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

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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 = 1.579, 95% CI: 1.274–1.956; MV/MV vs. VV, OR = 1.366, 95% CI: 1.165–1.603; MM vs. MV/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 Cl[−]-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

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CP; the results, however, are not definitive. Chang *et al.* (2007) reported that M470V alone was not associated with CP, but the haplotype of 125G/1001+11C/TG12/M470/2694T/4521G was associated with the risk of CP; Lee *et al.* (2003) reported that V470 with the Q1352H mutation was strongly associated with the risk of CP; and Steiner *et al.* (2011) reported that the haplotype of TG10-T7-M470 was associated with the risk of CP. The multiple haplotypes containing the M470V polymorphism examined in these studies make the analysis of this single marker's role difficult to assess in the pathogenesis of CP.

We therefore performed a systematic review of the literature and conducted a meta-analysis focused only on the M470V polymorphism to estimate its involvement in CP risk.

Methods

Identification of eligible studies

We carried out a systematic search of the PubMed and Embase databases through August 15, 2019. The following terms were used: “CF transmembrane conductance regulator,” “rs213950,” “M470V,” “Single Nucleotide Polymorphism” and “Chronic pancreatitis,” without any other limitation applied. The reference lists of the retrieved studies and reviews were manually searched for further relevant studies. Duplicate publications and irrelevant articles were excluded.

Inclusion and exclusion criteria

Studies included for the meta-analysis should conform to the following criteria: (1) case-control studies; (2) investigating the association between p.Met470Val (rs213950) and CP; (3) CP diagnoses via standard guidelines or diagnostic criteria; and (4) providing detailed genotypic frequencies of rs213950. Studies without detailed genotypic frequencies were excluded. Titles and abstracts of the search results were screened, followed by a full-text review of each article. Two reviewers (D.Z. and R.B.) extracted all eligible studies independently and the disagreements were resolved by discussion.

Data extraction

Data from the included studies were extracted by two reviewers (D.Z. and R.B.) independently. The following data were collected: name of first author, year of publication, country where the study was conducted, genotyping methods, ethnicity, and etiology of CP, number of cases and controls, genotype frequency in cases and controls. Different ethnicities were categorized as Asian or Caucasian. Data regarding ICP were also collected. Hardy-Weinberg equilibrium (HWE) in the controls was calculated and $p < 0.05$ was considered a significant departure from HWE. Two reviewers reached a consensus on each item and disagreements were solved via discussion with another reviewer (L.W.).

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 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.

Results

Characteristics of eligible studies

A total of six articles (Lee *et al.*, 2003; Fujiki *et al.*, 2004; Chang *et al.*, 2007; Tzetis *et al.*, 2007; de Cid *et al.*, 2010; Steiner *et al.*, 2011) were included based on the pre-determined inclusion and exclusion criteria. The detailed screening process is shown in Figure 1. In the article by Steiner *et al.* (2011), there were two separate studies, one was carried out in Switzerland consisting of persons from the Czech-Republic, France, Italy, and Switzerland, and the other study was from Germany and comprised only Germans, with these studies using a different genotyping methods. Thus, a total of seven case-control studies were included in this meta-analysis (shown in Table 1), which include a combined 1121 patients suffering from CP and 2209 controls. Four of the studies were carried out among Caucasians and three among Asians. The different genotyping methods used in each study and the presumed etiology of CP are shown in Table 1. In all the studies, the HWE of genotype distribution of the control group were calculated and found to be consistent with HWE.

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, $p_{\text{heterogeneity}} = 0.303$); the codominant model (MV vs. VV, OR = 1.292, 95% CI: 1.091–1.530, $p_{\text{heterogeneity}} = 0.153$; MM vs. VV, OR = 1.579, 95% CI: 1.274–1.956, $p_{\text{heterogeneity}} = 0.273$); the dominant model (MV/MV vs. VV, OR = 1.366, 95% CI: 1.165–1.603, $p_{\text{heterogeneity}} = 0.213$); and the recessive model (MM vs. MV/VV, OR = 1.346, 95% CI: 1.114–1.621, $p_{\text{heterogeneity}} = 0.219$). 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, $p_{\text{heterogeneity}} = 0.895$) and codominant models (MM vs. VV, OR = 1.666, 95% CI: 1.022–2.715, $p_{\text{heterogeneity}} = 0.937$). In the Caucasian group,

FIG. 1. Flowchart of the selection process for including studies.
*There were two separate studies in one article so that a total of seven studies were included.

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

Study first author (year)	Territory	Ethnicity	Genotype method	Case			Control			Etiology		
				M/M	M/V	V/V	M/M	M/V	V/V	ACP	ICP	p for HWE
de Cid (2010)	Spain	Caucasian	PCR+DGGE/SSCP	29	64	43	8	51	34	NA		0.187
Chang (2007)	Taiwan	Asian	Directing sequencing	18	38	22	37	84	79		+	0.23
Fujiki (2004)	Japan	Asian	PCR+RFLP	17	25	23	24	82	56	+	+	0.79
Lee (2003)	Korea	Asian	TDGS	6	14	8	23	52	42	NA		0.64
Tzetzis (2007)	Greece	Caucasian	PCR+DGGE/directing sequencing	3	18	4	24	104	83	NA		0.6
Steiner (2011) group 1	Switzerland	Caucasian	PCR+DGGE/dHPLC/SSCP	38	60	25	48	110	87		+	0.47
Steiner (2011) group 2	Germany	Caucasian	PCR+FRET probes	129	340	197	207	569	405		+	0.96

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

n	M vs. V		p _h	MV vs. VV		p _h	MM vs. VV		MM MV vs. VV		MM vs. MV VV	
	OR			OR			OR		OR		OR	p _h
Overall	7	1.260 (1.134–1.399)	0.303	1.292 (1.091–1.530)	0.153	1.579 (1.274–1.956)	0.273	1.366 (1.165–1.603)	0.213	1.346 (1.118–1.621)	0.219	
Ethnicities												
Asian	3	1.295 (1.009–1.662)	0.895	1.184 (0.792–1.769)	0.213	1.666 (1.022–2.715)	0.937	1.311 (0.902–1.907)	0.431	1.497 (0.979–2.290)	0.546	
Caucasian	4	1.392 (1.098–1.765)	0.075	1.433 (0.990–2.075)	0.106	2.011 (1.173–3.446)	0.061	1.567 (1.083–2.268)	0.084	1.533 (0.993–2.367)	0.081	
Etiology												
ICP	4	1.298 (1.020–1.653)	0.082	1.297 (1.074–1.566)	0.109	1.473 (1.165–1.862)	0.143	1.408 (0.954–2.078)	0.066	1.254 (1.023–1.538)	0.378	

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; p_h, 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.

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.

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. 5. Forest plot of homozygote comparison for ICP (MM vs. VV) using a random-effects model. Only ICP cases were analyzed in this comparison.

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

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 muta-

tions, 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

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 the 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* 470 M 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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