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Multisite Heterozygous Mutations of *PRSS1* Gene and Clinical Characterization of Patients With Hereditary Pancreatitis in The Chinese

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Abstract In four patients with chronic pancreatitis from two hereditary pancreatitis (HP) families and 63 normal controls, five exons of cationic trypsinogen gene (*PRSS1*) were amplified by PCR and it's products were analyzed by sequencing, related clinical data were also collected. All the four patients were found mutations in the *PRSS1* gene but their clinical feature is absolutely different. Six patients with diabetes mellitus were found in pedigree No. 1, it's members show pancreatitis symptom later, at about 29, the tumor markers (CA19-9, CA72-4) is obviously higher than the patients in pedigree No. 2, two patients with chronic pancreatitis in pedigree No. 2, show symptom earlier without diabetes mellitus, their clinical characterization are different too. The number of $CD4^+$T cell/ $CD8^+$T is very low in Ⅲ 8, but Ⅲ 7 is normal, and the level of anti-HBs of Ⅲ 8 is variable in the course of pancreatitis, but the phenomenon was not found in Ⅲ 7. In their *PRSS1* gene two guanosine (G) to adenosine (A) mutations were found in *PRSS1* exon 3 of pedigree No. 1, one was detected at 336 basyl, the other mutation occurs at 361 basyl. The results of the mutations were Lys →Lys and Ala →Thr. While thymine (T) to adenosine (A) and (guanosine) G→(adenosine) A mutation in *PRSS1* exon 3 was detected in the other patient of pedigree No. 2 (Ⅲ 8). One was 361 basyl, the other at 415 basyl. While c.415 T→A was not found in the proband of pedigree No. 2 *PRSS1* gene (Ⅲ 7). All of the mutations were heterozygous mutation, that is to say all of the trypsinogen were wild type and mutant type concomitance, the normal and abnormal pathway of active trypsinogen exist partially. At the same time, the mutations of *SPINK1* were not observed. Compared with the documents and registration of NCBI, it can be concluded that *PRSS1* gene had many kinds of mutations in hereditary pancreatitis, the heterozygous mutations (c.336 G→A, c.415 T→A) were the novel mutations and related with clinical phenotype. What's more, it's the first time that the multisite heterozygous mutations of *PRSS1* gene were reported. The presence of the mutations in four patients with chronic pancreatitis, it's absence in their relatives and the strong evolutionary conservation of the mutation, all indicate that the trypsinogen mutation is associated with hereditary pancreatitis and for the first time raises the question whether a gain or a loss of trypsin function participates in the onset of Chinese pancreatitis.

Key words hereditary pancreatitis, mutation of *PRSS1*, heterozygous mutation, multisite, clinical phenotype

Hereditary pancreatitis (HP) is an autosomal dominant condition with 80% penetrance [1]. Patients with this disease exhibit a 53 fold increased risk of pancreatic cancer with a cumulative risk of 40% by the age of 70 [2~4]. In most cases the disease begins with recurrent episodes of acute pancreatitis in children and young adults and progresses to chronic pancreatitis with exocrine and endocrine pancreatic insufficiency, for yet unknown reasons, unaffected mutation carriers neither develop pathological changes in the pancreas nor share the increased pancreatic cancer risk of their affected relatives. HP usually caused by mutations in the cationic trypsinogen (*PRSS1*) gene, especially R122H or N29I[5,6]. The R122C mutation eliminates the arginine autolysis site as with R122H mutations. Whitcomb proposed that Arg 122～Val123 autolytic peptide bond in trypsin plays an important role in the degradation of prematurely activated in the pancreas. Destruction of this "failsafe mechanism" by the R122H mutation would increase intrapancreatic trypsin activity and disturb the protease antiprotease equilibrium and eventually provoke pancreatitis [1].

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While N29I mutation may also enhance intrapancreatic trypsin activity as has been demonstrated *in vitro* [7]. Recently, we found that some patients with especial history (diarrhea and/or stomachache frequently, the same disease in the relative) and family, these phenomenons led us to study the genetic influences with inherited forms of pancreatitis. We sequenced the *PRSS1* gene and serine protease inhibitor Kazal type1 (*SPINK1*) gene in the proband and the affected patients of the two families without these common mutations. Novel mutations of *PRSS1* were detected in two Chinese HP families and related with clinical phenotype. All of the mutations were show wild and mutant type concomitance.

1 Materials and methods

1.1 Selection of patients

We collected the records of all the clinical data of the two hereditary pancreatitis families. The diagnosis of pancreatitis was based on the presence of a typical history (recurrent upper abdominal pain, radiating to the scapula tip, relieved by leaning forward or sitting upright and increased after eating), suggestive radiological findings, such as pancreatic calcifications or pseudocysts, and/or pathological findings (pancreatic ductal irregularities and dilatations) revealed by endoscopic retrograde pancreaticography. Including four patients with chronic pancreatitis and 63 normal controls. The information of their clinical history was corroborated by a questionnaire.

Pedigree No. 1: The 26 year old index patient (Ⅱ 10), with symptoms from age 13, was diagnosed with pancreatitis at age 18 years (see pedigree Figure 1). His parent (father) was diagnosed with chronic pancreatitis at age 47 years and died with pancreatic cancer ten years later, one of his seven sisters and brothers had pancreatitis at age 23 years. Both the patients (Ⅱ 5, Ⅱ 10) referred to our hospital for pancreatitis three times. Six patients with diabetes mellitus they were Ⅰ 1, Ⅱ 3, Ⅱ 5, Ⅱ 7, Ⅱ 10 and Ⅲ 8[8].

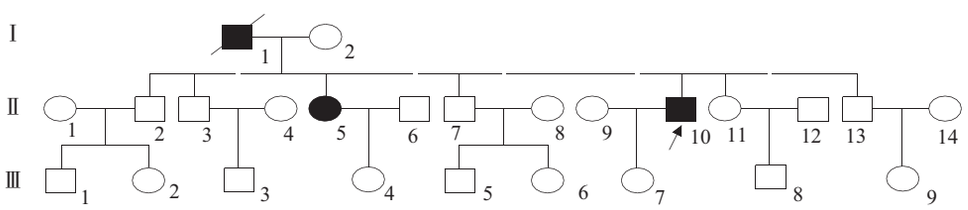

Fig. 1 Pedigree No. 1
↗: Proband; ●: Pancreatitis; ○: Normal.

Pedigree No. 2: The 29 year old proband (Ⅲ 8), it's the sixth time accept to our hospital with pancreatitis. She was suspected with the symptoms of pancreatitis because of diarrhea and stomachache frequently from eight, her brother (Ⅲ 7) had the same disease at 17, now he had develop to chronic pancreatitis (pedigree Figure 2).

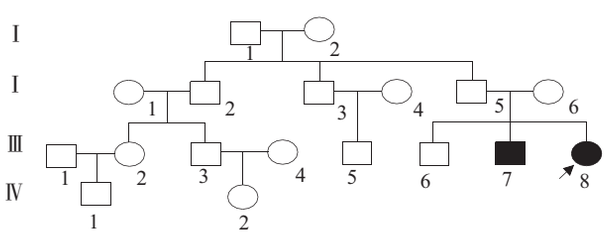

Fig. 2 Pedigree No. 2
↗: Proband; ●: Pancreatitis; ○: Normal.

1.2 DNA extraction and PCR

Whole DNA was extracted from 10 ml of venous blood using the Promega DNA isolation kit. The venous blood was anti-cognation by EDTA-2K.The mutation was detected using an allele specific polymerase chain reaction (PCR). Briefly, The 50 μl reaction mixture contained 200 ng of genomic DNA, 10 mmol/L Tris•HCl (pH 9.0), 50 mmol/L KCl, 0.1% Triton, 2 mmol/L $MgCl_2$, 0.25 mmol/L dNTPs, 100 ng of sense primer (5′ ggtcctgggtctcatacctt 3′), 100 ng of antisense primer (5′ gggtaggaggcttcacactt 3′) and 3.0 U Taq-DNA polymerase to generate an 898 bp fragment.

Cycling conditions were an initial step at 94℃ for 5 min, then 30 cycles at 94℃ for 1 min, 55℃ for 1 min, 72℃ for 1 min, and a final elongation step at 72℃ for 10 min. The PCR products were purified after electrophoresis on an agarose gel with the purified products we performed sequencing. For sequencing the Perkin Elmer Big Dye Sequencing kit and an ABI PRISM7700 sequencer were used. Shanghai Shenggong Limited Company did the work. While we

used multi-PCR to amply the serine protease inhitor Kazal type 1 (*SPINK* 1) gene, the sense primer and anti-sense primer as shown in Table 1.

Table 1　Primers used in PCR

	Sense primer	Antisense primer	*m*/ng
SPINK1 1	5′ tttgagttcatcttacaggtgag 3′	5′ gtttgcttttctcggggtgag 3′	50
SPINK1 2	5′ aagaaactcaagtttgtact 3′	5′ catgtttcaggcccacct 3′	100
SPINK1 3	5′ gtgttatgttttgaaaaatcggt 3′	5′ gtgttatgttttgaaaatcgg 3′	50

The 100 μl reaction mixture contained 400 ng of genomic DNA, 10 mmol/L Tris•HCl (pH 9.0), 50 mmol KCl, 0.1% Triton, 2.5 mmol $MgCl_2$, 0.30 mmol/L dNTPs, cycling conditions were the similar to *PRSS1*. But the renaturation temperature at 52℃.

2　Results

2.1　DNA sequencing revealed mutations in *PRSS1* gene

Genomic DNA from G →A carrying patients (pedigree No.1) was amplified into one long DNA fragments by long-distance PCR using *PRSS1* gene-specific primers (Figure 3a, b).

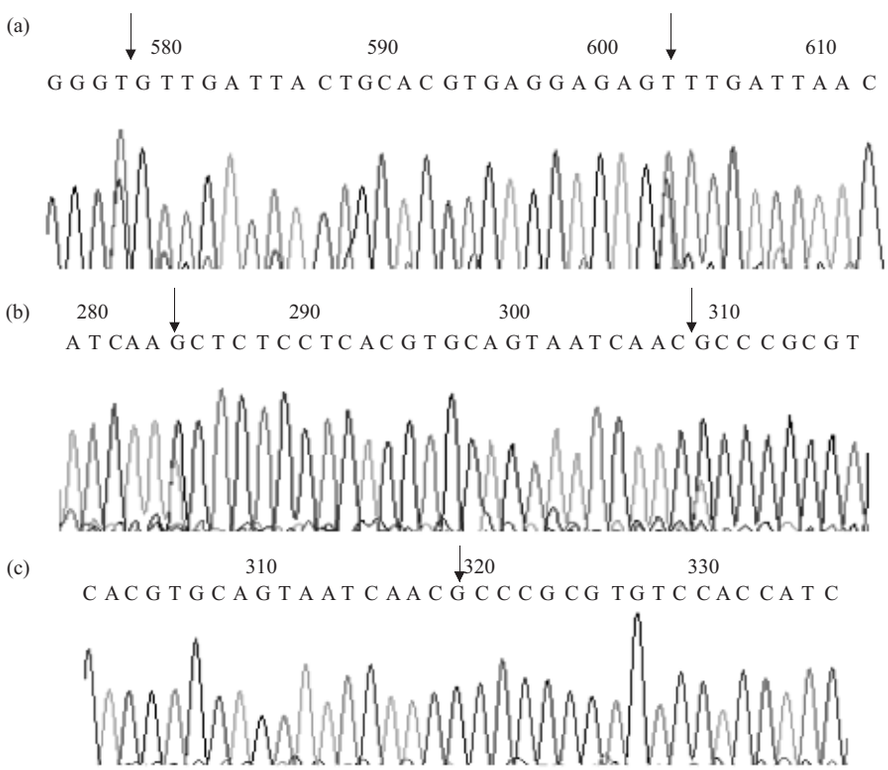

Fig. 3　The sequencing result of exon 3 of *PRSS1* gene of Pedigree No. 1

Arrows indicate the site at which G→A mutation occurs. (a) Forward sequencing result of Ⅱ5 and Ⅱ10. (b) Reverse sequencing result of Ⅱ5 and Ⅱ10. (c) The result of normal control.

The result of the mutations as follow:

Lys112
PRSS1 wt 5′ …A ATC AAG CTC…3′
Lys112
G→A 5′ …A ATC AAA CTC…3′
Ala121
PRSS1 wt 5′ …C AAC GCC CGC…3′
Thr121
G→A 5′ …C AAC ACC CGC…3′

Both the pantients of pedigree No. 1 (Ⅱ5 and Ⅱ10) were found to be carriers of a previously unreported G to A transition at the amino codon 112 (336 basyl of *PRSS1* exon3). This mutation is causing a Lys to Lys substitution. It was a neutral mutation.While another mutation was found in the amino codon 121 (361 basyl of *PRSS1* exon3) of the two effect patient. Based on these findings we concluded that both the patients of Ⅱ5 and Ⅱ10 carried a 361 G→A mutation must be hereditary from their father.

2.2 We found completely different result of sequence in pedigree No. 2

Patients of pedigree No. 2 were found basyl substitution at the amino codon 121, the *PRSS1* exon 3 sequence of the index patient (Ⅲ8) and her brother (Ⅲ7) realed a G→A change causing Ala to Thr substitution (Figure 4a, b).

In the Ⅲ8 of pedigree No. 2, at 415 of *PRSS1*, an A instead of the reported T constitutes the wild type sequence (Figure 5a, b). This point mutation was predicted to result in a Cys to Ser substitution. The mutation did not find in Ⅲ7.

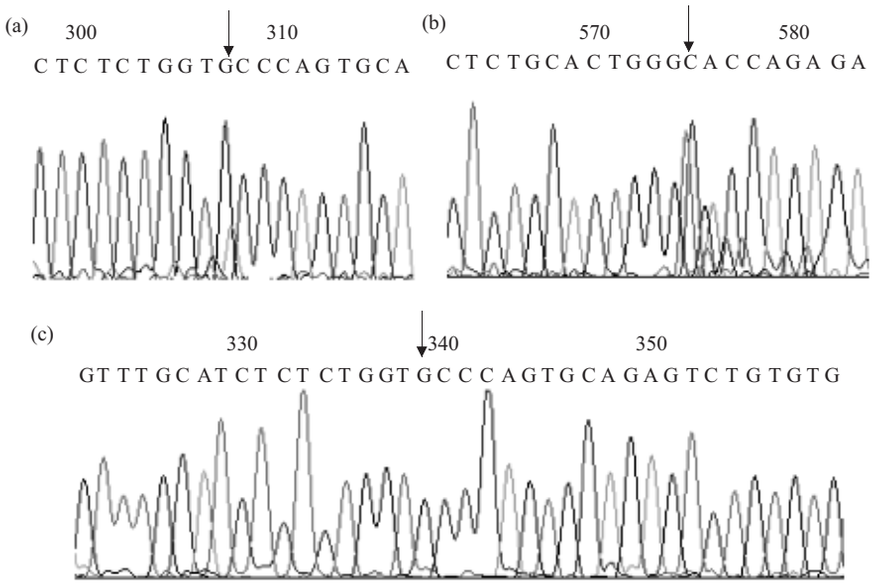

Fig. 4 The sequencing result of exon 3 of *PRSS1* gene of pedigree No.2

Arrows indicate the site at which G→A mutation occurs. (a) Forward sequencing result of the effect patients of pedigree No.2. (b) Reverse sequencing. (c) The result of normal control.

Ala121
PRSS1 wt 5′ …C AAC GCC CGC…3′
Thr121
G→A 5′ …C AAC ACC CGC…3′

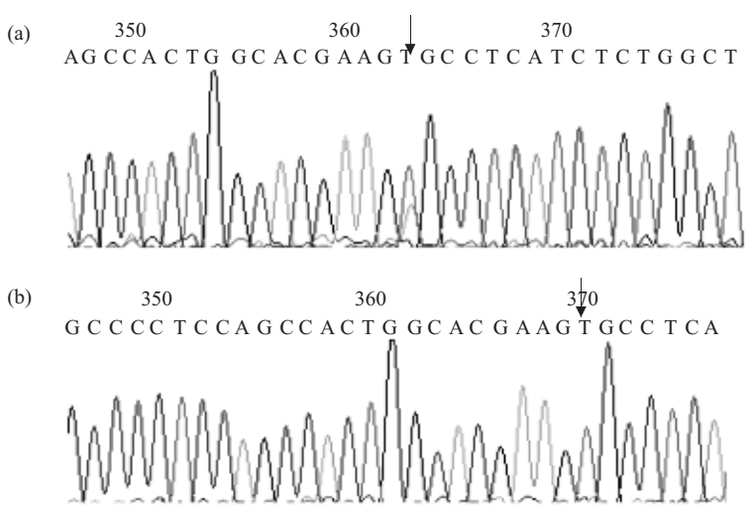

Fig. 5 DNA sequencing of the *PRSS1* exon 3 in the region of the mutation identified in patients with pancreatitis of pedigree No.2

Arrows indicate the site at which T→A mutation occurs. (a) The sequencing result of Ⅲ8(pedigree No.2). (b) The sequencing result of normal control. The result of the mutations as follow:

Cys139
PRSS1 wt 5′ …G AAG TGC CTC…3′
Ser139
T→A 5′ …G AAG AGC CTC…3′

2.3 Three-dimensional view of human *PRSS1* gene according to crystallographic structure

In order to show the structure of mutant trypsin is similar or different from the normal, R122H and between the two pedigrees. We get the three-dimensional view of human PRSS1 according to crystallographic structure by SWISS-MODEL (Figure 6a～f).

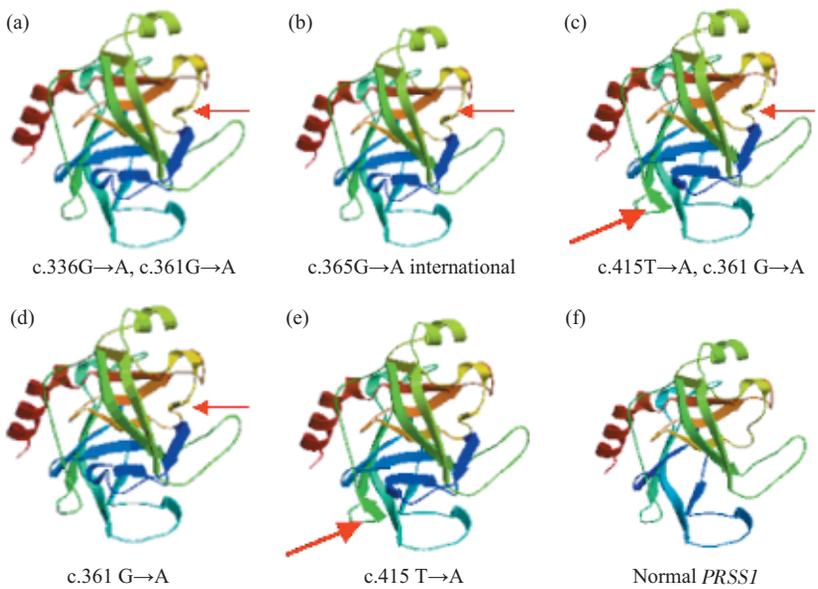

Fig. 6　Three-dimensional view of human PRSS1 according to crystallographic structure

A121 (c.361 G→A) is evolutionarily conserved in the trypsinogen gene of all terrestrial vertebrates and would thus seem of importance in the protein's structure and function. As A121 is only one amino acid distant from R122H (also numbered as R117H majority using the chymotrypsinogen amino acid numbering system), it's replacement by Thr may result in abnormal conformation of the peptide. It was not in relation to the active site and the activation domain, but in close proximity to the 70-loop (calciumbinding loop) of pedigree No.1 (a, c, d), this mutation is similar to the R122H (b), while the two point of the three-dimensional of pedigree No.2 (c) is diffident from the normal trypsin obviously (d) which arrows indicate.

2.4 Molecular modeling

The structure of the wild type PRSS1 in the front view (standard view, Figure 6f) shows that R122H is located at considerable distance from the active site or the activation domain of trypsin and would therefore be unlikely to directly affect its catalytic activity. Similarly, the mutant PRSS1 of pedigree No. 1 did not affect its catalytic activity because of its vicinity to the codon 122. In both orientations it can be seen in close proximity to the calcium-binding loop (70-loop) of trypsin. In view of this location at the exposed back of the molecule a replacement of A121 by T could not only affect a potential hydrolysis site of trypsin but would also permit the formation of disulfide bonds between two trypsinogen molecules or between trypsinogen and other proteins. But it was very surprising in the pedigree No. 2 (Figure 6c, d, e), the mutant trypsinogen of Ⅲ 7 isn't the same to index patient (Ⅲ 8), c.415 T→A mutation is near to the reported c.416 G→T (Teich *et al*. had found in the patient with idiopathic chronic pancreatitis in German [2001]). The amino acid sequence of PRSS1 in the four patients with pancreatitis was display (Figure 7).

The human beta T cell receptor (TCR) locus, comprising a complex family of genes, has been sequenced. The locus contains two types of coding elements——TCR elements (65 variable gene segments and two clusters of diversity, joining and constant segments) and eight trypsinogen genes——that constitute 4.6 percent of the DNA (Figure 8)[9].

…DRKTLNNDIMLIK(L/L)SSRAVIN(A/T)RVSTISLPTAPPATGTK(C/S)LISGWGNTA…

Fig. 7　Protein sequence of human PRSS1 in the two Chinese HP families

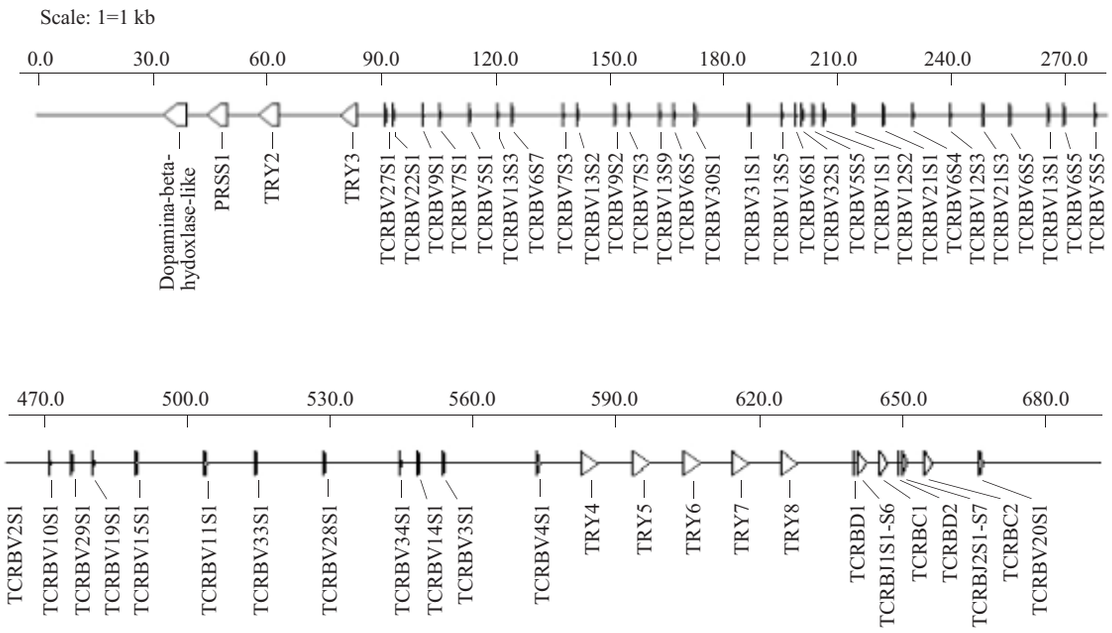

Fig. 8 The relationship between the human beta T cell receptor (TCR) and PRSS1

Human T cell receptor beta subunit map, Schematic organisation of the human T-cell receptor locus on chromosome 7 containing the trypsinogen genes *PRSS1*, *PRSS2* and *TRY6* and the pseudogenes *TRY5* and *TRY7*. The figure is not drawn to scale. Numbering according to NT_007914.14, NCBI build 36.The structure of TCR must be changed because of the mutation of *PRSS1*. It can explain some phenomenon like the number of $CD4^+T$/ $CD8^+T$ and the level of anti-HBs of Ⅲ8.

2.5 Population screening

To examine the linkage of c.336 G→A, c.361 G →A and c.415 T→A mutation, the *PRSS1* gene of 63 healthy individuals were tested at the same time to observe the mutation might be present in the Han population as a natural polymorphism. At the same time we didn't find the mutation of *SPINK1* in the patients with HP and the control members.

2.6 Characteristics of the mutation patient

The clinical of characteristics of the mutation patient is shown in Table 2.

From the Table 2 we can see that the level of HbA1c and CA19-9 of the pedigree No. 1 is obviously higher than the effect members of pedigree No. 2. What's more, the level of anti-HBs of Ⅲ8 is ranged from 0.02 to 110.5 in the course of pancreatitis.

Table 2 Clinical data of the patient with chronic pancreatitis

	c(GLU)/(mmol•L^{-1})[1)]	HbA1c /%	$CD4^+T$/ $CD8^+T$	CA72-4 /(U•ml^{-1})	CA19-9 /(U•ml^{-1})
Ⅱ5(pedigree No. 1)	8.96	6.80	1.820	7.680	396.5
Ⅱ10(pedigree No. 1)	9.15	5.96	1.564	41.870	123.6
Ⅲ7(pedigree No. 2)	5.32	3.69	1.623	0.961	15.3
Ⅲ8(pedigree No. 2)	4.98	4.23	0.332	1.570	22.1
Reference range	3.89～6.11	3.8～5.8	1.5～2.0	0～6.9	0～27

[1)] GLU: Plasma glucose; HbA1c: Glycosylated hemoglobin.

3 Discusion

The importa nce of trypsin was discovered in studies of hereditary pancreatitis (HP), most cases of HP are associated with mutations in the *cationic trypsinogen* gene (*PRSS1*)[10]. The R122H and N29I mutations (also numbered as R117H and N21I majority using the chymotrypsinogen amino acid numbering system) represent the vast of cases. These cause premature trypsinogen activation[10,11]. In families

with *PRSS1* R122H or N29I mutations, between 60% and 80% of individuals who inherit the mutation usually develop pancreatitis[11~13]. In addition, approximately half of individuals with acute pancreatitis will develop chronic pancreatitis, and up to 40% of individuals with chronic pancreatitis will develop pancreatic cancer[14~17]. Other mutations of this gene are rare, but may be of high interest concerning the role of *PRSS1* in development of hereditary pancreatitis. In accordance with previous reports, the substitutions are also the most correspond with HP.

It has been hypothesized that R122H mutation causes pancreatitis by altering a trypsin recognition site so preventing deactivation of trypsin within the pancreas and prolonging its action, resulting in autodigestion. However, to avoid incorrect interpretation, protein's structure and function analysis has to be done to investigate the influence of the suggested mutations on the activity of the *PRSS1* gene. Particularly, examination of the mutant protein demonstrated no change in its trypsin-binding ability differing from the wild-type protein[18]. Like IVS G→A, which destroys the splicing site of exon 3, consequently leads to activation of the gene. We found two of these G →A mutations (c.336 G→A, c.361 G→A) in Chinese patients with chronic pancreatitis. Another base substitution T→A was also detected in two mutant gene copies and for the first time in HP. Supported by the fact that this abnormality has never been found in healthy controls it has been considered to be an inactivating *PRSS1* gene mutation[19~23]. Furthermore, we found these patients had a family history of chronic pancreatitis. In our study, we detected the G→A or T→A substitution in the index patient and his/her relative. Therefore, our conclusion is that the associated base substitution c.336 G→A and c.415 T→A in the same *PRSS1* gene copy is a rare mutation.

In the past years, people believe absolutely those patients with HP are similar in clinical course, but in our study we may get the answer different. Six patients with diabetes mellitus were found in pedigree No. 1, it's members show pancreatitis symptom later, at about 29, the tumor markers (CA19-9, CA74-2) is obviously higher than the effect members of pedigree No. 2, and one member died with pancreatic cancer. But patients in pedigree No. 2 show symptom earlier without diabetes mellitus. Both of the families are large genealogies, 39 members included. Of course the different clinical courses of pancreatitis in individuals with hereditary pancreatitis appear to involve both environmental and genetic modifiers (unpublished observations) while the incomplete disease penetrance is complex[24~26] and likely reflects the effects of environmental triggers. Even identical twins with *PRSS1* mutations can be discordant for affected status (thus arguing for environmental triggers) but similar in clinical course when both are affected (arguing for modifier genes)[27]. Thus the major susceptibility factor for hereditary pancreatitis is known but the major disease modifying factors are yet to be identified. Compared with the documents and registration of NCBI, we can draw the conclusion that *PRSS1* gene had many kinds of mutation in hereditary pancreatitis, the heterozygous mutation is the novel mutations and related with clinical phenotype.

Very accurate diagnostic tests are now available for the major trypsinogen gene mutations. Genetic diagnosis of hereditary pancreatitis has significant implications for patients and their extended family[28], whereas diagnosis of other types of pancreatitis does not have the same impact. Therefore, the diagnosis of hereditary pancreatitis should be specifically limited to patients with gain of function *PRSS1* mutations[18] or otherwise unexplained pancreatitis in an individual from a family in which the pancreatitis phenotype appears to be inherited through a disease causing gene mutation expressed in an autosomal dominant pattern[28,29]. Familial pancreatitis refers to pancreatitis from any cause that occurs in a family with an incidence that is greater than would be expected by chance alone, given the size of the family and incidence of pancreatitis within a defined population[21]. This distinction is important from prognostic, genetic counseling, and family planning perspectives, and because of potential of insurance discrimination associated with a "genetic disease".

PRSS1 genetic testing is recommended in symptomatic patients with any of the following: (1) recurrent (two or more separate documented episodes of typical pain with hyper-amylasaemia) attacks of acute pancreatitis for which there is no explanation (for example, anatomical anomalies, ampullary or main pancreatic strictures, trauma, viral infection, gall stones, alcohol, drugs, hyperlipidaemia, etc.), (2) unexplained (idiopathic) chronic pancreatitis, (3) a family history of pancreatitis in a first degree (parent, sib, child) or second degree (aunt, uncle, grandparent)

relative, (4) an unexplained episode of documented pancreatitis occurring in a child that has required hospitalisation, and where there is significant concern that hereditary pancreatitis should be excluded, or (5) as part of an approved research protocol[21].

Family data were available for the patients with mutant *PRSS1* gene. In pedigree No. 2 this mutate gene was inherited from their parents who did not suffer from chronic pancreatitis, but from celiac disease. And the blood TG is very high (8.56～21.56, the reference range is 0～1.7) for twenty years. In some families the celiac disease was mapped close to the *SPINK1* locus on chromosome 5 (5q31)[15,16]. But in our study, we could not find *SPINK1* mutation in the patients with HP. While both the patients of pedigree No. 1 (Ⅱ5 and Ⅱ10) were found to be carriers of a previously unreported G to A transition at the amino codon 112. This mutation is causing a Lys to Lys neutral mutation. Another mutation was found in codon 121 in Ⅱ10 and Ⅱ5, Based on these findings we concluded that the father of Ⅱ5 and Ⅱ10 carried c.361 G→A mutation, but c.336 G→A must be hereditary either from his mother or father. Two effect patients of pedigree No. 2 (Ⅲ5 and Ⅲ8) were found two continuous basyl substitution at the amino codon 121 and 139, the *PRSS1* exon 3 sequence of the index patient (Ⅲ8) and her brother (Ⅲ7) realed a G→A change causing a Ala to Thr substitution. But another mutation c.415T→A only occur in Ⅲ8, this is the first report and near to the c.416 G→T(Teich *et al*.).

The underlying pathophysiological mechanisms through which carriers of trypsinogen mutations develop pancreatitis are unknown. The most intuitive explanation would changes in the HP is a fascinating condition that has provided new insight into the pathophysiology of pancreatitis. The structure of the wild type *PRSS1* in the front view shows that R122H is located at considerable distance from the active site or the activation domain of trypsin and would therefore be unlikely to directly affect its catalytic activity. Similarly, the mutant *PRSS1* of pedigree No. 1, because of its vicinity to the codon 122, only three amino distances. But it was very surprise in the pedigree No. 2 (Figure 6c, d, e), the mutant trypsinogen of Ⅲ7 isn't the same to index patient(Ⅲ8), that we think c.415 T→A may be hereditary either from their parent, but not Ⅲ7, and c.415 T→A mutation is near to the reported c.416 G→T (Teich *et al*. had found in the patient with idiopathic chronic pancreatitis in German [2001]). In both orientations it can be seen in close proximity to the calcium-binding loop (70-loop) of trypsin. In view of this location at the exposed back of the molecule a replacement of Arg-122 by cysteine could not only affect a potential hydrolysis site of trypsin but would also permit the formation of disulfide bonds between two trypsinogen molecules or between trypsinogen and other proteins.

Human beta T cell receptor (TCR) locus, comprising a complex family of genes, has been sequenced. The locus contains two types of coding elements——TCR elements (65 variable gene segments and two clusters of diversity, joining, and constant segments) and eight trypsinogen genes——that constitute 4.6 percent of the DNA. Genome-wide interspersed repeats and locus-specific repeats span 30 and 47 percent, respectively, of the 685-kilobase sequence. A comparison of the germline variable elements with their approximately 300 complementary DNA counterparts reveals marked differential patterns of variable gene expression, the importance of exonuclease activity in generating TCR diversity, and the predominant tendency for only functional variable elements to be present in complementary DNA libraries[9]. It can explain some phenomenon like the number of $CD4^+$T cell and and the level of anti-HBs of Ⅲ8.

There are however many unanswered questions particularly in relation to the ways in which these mutations relate to pancreatitis and cancer. A team should carry out management of these patients. HP improved and genetic research to be continued. Since the discovery that mutations in the cationic trypsinogen gene caused hereditary pancreatitis in 1996 it has become increasingly evident that acute and chronic pancreatitis are syndromes encompassing several complex disease mechanisms. The key genetic, environmental, and morphological factors that intersect to cause a complex pancreatic disease are slowly emerging, and knowledge of these factors will allow for disease models to be developed. These models will raise new research questions that alter current therapy and may limit progression of early disease. And if the factors and pathological pathways were identified early, then modifiable risk should be identified and addressed early so that rational preventative measures can be taken.

References

1 Applebaum-shapiro S E, Finch R, Pfutzer R H, *et al*. Hereditary pancreatitis in North America: the Pittsburg-Midwest Multi-Center Pancreatic Study Group Study. Pancreatology, 2001, **1**: 439～443

2 Pandya A, Blanton S H, Landa B, *et al*. Linkage studies in a large kindred with hereditary pancreatitis confirms mapping of the gene to c.16-Cm region on 7q. Genomics, 1996, **38**: 227～230

3 Whitcomb D C, Gorry M C, Preston R A, *et al*. Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. Nat genet, 1996, **14** (2): 141～145. PMID: 8841182

4 Creighton J E, Lyall R, Wilson D I, *et al*. Mutations of the cationic trypinogen gene in patients with hereditary pancreatitis. Br J Surg, 2000, **87** (2): 170～175

5 Farrow B, Evers B M. Inflammation and the development of pancreatic cancer. Surg Oncol, 2002, **10** (4): 153～169

6 Schneider A, Lamb J, Barmada M M, *et al*. Keratin 8 mutations are not associated with familial, sporadic and alcoholic pancreatitis in a population from the United States. Pancreatology, 2006, **6** (2): 103～108

7 Howes N, Greenhalf W, Neoptolemos J. Screening for early pancreatic ductal adenocarcinoma in hereditary pancreatitis. Med Clin North Am, 2000, **84** (3): 719～738

8 Liu Q C, Cheng Z J, Y Y, Ou Q S. A novel mutation of *PRSS1* gene in a Chinese hereditary pancreatitis family. Hereditas (Beijing), 2007, **29** (9): 1067～1070

9 Miyata S, Koshikawa N, Yasumitsu H, *et al*. Trypsin stimulates integrin alpha (5)beta (1)-dependent adhesion to fibronectin and proliferation of human gastric carcinoma cells through activation of proteinase-activated receptor-2. J Biol Chem, 2000, **275** (7): 4592～4598

10 Chen J M, Kukor Z, Le Maréchal C, *et al*. Evolution of trypsinogen activation peptides. Mol Biol Evol, 2003, **20** (11): 1767～1777

11 Sahin-Tóth M, Tóth M. Gain-of-function mutations associated with hereditary pancreatitis enhance autoactivation of human cationic trypsinogen. Biochem Biophys Res Commun, 2000, **278** (2): 286～289

12 Le Bodic L, Schnee M, Georgelin T, *et al*. An exceptional genealogy for hereditary chronic pancreatitis. Dig Dis Sci, 1996, **41** (7): 1504～1510

13 Lowenfels A B, Maisonneuve P, DiMagno E P, *et al*. Hereditary pancreatitis and the risk of pancreatic cancer. J Natl Cancer Inst, 1997, **89** (6): 442～446

14 Applebaum-Shapiro S E, Peters J A, O'Connell, *et al*. Motivations and concerns of patients with access to genetic testing for hereditary pancreatitis. Am J Gastroenterol, 2001, **96** (5): 1610～1617

15 Lee T Y, Oh H C, Kim M H, *et al*. Three cases of hereditary pancreatitis in two households in the same family associated with R122H mutation in cationic trypsinogen gene. Korean J Gastroenterol, 2007, **49** (6): 395～399

16 Klein A P, de Andrade M, Hruban R H, *et al*. Linkage analysis of chromosome 4 in families with familial pancreatic cancer. Cancer Biol Ther, 2007, **6** (3): 320～323

17 Liu Q C, Cheng Z J, Gao F, *et al*. Association of genetic polymorphisms in PRSS1 with risk of chronic pancreatitis in Han population of fujian. Int J Genet, 2007, **30** (06): 127～130

18 Le Marechal C, Masson E, Chen J M, *et al*. Hereditary pancreatitis caused by triplication of the trypsinogen locus. Nat Genet, 2006, **38** (12): 1372～1374

19 Sahin-Toth M. Biochemical models of hereditary pancreatitis. Endocrinol Metab Clin North Am, 2006, **35** (2): 303～312

20 Vitone L J, Greenhalf W, Howes N R, *et al*. Hereditary pancreatitis and secondary screening for early pancreatic cancer. Rocz Akad Med Bialymst, 2005, **50** (6): 73～84

21 Khalid A, Finke lstein S, Thompson B, *et al*. A 93 year old man with the PRSS1 R122H mutation, low SPINK1 expression, and no pancreatitis: insights into phenotypic non-penetrance. Gut, 2006, **55** (5): 728～731

22 Schneider A, Lamb J, Barmada M M, *et al*. Keratin 8 mutations are not associated with familial, sporadic and alcoholic pancreatitis in a population from the United States. Pancreatology, 2006, **6** (1～2): 103～108

23 Keim V. Genetics of pancreatitis. Scand J Surg, 2005, **94** (2): 103～107

24 Pho-Iam T, Thongnoppakhun W, Yenchitsomanus P T, *et al*. A Thai family with hereditary pancreatitis and increased cancer risk due to a mutation in PRSS1 gene. World J Gastroenterol, 2005, **11**(11): 1634～1638

25 Kenesi E, Katona G, Szilagyi L. Structural and evolutionary consequences of unpaired cysteines in trypsinogen. Biochem Biophys Res Commun, 2003, **309** (4): 749～754

26 Cheng Z J, Yang B, Liu Q C, *et al*. Study on a new point mutation of nt7444 G →A in the mitochondrial DNA in a type 2 diabetes mellitus family. Yi Chuan, 2007, **29** (4): 433～437

27 Keim V. Identification of patients with genetic risk factors of pancreatitis: impact on treatment and cancer prevention. Dig Dis, 2003, **21** (4): 346～350

28 Uygur-Bayramicli O, Dabak R, Orbay E, *et al*. Type 2 diabetes mellitus and CA 19-9 levels. World J Gastroenterol, 2007, **13** (40): 5357～5359

29 Chen J M, Le Maréchal C, Lucas D, *et al*. "Loss of function" mutations in the cationic trypsinogen gene (PRSS1) may act as a protective factor against pancreatitis. Mol Genet Metab, 2003, **79** (1): 67～70

中国遗传性胰腺炎患者胰蛋白酶原基因多位点杂合突变及其临床特征

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摘要 用基因产物直接测序法对 2 个遗传性胰腺炎家系中胰腺炎患者 (共有 4 例成员) 的胰蛋白酶原基因(cationic trypsinogen，*PRSS1*) 5 个外显子进行测序，并分析其各自的临床特征．在 4 例胰腺炎患者中均出现了 *PRSS1* 基因杂合突变，但两家系 *PRSS1* 基因突变的位点不同，且临床表现差异较大，其中家系 1 出现 6 例糖尿病患者且发病年龄较家系 2 明显延迟，平均发病年龄为 29 岁，分析其 *PRSS1* 基因发现 3 号外显子 336 位碱基存在 G→A 杂合性突变，为中性突变，表达的氨基酸从赖氨酸(Lys)→赖氨酸 (Lys)，同时在同一外显子的 361 位碱基还存在另一个 G→A 杂合性突变，造成 121 位的丙氨酸 (Ala) 被苏氨酸 (Thr) 所取代，胰蛋白酶原的空间结构发生改变，其与抑制因子的结合位点消失，“保护失败”而产生有活性的胰蛋白酶，造成胰腺自身的消化．而家系 2 未发现糖尿病患者，其胰腺炎患者的血清肿瘤标志物不增高，先证者 (Ⅲ8) 在胰腺炎发病过程中表现为 $CD4^+$T /$CD8^+$Tcell 和乙肝表面抗体 (anti-HBs) 随病程进展逐渐降低，而Ⅲ7 不表现出此现象，分析其 *PRSS1* 基因发现 3 号外显子 361 位碱基同样存在 G→A (c.361 G→A) 突变，而且在 415 位还存在一个杂合性突变点 T→A (c.415 T→A)，其中 c.415 T→A 不存在于Ⅲ7．胰蛋白酶原基因存在多种形式的突变，而且与临床表型相关．

关键词 遗传性胰腺炎，PRSS1 基因突变，杂合子突变，多位点，临床表型
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