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# CFTR POLYMORPHISMS OF HEALTHY INDIVIDUALS IN TWO CHINESE CITIES—CHANGCHUN AND NANJING

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## ABSTRACT

Background and Aim Mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, which encodes a chloride channel, cause cystic fibrosis. In order to investigate the polymorphic backgrounds of CFTR genes of healthy populations in different Chinese cities (Changchun and Nanjing), we analyzed 119 blood samples (Changchun 64, Nanjing 55) of randomly selected healthy individuals for poly T, TG-repeats and M470V polymorphisms. We analyzed the differences of CFTR polymorphic distributions between the two Chinese cities from the south and the north. Methods Genomic DNA was extracted from whole blood. DNA fragments of CFTR gene were amplified by polymerase chain reaction (PCR). Poly-T and TG repeats were directly sequenced by auto sequencer (ABI 310). M470V was detected by a *Hph*I restriction enzyme. Results The T7 allele was the most common haplotype in Changchun (0.938) and Nanjing (0.927) populations. The T5 allele was present in only 7 Changchun and 3 Nanjing subjects. The TG11 and TG12 alleles were dominant haplotypes in Changchun (TG11 0.500, TG12 0.453) and Nanjing (TG11 0.345, TG12 0.609). The frequency of the V470 allele was 0.633 in Changchun, which was higher than that in Nanjing (0.500) ($p<0.05$). There were three major haplotypes: T7-TG11-V470, T7-TG12-M470 and T7-TG12-V470. The T7-TG11-V470 was the most common haplotype in Changchun (0.514), while T7-TG12-M470 was the most common haplotype in Nanjing (0.500). Conclusion Though Changchun and Nanjing are in the same country, their polymorphic backgrounds of CFTR gene are very different. Most of the two populations have genotypes that cause lower CFTR function.

Key Words: CFTR, Polymorphisms, Poly-T, TG repeats, Chinese

## INTRODUCTION

Cystic fibrosis (CF) is the most common fatal autonomic recessive disease in Caucasian populations, with a prevalence of 1 in 2000–3000 whites.[1)] The disease affects multiple organs

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manifesting as pancreatic insufficiency and fibrosis, meconium ileus in newborns, recurrent respiratory infections and bronchiectasis, male infertility due to congenital bilateral absence of vas deferens, etc. The gene which encodes a membrane protein (the cystic fibrosis transmembrane conductance regulator (CFTR)) is responsible for CF and was cloned in 1989.[2] CFTR is a cAMP-regulated $Cl^-$ channel that is expressed in many epithelial tissues. Currently, over 1000 mutations and 200 polymorphic loci in CFTR have now been identified.[3] These mutations and polymorphisms confer somewhat variable phenotypes from classic CF to atypical CF with less severe pulmonary lesions, pancreatic sufficiency, and normal or borderline sweat chloride concentration.[4] It is now well recognized that the spectrum of CFTR-related disease is much broader than previously thought.[5] Some polymorphic loci may affect the transcription or function of the CFTR protein. Bombieri et al. found that poly-thymidine (poly-T), TG-repeats, and M470V polymorphisms play a role in developing CF-like diseases. Poly-T and TG-repeats at the junction of intron 8 and exon 9 affect the transcription of exon 9 mRNA by exon skipping, and alter the level of normal CFTR protein. On the other hand, an M470V polymorphism on exon 10 influences the intrinsic chloride activity.[6] It is estimated that about 1 in 20 to 25 caucasians carry the mutation of CFTR. However, CF is rare in Asian races including Chinese.[7] Naruse et al.[8] developed a simple method for measuring finger sweat chloride concentrations to test whether CFTR dysfunction underlies chronic pancreatitis in Japan. Fujiki[9] and Nam[10] investigated the CFTR polymorphisms in Japanese and Vietnamese groups. To our knowledge, there have been no reported CFTR mutations among the normal Chinese population, especially in Changchun and Nanjing city. The total population of China is about 1.34 billion (2001, WHO), around 20% of the total world population, and is distributed unevenly across 9,600,000 $km^2$. There are many differences among each region. In order to investigate the polymorphic backgrounds of CFTR genes of healthy populations in China, we randomly selected 119 healthy individuals from Changchun and Nanjing for an analysis of poly-T, TG-repeats and M470V polymorphisms.

## MATERIALS AND METHODS

*Subjects*

The medical department of Jilin University approved this study, and written informed consent was obtained from each subject prior to the study. 119 healthy unrelated subjects (68 males, 51 females) were arbitrarily selected from the cities of Changchun (64 subjects, 30 males, 34 females) and Nanjing (55 subjects, 38 males, 17 females). Mean age was 44.8 years (range 10–85 years). All the subjects were healthy volunteers who showed no disease at their regular annual health checkups, which included physical examinations, urinalysis, blood cell counts and blood biochemistry, radiographic examinations of the chest and upper gastrointestinal tract, and abdominal ultrasonography. 2 ml of blood samples were collected for genotyping.

*Methods*

DNA was extracted from 119 samples of whole blood with the QIAamp DNA Blood Mini kit (Qiagen, Hilden, Germany). PCR was carried out using ExTaq (TaKaRa, Japan) for analysis of the three polymorphisms. The PCR protocol was 35 cycles using a GeneAmp PCR system (model 9700; Applied Biosystems, Foster, CA, USA) at 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds. The oligonucleotide primers used were: intron 8 and exon 9 junction sense 5'-CCA TGT GCT TTT CAA ACT AAT TGT-3', antisense 5'-CAA CCG CCA ACA ACT GTCCT-3'; and exon 10 sense 5'-TTG TGC ATA GCA GAG TAC CTG AAA-3', antisense 5'-ATT GAT CCA TTC ACA GTA GCT -3'. PCR products were purified using a

High Pure PCR Product Purification kit (Roche Diagnostics, Mannheim, Germany) and were sequenced directly by an automated sequencer (ABI 310) using a dGTP BigDye™ Terminator Ready Reaction kit (Applied Biosystems). The M470V mutation was detected by *Hph*I restriction enzyme digestion.

*Statistical analysis*

Conformity of the genotype distribution of the polymorphisms to the Hardy-Weinberg equilibrium was examined in each population under study. Poly-T and TG-repeats are continuous in sequence, hence their haplotypes were identified by direct sequence analysis. The M470V polymorphisms were estimated using AGE photos of pure PCR products after *Hph*I restriction enzyme digestion. Only those genotypes with M/M470, V/V470, TG11/11, and TG12/12 could be estimated for each haplotype of TG-repeats and M470V. An $X^2$ test was used for statistical analysis, and $p<0.05$ was considered significant.

## RESULTS

The T7 allele was the most common haplotype in Changchun (0.938) and Nanjing (0.927) populations (Table 1). The T7/T7 was a dominant genotype in the two populations. The T5, T6 and T9 alleles were much less frequent than the T7 allele. The T5 allele was present in only 7 Changchun and 3 Nanjing subjects, and all of their genotypes were T5/T7.

The TG11 and TG12 alleles were dominant haplotypes in Changchun (TG11 0.500, TG12 0.453) and Nanjing (TG11 0.345, TG12 0.609) (Table 2). In Changchun, there were no significant differences between the incidences of the two alleles. However, in Nanjing the frequency of the TG12 allele was significantly higher than that of the TG11 allele.

The M470V polymorphic site in exon 10 was also genotyped. The frequency of the V470 allele was 0.633 in Changchun which was higher than that in Nanjing (0.500) ($p<0.05$) (Table 3). The frequency distribution of M/M, M/V and V/V genotypes in Changchun and Nanjing fit

**Table 1** Allele Frequency of Poly-T

| Ethnic groups | Number (frequency) of individuals with alleles of poly-T tract | | | |
|---|---|---|---|---|
| | T5 | T6 | T7 | T9 |
| Changchun (n=64) | 7 (0.055) | 0 (0.000) | 120 (0.938) | 1 (0.008) |
| Nanjing (n=55) | 3 (0.027) | 2 (0.018) | 102 (0.927) | 3 (0.027) |

**Table 2** Allele Frequency of TG Repeat

| Ethnic groups | Number (frequency) of individuals with alleles of TG-repeats | | | |
|---|---|---|---|---|
| | TG10 | TG11 | TG12 | TG13 |
| Changchun (n=64) | 2 (0.016) | 64 (0.500) | 58 (0.453) | 4 (0.031) |
| Nanjing (n=55) | 3 (0.027) | 38 (0.345) | 67 (0.609) | 2 (0.018) |

**Table 3** Genotypes and Allele Frequencies at the M470V Polymorphic Site

| Ethnic groups | No. (%) of haplotypes | | | | |
|---|---|---|---|---|---|
| | M/M | M/V | V/V | M | V |
| Changchun (n=64) | 8 (12.5) | 31 (48.44) | 25 (39.06) | 47 (36.72) | 81 (63.28) |
| Nanjing (n=55) | 15 (27.2) | 25 (45.45) | 15 (27.2) | 55 (50) | 55 (50) |

**Table 4** Estimated Frequencies of Three-Locus Haplotypes

| Haplotype | Frequency | | | | |
|---|---|---|---|---|---|
| | Changchun | Nanjing | Vietnamese | Japanese | Caucasians |
| T7-TG12-M470 | 0.257 | 0.500 | 0.424 | 0.340 | 0.060 |
| T7-TG11-V470 | 0.514 | 0.318 | 0.391 | 0.530 | 0.540 |
| T7-TG12-V470 | 0.162 | 0.152 | 0.108 | 0.110 | 0.010 |
| T5-TG12-V470 | 0.041 | 0.015 | 0.029 | 0 | 0 |
| T5-TG13-M470 | 0.014 | 0 | 0.006 | 0 | 0 |
| T5-TG13-V470 | 0.014 | 0 | 0.001 | 0 | 0 |
| T6-TG12-V470 | 0 | 0.015 | 0 | 0 | 0 |
| others | 0 | 0 | 0.002 | 0 | 0.389 |

Data on Japanese Vietnamese and Caucasians were cited from the paper of Nam et al[10].

the Hardy-Weindey distribution.

We linked the three loci poly T, TG-repeat and M470V to investigate the haplotypes of CFTR in Changchun and Nanjing populations (Table 4). There were three major haplotypes (T7-TG11-V470, T7-TG12-M470 and T7-TG12-V470) in Changchun and Nanjing. The T7-TG11-V470 was the most common haplotype in Changchun (0.514), while the T7-TG12-M470 was the most common haplotype in Nanjing (0.500).

## DISCUSSION

In order to investigate the polymorphic backgrounds of CFTR genes of healthy populations in Changchun and Nanjing, we analyzed 119 blood samples of randomly selected healthy individuals for poly T, TG-repeats and M470V polymorphisms. It was the first attempt at a systematic analysis of the functional polymorphisms of CFTR in the two cities. Moreover, we compared the CFTR polymorphic distributions of Changchun and Nanjing populations with populations of Caucasian, Japanese and Vietnamese. Those data were cited from the paper of Fujiki et al.[9] and Nam et al.[10]

Four alleles (T5, T6, T7, and T9) can be found at the polymorphic CFTR intron 8-exon 9 junction. In both the Changchun and Nanjing populations, the T7 allele was the most common haplotype. The frequency of the T5 allele in Changchun was 0.055, while in Nanjing it was 0.027. Those four alleles (T5, T6, T7, and T9) determine the efficiency with which the intron 8 splice acceptor site is used. That efficiency will decrease when a shorter stretch of thymidine

residues is found. A higher proportion of CFTR transcripts that lack exon 9 sequences (which encode part of the functionally important first nucleotide-binding domain), will therefore be found when a shorter stretch of thymidine residues is present.[11,12] Such transcripts are known to be translated in CFTR proteins that do not mature, and will therefore not result in apical chloride channel activity.[13,14] Homozygotes for the T5 allele are known to produce non-functional exon 9-CFTR mRNA, thus reducing the amount of normal CFTR protein to less than 10%.[12] In the Changchun and Nanjing populations we studied, the individuals who had the T5 allele were all heterozygotes as well as healthy persons without CF or CF-related disease.

In our study, both the Changchun and Nanjing populations had strikingly high frequencies of the TG12 allele compared with TG10, TG11 and TG13, which is the same for Japanese and Vietnamese.[10] However, in Caucasians, TG10 was predominant and the frequency of TG12 allele was only 1%–8%.[15] On a T7 background, the TG11 allele yielded a 2.8-fold increase in the proportion of CFTR transcripts that lacked exon 9, while TG12 gave a six-fold increase compared with the TG10 allele.[16] Thus we can conclude that the frequency of intact CFTR protein is lower in Asia. This may be because of the result of selective pressure. In Asian countries, infectious diarrhea diseases are the major cause of mortality among children. Half of the diarrhea related diseases are due to bacterial toxins that can cause secretory diarrhea via the activation of the CFTR chloride channel. Hence, decreased production of intact CFTR proteins in the TG12/12 genotype compared with the TG10/10, 10/11, and 11/11genotypes would lead to less fluid loss and help survival in diarrhea diseases.[9]

In the congenital bilateral absence of vas deferens (CBAVD) patients and normal subjects, several studies have established a good correlation between the number of TG, and particularly T repeats, in the polymorphic locus and the amount of CFTR mRNA lacking exon 9. A high number of TG repeats and a low number of T repeats have been shown to favor the exclusion of exon 9 in the mRNA.[16] In our study, T5 was found in cis with two different TG repeats (T5-TG12, T5-TG13). Groman et al. found that, when T5 is in trans with a severe CF mutation, the odds of pathogenicity are 28 and 34 times greater for T5-TG12 and T5-TG13, respectively, than for T5-TG11.[17] The 7 individuals from Changchun and 3 from Nanjing who had the T5-allele were all heterozygotes. They were healthy possibly only because severe CF mutations cannot be found in *trans* with T5. Further studies are required to investigate other mutations and polymorphic loci in CFTR of the 10 individuals.

The M470V polymorphisms appear to affect chloride channel activity. The M470 CFTR protein showed a 1.7-fold activity of V470 CFTR protein.[17] The frequency of the V470 allele was 0.633 in Changchun, which was higher than in Nanjing (0.500) ($P<0.05$).

The overall CFTR function in vivo is determined by its genotype. Based on TG repeats, which determine the level of functional CFTR protein, and M470V, which determines intrinsic chloride channel activity, genotypes such as M/M470 with TG11/11 or 11/12 and M/V470 with TG11/11, produce proteins with a higher CFTR function.[9] These genotypes are absent in Nanjing and significantly smaller in Changchun. Most M/M470 genotypes were associated with TG12/12, which reduces the amount of intact CFTR proteins from both genes. In the genotype (TG) 11/11-V/V470, both genes express proteins with low intrinsic channel activity. Most M/V470 genotypes consisted of the TG12-M470 and TG11-V470 haplotypes, which result in a lower CFTR function owing to a decrease in the amount of intact protein from one gene, and a lower intrinsic channel activity of the protein coded by the other. Thus the majority of Changchun and Nanjing populations have genotypes that cause a lower CFTR function.

We compared the polymorphisms of CFTR gene in Changchun and Nanjing populations with those in Japanese, Vietnamese and Caucasians. TG11 and TG12 were dominant haplotypes in Changchun and Japanese, with a ratio of roughly 1:1. The Vietnamese and Nanjing populations

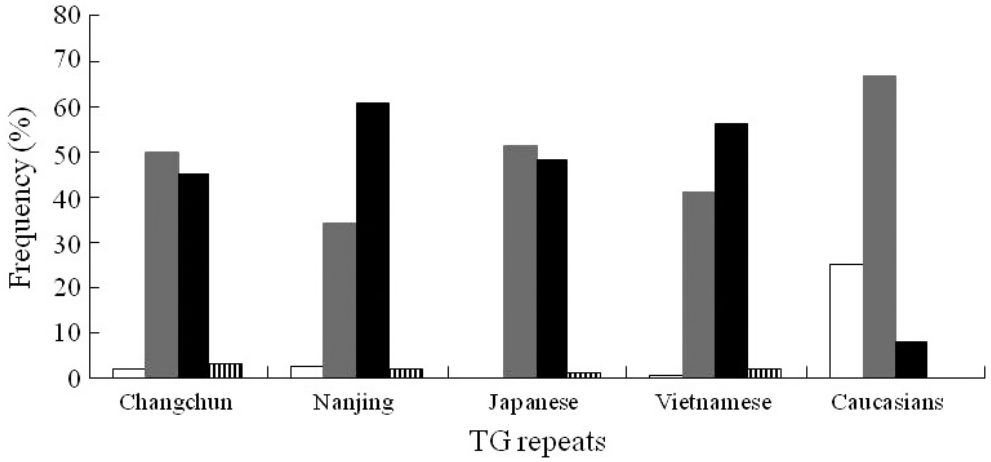


**Fig. 1** Frequency of TG repeats in different populations (The data of Japanese were cited from the paper of Fujiki et al.[9] and the data of Vietnamese and Caucasians were cited from the paper of Nam et al.[10]). White bars are TG10, hatched bars TG11, black bars are TG12, and strip bars are TG13.

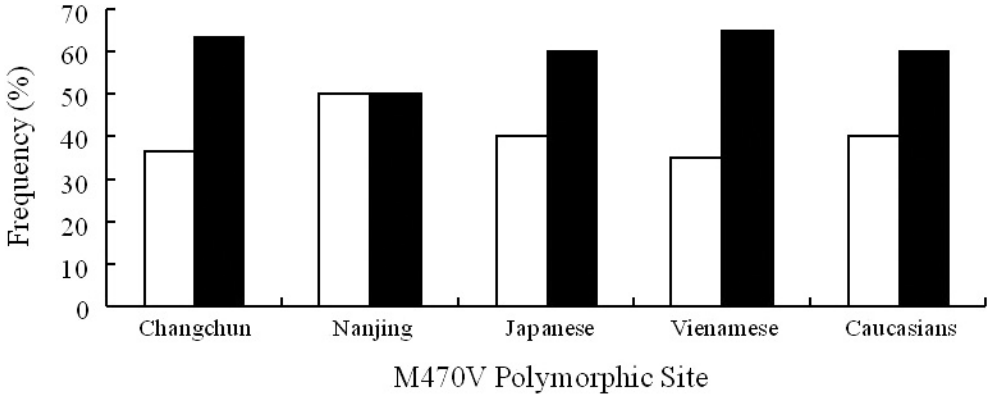


**Fig. 2** Frequency of M470V in different populations (The data of Japanese were cited from the paper of Fujiki et al.[9] and the data of Vietnamese and Caucasians were cited from the paper of Nam et al.[10]). White bars are M and black bars are V.

had a higher frequency of the TG12 allele. In Caucasians, the TG10 and TG11 repeats were predominant (Figure 1). The ratio of methionine (M) and valine (V) at position 470 in exon 10 was roughly 1:2 in Changchun, Japan and Caucasians. In Nanjing and Vietnamese the ratio was roughly 1:1 (Figure 2). There are three major haplotypes (T7-TG11-V470, T7-TG12-M470 and T7-TG12-V470) in Changchun, Nanjing, Japan and Vietnam, respectively. T7-TG11-V470 was the most common haplotype among Changchun and Japanese. In Nanjing and Vietnamese, the frequency of T7-TG12-M470 was higher than in the others. In Caucasians, two haplotypes (T7-TG11-V470 and T7-TG10-M470) were predominant (Table 4). Thus, we can conclude that there are marked differences among the frequencies of poly T, TG repeats and M470V polymorphisms of CFTR in Asian countries compared with Caucasian populations. Though Changchun and Nanjing are in the same country, their polymorphic backgrounds of CFTR gene

are quite different. On the contrary, Changchun is similar to Japanese, and Nanjing is the same as in Vietnamese. Are there any homologies between the origins of Changchun and Japanese populations, or Nanjing and Vietnamese? Although further analysis is required to test the validity of the hypothesis, according to the studies of Chan Hl et al., from the point of view of the geographical distribution of HBV subgenotypes, Southeast Asian countries such as southern China, Vietnam, Myanmar and Thailand have the same subgenotypes (C1 type), while Far East countries such as northern China, Japan and Korea, have another subgenotype (C2 type).[18-20)]

Genetic studies of the past two decades have served to enhance our understanding of pathogenesis in many diseases including pancreatic diseases, especially chronic pancreatitis.[21,22)] Several mutations have been identified that predispose carriers to develop chronic pancreatitis.[23-34)] CFTR mutations are one of the most interesting mutations associated with chronic pancreatitis. Sharer et al.[24)] revealed the association of CFTR mutation with chronic pancreatitis in 1998. Underlying mechanisms leading to the development of chronic pancreatitis still remain poorly understood, although recent publications confirm the association of chronic pancreatitis and CFTR mutations.[27-29,34)] However, those mutations alone are not sufficient for the pathogeneses of chronic pancreatitis in most patients, and further studies will be necessary to clarify the pathogenetic role of CFTR in combination with the risk factors of environment, ethnic, constitution, nutrition and other genetic factors.

Accumulations of data like those of the present study are also important for this pathogenetical analysis in the disease, because Changchun and Nanjing are ethnically identical, but differ in environment, mutation, etc.

In summary, we have investigated the polymorphic backgrounds of the CFTR gene of healthy populations from two different Chinese cities (Changchun and Nanjing). There are some differences between them. Although the origins of the two populations may differ, but we cannot draw this conclusion merely through our study, because our subjects and genetic analyses are so limited. This hypothesis should be verified further. The majority of the Changchun and Nanjing populations have genotypes that lower the CFTR function. To understand the association of CFTR dysfunction and CFTR- related disease in China, we should continue to study the genetic backgrounds of CFTR in unhealthy Chinese populations.

## REFERENCES

1) Welsh MJ. Cystic fibrosis. In: Glodman L, Bennett JC. Lecil textbook of medicine. 21st ed. Philadelphia, Pennsylvania, 2000: 401–405.
2) Rommens JM, Iannuzzi MC, Kerem B, Drumm ML, Melmer G, Dean M, et al. Identification of the cystic fibrosis gene: chromosome walking and jumping. *Science*, 1989; 245: 1059–1065.
3) Cystic Fibrosis Mutation Database (online). Available at: www. Genet. sickkids. On. ca/ cftr/. Accessed June 1, 2005.
4) Zielenski J, Patrizio P, Corey M, Handelin B, Markiewicz D, Asch R, Tsui LC. CFTR gene variant for patients with congenital absence of vas deferens. *Am J Hum Genet*, 1995; 57: 958–960.
5) Knowles MR, Durie PR. What is cystic fibrosis? *N Engl J Med*, 2002; 347: 439–442.
6) Bombieri C, Benetazzo M, Saccomani A, Belpinati F, Gilè LS, Luisetti M, Pignatti PF. Complete mutational screening of the CFTR gene in 120 patients with pulmonary disease. *Hum Genet*, 1998; 103: 718–722.
7) Yamashiro Y, Shimizu T, Oguchi S, Shioya T, Nagata S, Ohtsuka Y. The estimated incidence of cystic fibrosis in Japan. *J Pediatr Gastroenterol Nutr*, 1997; 24: 544–547.
8) Naruse S, Ishiguro H, Suzuki Y, Fujiki K, Ko SB, Mizuno N, et al. A finger sweat chloride test for the detection of a high-risk group of chronic pancreatitis. *Pancreas*, 2004; 28: e80–e85.
9) Fujiki K, Ishiguro H, Ko SB, Mizuno N, Suzuki Y, Takemura T, et al. Genetic evidence for CFTR dysfunction in Japanese: Background for chronic pancreatitis. *J Med Genet*, 2004; 41: e55–e60.
10) Nam MH, Hijikata M, Tuan LA, Lien LT, Shojima J, Horie T, et al. Variations of the CFTR gene in the

Hanoi-Vietnamese. *Am J Med Genet*, A 2005; 136: 249–253.
11) Chu CS, Trapnell BC, Murtagh JJ Jr, Moss J, Dalemans W, Jallat S, et al. Variable deletion of exon 9 coding sequences in cystic fibrosis transmembrane conductance regulator gene mRNA transcripts in normal bronchial epithelium. *EMBO J*, 1991; 10: 1355–1363.
12) Chu CS, Trapnell BC, Curristin S, Cutting GR, Crystal RG. Genetic basis of variable exon 9 skipping in cystic fibrosis transmembrane conductance regulator mRNA. *Nat Genet*, 1993; 3: 151–156.
13) Strong TV, Wilkinson DJ, Mansoura MK, Devor DC, Henze K, Yang Y, et al. Expression of an abundant alternatively spliced form of the cystic fibrosis transmembrane conductance regulator (CFTR) gene is not associated with a cAMP-activated chloride conductance. *Hum Mol Genet*, 1993; 2: 225–230.
14) Delaney SJ, Rich DP, Thomson SA, Hargrave MR, Lovelock PK, Welsh MJ, et al. Cystic fibrosis transmembrane conductance regulator splice variants are not conserved and fail to produce chloride channels. *Nat Genet*, 1993; 4: 426–431.
15) Tzetis M, Efthymiadou A, Strofalis S, Psychou P, Dimakou A, Pouliou E, et al. CFTR gene mutations including three novel nucleotide substitutions and haplotype background in patients with asthma, disseminated bronchiestasis and chronic obstructive pulmonary disease. *Hum Genet*, 2001; 108: 216–221.
16) Cuppens H, Lin W, Jaspers M, Costes B, Teng H, Vankeerberghen A, et al. Polyvariant mutant cystic fibrosis transmembrane conductance regulator genes: the polymorphic (Tg) m locus explains the partial penetrance of the T5 polymorphism as a disease mutation. *J Clin Invest*, 1998; 101: 487–496.
17) Groman JD, Hefferon TW, Casals T, Bassas L, Estivill X, Des Georges M, et al. Variation in a repeat sequence determines whether a common variant of the cystic fibrosis transmembrane conductance regulator gene is pathogenic or benign. *Am J Hum Genet*, 2004; 74: 176–179.
18) Chan HL, Tsui SK, Tse CH, Ng EY, Au TC, Yuen L, et al. Epidemiological and virological characteristics of 2 subgroups of hepatitis B virus genotype C. *J Infect Dis*, 2005; 191: 2022–2032.
19) Huy TT, Ushijima H, Quang VX, Win KM, Luengrojanakul P, Kikuchi K, et al. Genotype C of hepatitis B virus can be classified into at least two subgroups. *J Gen Virol*, 2004; 85: 283–292.
20) Tanaka Y, Orito E, Yuen MF, Mukaide M, Sugauchi F, Ito K, et al. Two subtypes (subgenotypes) of hepatitis B virus genotype C: a novel subtyping assay based on restriction fragment length polymorphism. *Hepatol Res*, 2005; 33: 216–224.
21) Keim V. Role of genetic disorders in acute recurrent pancreatitis. *World J Gastroenterol*, 2008; 14: 1011–1015.
22) Lerch MM, Mayerle J, Aghdassi AA, Budde C, Nitsche C, Sauter G, et al. Advances in the etiology of chronic pancreatitis. *Dig Dis*, 2010; 28: 324–329.
23) Sharer N, Schwarz M, Malone G, Howarth A, Painter J, Super M, Braganza J. Mutations of the cystic fibrosis gene in patients with chronic pancreatitis. *N Engl J Med*, 1998; 339: 645–652.
24) Cohn JA, Friedman KJ, Noone PG, Knowles MR, Silverman LM, Jowell PS. Relation between mutations of the cystic fibrosis gene and idiopathic pancreatitis. *N Engl J Med*, 1998; 339: 653–658.
25) Gelrud A, Sheth S, Banerjee S, Weed D, Shea J, Chuttani R, et al. Analysis of cystic fibrosis gener product (CFTR) function in patients with pancreas divisum and recurrent acute pancreatitis. *Am J Gastroenterol*, 2004; 99: 1557–1562.
26) Cantin AM, Hanrahan JW, Bilodeau G, Ellis L, Dupuis A, Liao J, et al. Cystic fibrosis transmembrane conductance regulator function is suppressed in cigarette smokers. *Am J Respir Crit Care Med*, 2006; 173: 1139–1144.
27) Chang YT, Chang MC, Su TC, Liang PC, Su YN, Kuo CH, et al. Association of cystic fibrosis transmembrane conductance regulator (CFTR) mutation/variant/haplotype and tumor necrosis factor (TNF) promoter polymorphism in hyperlipidemic pancreatitis. *Clin Chem*, 2008; 54: 131–138.
28) Felderbauer P, Karakas E, Fendrich V, Bulut K, Horn T, Lebert R, et al. Pancreatitis risk in primary hyperparathyroidism: relation to mutations in the SPINK1 trypsin inhibitor (N34S) and the cystic fibrosis gene. *Am J Gastroenterol*, 2008; 103: 368–374.
29) Felderbauer P, Karakas E, Fendrich V, Lebert R, Bartsch DK, Bulut K. Multifactorial genesis of pancreatitis in primary hyperparathyroidism: evidence for “protective” (PRSS2) and “destructive” (CTRC) genetic factors. *Exp Clin Endocrinol Diabetes*, 2011; 119: 26–29.
30) Ko SB, Mizuno N, Yatabe Y, Yoshikawa T, Ishiguro H, Yamamoto A, et al. Corticosteroids correct aberrant CFTR localization in the duct and regenerate acinar cells in autoimmune pancreatitis. *Gastroenterology*, 2010; 138: 1988–1996.
31) Maruyama K, Harada S, Yokoyama A, Mizukami S, Naruse S, Hirota M, et al. Association analyses of genetic polymorphisms of GSTM1, GSTT1, NQO1, NAT2, LPL, PRSS1, PSTI, and CFTR with chronic alcoholic pancreatitis in Japan. *Alcohol Clin Exp Res*, 2010; 34: S34–S38.

32) Midha S, Khajuria R, Shastri S, Kabra M, Garg PK. Idiopathic chronic pancreatitis in India: phenotypic characterisation and strong genetic susceptibility due to SPINK1 and CFTR gene mutations. *Gut*, 2010; 59: 800–807.
33) Cavestro GM, Zuppardo RA, Bertolini S, Sereni G, Frulloni L, Okolicsanyi S, et al. Connections between genetics and clinical data: role of MCP-1, CFTR, and SPINK-1 in the setting of acute, acute recurrent, and chronic pancreatitis. *Am J Gastroenterol*, 2010; 105: 199–206.
34) Schneider A, Larusch J, Sun X, Aloe A, Lamb J, Hawes R, et al. Combined bicarbonate conductance-impairing variants in CFTR and SPINK1 variants are associated with chronic pancreatitis in patients without cystic fibrosis. *Gastroenterology*, 2011; 140: 162–171.