The Cystic Fibrosis Transmembrane Conductance Regulator 470 Met Allele Is Associated with an Increased Risk of Chronic Pancreatitis in Both Asian and Caucasian Populations: A Meta-Analysis

Donger Zhou,1 Rui Bai,2 and Liang Wang1

Background: The Met470Val polymorphism (1540A>G [rs213950]) within the cystic fibrosis transmembrane conductance regulator (CFTR) protein has been reported to be associated with chronic pancreatitis (CP). The results remain inconclusive, and therefore, we performed this meta-analysis to clarify the association between M470V and CP risk.

Methodology/Results: We conducted a meta-analysis of 7 case–control studies, including a total of 1121 CP patients and 2209 controls from Asian and Caucasian populations. We calculated the odds ratio (OR) and 95% confidence intervals (95% CI). Met470Val was found to be significantly associated with an increased risk of CP under all the genetic models (M vs. V, OR = 1.260, 95% CI: 1.134–1.399; MV vs. VV, OR = 1.292, 95% CI: 1.091–1.530; MM vs. VV, OR = 1.346, 95% CI: 1.114–1.621). Met470Val was also found to be significantly associated with an increased risk of idiopathic CP (ICP) in allele contrast, codominant, and recessive models (M vs. V, OR = 1.298, 95% CI: 1.020–1.653; MV vs. VV, OR = 1.297, 95% CI: 1.074–1.566; MM vs. VV, OR = 1.473, 95% CI: 1.165–1.862; MM vs. MV/VV, OR = 1.254, 95% CI: 1.023–1.538).

Conclusions: The CFTR 470 M allele is significantly associated with an increased risk of CP in both Asian and Caucasian populations. The CFTR 470 M allele is also significantly associated with risk of ICP.

Keywords: CFTR, polymorphism, chronic pancreatitis, meta-analysis

Introduction

Chronic pancreatitis (CP) is a chronic inflammatory syndrome of the pancreas, characterized by recurrent abdominal pain; fibrosis and atrophy of the pancreatic parenchyma; and loss of exocrine and endocrine tissue (Witt et al., 2007; Issa et al., 2014). The etiologic factors underlying CP, including alcohol toxicity, anatomic abnormalities, autoimmunity, and genetic predisposition, interact with each other leading to a complex pathogenesis. CP is frequently subclassified according to epidemiologic factors including but not limited alcoholism, familial history, and autoimmune disorders. When no specific factor can be identified, it is classified as idiopathic chronic pancreatitis (ICP).

Although the pathogenesis of CP remains unclear, certain genetic factors have been shown to contribute to CP progression (Hanck et al., 2003; Thrower et al., 2008). Multiple studies have suggested a link between the cystic fibrosis transmembrane conductance regulator (CFTR) gene and CP (Audrezet et al., 2002; Keiles and Kammesheidt, 2006). The CFTR gene is positioned on chromosome 7q31 and contains 27 exons, encoding a cAMP regulated Cl2−channel protein expressed on the epithelial cell membrane and regulating other membrane transport proteins (Moskowitz et al., 2008; Tomaiuolo et al., 2015). More than 2000 mutations and polymorphic loci have been characterized in the CFTR gene (Cystic Fibrosis Mutation Database). Among them is an amino acid changing polymorphism (Met470Val; 1540A>G [rs213950]) in exon 10, which has been associated with CP (Lee et al., 2003; Fujiki et al., 2004).

The M470V polymorphism is often investigated with other polymorphisms such as the 5-thymidine (5T) repeats and the TG repeats. Haplotypes defined by various combinations of M470V, 5T, and TG repeats were found to be associated with...
Statistical analysis

Data extraction

Characteristics of eligible studies

Results

Statistical analysis

We conducted and reported our meta-analysis in accordance with the guideline of the Meta-analysis of Observational Studies in Epidemiology (MOOSE) Group (Stroup et al., 2000). The association strength between p.V470M (rs213950) and CP was measured by odds ratio (OR) with 95% confidence intervals (95% CI) (Lau et al., 1997). The pooled ORs were calculated for allele contrast (M vs. V), codominant model (MM vs. VV, MV vs. VV), dominant (MM MV vs. VV), and recessive (MM vs. MV VV) models, assuming dominant and recessive effects of the variant M allele, respectively. Subgroup analyses were also applied based on ethnicities. Sensitivity analyses were performed to identify an individual study’s effect on the pooled results. The chi-square based Q-test was used to check the statistical heterogeneity among studies, and heterogeneity was considered statistically significant when \( p < 0.10 \). The fixed-effects model (based on Mantel-Haenszel method) was used when there was no significant heterogeneity; otherwise, the random-effects model (based on DerSimonian-Laird method) was applied (DerSimonian and Laird, 2015). Publication bias was detected using Begg’s funnel plot and the Egger’s linear regression test, and a \( p < 0.05 \) was considered statistically significant (Egger et al., 1997). All statistical analyses were calculated with STATA software (version 14.0; StataCorp, College Station, TX). Also, all \( p \)-values were two-sided.

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Meta-analysis results

The results showed that M470 had a significant association with increased risk of CP under all models of inheritance evaluated, including the allele contrast (M vs. V, \( OR = 1.260, 95\% CI: 1.134–1.399, \chi^2_{\text{heterogeneity}} = 0.303 \)); the codominant model (MM vs. VV, \( OR = 1.292, 95\% CI: 1.091–1.530, \chi^2_{\text{heterogeneity}} = 0.153 \)); MM vs. VV, \( OR = 1.579, 95\% CI: 1.274–1.956, \chi^2_{\text{heterogeneity}} = 0.273 \)); the dominant model (MV/MV vs. VV, \( OR = 1.366, 95\% CI: 1.165–1.603, \chi^2_{\text{heterogeneity}} = 0.213 \)); and the recessive model (MM vs. MV VV, \( OR = 1.346, 95\% CI: 1.114–1.621, \chi^2_{\text{heterogeneity}} = 0.219 \)). The results are shown in Table 2 and Figures 2 and 3.

In the subgroup analysis, ethnicity was added to the investigation. Among the Asian studies, a significant association between M470 and CP was observed in the allele contrast (M vs. V, \( OR = 1.295, 95\% CI: 1.009–1.662, \chi^2_{\text{heterogeneity}} = 0.895 \)) and codominant models (MM vs. VV, \( OR = 1.666, 95\% CI: 1.022–2.715, \chi^2_{\text{heterogeneity}} = 0.937 \)). In the Caucasian group,
significant heterogeneity was observed; therefore, we applied a random-effects model when $p_{\text{heterogeneity}} < 0.1$. A significant association was also observed in the Caucasian group under the allele contrast model ($M$ vs. $V$, OR = 1.392, 95% CI: 1.098–1.765, $p_{\text{heterogeneity}} = 0.075$); the codominant model ($MM$ vs. $VV$, OR = 2.011, 95% CI: 1.083–2.268, $p_{\text{heterogeneity}} = 0.084$); and the dominant model ($MV/MV$ vs. $VV$, OR = 1.567, 95% CI: 1.083–2.268, $p_{\text{heterogeneity}} = 0.084$). Results are shown in Table 2.

We also performed an analysis of the ICP cases, that is, no etiology reported; and we observed significant heterogeneity. Therefore, we applied the random-effects model when $p_{\text{heterogeneity}} < 0.1$ and found that a strong association existed between $M470$ and ICP under the allele contrast model ($M$ vs. $V$, OR = 1.298, 95% CI: 1.020–1.653, $p_{\text{heterogeneity}} = 0.082$); the codominant model ($MV$ vs. $VV$, OR = 1.297, 95% CI: 1.074–1.566, $p_{\text{heterogeneity}} = 0.109$); $MM$ vs. $VV$, OR = 1.473, 95% CI: 1.165–1.862, $p_{\text{heterogeneity}} = 0.143$); and the recessive model ($MM$ vs. $MV/VV$, OR = 1.254, 95% CI: 1.023–1.538, $p_{\text{heterogeneity}} = 0.378$). The results are shown in Table 2 and Figures 4 and 5.

**Sensitivity analysis**

A sensitivity analysis was performed using the method of deleting a single study each time and recalculating the pooled OR to explore the excluded study’s influence on the final result. We performed the analysis of overall comparison for all of the inheritance models. As shown in Figure 6, after deleting the study by Steiner group 2, the recalculated OR was more than the upper CI limit (95% CI) of the original one in allele contrast. But the same trend could be drawn and the risk was even more significant after deleting the last study. Similar situations were seen in other model comparison (data not shown). So the results are robust.

**Publication bias**

Publication bias was assessed by Begg’s funnel plot and Egger’s test. No evidence of publication bias was observed (Fig. 7).

**Discussion**

The $M470V$ polymorphism is a missense single nucleotide variant located in exon 10 of the $CFTR$ gene, in which the $M$ allele is the ancestral allele. However, while the $M$ allele is the major allele in African populations, it has been reported to be the minor allele in non-African populations, including the European and Asian populations (Kosova et al., 2010) according to the International HapMap Project (International HapMap, 2005) and the Human Genome Diversity Project (HGDP) (Cann et al., 2002). The frequencies of the $V$ allele were 0.93 in Tuscans and 0.80 in Mongolians in the database (Kosova et al., 2010). In our study, the combined frequency of the $V$ allele in the control group is 0.59 in total (0.60 in Asian group and 0.59 in European group), which is consistent with previous reports (Chang et al., 2007; Rosendahl et al., 2013). Kosova et al. (2010) postulated that Val470 might provide a positive selection because the $M$ allele was
### Table 1. Characteristics of Included Studies and Genotype Frequencies in Case and Control Groups

<table>
<thead>
<tr>
<th>Study first author (year)</th>
<th>Territory</th>
<th>Ethnicity</th>
<th>Genotype method</th>
<th>Case</th>
<th>Control</th>
<th>Etiology</th>
<th>p for HWE</th>
</tr>
</thead>
<tbody>
<tr>
<td>de Cid (2010)</td>
<td>Spain</td>
<td>Caucasian</td>
<td>PCR+DGGE/SSCP</td>
<td>29</td>
<td>64</td>
<td>43 8 51 34</td>
<td>NA</td>
</tr>
<tr>
<td>Chang (2007)</td>
<td>Taiwan</td>
<td>Asian</td>
<td>Directing sequencing</td>
<td>18</td>
<td>38</td>
<td>22 37 84 79</td>
<td>+</td>
</tr>
<tr>
<td>Fujiki (2004)</td>
<td>Japan</td>
<td>Asian</td>
<td>PCR+RFLP</td>
<td>17</td>
<td>25</td>
<td>23 24 82 56</td>
<td>+</td>
</tr>
<tr>
<td>Lee (2003)</td>
<td>Korea</td>
<td>Asian</td>
<td>TDGS</td>
<td>6</td>
<td>14</td>
<td>8 23 52 42</td>
<td>NA</td>
</tr>
<tr>
<td>Tzetis (2007)</td>
<td>Greece</td>
<td>Caucasian</td>
<td>PCR+DGGE/directing sequencing</td>
<td>3</td>
<td>18</td>
<td>4 24 104 83</td>
<td>NA</td>
</tr>
<tr>
<td>Steiner (2011) group 1</td>
<td>Switzerland</td>
<td>Caucasian</td>
<td>PCR+DGGE/dHPLC/SSCP</td>
<td>38</td>
<td>60</td>
<td>25 48 110 87</td>
<td>+</td>
</tr>
<tr>
<td>Steiner (2011) group 2</td>
<td>Germany</td>
<td>Caucasian</td>
<td>PCR+FRET probes</td>
<td>129</td>
<td>340</td>
<td>197 207 569 405</td>
<td>+</td>
</tr>
</tbody>
</table>

ACP, alcoholic chronic pancreatitis; DGGE, denaturing gradient gel electrophoresis; dHPLC, denaturing high performance liquid chromatography; FRET, fluorescence resonance energy transfer; HWE, Hardy–Weinberg equilibrium; ICP, idiopathic chronic pancreatitis; NA, not applicable; PCR, polymerase chain reaction; RFLP, restricted fragment length polymorphism; SSCP, single-strand conformation polymorphism; TDGS, two-dimensional gene scanning.

### Table 2. Results of Meta-Analysis

<table>
<thead>
<tr>
<th></th>
<th>M vs. V</th>
<th>MV vs. VV</th>
<th>MM vs. VV</th>
<th>MM MV vs. V</th>
<th>MM vs. MV VV</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>OR</td>
<td>pH</td>
<td>OR</td>
<td>pH</td>
<td>OR</td>
</tr>
<tr>
<td>Overall</td>
<td>7</td>
<td>1.260 (1.134–1.399)</td>
<td>0.303</td>
<td>1.292 (1.091–1.530)</td>
<td>0.153</td>
</tr>
<tr>
<td>Ethnicities</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>3</td>
<td>1.295 (1.009–1.662)</td>
<td>0.895</td>
<td>1.184 (0.792–1.769)</td>
<td>0.213</td>
</tr>
<tr>
<td>Caucasian</td>
<td>4</td>
<td>1.392 (1.098–1.765)</td>
<td>0.075</td>
<td>1.433 (0.990–2.075)</td>
<td>0.106</td>
</tr>
<tr>
<td>Etiology</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICP</td>
<td>4</td>
<td>1.298 (1.020–1.653)</td>
<td>0.082</td>
<td>1.297 (1.074–1.566)</td>
<td>0.109</td>
</tr>
</tbody>
</table>

All CP cases including ICP were analyzed in overall comparison and ethnicities comparison; only ICP cases were analyzed in the ICP comparison. Bold OR with statistical significance.

n, number of studies included; OR, odds ratio; pH, p-value for heterogeneity.
FIG. 2. Forest plot of allele contrast for overall comparison (M vs. V) using a fixed-effect model. All CP cases including ICP were analyzed in overall comparison. ICP, idiopathic chronic pancreatitis.

FIG. 3. Forest plot of homozygote comparison for overall comparison (MM vs. VV) using a fixed-effect model. All CP cases including ICP were analyzed in overall comparison.
associated with disadvantageous fertility effects. In addition, there have been several studies examining the relationship between the M470 allele and other diseases. Ciminelli et al. (2007) reported that the M allele was highly associated with CF-causing mutations in CF. Steiner et al. (2011) reported that the TG10-T7-M470 haplotype increased the risk of ICP and congenital bilateral absence of the vas deferens (CBAVD). Our results showed that M470 independently increases the risk of CP, including ICP, in both Asian and European populations.

FIG. 4. Forest plot of allele contrast for ICP (M vs. V) using a random-effects model. Only ICP cases were analyzed in this comparison.

FIG. 5. Forest plot of homozygote comparison for ICP (MM vs. VV) using a random-effects model. Only ICP cases were analyzed in this comparison.
Little is understood about the pathophysiological role of M470V in the CFTR protein in CP. Val470 causes the CFTR protein to mature quickly but with a lower activity, while Met470 results in an intact protein with normal activity that matures slowly (Cuppens et al., 1998). Several studies have shown that M470V may act like a modifier by increasing the penetrance of other mutations or polymorphisms (de Meeus et al., 1998). For example, Lee et al. (2003) reported that E217G and Q1352H on the M470 background can both decrease membrane protein expression and anion transporting activities by 60–70%, and that Q1352H in the V470 background can also arise in the low activity type of V470, so that either M or V can combine with other polymorphisms to confer a dysfunction of the CFTR protein. However, it has been reported (Pompei et al., 2006; Ciminelli et al., 2007) that the majority of the disease-causing mutations, including but not limited to F508del, N1303K, and W1282X, occur in the M470 background, and that this may be one of the reasons that M470 was associated with an increased risk of CP. There was also conjecture (Steiner et al., 2011) that the slower maturation of the M allele may not affect the intrinsic chloride channel activity, but may impair the tissue- and development-specific maturation and turnover of a functional CFTR channel. As we do not know the function of the CFTR clearly, additional mechanistic work needs to be done to functionally characterize the physiological role of the various CFTR protein polymorphisms and their contribution to in CP.

There are some advantages to this meta-analysis. This is the first meta-analysis to focus solely on the association between M470V and CP. Previous meta-analyses concerning the CFTR gene and CP usually focused on known deleterious

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**FIG. 6.** Sensitivity test of allele contrast for overall comparison (M vs. V).

**FIG. 7.** Begg’s funnel plot of allele contrast for overall comparison (M vs. V).
mutations and not structural polymorphisms that were not predicted to interfere with protein function. In this study, we demonstrated for the first time that het M470V polymorphism in the CFTR gene was independently associated with the risk of CP. In addition, there were no limitations in the literature search, and thus selection bias was well controlled. There was also no publication bias identified by either Begg’s funnel plot or Egger’s regression test.

This meta-analysis was not without its limitations. First, in the subgroup analysis, the number of studies in each subgroup was limited and the sample size among the studies was significant. These may contribute to heterogeneity in some genetic models. Second, 932 (83%) of 1121 CP patients were ICP patients, and therefore ICP contributes to the majority of the patient group. ICP was defined as CP without obvious epidemiologic factors, which might lead the ICP more likely to be caused by genetic predisposition. We should be cautious when interpreting the relationship between M470 and CP with mixed etiology. More samples of CP with mixed etiology should be included in a future study. Third, the ethnicities in this study were Asian and Caucasian and these populations have a similar genetic background where the M allele is the minor allele. The M allele, however, is the major allele in African populations; there have been no studies on the CFTR genes effects on CP among this population to our knowledge. It would be interesting to also investigate the role of M470V in CP in other ethnic groups.

In summary, we analyzed seven case–control studies, which included 1121 CP patients and 2209 controls for this meta-analysis. We demonstrated that the CFTR 470M allele independently is strongly associated with an increased risk of CP both in Asian and Caucasian populations. The same significant association was also observed in ICP patients.

Authors’ Contributions

D.Z. and R.B. confirmed the topic of this article. D.Z., R.B., and L.W. performed the statistical analysis and analyzed the results. R.B. and L.W. contributed to revising the article. D.Z. wrote the article. All authors approved the final version of the article.

Author Disclosure Statement

No competing financial interests exist.

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