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RAPID COMMUNICATION

Comparative analysis of common *CFTR* polymorphisms poly-T, TG-repeats and M470V in a healthy Chinese population

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Abstract

AIM: To investigate the three important cystic fibrosis transmembrane conductance regulator (*CFTR*) haplotypes poly-T, TG-repeats and the M470V polymorphisms in the Chinese population, and to compare their distribution with that in Caucasians and other Asian populations.

METHODS: Genomic DNA was extracted from blood leukocytes. Exons 9 and 10 of the *CFTR* gene were obtained through polymerase chain reaction (PCR). Exon 9 DNA sequences were directly detected by an automated sequencer and poly-T and TG-repeats were identified by direct sequence analysis. Pure exon 10 PCR-amplified products were digested by *Hph* I restriction enzyme and the M470V mutation was detected by the AGE photos of digestion products.

RESULTS: T7 was the most common (93.6%) haplotype and the (TG)11 frequency of 57.2% and (TG)12 frequency of 40.9% were dominant haplotypes in the junction of intron 8 (IVS-8) and exon 9. The frequency of T5 was 3.8% and all T5 allele tracts (10 alleles) were joined with (TG)12. Four new alleles of T6 (1.5%) were found in three healthy individuals. In exon 10, the V allele (56.1%) was slightly more frequent than the M allele (43.9%), and the M/V (45.5%) was the dominant genotype in these individuals. The three major haplotypes T7-(TG)11-V470, T7-(TG)12-M470 and T7-TG11-M470 were related to nearly 86.0% of the population.

CONCLUSION: The polymorphisms of poly-T, TG-repeats, and M470V distribution were similar to those in other East Asians, but they had marked differences in frequency from those single haplotype polymorphisms or linkage haplotypes in Caucasians. Thus, they may be able to explain the low incidence of CF and CF-like diseases in Asians.

Key words: Cystic fibrosis transmembrane conductance regulator gene; Gene polymorphism; Poly-T; TG-repeats; M470V

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INTRODUCTION

The cystic fibrosis transmembrane conductance regulator (CFTR) gene on chromosome 7q31 spans approximately 250 kb of DNA and encodes 27 exons encodes[1]. The *CFTR* gene encodes a cAMP- and ATP-dependent chloride channel that is present in the apical membrane of the epithelial cells that line most exocrine glands[2]. Phosphorylation of the regulatory domain by protein kinase A, followed by binding and hydrolysis of ATP at both nucleotide-binding domains, regulates the transport of chloride ions through the channel[3]. Absence, reduced levels, or malfunction of the CFTR protein results in cystic fibrosis (CF), and CF-like diseases such as congenital bilateral absence of the vas deferens (CBAVD)[4,5], bronchiectasis[6] and chronic pancreatitis[7]. Since the discovery of the *CFTR* gene, more than 1000 mutations and 200 polymorphisms have been identified[8]. CF is one of the most common autosomal recessive disorders in Caucasians, with an incidence of approximately 1 in 2500 Caucasian births and a carrier frequency of approximately 1 in 25. However, in Asians, the prevalence of CF is very low, with an incidence of approximately 1 in 100 000, and in particular, the severe mutations, such as ΔF508, G542X and N1303K, are rarely found in Asians. Previous studies have demonstrated that polymorphisms outside the *CFTR* gene[9,10], as well as within the gene, may affect transcription or function of the CFTR protein and modify the phenotype of some CF mutations. It has been mentioned that poly-T, TG-repeats and M470V polymorphisms play a role in the development of CF-like diseases. The poly-T tract located at the junction of intron

8 (IVS-8) and exon 9 influence transcription, and thereby reduce the amount of normal CFTR protein. The number of T residues present, five, seven or nine, affects the splicing efficiency of exon 9. If the T5 allele is present, a proportion of CFTR transcripts will lack exon 9, which produces a non-functional protein and variable CF symptoms[11]. The TG-repeats, 5' of the poly-T, also influence splicing of exon 9[12], and when present on the same allele as a 5T repeat, the longer the TG-repeats, the higher the proportion of CFTR transcripts that will lack exon 9. On the other hand, the M470V polymorphism on exon 10 affects the intrinsic chloride activity, and thereby affects the function of the CFTR protein[12,13].

Although mutations and polymorphisms of CFTR have been extensively studied in Western populations, their importance is less well studied in East Asia because of the rare presentation of classical CF. There are just a few data on *CFTR* in Asia, especially in China. No reports on CFTR genetic background among the normal Chinese population have been published, except for sporadic reports on *CFTR* mutations in CF-like patients. To explore polymorphic backgrounds of CFTR in the Chinese population, we analyzed polymorphisms of poly-T, TG repeats and M470V in 132 healthy individuals among the general population in Jiangsu Province.

MATERIALS AND METHODS

Subjects

A total of 132 healthy unrelated subjects were randomly selected from the population of Jingsu Province (78 males, 54 females; mean age 44 years, range 16-85 years). Four milliliters of blood were collected for genotyping. The blood was mixed with EDTA and stored at -80℃.

DNA analysis

Genomic DNA was extracted from blood leukocytes using the QiaAmp DNA Blood Mini kit (Qiagen, Hilden, Germany). Polymerase chain reaction (PCR) was carried out using Ex *Taq* Polymerase (Takara, Japan). The PCR used a GeneAmp PCR system (Model PTC-200, Bio-Red, Foster City, CA, USA). Cycling for both reactions was performed as follows: 94℃ for 5 min for preheating, 35 cycles at 94℃ for 30 s, 60℃ for 60 s, and 72℃ for 30 s, followed by one cycle at 72℃ for 10 min for extension. The oligonucleotide primers used were: Intron 8 and exon 9 junction, sense 5'-CCATGTGCTTTTCAAACTAAT TGT-3', anti-sense 5'-TAAAGTTATTGAATGCTCGC CATG-3'; and exon 10, sense 5'-TTGTGCATAGCAG AGTACCTGAAA-3', anti-sense 5'-GCTTCTTAAAGC ATAGGTCATGTG-3'. The sequences of exon 9 PCR-amplified products were sequenced by Shanghai Invitrogen Company using an automated sequencer (ABI 737). Exon 10 PCR-amplified products were purified using the High Pure PCR Product Purification Kit (Roche Diagnostics, Mannheim, Germany). The M470V mutation was detected by *Hph*I restriction enzyme digestion.

Haplotype analysis

Haplotypes consisting of three loci were investigated, that is, poly-T, TG-repeats, and the M470V. The M470V polymorphisms were estimated by the AGE photos of pure PCR products after *Hph*I restriction enzyme digestion (Figure 1). Poly-T and TG-repeats are continuous in sequence, hence their haplotypes were identified by direct sequence analysis (Figure 2). The frequency of each haplotype of TG-repeats and M470V (P_m) was estimated by the following equation derived from the Hardy-Weinberg law: $(P_1 + P_2 + P_3 + .P_m)^2 = 1$, where $P_1 + P_2 + P_3 + .+P_m = 1$, and P_1^2, P_2^2, P_3^2, P_m^2 are the frequencies of homozygous for either locus or both loci.

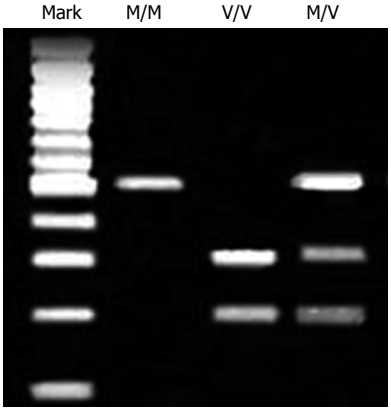

Figure 1 M470V polymorphism in exon 10.

Table 1 Allele frequency of poly-T tract

Number (n)	Number (frequencies) of poly-T tract			
	T5	T6	T7	T9
$2n$ = 264	10 (0.0379)	4 (0.0152)	247 (0.9356)	3 (0.0114)

RESULTS

Poly-T

T7 was the most common haplotype (93.6%), hence, T7/T7 was a dominant genotype in Chinese individuals (Table 1). Four alleles of T6 were newly found in the normal subjects. Ten alleles of T5 with a frequency of 3.8% were found. The T9 alleles were very rare at just 1.1%. The sequence analysis indicated that four alleles of T6 probably resulted from a T deletion from T7.

TG-repeats

(TG)11 and (TG)12 were the dominant haplotypes in the Chinese population, with a frequency of 57.2 and 40.9%, respectively. The frequency distribution of genotypes (TG)11/(TG)11, (TG)11/(TG)12 and (TG)12/(TG)12 was 46.2, 21.2 and 29.6%, respectively (Table 2). The relative ratio of the (TG)11/(TG)11, (TG)11/(TG)12 and (TG)12/(TG)12 was roughly 2:1:1.

M470V

The V allele was slightly more frequent than the M allele in the Chinese population, and the frequencies were 56.1 and 43.9%, respectively. The dominant genotype was M/V, followed by V/V and M/M (Table 3).

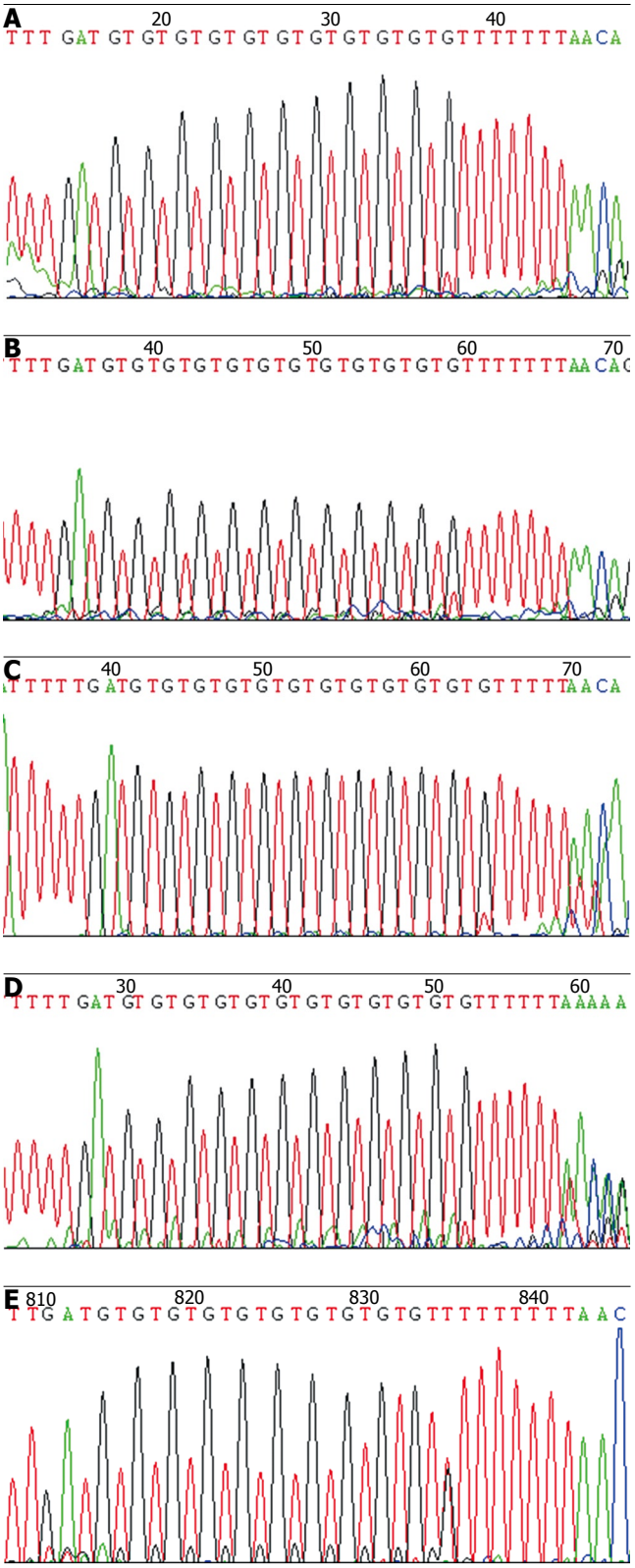

Figure 2 The polymorphisms of TG-repeats and Poly-T haplotype in IVS-8. **A**: TG11-T7; **B**: TG12-T7; **C**: TG12-T5; **D**: TG12-T6; **E**: TG10-T9.

Three-locus haplotype analysis

T7-TG11-V470 was the dominant linkage haplotype in the Chinese population, and the frequency was 47.4%, which was almost half of all the types of linkage. Other frequencies were: T7-TG12-M470, 29.2%; T7-TG11-M470, 9.9%; and T7-TG11-M470, 6.8%, and others were very rare (Table 4). The T5 alleles were all linked with (TG)12, and its distribution ratio at M470 and V470 loci was 4:1.

Table 2 Allele frequency of TG-repeat polymorphisms

Number ($2n$)	Number (frequencies) of TG-repeats			
	TG10	TG11	TG12	TG13
264	3 (0.0114)	151 (0.572)	108 (0.4091)	2 (0.0076)
	TG-repeat number (frequencies) in individuals with alleles			
	11/11	11/12	12/12	11/13	10/12
132	61 (0.4621)	28 (0.2121)	39 (0.2955)	2 (0.0152)	2 (0.0152)

Table 3 Genotypes and allele frequencies at M470V polymorphic site

Number ($2n$)	Number (frequencies) of individuals with genotypes			Number (frequencies) of individuals with alleles	
	MM	MV	VV	M	V
264	28 (0.2121)	60 (0.4545)	44 (0.3333)	116 (0.4394)	148 (0.5606)

Table 4 Linkage of poly-T, TG-repeats and M470V

Linkage haplotypes	$2n$ (frequencies) ($2n$ = 264)
T7-TG11-V470	125 (0.4735)
T7-TG12-M470	77 (0.2917)
T7-TG11-M470	26 (0.0985)
T7-TG12-V470	18 (0.0682)
T5-TG12-M470	8 (0.0303)
T5-TG12-V470	2 (0.0076)
T7-TG13-M470	2 (0.0076)
T9-TG10-M470	1 (0.0038)
T9-TG10-V470	1 (0.0038)
T6-TG12-M470	2 (0.0076)
T6-TG12-V470	1 (0.0038)
T6-TG11-V470	1 (0.0038)

DISCUSSION

The present study is believed to be the first comprehensive report on the functional polymorphisms of CFTR in the Chinese population. Analysis of three polymorphic loci with frequent alleles in the general population showed the poly-T, TG-repeats and M470V distributions were similar to those reported for other East Asians[14-16]. T7 was the most common haplotype (93.6%), and (TG)11 and (TG)12 were the dominant haplotypes in the junction of intron 8 (IVS-8) and exon 9. In exon 10, the V allele was slightly more frequent than the M allele, and the M/V genotype was the dominant genotype. The three major haplotypes T7- (TG)11-V470, T7- (TG)12- M470 and T7-TG11-M470 were found in nearly 86.0% of the population.

Similar to other populations, the T7 allele was the most common haplotype (93.6%) in IVS-8, and T7/T7 was the dominant genotype in Chinese individuals. The T6 allele, in addition to the well-known T5, T7 and T9 alleles, was

found. It has not been reported in Caucasians, but it has been reported in Asians, including Vietnamese[14], Japanese[15] and Koreans[16]. However, its functional transcript and disease association is uncertain at this time. Four T6 alleles (1.5%) were found in three normal individuals, and the frequency was higher than that in Japanese (1.2%)[15] and Vietnamese (0.1%)[14]. The T9 allele was found in three alleles, and its frequency of 1.1% was higher than that in Japanese (0.6%-1.0%)[14,15], Vietnamese (0.6%)[14] and Koreans (0.52%)[16] but lower than in Caucasians (7.0%)[14]. It is known that polymorphisms in IVS-8 Tn tract affect the RNA splicing of exon 9, and the T9 allele is associated with the most efficient usage of the IVS-8 splice acceptor site[11]. This efficiency decreases with shorter poly-T tract T5, which results in a lower than normal level of full-length CFTR mRNA, and presumably a decrease in mature, functional CFTR protein. T5 may be the most common atypical CF mutation worldwide. Prior studies have demonstrated that some individuals who carry T5 with a severe CF-causing mutation may have non-classic CF; others may have male infertility due to CBAVD[5,17-20], lung disease such as bronch iectasis[6,21,22]and chronic pancreatitis[23,24]; and approximately 40% may be healthy and fertile as a consequence of incomplete penetrance[5,17]. In our study, the frequency of the T5 allele in the Chinese population was 3.8%, which is similar to the Vietnamese (3.7%)[14], but lower than that in Caucasians (7.0%)[5,13], and higher than in Japanese (0.6-1.0%)[14,15] and Koreans (1.7%)[16].

The main TG-repeat was (TG)11/11, with 61 (46.21%) individuals having the (TG)11/11 haplotype. (TG)11 was the dominant haplotype in the Chinese population, with a high frequency of 57.2%. This was higher than in Vietnamese (41%)[14] and Japanese (51%-54%)[14,15], but lower than in Caucasians (67%)[14]. The main dominant haplotypes were (TG)11 and (TG)12 in the Chinese population, as well as other Asian populations, however, in Caucasians, after the (TG)11 haplotype, the most common was the (TG)10 haplotype[14,25]. As previously reported, the TG-repeats that join with poly-T tracts also influence splicing of exon 9[12], and when present on the same allele as a T5 tract, the longer the TG-repeats, the higher the proportion of CFTR transcripts that will lack exon 9. T5 allele adjacent to either (TG)12 or (TG)13 repeats is more likely to exhibit an abnormal phenotype than T5 adjacent to (TG)11[26]. In our study, all T5 allele tracts (10 alleles) were joined with (TG)12 repeats. The TG repeat number also exerts an effect on a T7 background. Compared with (TG)10 allele, TG (11) increases almost threefold the proportion of CFTR transcripts that lack exon 9[12].

At the M470V locus on exon 10, similar to other Asian populations and Caucasians, the V allele (56.1%) was more frequent than the M allele (43.9%) in the Chinese population. The M470V polymorphism is a missense mutation caused by a particular amino acid alteration in the exon 10 M470V locus. It has been shown the M470 allele causes a delay in CFTR protein maturation and gives rise to a chloride channel with an increased probability of being open, compared with the V470 CFTR protein[12]. It has also been shown that the variability of European random *CFTR* genes is almost completely restricted to those who carry the M allele of the M470V polymorphic site[27].

Table 5 Frequencies of TG-repeats and M470V haplotypes

M470V	(TG) *n*	*n*/frequencies (*n* = 132)
V/V	11/11	35 (0.2625)
M/M	12/12	27 (0.2045)
M/V	11/11	26 (0.1970)
M/V	11/12	20 (0.1515)
M/V	12/12	11 (0.0833)
V/V	11/12	8 (0.0606)
M/V	11/13	2 (0.0152)
V/V	12/12	1 (0.0076)
M/M	10/12	1 (0.0076)
M/V	10/12	1 (0.0076)

However, interestingly, the M allele has been reported for some CF mutations, and particularly for ΔF508[28-30], most mutations have been found to be associated with the M470 allele, while the V470 allele shows an extended haplotype homozygosity[27,31,32].

The T7 allele tracts were combined with (TG)11 and (TG)12 repeats, but the T7-(TG)10 haplotype was not found in our study. In the T7 background, the (TG)11/(TG)11-V/V, (TG)12/(TG)12-M/M, (TG)11/(TG)11-M/V and (TG)11/(TG)12-M/V were the four main haplotypes and almost equally distributed in the Chinese population (Table 5). We found that TG-repeats and M470V had a linkage distribution in that V/V was combined with (TG)11/(TG)11, and M/M was combined with (TG)12/(TG)12 haplotype. The major haplotypes were T7-(TG)11-V470 and T7-(TG)12- M470, similar to Japanese and Vietnamese, but in the Chinese and Japanese, the main haplotype was T7-TG11-V470. Conversely, T7-TG12- M470V was the main haplotype in Vietnamese. However, in Caucasians, two main haplotypes, T7-(TG)11- V470 and T7-(TG)10-M470 are predominant[14,25]. As previously reported, TG-repeats also influence the function of CTFR protein, and longer TG repeats increase the proportion of CFTR transcripts that lack exon 9[12]. Therefore, the two major (TG)11/(TG)12-bearing haplotypes may have a corresponding low CFTR activity in Asian populations compared with the dominant (TG)11/(TG)10-bearing haplotypes in Caucasians. This may explain the low incidence of CF and CF-like diseases in Asians.

This report provides evidence for a poly-T, TG-repeat and M470V haplotype background in the Chinese population. We found polymorphisms of poly-T, TG-repeats and M470V were similarly distributed in other East Asians, and have marked differences from the frequencies of single haplotype polymorphisms or linkage haplotypes in Caucasians. Further study of the relationship between polymorphisms of poly-T, TG repeats and M470V haplotypes in CF and CF-like diseases in the Chinese population should be undertaken.

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COMMENTS

Background

The cystic fibrosis transmembrane conductance regulator (*CFTR*) gene encodes a cAMP- and ATP-dependent chloride channel that is present in the apical membrane of the epithelial cells that line most exocrine glands. Absence, reduced levels, or malfunction of the CFTR protein results in CF and CF-like diseases, such as CBAVD, bronchiectasis and chronic pancreatitis. It has been reported that poly-T, TG-repeats and M470V polymorphisms play a role in the development of CF-like diseases. The present study is believed to be the first comprehensive report on functional polymorphisms of *CFTR* in the Chinese population.

Research frontiers

The *CFTR* gene is a cAMP- and ATP-dependent chloride ion transport channel. The three haplotypes poly-T, TG-repeats and M470V polymorphisms play a role in the development of CF-like diseases. Although mutations and polymorphisms of *CFTR* have been extensively studied in Western populations, their importance is less well-established in East Asia. The present study is believed to be the first comprehensive report on functional polymorphisms of *CFTR* in the Chinese population. This study provides evidence for poly-T, TG-repeat and M470V haplotype backgrounds in the Chinese population.

Innovations and breakthroughs

There are just a few data on *CFTR* in Asia, especially in China. No reports on *CFTR* genetic background among the normal Chinese population have been published, except for sporadic reports on *CFTR* mutations in CF-like patients. The present study is believed to be the first comprehensive report on functional polymorphisms of *CFTR* in the Chinese population. This study provides evidence for poly-T, TG-repeat and M470V haplotype backgrounds in the Chinese population.

Applications

Comparative analysis of common *CFTR* polymorphisms in poly-T, TG-repeats and M470V in the healthy Chinese population sheds light on the situation of *CFTR* gene mutations and polymorphisms in the Chinese population. This study provides a polymorphic background of *CFTR* in the Chinese population, and helps to understand CF-like diseases such as CBAVD, bronchiectasis and chronic pancreatitis in China.

Terminology

CFTR gene on chromosome 7q31 spans approximately 250 kb of DNA and encodes 27 exons. The *CFTR* gene encodes a cAMP- and ATP-dependent chloride channel that is present in the apical membrane of the epithelial cells which line most exocrine glands.

Peer review

This interesting study investigated three important *CFTR* haplotypes, poly-T, TG-repeats and M470V polymorphisms in the Chinese population. The results may explain the low incidence of CF and CF-like diseases in Asians.

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