * *c1*/* *c2* polymorphism and alcohol use disorders, and the potential influence of the *CYP2E1* * *c1*/* *c2* polymorphism on the severity and dimensions of alcohol use disorders in Tibetan. **Methods** Three hundred and forty Tibetans with Alcohol Use Disorders Identification Test (AUDIT) score ≥10 and another 315 matched control subjects with AUDIT score ≤5 were enrolled. The *CYP2E1* * *c1*/* *c2* polymorphism was determined by the standard PCR-RFLP method. **Results** The frequency of the *CYP2E1* * *c2* allele in subjects with alcohol use disorders (16.2%) was significantly higher than that of the controls (10.8%), with a *P* value of 0.005 and *OR* value of 1.60 (95% CI: 1.15~2.21). There was also a significant difference in genotype frequencies between the 2 groups ($\chi^2 = 8.75$, $P = 0.01$). Subjects with alcohol use disorders had higher frequencies of genotypes with at least one copy of allele *c2* (28.5% vs. 18.7%; $\chi^2 = 8.65$, $P = 0.003$; $OR = 1.73$) than the control group. The association of *CYP2E1* * *c2* allele with alcohol use disorders was much stronger in males than in females, with a male *OR* value of 2.30. *CYP2E1* * *c2* allele was associated with increased alcohol consumption and alcohol use disorders in males. **Conclusion** There is the positive association among *CYP2E1* * *c2* allele, alcohol use disorders, and the amount of alcohol consumption in Tibetan population.

**Key words**: alcohol use disorders; *CYP2E1* gene; Tibetan

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**Biography** GUO Wan-jun(1973-), male, Ph. D. student, attending psychiatrist, mainly engaged in molecular genetics of mental disorders and clinical mental health service in general hospital.

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# 西藏藏族人群 *CYP2E1* 基因 * *c1*/ * *c2* 功能多态性与酒精使用障碍之间的关联分析

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[摘要] 目的：用病例对照研究的方法，对拉萨郊县藏族人群中 *CYP2E1* 基因的 * c1/ * c2 多态性与酒精使用障碍之间进行关联分析，以期发现他们之间的相关性以及该基因多态性对酒精使用障碍严重程度及相关因子的影响。方法：使用聚合酶链反应-限制性片段长度多态性（PCR-RFLP）方法对 655 份 DNA 标本的 *CYP2E1* * *c1*/ * *c2* 多态性进行基因分型。其中 340 份 DNA 来自酒精使用障碍鉴别测试（AUDIT）总分≥10 的酒精使用障碍被试，另 315 份 DNA 来自 AUDIT≤5 的正常对照被试。将基因分型结果与酒精使用障碍进行关联分析。结果：不论在病例组还是对照组，该等位基因频率的分布均符合 Hardy-Weinberg 平衡定律。病例组 *CYP2E1* * *c2* 等位基因的频率（16.2%）明显高于对照组（10.8%），其 *P* 值为 0.005，*OR* 值为 1.60（95% 可信区间为 1.15～2.21）。病例组带有 * *c2* 等位基因的基因型（ * *c1* * */c2* + * *c2* * */c2*）频率明显比对照组高（28.5% vs. 18.7%；$\chi^2 = 8.65$，$P = 0.003$；$OR = 1.73$）；在男性中，这样的差异性更显著，其 *OR* 值高达 2.30；但在女性中，差异却无统计学意义。男性病例组中的 * *c2* 等位基因能使 AUDIT 及酒精依赖严重程度问卷（SADQ）中描述个体饮酒量的因子得分增加。结论：在该藏族人群中，特别是男性中，*CYP2E1* * *c2* 等位基因与酒精使用障碍及饮酒量的增加均有关联。

[关键词] 酒精使用障碍； *CYP2E1* 基因； 藏族
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Family, twin, and adoption studies have shown that genetic factors are implicated in the etiology of alcohol use disorders. It has been estimated that genes may account for up to 50% ~ 60% of variance in the population [1-2]. Multiple genes and/or interaction between genes and psycho-socio-cultural factors contribute to the overall liability to develop the disorders. Furthermore, studies suggest that genetic factors may also influence the patterns of alcohol use, such as the quantity of alcohol consumed on a typical drinking occasion, drinking frequency, the frequency, and severity of intoxication [1,3-4].

Association analysis of candidate genes is one of the most robust strategies in the genetic study of alcohol use disorders and related phenotypic traits, as the enzymes which process alcohol in the body are well understood. For example, the protective effect of *ALDH2* genotype against alcohol abuse is well established [3]. Many other candidate genes from the alcohol metabolism pathway (e.g. alcohol dehydrogenase, ADH; cytochrome P4502E1, CYP2E1) [3,5-9], genes from the dopaminergic (e.g. *DRD2*, *DRD3*, *DRD4*, *DAT1* genes) and GABAergic systems (e.g. *GABAA* and *GABAB* receptor gene cluster) [3,10-12] have been extensively studied. Some of findings, such as those of alcohol-metabolizing enzymes, are promising and in need of replication [3,5-9,13-16].

Alcohol metabolism is one of the biochemical determinants that can significantly influence drinking behavior, the development of alcoholism, and alcohol-induced organ damage. Several enzymes are involved in the process of alcohol metabolism. ADH oxidizes most alcohol to acetaldehyde in the cytoplasm of hepatic cells, and then aldehyde dehydrogenase-2 (ALDH2), which is mainly located in mitochondria of hepatic cells, will convert acetaldehyde to acetate by its oxidization [17-18]. In addition to the ADH metabolism pathway of ethanol, cytochrome P4502E1 (CYP2E1)-catalyzed metabolism also has an important role in oxidizing alcohol to acetaldehyde, which is induced by alcohol and is responsible for 10% of ethanol metabolism after ingestion of moderate alcohol doses [19-20]. The capacity to oxidize ethanol is elevated up to 10-fold in heavy consumers [19-21]. During the CYP2E1 metabolism process, oxygen-derived free radicals is released as a by-product and thus causes oxidative stress to cells[22-24]. The *CYP2E1* genes have also been studied as important candidate genes for alcohol related liver diseases as acetaldehyde and oxygen-derived free radicals from alcohol metabolism may injure liver cells [5,7-9,25-27].

It has been reported that the *CYP2E1* * *c1*/* *c2* polymorphism, located in the 5'-transcription regulatory region of the gene, can significantly alter the activity of the CYP2E1 enzyme. The wild type allele (*c1*) is associated with lower expression than the mutant allele (*c2*). Thus the *c2*/*c2* genotype, which lacks the *Rsa*I restriction site, is associated with an up to 10-fold higher transcriptional activity than the *c1*/*c1* genotype [5,7,22-24]. Several studies have reported an association between the *CYP2E1* * *c1*/* *c2* polymorphism and alcohol consumption or related mental disorders but results from other studies have been inconsistent [5-9,13-14,25,27-32]. Studies have also reported that the *CYP2E1* * *c2* allele is associated with an increased risk of alcohol related liver diseases and other alcohol related diseases [24,26,33-37], although other studies failed to replicate the findings [24,27,29-30,32,37-38].

In the present study, we used a sample collected during an epidemiological study of alcohol use disorders in a Tibetan population in China, to perform a case-control study investigating the relationship between the *CYP2E1* * *c1*/* *c2* polymorphism and alcohol use disorders, and the potential influence of *CYP2E1* * *c1*/* *c2* polymorphism on the severity and dimensions of alcohol use disorders in this population.

# 1 SUBJECTS AND METHODS

**1.1 Subjects** We performed an epidemiological survey in 3 171 individuals from 4 suburb counties of Lasa city in Tibet using the Chinese Interview Version of Alcohol Use Disorders Identification Test (AUDIT) [39]. During the survey, 340 individuals (193 males and 147 females) with an AUDIT score ≥10 (the cut-off point for diagnosing alcohol use disorders) and 315 (172 males and 143 females) controls with the AUDIT score ≤5 were invited to participate in the genetic study. Their average age was 48.06±11.90 for the alcohol use disorder group with a range from 17 to 77 years and 47.09±12.23 in the control group with a range from 16 to 84 years.

In current study, the 10-item AUDIT was used to assess 3 aspects related to alcohol drinking which include Factor-I (alcohol intake), Factor-II (alcohol dependence) and Factor-III (alcohol-related problems)[40]. We also used the Severity of Alcohol Dependence Questionnaire (SADQ) [41-42] to assess the severity of alcohol use disorders and to measure 5 aspects (dimensions) of alcohol dependence syndrome including Dimension-I (physical withdrawal symptoms), Dimension-II (affective withdrawal symptoms), Dimension-III (craving and withdrawal-relief drinking), Dimension-IV (typical daily consumption) and Dimension-V (reinstatement of withdrawal symptoms after a period of abstinence), and a companion scale, the Impaired Control Scale (ICQ), to measure the extent to which a client perceives themselves to be out of control with regard to their alcohol use. Genomic DNA for each subject was extracted from buccal swabs. All participants gave written informed consent before the investigation.

**1.2 Genotyping** The functional polymorphism in the *CYP2E1* gene was determined by PCR-RFLP method with the oligonucleotides 5′- CCA GTC GAG TCT ACA TTG TCA -3′ and 5′- TTC ATT CTG TCT TCT AAC TGG -3′ as described [5]. The PCR amplification was performed by initial denaturation at 94 ℃ for 5 min, following by 35 cycles of 94 ℃ for 30 s, 55 ℃ for 30 s and 72 ℃ for 60 s, with a final extension at 72 ℃ for 6 min. The 413 bp PCR products were digested with *Rsa* I with the *c1* allele cut into 352 bp and 61 bp fragments and the *c2* allele uncut. The digested fragments were separated in 3% agarose gel and visualized with ethidium-bromide staining.

**1.3 Analysis of data** All genotype data were tested for Hardy-Weinberg disequilibrium by chi-squared analysis. Comparison of allele and genotype frequencies between cases and controls was carried out using the Chi-squared test with program UNPHASED (allele-wise) or performed using SPSS11.0 (Statistical Package for the Social Sciences) (genotype-wise). We also used the scores of the AUDIT and the SADQ as quantitative traits to estimate the allelic (by program UNPHASED) and genotypic (Student T test) effects of *CYP2E1* * *c1*/* *c2* polymorphism on the severity and dimensions of alcohol use disorders. We set the significant difference at 0.05.

# 2 RESULTS

**2.1 Comparison of allelic and genotypic frequencies of *CYP2E1* * *c1*/* *c2* polymorphism between cases and controls** The allele and genotype frequencies of the *CYP2E1* * *c1*/* *c2* polymorphism are shown in Table 1. The Hardy-Weinberg disequilibrium (HWE) test confirmed that the genotype distribution of the polymorphism was in HWE in both case and control groups. The frequency of the allele *c2* in the *CYP2E1* * *c1*/* *c2* polymorphism in subjects with alcohol use disorders (16.2%) was significant higher than controls (10.8%), with a $P$ value of 0.005 and $OR$ value of 1.60 (95% CI: 1.15 ~ 2.21). There was also significant difference in genotype frequencies between two groups ($\chi^2 = 8.75$, $P = 0.01$). Subjects with alcohol use disorders had a higher frequency of genotypes with at least one copy of allele *c2* (28.5% vs. 18.7%; $\chi^2 = 8.65$, $P = 0.003$; $OR = 1.73$) than the control group.

Previous studies showed that males have a higher prevalence of alcohol use disorders than females[39,43-45]. We, thus, assumed that gender might modify the effect (odds ratio) of the haplotype/allele if this is one of risk factors. By using gender as effect modifier in the program UNPHASED, we found that males with the allele *c2* in the *CYP2E1* * *c1*/* *c2* polymorphism had a much higher risk for alcohol abuse disorders than females ($\chi^2 = 6.73$, $P = 0.01$; $OR = 2.30$, 95% CI: 1.23 ~ 4.32). We subsequently compared the allele and genotype frequencies of the *CYP2E1* * *c1*/* *c2* polymorphism between cases and controls in males and females respectively. The frequency of the *CYP2E1* * *c2* allele in males with alcohol use disorders (17.1%) was significantly higher than the male controls (7.8%) with a $P$ value of 0.0001 and $OR$ value of 2.42 (95% CI: 1.51 ~ 3.89), and the frequencies of genotypes were also significantly different between male cases and controls ($\chi^2 = 12.18$, $P = 0.001$). Genotype frequencies with at least one copy of the *c2* allele in males with alcohol use disorders (29.0%) was significantly higher than male controls (14.6%) ($\chi^2 = 11.04$, $P = 0.001$; $OR = 2.40$). However, there was no significant difference in either allele or genotype frequencies between females with alcohol use disorders and female controls (Table 1).

**2.2 Influence of *CYP2E1* * *c1*/* *c2* polymorphism on the severity and dimensions of alcohol use disorders** The results of the allelic and genotypic effects of *CYP2E1* * *c1*/* *c2* polymorphism on the severity and dimensions of alcohol use disorders are shown in Table 2. In general, there were no significant effects of *CYP2E1* * *c1*/* *c2* polymorphism (allele-wise and genotype-wise) on the scores of the total AUDIT and its 3 factors, the scores of total SADQ, and its 5 dimensions in the overall samples. However, after the samples were divided into male and female groups, we found that, in male group only, the allele *c2* was associated with the increased scores of Factor-I of the AUDIT (alcohol intake) ($\chi^2 = 4.67$, $P = 0.03$) and Dimension-IV of the SADQ (typical daily consumption)

($\chi^2 = 3.65$, $P = 0.05$). Male subjects possessing a genotype with at least one copy of the *c2* allele (i.e. *c1/c1 + c1/c2*) had significantly higher scores on Factor-I of the AUDIT ($t = -2.06$, $P = 0.04$) and Dimension-IV of the SADQ ($t = -2.40$, $P = 0.02$) than male subjects without a copy of c2 allele (Table 2, Figure 1). There were no such effects of the *CYP2E1 * c1/ * c2* polymorphism in the female group.

**Tab. 1 *CYP2E1 c1/c2* genotype and allele frequency**

| Genetic Polymorphism | | AUDIT≥10 Frequencies (%) | AUDIT≤5 Frequencies (%) | $\chi^2$ | *P* | *OR*(95% CI) |
|---|---|---|---|---|---|---|
| Total | | | | | | |
| Allele | *c1* | 570 (83.8) | 562 (89.2) | 8.07 | 0.005 | 1.60 |
| | *c 2* | 110 (16.2) | 68 (10.8) | | (1.15~2.21) | |
| Genotype | *c1/c1* | 243 (71.5) | 256 (81.3) | 8.75 | 0.01 | |
| | *c1/c2* | 84 (24.7) | 50 (15.9) | (8.65)* | (0.003)* | 1.73* |
| | *c2/c2* | 13 (3.8) | 9 (2.9) | | (1.20~2.50) | |
| Females | | | | | | |
| Allele | *c1* | 250 (85.0) | 245 (85.7) | 0.05 | 0.83 | 1.052 |
| | *c2* | 44 (15.0) | 41 (14.3) | | (0.66~1.67) | |
| Genotype | *c1/c1* | 106 (71.5) | 109 (76.2) | 3.45 | 0.18 | |
| | *c1/c2* | 38 (25.9) | 27 (18.9) | (0.64)* | (0.43)* | 1.24* |
| | *c2/c2* | 3 (2.0) | 7 (4.9) | | (0.73~2.10) | |
| Males | | | | | | |
| Allele | *c1* | 320 (82.9) | 317 (92.2) | 14.00 | 0.0001 | 2.42 |
| | *c2* | 66 (17.1) | 27 (7.8) | | (1.51~3.89) | |
| Genotype | *c1/c1* | 137 (71.0) | 147 (85.5) | 12.18 | 0.001 | |
| | *c1/c2* | 46 (23.8) | 23 (13.4) | (11.04)* | (0.001)* | 2.40* |
| | *c2/c2* | 10 (5.2) | 2 (1.2) | | (1.42~4.07) | |

* Comparison of genotype frequencies between cases and controls after combining genotypes with at least one copy of *CYP2E1 * c2* (i.e. *c1/c2* and *c2/c2*).

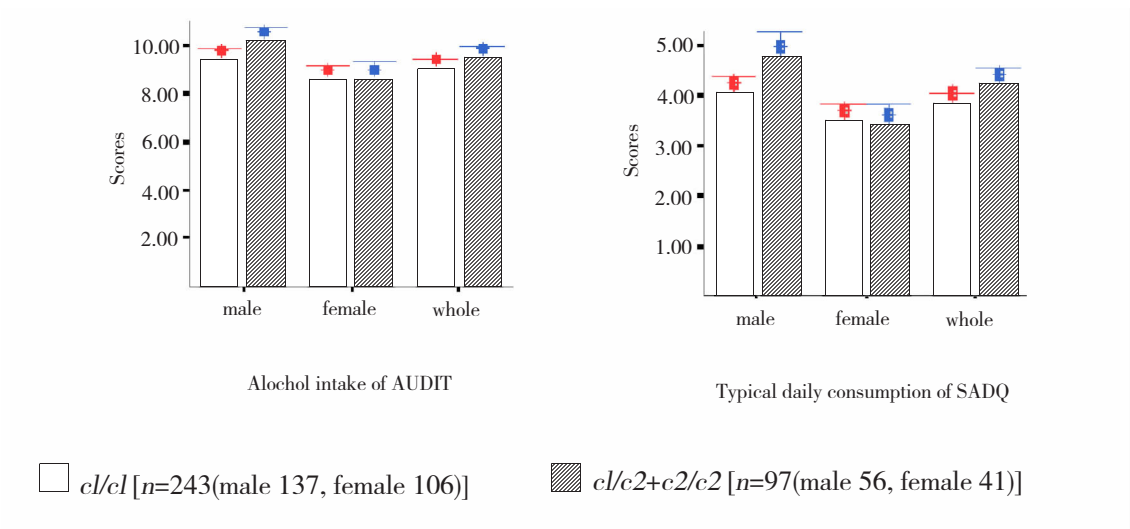


**Fig. 1 Influence of *CYP2E1 * c1/ * c2* polymorphism on the alcohol consumption factor (dimension) of AUDIT and SADQ.**

**Tab. 2 Effects of *CYP2E1* * *c1*/ * *c2* on the severity and dimensions of alcohol use disorders**

| Scores | Genotype-/ Allele-wise | Total sample ($n=340$) | | | Female ($n=193$) | | | Male ($n=147$) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $t/\chi^{2\#}$ | $P$ | $\bar{x}\pm s$ | $t/\chi^{2*}$ | $P$ | $\bar{x}\pm s$ | $t/\chi^{2\#}$ | $P$ | $\bar{x}\pm s$ |
| AUDIT/ | Genotype-wise | −0.51 | 0.61 | | 0.10 | 0.92 | | −0.68 | 0.50 | |
| Total | *c1/c1* | | | 17.47 ±5.60 | | | 16.07 ±5.22 | | | 18.55 ±5.67 |
| | *c1/c2* + *c2/c2* | | | 17.81 ±5.00 | | | 15.98 ±4.41 | | | 19.14 ±5.01 |
| | Allele-wise | 0.06 | 0.81 | | 0.02 | 0.90 | | 0.03 | 0.86 | |
| AUDIT/ | Genotype-wise | −1.49 | 0.14 | | 0.08 | 0.94 | | −2.06 | 0.04 | |
| I | *c1/c1* | | | 9.13 ±2.46 | | | 8.67 ±2.51 | | | 9.49 ±2.36 |
| | *c1/c2* + *c2/c2* | | | 9.57 ±2.37 | | | 8.63 ±2.23 | | | 10.25 ±2.24 |
| | Allele-wise | 2.70 | 0.11 | | 0.02 | 0.89 | | 4.67 | 0.03 | |
| AUDIT/ | Genotype-wise | 0.20 | 0.84 | | −0.03 | 0.98 | | 0.32 | 0.75 | |
| II | *c1/c1* | | | 4.07 ±2.83 | | | 3.50 ±2.48 | | | 4.50 ±3.01 |
| | *c1/c2* + *c2/c2* | | | 4.00 ±2.55 | | | 3.51 ±2.29 | | | 4.36 ±2.68 |
| | Allele-wise | 0.07 | 0.79 | | 0.07 | 0.80 | | 0.46 | 0.50 | |
| AUDIT/ | Genotype-wise | 0.09 | 0.93 | | 0.12 | 0.91 | | 0.05 | 0.96 | |
| III | *c1/c1* | | | 4.27 ±3.14 | | | 3.90 ±3.23 | | | 4.56 ±3.04 |
| | *c1/c2* + *c2/c2* | | | 4.24 ±3.19 | | | 3.83 ±2.97 | | | 4.54 ±3.34 |
| | Allele-wise | 0.35 | 0.55 | | 0.09 | 0.77 | | 0.41 | 0.52 | |
| SADQ/ | Genotype-wise | −0.65 | 0.51 | | −0.38 | 0.71 | | −0.51 | 0.61 | |
| Total | *c1/c1* | | | 10.90 ±8.31 | | | 10.09 ±7.57 | | | 11.53 ±8.82 |
| | *c1/c2* + *c2/c2* | | | 11.53 ±7.23 | | | 10.58 ±6.57 | | | 12.21 ±7.66 |
| | Allele-wise | 0.20 | 0.66 | | 0.0002 | 0.99 | | 0.22 | 0.64 | |
| SADQ/ | Genotype-wise | −0.37 | 0.71 | | 0.99 | 0.33 | | −1.33 | 0.19 | |
| ICQ | *c1/c1* | | | 7.03 ±3.04 | | | 6.41 ±3.12 | | | 7.51 ±2.90 |
| | *c1/c2* + *c2/c2* | | | 7.16 ±2.85 | | | 5.88 ±2.57 | | | 8.11 ±2.69 |
| | Allele-wise | 0.26 | 0.61 | | 0.75 | 0.39 | | 1.45 | 0.23 | |
| SADQ/ | Genotype-wise | −0.38 | 0.71 | | −1.17 | 0.24 | | 0.53 | 0.60 | |
| I | *c1/c1* | | | 1.89 ±2.69 | | | 1.75 ±2.59 | | | 1.99 ±2.76 |
| | *c1/c2* + *c2/c2* | | | 2.01 ±2.72 | | | 2.34 ±3.06 | | | 1.77 ±2.43 |
| | Allele-wise | 0.14 | 0.71 | | 0.51 | 0.47 | | 0.02 | 0.90 | |
| SADQ/ | Genotype-wise | 0.51 | 0.61 | | −0.52 | 0.60 | | 1.09 | 0.28 | |
| II | *c1/c1* | | | 0.79 ±1.51 | | | 0.90 ±1.44 | | | 0.71 ±1.57 |
| | *c1/c2* + *c2/c2* | | | 0.70 ±1.49 | | | 1.05 ±1.66 | | | 0.45 ±1.32 |
| | Allele-wise | 1.12 | 0.29 | | 0.03 | 0.85 | | 2.25 | 0.13 | |
| SADQ/ | Genotype-wise | −0.14 | 0.89 | | 1.24 | 0.22 | | −0.81 | 0.42 | |
| III | *c1/c1* | | | 1.92 ±2.94 | | | 1.53 ±2.66 | | | 2.22 ±3.12 |
| | *c1/c2* + *c2/c2* | | | 1.97 ±2.77 | | | 1.07 ±1.70 | | | 2.63 ±3.20 |
| | Allele-wise | 0.003 | 0.95 | | 1.55 | 0.21 | | 0.28 | 0.59 | |
| SADQ/ | Genotype-wise | −1.75 | 0.08 | | 0.39 | 0.70 | | −2.40 | 0.02 | |
| IV | *c1/c1* | | | 3.81 ±1.90 | | | 3.50 ±1.66 | | | 4.05 ±2.05 |
| | *c1/c2* + *c2/c2* | | | 4.27 ±1.78 | | | 3.39 ±1.43 | | | 4.80 ±1.78 |
| | Allele-wise | 0.28 | 0.13 | | 0.15 | 0.70 | | 3.66 | 0.05 | |
| SADQ/ | Genotype-wise | −0.52 | 0.60 | | −0.76 | 0.45 | | −0.03 | 0.98 | |
| V | *c1/c1* | | | 2.48 ±2.63 | | | 2.38 ±2.53 | | | 2.56 ±2.71 |
| | *c1/c2* + *c2/c2* | | | 2.64 ±2.23 | | | 2.73 ±2.40 | | | 2.57 ±2.12 |
| | Allele-wise | 0.32 | 0.58 | | 0.19 | 0.66 | | 0.13 | 0.72 | |

#: $t$ value for genotype-wise, $\chi^2$ for allele-wise analysis.

## 3 Discussion

The *c2* allele of the *CYP2E1 * c1/ * c2* polymorphism is associated with higher activities of the CYP2E1 enzyme, which is linked to ethanol metabolism [5,7,22-24]. In the present study, we found that the frequency of *CYP2E1 * c2* allele (13.6%) in the Tibetan population was lower than that of other East Asian populations (18.7% ~ 24.0%)[5,8-9,14,25,27,30], but was higher than that of Caucasian populations (less than 5.0%) [28-29,31-32]. Furthermore, our results showed that the *CYP2E1 * c2* allele was significantly associated with alcohol use disorders, especially in males, in the Tibetan population. The *CYP2E1 * c2* allele was also implicated in increasing alcohol consumption in males with alcohol use disorders. This is in line with previous studies, which have shown the *c2* allele of *CYP2E1* to be associated with greater alcohol consumption [8], the development of alcoholism [7] and the earlier onset of alcohol abuse[28].

It is commonly acknowledged that elevated acetaldehyde levels in blood may protect individuals from heavy drinking due to its aversive effects such as face flushing and other unpleasant symptoms [9]. Previous studies have reported that the low activity allele *ALDH2 * 2*, which provides protection against alcohol dependence, is present at a relatively low frequency in the Tibetan population (< 5%) [46]. Most Tibetan population possess the *ALDH2 * 1/ALDH2 * 1* genotype and convert acetaldehyde to acetate with higher efficiency. Thus, individuals with the *CYP2E1 * c2* allele may not easily experience the aversive effects of excessive alcohol consumption, and subsequently drink more. Studies, including the present one, have confirmed the hypothesis that subjects with *CYP2E1 * c2* allele, especially those carrying both *CYP2E1 * c2* allele and the *ALDH2 * 1/ALDH2 * 1* genotype, have a significantly higher alcohol consumption [6-9].

However, other studies have reported that *CYP2E1 * c2* had no significantly influence on alcohol consumption and/or related mental problems. It should be noted that some of the studies were performed in populations with very low frequencies of the *CYP2E1 * c2* allele (less than 5%) which may have limited the power of the study [29,31-32]. Other studies in East Asian population with relative high frequencies of *CYP2E1 * c2* allele have not considered the interaction between *CYP2E1* and *ALDH2* genes [5,13-14,25,27,30].

It should be pointed out that, in this present study, the association of *CYP2E1 * c2* allele with higher risk of alcohol use disorders was much stronger in males than in females with an odds ratio of 2.30. In addition, the involvement of *CYP2E1 * c2* allele with increased alcohol consumption was observed in males with alcohol use disorders only. There are some possible reasons: Heavy drinking in women is strongly against cultural expectation in Tibet. Thus it is possible that the protective environment for women masks any genetic effects of genes involved in ethanol metabolism by reducing exposure. In addition, it has been reported that the contribution of CYP2E1 to ethanol metabolism increases significantly when ADH is saturated, i.e. at high alcohol dose levels (100 g/d), or after long-term ethanol intake[19,21]. Thus CYP2E1 alleles may have a functionally limited contribution in the process of ethanol metabolism in females as generally they drink less. However, we are unable to compare this finding with previous studies as most of them focused on samples collected from males [8-9,13,27], or used the samples as a whole without analyzing males and females separately [5-7,25,28].

In conclusion, the present study confirms the positive association among *CYP2E1 * c2* allele, alcohol use disorders, and the amount of alcohol consumption, in the Tibetan population, especially in males. Further study is warranted to explore the interaction among genes involving in the ethanol metabolism (e.g. *ADH* genes and *ALDH* genes), and to identify other susceptibility genes which may responsible for addiction behaviors.

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