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Association of Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR*) Mutation/Variant/Haplotype and Tumor Necrosis Factor (*TNF*) Promoter Polymorphism in Hyperlipidemic Pancreatitis

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BACKGROUND: The mechanism by which hypertriglyceridemia (HTG) leads to pancreatitis is not clear. We sought to determine whether the genes involved in pancreatic ductal or acinar cell injury, including the cationic trypsinogen gene [protease, serine, 1 (trypsin 1) (*PRSS1*)], the pancreatic secretory trypsin inhibitor gene [serine peptidase inhibitor, Kazal type 1 (*SPINK1*)], the cystic fibrosis transmembrane conductance regulator gene [cystic fibrosis transmembrane conductance regulator (ATP-binding cassette subfamily C, member 7) (*CFTR*)], and inflammation genes such as tumor necrosis factor [tumor necrosis factor, TNF superfamily, member 2 (*TNF*)] are associated with hyperlipidemic pancreatitis (HLP) in patients with HTG.

METHODS: We performed genetic analysis of 126 HTG patients in Taiwan (46 with HLP and 80 without HLP). The entire coding and intronic regions of the *PRSS1*, *SPINK1*, and *CFTR* genes were identified by heteroduplex analysis techniques and were confirmed by sequencing analysis. The presence of 125G/C, 1001 + 11C>T, 1540A>G (Met470Val), 2694T>G, and 4521G>A in *CFTR*, the presence of 272C>T in *SPINK1*, and *TNF* promoter polymorphisms (nucleotide positions 1031, 863, 857, 308, and 308) were measured by direct sequencing.

RESULTS: Of the 126 HTG patients, 13 (10.3%) carried a *CFTR* mutation. No *PRSS1* or *SPINK1* mutations were detected in our patients or in HTG controls. The *CFTR* gene mutation rates in HTG with and without HLP were 26.1% (12 of 46) and 1.3% (1 of 80), respectively ($P < 0.0001$). The *CFTR* gene mutations were all Ile556Val. A multivariate analysis of HTG patients indicated that triglycerides, *CFTR* 470Val, and *TNF* promoter 863A were independent risk markers for HLP.

CONCLUSIONS: This genetic study is the first one to address the association of HLP with the *CFTR* mutation/variant/haplotype and *TNF* promoter polymorphism in a Chinese HTG population. The results suggest that the occurrence of HLP is multifactorial and polygenic.

Hypertriglyceridemia (HTG)[5] is associated with acute pancreatitis and is found in 3%–38% of patients with acute pancreatitis *(1–5)*. HTG is the 3rd most frequent etiology of acute pancreatitis in Taiwan *(6)*, accounting for about 10%–15% of patients with acute pancreatitis. In the absence of other etiologic factors and in the presence of high serum triglyceride (TG) concentrations (>11.3 mmol/L), acute pancreatitis is considered to be secondary to HTG, *(5, 7)*. Patients who present with significant HTG and pancreatitis usually have a preexisting abnormality in lipoprotein metabolism, such as lipoprotein lipase deficiency or apolipoprotein C deficiency *(8)*. Mutations, such as in genes for lipoprotein lipase and apolipoprotein, have been reported to be associated with HTG, but their occurrence does not explain why some patients develop acute pan-

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[5] Nonstandard abbreviations: HTG, hypertriglyceridemia; TG, triglyceride; OR, odds ratio; CI, confidence interval; HLP, hyperlipidemic pancreatitis.

creatitis and others do not. Previous studies have been inconclusive regarding which HTG patients will develop pancreatitis *(9–11)* and in explaining why some HTG patients seldom develop pancreatitis. The mechanism by which HTG leads to pancreatitis is not clear. Unbound free fatty acids are toxic and may cause trypsinogen activation, which could then lead to injury of acinar cells or capillaries and thereby initiate acute pancreatitis *(12, 13)*. Some HTG patients, however, seldom develop pancreatitis, even with marked increases in TG concentrations *(2)*.

Genetic factors may play important roles in susceptibility to pancreatic injury, as well as in the severity and evolution of the inflammatory process *(8)*. In humans, the genes *PRSS1*[6] [protease, serine, 1 (trypsin 1)], *SPINK1* (serine peptidase inhibitor, Kazal type 1) *(14)*, and *CFTR* [cystic fibrosis transmembrane conductance regulator (ATP-binding cassette sub-family C, member 7)] *(15–17)* and genes encoding factors that modulate the inflammatory response to pancreatic injury, such as *TNF* [tumor necrosis factor (TNF superfamily, member 2)] *(18)*, are reportedly associated with acute recurrent pancreatitis *(19)* and chronic pancreatitis *(20)*. In addition, mutations in these genes have enhanced the pancreatic inflammatory response in animal models. A transgenic mouse model expressing the Arg122His mutation in mouse trypsinogen displayed early-onset acinar injury, inflammatory cell infiltration, and enhanced response to cerulein-induced pancreatitis *(21)*. Similarly, *cftr*(−/−) mice showed constitutive expression of proinflammatory cytokines and developed more severe pancreatitis episodes after cerulein stimulation *(22)*. We evaluated the hypotheses that sensitization of ductal or acinar pancreas cells or cofactors is involved in the HTG process to induce acute pancreatitis and high serum TG concentrations and that mutations and functional variations of *PRSS1*, *SPINK1*, and *CFTR*, and *TNF* promoter polymorphisms are associated with hyperlipidemic pancreatitis (HLP).

Materials and Methods

CASES (HTG WITH HLP) AND CONTROLS (HTG WITHOUT HLP)

Patients and control individuals were recruited at a tertiary referral center (National Taiwan University Hospital) from September 2004 through December 2006. All patients and control individuals were Taiwanese or Taiwan Chinese from the same narrowly confined area in Taiwan *(18)*. Most study participants were not aboriginal Taiwanese, i.e., individuals with ancestors who had moved to Taiwan from southeastern China about 500 years ago. The control HTG patients without HLP were from the same areas as the HLP patients, were recruited from the hospital during the same time period, and had no history of acute pancreatitis, chronic pancreatitis, pancreatic adenocarcinoma, or any apparent biliary or pancreatic diseases. The control individuals also had no family history of pancreatitis or pancreatic adenocarcinoma. A diagnosis of HLP was based on the presence of a typical history (upper-abdominal pain with radiation to the back that was relieved by leaning forward or sitting upright and was increased after eating) and the following: suggestive radiologic findings and/or a 3-fold increase in amylase and/or lipase concentrations. Excluded from the study were patients who reported any other known risk factors for pancreatitis (either singly or in combination), including the following: a history of alcohol consumption of more than 2 drinks/day (20 g); biliary, metabolic (such as increased calcium), or autoimmune factors; medication with known pancreatic toxicity; pancreatic or periampullary tumors; a pancreatic duct anomaly or endocrine disorders; or a family history of pancreatitis.

Demographic and laboratory data were collected from medical chart records by means of a questionnaire that included information regarding clinical and family history. The clinical histories, which included the age of onset of symptoms, smoking history, and episodes of acute pancreatitis, were collected and reviewed.

ANTHROPOMETRICS AND BIOMEDICAL MEASUREMENTS

We measured fasting concentrations of glucose, total cholesterol, and TGs. Diabetes mellitus was diagnosed on the basis of a fasting plasma glucose value of >6.99 mmol/L and a postprandial 2-h plasma glucose value of >11.10 mmol/L, or by requirements for insulin or oral hypoglycemic drugs. Dyslipidemia was measured as a fasting TG concentration ≥2.25 mmol/L; hypercholesterolemia was indicated by a total-cholesterol concentration ≥6.0 mmol/L. We obtained weight and height measurements and calculated the body mass index.

We studied 126 patients (46 HLP patients and 80 HTG patients without HLP). Blood samples were drawn into EDTA anticoagulant after patients had provided written informed consent. The institutional ethics committee approved the study.

DNA EXTRACTION

We used the Viogene Blood & Tissue Genomic DNA Extraction System reagent set according to the manu-

[6] Human genes: *PRSS1*, protease, serine, 1 (trypsin 1); *SPINK1*, serine peptidase inhibitor, Kazal type 1; *CFTR*, cystic fibrosis transmembrane conductance regulator (ATP-binding cassette sub-family C, member 7); and *TNF*, tumor necrosis factor (TNF superfamily, member 2).

facturer's instructions to extract genomic DNA from leukocytes and stored the extracts at –20 °C until analysis *(15)*.

MUTATION/VARIANT SCREENING OF THE *PRSS1*, *SPINK1*, AND *CFTR* GENES

We amplified the coding and flanking noncoding regions of these 3 genes with primers *(14, 23)* and protocols *(24)* that have previously been described.

PCR AMPLIFICATION AND DENATURING HPLC HETERODUPLEX ANALYSIS

Mutations in the *PRSS1*, *SPINK1*, and *CFTR* genes were examined by PCR analysis and then analyzed with denaturing HPLC, according to procedures modified from those described in previous reports *(15, 25)*. Denaturing HPLC was performed on a WAVE DNA fragment analysis system equipped with a DNASep column and an ultraviolet-C scanner to detect eluted DNA (Transgenomic) *(26)*. Before loading onto the column, we denatured the PCR product by heating to 94 °C for 5 min and reannealing slowly by decreasing the temperature 1 °C/min until the temperature reached 25 °C (70 min total). The resolution temperature for each sequencing analysis was determined with DHPLC Melt software (http://insertion.stanford.edu/melt.html). PCR products displaying abnormal elution profiles were sequenced.

SEQUENCE ANALYSIS

We sequenced purified PCR products with BigDye Terminator sequencing chemistry (Applied Biosystems) and primers used in the initial amplification step and analyzed the sequencing reaction products with an ABI 3100 automated DNA sequencer (Applied Biosystems). Sequences were compared with the published sequence database (updates at http://www.uni-leipzig.de/pancreasmutation/db.html and http://www.genet.sickkids.on.ca/cftr/app).

VARIANTS, POLYMORPHISMS, AND GENOTYPING FOR *CFTR*, *SPINK1*, AND THE *TNF* PROMOTER

We evaluated the presence of 125G/C, 1001 + 11>CT, 1540A>G (Met470Val), 2694T>G, and 4521G>A variants in *CFTR* and the 272C>T polymorphism in *SPINK1* by direct sequencing. *TNF* promoter polymorphisms (nucleotide positions 1031, 863, 857, 308, 308) were evaluated via direct sequencing.

HAPLOTYPE ANALYSIS

We used a haplotype-based analytical approach to enhance investigation of disease-associated *CFTR* variants and studied 5 *CFTR* diallelic polymorphic loci to determine which haplotypes were associated with HLP risk. Expectation-maximization–based estimations of haplotype frequency and permutation-based hypothesis-testing procedures were based on previous work at our institution *(18)*. The level of statistical significance was set at $P < 0.05$ for the omnibus test and 1×10^{-3} (0.05/32) for individual haplotype analyses (32 haplotypes for 5 loci).

STATISTICAL ANALYSIS

To compare the between-group demographic data, we used the Student unpaired *t*-test for continuous data and the χ^2 test for categorical data. Statistical analysis of genotype distribution and allele frequencies was performed with the χ^2 test or the Fisher exact test. We applied multiple stepwise logistic regression analysis to determine the independent risk factors related to the presence of HLP, with adjustments made for age at study enrollment, sex, and anthropometric and biomedical characteristics to prevent confounding bias. All tests were 2-tailed with statistical significance set at $P < 0.05$ and were performed with SPSS software (SPSS for Windows 11.5).

Results

We studied 126 unrelated HTG patients, 90 of whom were men [mean (SD) age 45.1 (9.9) years]; 46 of the patients had HLP (36 men and 10 women) and 80 patients did not (54 men and 26 women).

The mean age of the HLP patients was younger than that of patients without HLP ($P = 0.006$). The difference in sex distribution between these 2 groups was not statistically significant. The clinical characteristics of the patients are summarized in Table 1. TG concentrations, ever-measured maximum TG concentrations, fasting glucose concentrations, hemoglobin A1c concentrations, and body mass indices were higher in HLP patients than in HTG patients without HLP.

PRSS1, *SPINK1*, AND *CFTR* GENE MUTATIONS IN HTG PATIENTS WITH AND WITHOUT HLP

For the patient cohort in this study, we analyzed all exons, including flanking intronic regions of *PRSS1*, *SPINK1*, and *CFTR*, by denaturing HPLC and confirmed the sequences by direct sequencing if heteroduplexing occurred.

Thirteen HTG patients (10.3%) had *CFTR* mutations. The only *CFTR* mutation hot spot was in codon 556 (Ile556Val), and of the 13 patients with this substitution, 12 had HLP. The spectrum and distribution of the mutations are presented in Table 1. The frequency of *CFTR* mutation was much higher in HLP patients

Table 1. Clinical characteristics of HTG patients with with and without HLP.[a]

Patient Characteristics[b]	HTG total n = 26	HTG without HLP n = 80	HTG with HLP n = 46
Age, years*	45.1 (9.9)	47.0 (10.1)	42.0 (8.9)
Male sex, %	71.4%	67.5%	78.3%
Smoking	32 (25.4%)	17 (21.3%)	15 (32.6%)
Alcohol	28 (22.2%)	14 (17.5%)	14 (30.4%)
Diabetes*	34 (27.0%)	5 (6.3%)	29 (63.0%)
Fasting glucose, mmol/L*	6.17 (2.37)	5.21 (0.92)	7.86 (3.09)
HbA1c[c], %*	6.3 (1.5)	5.8 (0.8)	7.1 (1.9)
AST, U/L	29.9 (19.2)	28.5 (14.5)	32.3 (25.3)
ALT, U/L	34.7 (23.1)	33.8 (24.7)	36.3 (20.4)
Total cholesterol, mmol/L	5.72 (2.21)	5.69 (1.45)	5.77 (3.13)
TG, mmol/L*	7.82 (10.82)	4.26 (3.69)	14.07 (1.55)
Maximum TG, mmol/L*	13.50 (32.26)	6.65 (4.86)	25.48 (15.71)
BMI, kg/m^2	25.8 (3.0)	25.2 (3.1)	26.9 (2.6)
CFTR mutation			
Ile556Val*	13 (10.3%)	1 (1.3%)	12 (26.1%)
CFTR variation			
125G>C*	7 (5.6%)	1 (1.3%)	6 (13.0%)
470Val*	64 (50.8%)	28 (35.0%)	36 (78.3%)
1001 + 11>CT	0 (0.0%)	1 (1.3%)	1 (2.2%)
2694T>G	0 (0.0%)	0 (0.0%)	0 (0.0%)
4521G>A	2 (1.6%)	0 (0.0%)	2 (4.3%)
SPINK1 variation			
c.0.272C>T (3′ UTR)	14 (11.1%)	12 (15.0%)	2 (4.3%)
TNF promoter			
1031C	47 (37.3%)	25 (31.3%)	22 (47.8%)
863A*	61 (48.4%)	28 (35.0%)	33 (71.7%)
857T	32 (35.4%)	19 (23.8%)	13 (28.3%)
308A	44 (34.9%)	25 (31.3%)	19 (41.3%)
238A	12 (9.5%)	10 (12.5%)	2 (4.3%)

[a] Data are presented as the mean (SD) or as the number of patients (percent).
[b] * indicates $P < 0.05$ for comparisons of hyperlipidemic patients with and without HLP.
[c] HbA1c, major fraction of glycosylated hemoglobin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; BMI, body mass index; UTR, untranslated region.

than in patients without HLP (26.1% and 1.3%, respectively, $P < 0.001$). All of the mutations were present in the heterozygous state. No *PRSS1* or *SPINK1* mutations were detected in these 2 groups.

ASSOCIATION OF THE *CFTR* MET470VAL POLYMORPHISM WITH HLP

Our results showed a significant difference in the frequency of the 470Val variant between HLP patients (78.3%) and HTG patients without HLP (35.0%, $P < 0.0001$) (Table 1).

CFTR HAPLOTYPE ANALYSIS AND ASSOCIATION OF *CFTR* HAPLOTYPE WITH HLP

Haplotypes were assembled with the genotype data obtained from the 126 tested samples and with the permutation test–based haplotype program that we used in our previous studies *(15, 18)*. We analyzed 5 loci, consisting of 125G/C, 1001 + 11>CT, 1540A>G (Met470Val), 2694T>G, and 4521G>A variants. Table 2 displays the results of our analyses of the estimated frequencies for 5-locus haplotypes for the *CFTR* polymorphic sites for patients and control indi-

Table 2. *CFTR* haplotype analysis in HTG patients with and without HLP and *P* values for permutation test comparisons.

5′ UTR[a]	Intron 6b	Exon 10	Exon 14a	Exon 24	Overall, %	HTG without HLP, %	HTG with HLP, %	*P*	OR[b]
125GC	1001+11>CT	Met470Val	2694T>G	4521G>A					
G	C	Met	T	G	0.72703	0.8125	0.56287	0.001	
G	C	Val	T	G	0.20551	0.1625	0.29582	0.014	2.47 (1.17–5.21)
C	C	Val	T	G	0.03656	0	0.08461	0.004	21.94 (2.70–177.96)
C	C	Met	T	G	0.01106	0	0.04582	0.302	
G	C	Met	T	G	0.00794	0.0125	0	0.334	
G	C	Val	T	G	0.00794	0.0125	0	0.334	
G	T	Val	T	G	0.00397	0	0.01087	0.072	
Likelihood ratio statistic			38.74909				0.001		

[a] UTR, untranslated region.
[b] ORs are presented as the mean (95% CI).

viduals in the entire population. With 5 loci, there should be 32 (i.e., 2^5) haplotypes. Because of linkage disequilibrium in this small region, however, some haplotypes did not exist or were present at very low frequencies, so we have listed only haplotypes with frequencies >0.001. The results of the omnibus haplotype profile test *(27)* were statistically significant (χ^2 = 38.74909; $P = 0.001$), indicating that the difference in the overall haplotype frequency profile between the patients and control individuals was significant and that some disease-predisposing haplotypes may occur in HTG patients with HLP. The 125G/1001 + 11C/470Met/2694T/4521G haplotype was a dominant haplotype in HTG patients. The 125C/1001 + 11C/470Val/2694T/4521G haplotype was associated with highest risk of HLP, with a large association effect [$P = 0.001$; odds ratio (OR), 21.94; 95% confidence interval (CI), 2.7–177.9] (Table 2).

TNF PROMOTER POLYMORPHISM ASSOCIATED WITH HLP

Of the 1031, 863, 857, 308, and 238 variable nucleotide positions in the *TNF* promoter, only the 863A variant was associated with HLP among the 126 HTG patients (71.7% vs 31.3%, $P < 0.001$) (Table 1).

GENE-GENE INTERACTION: *CFTR* MET470VAL AND *TNF* PROMOTER 863C/A

Out result demonstrated that the functional *CFTR* variant 470Val and the *TNF* promoter 863A variant were both associated with HLP, with ORs of 3.70 (95% CI, 2.90–15.50) and 4.71 (95% CI, 2.14–10.38), respectively. In patients with *CFTR* 470Val and *TNF* 863A, the OR was higher (OR, 5.18; 95% CI, 2.21–12.16) (Table 3).

ANTHROPOMETRICS AND BIOMEDICAL VARIABLES ASSOCIATED WITH HLP AMONG HTG PATIENTS

Higher fasting glucose concentrations, hemoglobin A1c concentrations, ever-measured maximum TG concentrations, TG concentrations and body mass indices were associated with the occurrence of HLP in univariate analyses (Table 4). According to a multivariate analysis adjusted for age and sex, however, only fasting glucose concentration, ever-measured maximum TG concentration, and TG concentration were

Table 3. Multivariate analysis of predictors of HLP among HTG patients.

Characteristic[a]	*P*	OR[b]
Fasting glucose*	0.001	1.06 (1.0.–1.08)
HbA1c[c]	0.141	
Total cholesterol	0.101	
TG*	0.030	1.002 (1.001–1.003)
Maximum TG*	0.000	1.004 (1.003–1.006)
BMI	0.861	
CFTR 125G>C	0.202	
CFTR 470Val*	0.007	3.70 (2.90–15.50)
SPINK1 c.0.272T	0.374	
TNF 863A*	0.011	4.71 (2.14–10.38)
CFTR 470Val × *TNF* 863A*	0.002	5.18 (2.21–12.16)

[a] * indicates $P < 0.05$ for comparison of hyperlipidemic patients with and patients without HLP.
[b] ORs are presented as the mean (95% CI).
[c] HbA1c, major fraction of glycosylated hemoglobin; ALT, alanine aminotransferase; BMI, body mass index.

Table 4. Univariate analyses of predictors of HLP among HTG patients.

Characteristic[a]	Regression coefficient	SE	Wald	*P*
Fasting glucose*	0.057	0.011	24.482	0.000
HbA1c*[b]	0.953	0.236	16.345	0.000
ALT	0.005	0.008	0.359	0.549
Total cholesterol	0.000	0.002	0.044	0.834
TG*	0.002	0.001	17.979	0.000
Maximum TG*	0.004	0.001	27.450	0.000
BMI*	0.208	0.071	8.608	0.003
125G>C*	3.328	1.061	9.481	0.002
470Val*	1.900	0.427	19.756	0.000
SPINK1 c.0.272T	1.356	0.788	2.964	0.085
TNF 863A*	1.551	1.403	14.826	0.000

[a] * indicates $P < 0.05$ for comparisons of HTG patients with and without HLP.
[b] HbA1c, major fraction of glycosylated hemoglobin; ALT, alanine aminotransferase; BMI, body mass index.

independent risk factors for the occurrence of HLP in HTG patients (Table 3).

GENOTYPE-PHENOTYPE ASSOCIATION

Within the HLP group, patients with *CFTR* mutations, patients without *CFTR* mutations, and patients with *CFTR* Met470Val and *TNF* promoter variants demonstrated no significant differences with respect to the values for demographic, anthropometric, and biomedical variables.

Discussion

It is important and difficult to predict which hyperlipidemic patient is likely to develop an episode of pancreatitis. Comparative profiling of anthropometric and biomedical variables, coupled with a genetic host-susceptibility approach may revolutionize medical practice. We compared the clinical and genetic characteristics of HLP patients and HTG patients without HLP to identify the susceptibility to HLP within a small geographic area. This study is the first to address the role of genetic factors (other than genes involved in lipid metabolism) in HLP and is the first to demonstrate an association of *CFTR* mutation with HLP. It is the largest study to investigate HLP patients with patients with HTG alone as a control group; other studies have been case reports or case series *(9–11)*. Our results revealed that the TG concentration, the fasting glucose concentration, and genetic factors (including *CFTR* mutation/variant/haplotype and *TNF* promoter polymorphism) may contribute to the development of HLP in HTG patients. Our results suggest that the occurrence of HLP in HTG patients is a multifactorial and polygenic event.

Our previous studies demonstrated that the spectrum of *SPINK1*, *PRSS1* (unpublished data), and *CFTR* mutations/variants in our study patients with idiopathic chronic pancreatitis is very different from the spectrums in our healthy controls and pancreatitis patients from Western countries *(15)*. In our current study, we detected no *PRSS1* or *SPINK1* gene mutations in HLP patients and in HTG control individuals. In contrast, the Ile556Val *CFTR* missense mutation was the most common mutation in our HLP patients. The Ile556Val mutation has a mild effect among *CFTR* mutations and appears to be associated with the pancreatitis phenotype *(28)*. It was originally identified in patients with moderate pulmonary disease and insufficient pancreatic function *(29)*. Ile556Val was also reported to be associated with chronic pulmonary disease in Singapore *(30)*. The frequency of the Ile556Val mutation in our HLP patients was 26.1% (12 of 46). Interestingly, Ile556Val was also the most frequent *CFTR* mutation in our patients with idiopathic chronic pancreatitis *(15)*. The frequency of the Ile556Val *CFTR* mutation in our healthy control population was about 1% (1 in 200) *(15)*, similar to the frequency in our HTG patients without HLP (1.3%, 1 of 80). Because the TG concentrations in our chronic pancreatitis cohort were within the TG reference interval, HTG is not likely associated with the *CFTR* Ile556Val mutation. Furthermore, the mean TG concentration in our HLP patients with the Ile556Val mutation was not significantly different from that of patients without the Ile556Val mutation.

CFTR is a human gene with many mutations and variants; it is thus advisable to use a comprehensive method to analyze genetic alterations and to use a haplotype-based approach to address the role of *CFTR* variants in assessing the risks for distinct phenotypes of *CFTR*-related disease in different populations. Functional *CFTR* polymorphisms may also affect the expression of the CFTR protein. Met470Val, a common functional polymorphism in *CFTR* exon 10, affects intrinsic chloride channel activity *(31)*. The cDNA single-nucleotide polymorphisms 2694T>G and 4521G>A may affect pre-mRNA splicing by changing regulatory-sequence motifs of exonic splice enhancers, leading to increased skipping of exons 9 and 12 and lower amounts of normal transcripts *(32)*. The 125G/C polymorphism is associated with susceptibility to pancreatitis in Chinese individuals *(15)*. Our study has shown that 470Val is associated with HLP among patients with HTG. In contrast, the 470Val variant is not associated with susceptibility to idiopathic chronic

pancreatitis in Chinese individuals. Lee et al., however, reported that *CFTR* mutation with a 470Val background was associated with a pancreatic phenotype (pancreatitis) in the Korean population *(33)*. Our haplotype analysis demonstrated a distinct haplotype (125C/1001 + 11C/470Val/2694T/4521G) that conferred a high risk for HLP among HTG patients and the haplotype differed from the one in the risk for idiopathic chronic pancreatitis in the Chinese population *(15)*. These observations suggest that the role of a polymorphism such as Met470Val might be different in different disease subsets and in different ethnic populations.

Proinflammatory cytokines also play an important role in the development of pancreatitis. Different mutations in different genes may lead to different phenotypic presentations of pancreatitis, and even the same mutation may have different consequences depending on the interaction of the genetic background with environmental factors *(20)*. *TNF* promoter polymorphism has been reported to be associated with the manifestation of hereditary pancreatitis *(21)* in Caucasians and of chronic pancreatitis in Chinese *(18)*. Our study also has demonstrated that *TNF* promoter polymorphism (i.e., the 863A variant) is associated with the development of HLP in HTG patients. In an animal model, multiple proinflammatory cytokine genes were constitutively overexpressed in the pancreas of *cftr*(–/–) mice compared with wild-type mice *(22)*. Furthermore, more severe pancreatitis attacks were observed after cerulein stimulation in *cftr*(–/–) mice *(22)*. These findings may partially explain the susceptibility to acute pancreatitis in HTG patients with the *CFTR* variant. Our study has shown that gene-gene interaction between *CFTR* and *TNF* increased the risk of HLP development in HTG patients, in addition to producing high serum TG concentrations. Further studies including larger sample sizes and evidence from different ethnic groups are needed, along with investigations of the roles of these genes in the pathogenesis of HLP.

In conclusion, *CFTR* mutations are associated with a broad spectrum of pancreatic phenotypes. Identification of the association of *CFTR* and other genes with HLP have provided evidence that HTG patients with *CFTR* mutations/variants are more susceptible to developing HLP. This susceptibility increased after *CFTR* mutation/variant interaction with proinflammatory cytokines such as TNF. The development of HLP in HTG patients appears to be a multifactorial and polygenic event.

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