*Title*

**The TA allele of rs2070673 in the *CYP2E1* gene is associated with lobular inflammation and nonalcoholic steatohepatitis in patients with biopsy-proven nonalcoholic fatty liver disease**

**Short title:** *CYP2E1* variant and NAFLD severity

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**List of Abbreviations**

ALD, alcoholic liver disease; ALT, alanine aminotransferase; CYP2E1, cytochrome P450 2E1; IL, interleukin; IL-1RA, interleukin-1 receptor antagonist, IP-10, interferon-inducible protein-10; MAF, minor allele frequency; NAFLD, nonalcoholic fatty liver disease; NAS, nonalcoholic fatty liver disease activity score; NASH, nonalcoholic steatohepatitis; NF-kB, nuclear factor kappa B; OR, odds ratio;

PNPLA3, patatin-like phospholipase domain containing protein 3; SNP, single nucleotide polymorphism; TM6SF2, trans-membrane protein 6 superfamily member 2.

**Abstract**

**Background:** Cytochrome P450 2E1 (CYP2E1) plays a role in lipid metabolism, and by increasing hepatic oxidative stress and inflammation, the up-regulation of CYP2E1 is involved in development of nonalcoholic steatohepatitis (NASH). We aimed to explore the relationship between *CYP2E1*-333A>T (rs2070673) and the histological severity of nonalcoholic fatty liver disease (NAFLD).

**Methods:** We studied 438 patients with biopsy-proven NAFLD. NASH was defined as NAFLD Activity Score ≥5 with existence of steatosis, ballooning and lobular inflammation. *CYP2E1*-333A>T (rs2070673) was genotyped by MALDI-TOF mass spectrometry. Serum cytokines related to inflammation were measured by the Bio-plex 200 system to investigate possible mediating factors involved in the process.

**Results:** TA genotype of rs2070673 had a higher prevalence of moderate/severe lobular inflammation (27.6% vs. 20.3% vs. 13.3%, P<0.01) and NASH (55.7% vs. 42.4% vs. 40.5%, P<0.01) compared with the AA and TT genotypes, respectively. In multivariable regression modelling the heterozygote state TA was associated with moderate/severe lobular inflammation (adjusted-OR: 2.31, 95%CI 1.41-3.78, P<0.01) or NASH (adjusted-OR, 95%CI: 1.82, 1.22-2.69, P<0.01), independently of age, sex, common metabolic risk factors and presence of liver fibrosis. Compared with no-NASH, NASH patients had significantly higher levels of serum interleukin-1 receptor antagonist, interleukin-18 and interferon-inducible protein-10 (IP-10), whereas only IP-10 was increased with the rs2070673 TA variant (P=0.01). Mediation analysis showed that IP-10 was responsible for ~60% of the association between the

rs2070672 and NASH.

**Conclusions:** The TA allele of rs2070673 is strongly associated with lobular inflammation and NASH and this effect appears to be largely mediated by serum IP-10 levels.

**Keywords:** cytochrome P450 2E1; nonalcoholic fatty liver disease; nonalcoholic steatohepatitis; single nucleotide polymorphism; cytokines.

## INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) has become the leading cause of chronic

liver diseases in many parts of world [1]. Approximately 25% of adults in Asia have NAFLD, which causes considerable morbidity and mortality and also has important economic consequences [2]. Research has clearly shown that approximately 20%-30% of persons with simple steatosis can develop NASH, and NASH is an important risk factor for subsequent development of cirrhosis or hepatocellular carcinoma [3]

As a member of the cytochrome P450 superfamily that is mainly expressed in the liver, cytochrome P450 2E1 (CYP2E1) is involved in metabolism of xenobiotics (including drugs, carcinogens and toxins) and endogenous substrates (including steroids and fatty acids) [4]. Compared with other cytochrome P450 proteins, CYP2E1 is more likely to produce reactive oxygen species that play a key role in the development and progression of both alcoholic liver disease (ALD) and NAFLD [5-6]. Some studies have shown that CYP2E1 can promote hepatic macrophage activation and induction of inflammatory cells, mainly through increasing levels of oxidative stress in NAFLD [5-7]. In NAFLD, CYP2E1 can also increase the release of proinflammatory factors mainly through the activation of the c-Jun N-terminal kinase (JNK) pathway [8]. NASH may be improved with some drugs or weight loss that reduce lobular inflammation and decrease the levels of CYP2E1 [9-10].

With the progress of genome-wide association studies, more and more single nucleotide polymorphisms (SNPs) have been detected that are associated with the presence of NASH, such as G alleles of *patatin-like phospholipase domain containing*

*protein 3* (PNPLA3) and T alleles of *transmembrane protein-6 superfamily member 2* (*TM6SF2*), which are the two genetic risk factors most robustly associated with higher susceptibility for NASH [11-12]. Polymorphisms within the *CYP2E1* gene can also affect metabolism of fatty acids, which leads to increased levels of toxic lipid peroxides. The link between *CYP2E1* -333A>T (rs2070673) and drug metabolism has been studied in many diseases [13-14]. To date, however, there are few studies examining the association between the *CYP2E1*-333A>T (rs2070673) polymorphism and NASH in humans.

Thus, the main aim of this cross-sectional study was to examine the association between *CYP2E1*-333A>T (rs2070673) and the presence of NASH in a large cohort of individuals with biopsy-confirmed NAFLD.

## MATERIALS AND METHODS

### Study population

In this research, all participants were consecutively recruited from the PERSONS cohort at the First Affiliated Hospital of Wenzhou Medical University during a period of three years (from 2016 to 2019). The main exclusion criteria were as follows: (1) liver fat content less than 5% on liver biopsy; (2) excessive alcohol consumption (defined as > 140 g/week for men or > 70 g/week for women, respectively); and (3) presence of chronic liver diseases other than NAFLD, such as viral hepatitis, hemochromatosis, drug-induced hepatitis and autoimmune hepatitis. As a consequence of these exclusion criteria, 438 Chinese individuals with biopsy-

confirmed NAFLD were included in the study. All participants were unrelated. The protocol of the research was approved by the ethics committee of the First Affiliated Hospital of Wenzhou Medical University (2016-246, 1 December 2016). The study was registered in the Chinese Clinical Trial Registry (ChiCTR-EOC-17013562). Informed written consent was given by each participant.

**Clinical and laboratory data**

Clinical and laboratory data were collected from all participants within 24 hours of liver biopsy. Body mass index was calculated as body weight (kg)/height$^2$ ($m^2$). Laboratory parameters were measured centrally by an automated analyzer (Abbott AxSYM) using standard methods. Homeostasis model assessment insulin resistance (HOMA-IR) was calculated as fasting glucose (mmol/L) * fasting insulin (mIU/L) /22.5.

**Comorbidity definitions**

Hypertension was diagnosed when blood pressure was more than 140/90 mmHg or when the patient was treated with any anti-hypertensive agents. The diagnosis of diabetes was based on fasting glucose levels ≥ 7 mmol/L, hemoglobin A1c ≥ 6.5% (≥ 48 mmol/mol) and/or the use of any anti-hyperglycemic agents. Dyslipidemia was defined as total cholesterol ≥ 5.2 mmol/L, triglycerides ≥ 1.7 mmol/L, high-density lipoprotein (HDL)-cholesterol ≤ 1.0 mmol/L for men or ≤ 1.3 mmol/L for women, low-density lipoprotein (LDL)-cholesterol ≥ 3.4 mmol/l and/or use of any lipid-

lowering drugs. Central obesity was diagnosed as waist circumference ≥ 90 cm for men or ≥ 80 cm for women.

**Liver histology assessment**

In all participants percutaneous liver biopsy examination was performed with ultrasound guidance. Each liver specimen was assessed by an experienced liver pathologist (who was blinded to participants' clinical data) in accordance with the validated histological scoring system for NAFLD, which was proposed by Kleiner et al [15]. The presence of definite NASH was defined as NAFLD Activity Score (NAS) ≥ 5; alternatively, NASH was also defined as a steatosis, activity, and fibrosis (SAF) score ≥ 5 with the presence of hepatic steatosis, ballooning and lobular inflammation. Moderate/severe steatosis and lobular inflammation were defined as the presence of their respective histologic scores ≥ 2[16]. Liver fibrosis was staged from zero to 4 and the presence of fibrosis was defined as stage F ≥ 1.

**Genetic analysis**

All whole blood samples were stored in -80°C. A total of approximately 20 ng genomic DNA was extracted from peripheral blood cells for each patient using the TIANamp Blood DNA Kit (TIANGEN BIOTHCH, Beijing, China).

The rs2070673 polymorphisms were CYP2E1:2KB upstream variants. The primer sequences were the following: forward 5'-

ACGTTGGATGACTCCAAACAAATGCATGGG-3', reverse 5'-ACGTTGGATGACTCCAAACAAATGCATGGG-3' (rs2070673). Genotyping for them were detected by MassARRAY platform (Agena Bioscience, San Diego, CA, USA). The main steps include PCR amplification reaction, alkaline phosphatase reaction, single base extension reaction, resin purification reaction, chip spotting and mass spectrometry detection. Assay Design v3.1 was used to design locus-specific polymerase chain reaction and detection primers. Allele type of DNA amplified by multiplex polymerase chain reaction was detected by using Matrix-assisted laser desorption ionization-time of flight mass spectrometry.

Using the CaTS power calculator, the cohort had 98% power to detect a significant association [odds ratio (OR) of 1.74] for an additive genetic model (rs2070673 AA vs. TA vs. TT) with the prevalence of NASH in NAFLD of 0.30 and a minor allele frequency (MAF) for rs2070673 of 0.39.

**Serum cytokine analysis**

Serum samples were collected from patients in the fasting state on the day of liver biopsy and stored at -80°C prior to testing. Serum cytokines were measured using the Bio-plex 200 system and matched kits (Bio-Rad, Hercules, California, USA). All measurements were performed only once. The intra-and inter-assay coefficients of variations were $\leqslant$15% and $\leqslant$25%, respectively. Specifically, we tested the circulating levels of interleukin-1β (IL-1β), interleukin-1 receptor antagonist (IL-1RA), IL-6, IL-7, IL-8, IL-9, IL-13, IL-16, IL-18 and interferon-inducible protein-10

(IP-10).

**Immunohistochemical analysis**

Hepatic paraffin sections were deparaffinized with xylene and rehydrated with alcohol. Deparaffinized sections were incubated in 3% hydrogen peroxide ($H_2O_2$) for 30 min and normal goat serum to block nonspecific protein binding for 30 min. Hepatic sections were then incubated overnight with rabbit anti-CYP2E1 and anti-IP-10 antibodies at a 1:100 dilution (Protein Tech Group, Wuhan, China). Two-Step Immuno-histochemical (IHC) broad spectrum detection reagent for 30 min at 37°C was applied. DAB, used as the chromogen, was counterstained with hematoxylin. The brown-stained areas were considered positive.

**Statistical analysis**

Continuous variables were presented as means ± SD or medians (Q1-Q3) and categorical variables were presented as frequencies (%). Continuous variables were compared by the Student's *t* test (for variables normally distributed) or the nonparametric Kruskal-Wallis test (for variables not normally distributed), whilst categorical variables were compared using the chi-square test. Multivariable logistic regression analysis was undertaken to study the relationships between the SNPs of rs2070672 and rs2070673 variants (using dominant genetic models) and liver histological features. Causal mediation analysis was performed to calculate the indirect effect of these two genotypes on risk of having NASH mediated by cytokines.

P< 0.05 was considered to be statistically significant. We used R software (version 3.5.2, R Foundation for Statistical Computing, Vienna, Austria) for all data analysis.

## RESULTS

### Genotype distribution of *CYP2E1* polymorphism

Among the 438 adult patients with NAFLD included in the study, the distribution of *CYP2E1*-333T>A (rs2070673) TT, TA and AA genotypes was 36%, 50% and 14%, respectively. Our patient cohort met the Hardy-Weinberg equilibrium, and MAF of *CYP2E1* variants were consistent with the data of 1000 genomes project in Asia.

### *CYP2E1* variants and clinical characteristics of NAFLD

**Table 1** shows the main clinical and histopathological characteristics of participants stratified by different genotypes of rs2070673. The mean age and the proportion of men between rs2070673 AA, TA and TT were 40±13 vs. 41±13 vs. 42±13 years (P=0.40) and 71.2% vs. 73.8% vs. 69.6% (P=0.67), respectively. The percentage of participants with insulin resistance (i.e. defined as HOMA-IR score ⩾2.7) was 76.8%, 71.7% and 68.8% among the rs2070673 AA, TA and TT variants (P=0.54). As also shown in **Table 1**, there were no significant differences in biochemical characteristics among the rs2070673 AA, TA and TT genotypes, except for serum triglyceride levels that were 2.28 (1.69-3.28), 1.90 (1.37-2.84) and 1.88 (1.37-2.59) mmol/L, respectively, in the carriers of these three genotypes (P=0.04). Hypertension, diabetes, dyslipidemia and central obesity were similar among the three different

genotypes. In **Supplementary Table 1**, we undertook comparisons between rs2070673 AA genotype and TA genotype, AA genotype and TT genotype, and TA and TT genotype, respectively. These results showed that carriers of the rs2070673 AA genotype had higher levels of triglycerides than those carrying the TA genotype (2.28 (1.69-3.28) vs. 1.90 (1.37-2.84) mmol/L, P=0.03) and the TT genotype (2.28 (1.69-3.28) vs. 1.88 (1.37-2.59) mmol/L, P=0.01). Conversely, plasma triglycerides did not differ between carriers of the TA and TT genotypes (1.90 (1.37-2.84) vs. 1.88 (1.37-2.59) mmol/L, P=0.64). Other clinical characteristics did not significantly differ among the three different genotypes.

### *CYP2E1* variants and histologic features of NAFLD

As shown in **Table 1,** carriers of the TA genotype of *CYP2E1* (rs2070673) had a higher prevalence of moderate/severe lobular inflammation and definite NASH (55.7% vs. 42.4% vs. 40.5%, P<0.01) compared with carriers of the AA and TT genotypes, respectively. In **Supplementary Table 2**, 415 participants were also assessed by SAF score. Similarly, carriers of the rs2070673 TA variant had higher proportions of moderate/severe lobular inflammation and definite NASH than those carrying the AA and TT variants. Presence of any stage of liver fibrosis was not significantly different between the three genetic variants (**Table 1**). Similarly, the proportion of significant fibrosis (stage F≥2) was essentially comparable (20.7% vs. 24.5% vs. 27.2%) in those carrying the rs2070673 AA, TA and TT variants, respectively.

To further examine the association between *CYP2E1* genetic variants and liver histology, we performed multivariable logistic regression models that were adjusted for age, sex, BMI, diabetes, hypertension, dyslipidemia, central obesity and presence of fibrosis. As shown in **Table 2,** in the additive model, presence of rs2070673 TA variant significantly increased the risk of moderate/severe lobular inflammation with an adjusted-OR of 2.94, 95% CI 1.63-5.30, and definite NASH with an adjusted OR of 1.94, 95% CI 1.23-3.05 for TA vs. TT carriers, respectively. The rs2070673 AA variant was not associated with moderate/severe steatosis (OR 0.86, 0.44-1.68, AA vs. TT), presence of ballooning (OR 0.79, 0.34-1.85, AA vs. TT), moderate/severe lobular inflammation (OR 2.04, 0.89-4.65, AA vs. TT) or definite NASH (OR 1.06, 0.55-2.06, AA vs. TT). In the heterozygous model, rs2070673 TA variant significantly increased the risk of moderate/severe steatosis with an OR of 1.59, 95%CI 1.06-2.37 (TA vs. AA+TT), moderate/severe lobular inflammation with an OR of 2.31, 95%CI 1.41-3.78 (TA vs. AA+TT) and definite NASH with an OR of 1.82, 95%CI 1.22-2.69 (TA vs. AA+TT). As shown in the **Supplementary Table 1**, no significant differences in liver histology features were found between rs2070673 AA and TA genotypes or AA and TT genotypes. Comparing those carrying the TT genotype, carriers of the TA genotype had higher proportion of moderate/severe steatosis (67.0% vs. 55.7%, P=0.03), moderate/severe lobular inflammation (27.6% vs. 13.3%, P<0.01) and definite NASH (55.7% vs. 40.5%, P<0.01). Further analysis showed that the allele frequency of the rs2070673:A of *CYP2E1* gene was higher in moderate/severe lobular

inflammation than in mild lobular inflammation (45.2% vs. 36.9%, P=0.03). However, the allele frequency of the rs2070673:A was not significantly different between no-NASH and NASH (36.7% vs. 40.8%, P=0.22) (**Supplementary Table 3**).

### *CYP2E1*-352A>G (rs2070672) polymorphism and serum cytokines

As it is known that cytokines play an important role in proinflammatory and anti-inflammatory effects in NAFLD [17-19], we measured a variety of serum cytokines in order to better investigate the potential mediators explaining the aforementioned significant association between the rs207673 variant and NASH. In particular, we measured serum levels of IL-1β, IL-1RA, IL-6, IL-7, IL-8, IL-9, IL-13, IL-16, IL-18 and IP-10 in a subset of 229 patients. Serum cytokine levels stratified by NASH status are shown in **Table 3**. Compared to those with no-NASH, patients with NASH had significantly higher levels of serum IL-1RA, IL-18 and IP-10 (**Table 1** and **Figure 1A**).

Serum cytokine levels stratified by different rs2070673 variants are summarized in **Table 4**. Only serum IP-10 levels were significantly (P=0.01) higher in TA alleles than in AA+TT alleles (1107.09 (737.61-1521.95) vs. 914.38 (650.83-1251.43) pg/ml) (**Table 4** and **Figure 1B**). Serum levels of IL-1RA and IL-18 were not significantly different between TA and AA+TT alleles (**Table 4** and **Figure 1B**). Interestingly, the levels of serum IP-10 markedly increased according to the histological severity of steatosis, hepatocyte ballooning and lobular inflammation

(**Figure 1C**).

A causal mediation analysis was performed to examine the effects of serum IL-1RA, IL-18 and IP-10 levels between rs2070673 and NASH. This analysis showed that serum IP-10 levels largely mediated the association between the rs2070673 variant and NASH (average casual mediation effect: 0.08, 0.01-0.13, P=0.02) (**Table 5**). Indeed, 61% of the observed association between NASH and the rs2070673 variant was mediated by IP-10 levels (**Table 5**).

***CYP2E1* and IP-10 with immunohistochemical analysis**

Immunohistochemistry was performed to examine the distribution of CYP2E1 into the liver. As shown in **Figure 2A**, CYP2E1 was widely present in hepatocyte cytoplasm. In addition, we also explored the distribution of IP-10 within the liver parenchyma in different genotypes by using immunohistochemistry. Our results show that regardless of the genotype, IP-10 was mainly expressed around steatotic hepatocytes and inflammatory cells.

## DISCUSSION

The main and novel findings of our cross-sectional study, which was performed in a cohort of Chinese middle-aged individuals with biopsy-proven NAFLD, were as follows: 1) carriers of the TA genotype of *CYP2E1* (rs2070673) had a higher prevalence of moderate/severe lobular inflammation and definite NASH compared to those carrying the AA and TT genotypes, independently of the presence of liver fibrosis, age, sex, obesity, diabetes and other metabolic risk factors; and 2) the association between rs2070673 TA allele and NASH was largely mediated by higher levels of serum IP-10.

To the best of our knowledge, this is one of the largest observational studies to show that the *CYP2E1* rs2070673 variant is related to human NASH. Our cohort met the Hardy-Weinberg equilibrium and the allele frequencies of the rs2070673: A of CYP2E1 gene was 0.39 in NAFLD patients. A previous study has shown that the allele frequencies of the rs2070673: A of CYP2E1 gene was 38%-59% in the Chinese general population [20]. In the meantime, the results in 1000 genomes have shown that the allele frequencies of the rs2070673: A was 46% in East Asian people It is interesting that the genotype distribution of CYP2E1 gene in NAFLD was very similar to the distribution in the general population. At the same time, in our study, the population characteristics, comorbidities, and main biochemical characteristics were not significantly different among the three different *CYP2E1* rs2070673 genotypes, thereby suggesting that there were no other measured external factors affecting our results. Interestingly, some patients with NAFLD may not have insulin resistance[16].

However, we did not find any significant relationship between *CYP2E1* genetic variants and NASH in subjects without HOMA-estimated insulin resistance in this study.

Compared with those carrying the AA and TT alleles, carriers of the TA allele had a higher prevalence of definite NASH. The difference between the AA and TT genotypes was not significant. Furthermore, by using multiple logistic regression analyses with an additive model (TA vs. TT) or heterozygous model (TA vs. AA+TT), the rs2070673 TA variant was closely associated with moderate/severe lobular inflammation and NASH. In a dominant model (AA+TA vs. TT), the rs2070673:A also increased the risk of moderate/severe lobular inflammation and definite NASH. However, the frequency of rs2070673:A did not show any significant difference between no-NASH and NASH. It further confirms that rs2070673 TA variant may be a risk factor of NASH. Although some experimental evidence supports an association between CYP2E1 levels and liver fibrosis in mice [21-22], we did not find any association between the histologic stage of liver fibrosis and the gene of *CYP2E1* (rs2070673). However, the pathogenesis of liver fibrosis also involves hepatocyte insulin resistance that was not significantly different between different genotypes of CYP2E1 gene [23]. In the meantime, it should also be noted that levels of liver fibrosis were relatively low among participants of our study and further research is needed in subjects with greater amounts of liver fibrosis.

In a subgroup analysis, we found that serum levels of IL-1RA, IL-18 and IP-10 were significantly increased in NASH patients, which is consistent with some published data [19-24-25]. IL-1RA was positively correlated with steatosis and lobular inflammation in another study [18]. It has been reported that IP-10 can promote the development of NAFLD mainly through nuclear factor kappa B (NF-kB) [25]. When NF-kB is inhibited, it is often accompanied by a decrease in the expression of CYP2E1 [26-27]. Therefore, it is plausible that there may be a connection between CYP2E1 and NF-kB, and our mediation analysis also suggests that IP-10 may play a key role in the association between the rs2070673 variant and NASH.

IP-10, also known as CXC motif chemokine 10, is a chemokine that can induce targeted chemotaxis of specific cells to mediate the development of infectious diseases, autoimmune diseases and metabolic diseases [28-29]. The receptor for the chemokine IP-10 is the CXC chemokine receptor 3, which is expressed in several immune cell types, such as T cells, monocytes/macrophages and resident cells [30]. Therefore, IP-10 potentiates T-cell recruitment at different sites of inflammation. At the same time, T cells also play an important role in the development of NASH. The infiltration of T cells can often be observed in NASH [30]. A study has shown that in mice lacking *CYP2E1*, the content of T cells is significantly reduced, suggesting a possible connection between CYP2E1 and T cells [31]. CYP2E1 is widely present in the hepatocytes as one of monooxygenases in the liver, as confirmed in our immuno-histochemical study (**Figure 2A**) [32-33]. However, there is limited research studying

the distribution of IP-10 in NAFLD in man. With different *CYP2E1* genotypes, our data show that IP-10 is located around steatotic hepatocytes and inflammatory cells in AA, TA or TT variants. IP-10 around steatotic hepatocytes and inflammatory cells may play a vital role in the production of oxidative stress (**Figure 2B-D**). Therefore, we speculate that the rs2070673 TA variant may change the activity of CYP2E1 or the expression of *CYP2E1* mRNA, and may also affect IP-10 levels secreted by liver cells. Increased secretion of IP-10 may then promote the accumulation of T cells at sites of inflammation and increase the histological severity of hepatic inflammation, thereby promoting NASH development (**Figure 2E**). However, rs2070673 has been only studied in the researches of latent tuberculosis infection or industrial chemicals. There is no study to explore the function of rs2070673, so further research is required to confirm or refute this hypothesis[34].

One of the most important limitations of this study is the lack of ethnic diversity in our cohort, which is made up of Asian individuals. As the effect of genes is different in different ethnic groups, these results should be further validated in other cohorts of different ethnicities. Further experimental research is also needed to better understand the underlying mechanisms linking the *CYP2E1* rs2070673 variant and NASH.

In conclusion, the results of our study show that the *CYP2E1* rs2070673 TA variant is associated with an increased risk of moderate/severe lobular inflammation and NASH, independently of stage of liver fibrosis, age, sex, obesity, diabetes and other

common metabolic risk factors. These data also suggests that higher serum IP-10 levels mediate, in large part, the association between *CYP2E1* rs2070673 TA variant and NASH in our cohort of Chinese adults with biopsy-confirmed NAFLD.

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None.

**Conflict of Interest Statement**

None.

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**Author Contributions**

Authors' contributions: Hong-Lei Ma and Ming-Hua Zheng designed the study. Yue Yu, Xin-Xin Wang, Gang Li and Ou-Yang Huang collected the data. Hong-Lei Ma, Sui-Dan Chen and Liang-Jie Tang analyzed the data. Kenneth I. Zheng, Rafael S

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**Table legends**

**Table 1.** Baseline characteristics of patients with biopsy-proven NAFLD stratified by genotypes of *CYP2E1* rs2070673

**Table 2.** Association between *CYP2E1* rs2070673 variants and liver histology features in patients with biopsy-proven NAFLD.

**Table 3.** Subgroup of 229 patients with biopsy-proven NAFLD with available serum cytokine measurements, stratified by NASH status

**Table 4.** Subgroup of 229 patients with biopsy-proven NAFLD with available serum cytokine measurements, stratified by the rs2070673 *CYP2E1* genotype.

**Table 5.** Mediation effect analysis showing the effect of serum IL-1RA, IL-18 and IP-10 levels on the association between the *CYP2E1* rs2070673variant and definite NASH.

**Supplementary Table 1.** Baseline characteristics of patients with biopsy-proven NAFLD, stratified by genotypes of CYP2E1 rs2070673

**Supplementary Table 2.** Histology characteristics assed by SAF score of patients with biopsy-proven NAFLD, stratified by genotypes of *CYP2E1* rs2070673.

**Supplementary Table 3.** Allele frequency of *CYP2E1* rs2070673 according to liver histology features.

**Figure legends**

**Figure 1. (A)** Serum levels of IL-1RA, IL-18 and IP-10 in NASH and no-NASH. **(B)** Serum levels of IL-1RA, IL-18 and IP-10 in the TT and AA+TA genetic variants. **(C)** Serum levels of IP-10 stratified by histologic scores of steatosis, ballooning and inflammation. Each subgroup was compared using nonparametric Kruskal-Wallis test (*: P =0.01; **: P <0.01).

**Figure 2. (A)** CYP2E1 and IP-10 are shown with brown-stained areas in hepatocytes by immunohistochemical staining. **(B-D)** CYP2E1 and IP-10 are shown with brown-stained areas in hepatocytes by immunohistochemical staining between different genotype of *CYP2E1* rs2070673. **(E)** Hypothesis that carriage of rs2070673:A increases the presence of NASH and moderate/severe lobular inflammation by releasing more IP-10 to recruit more T cells in the liver.