Targeted Gene Next-Generation Sequencing in Chinese Children with Chronic Pancreatitis and Acute Recurrent Pancreatitis

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Objective To identify causal mutations in certain genes in children with acute recurrent pancreatitis (ARP) or chronic pancreatitis (CP).

Study design After patients were enrolled (CP, 55; ARP, 14) and their clinical characteristics were investigated, we performed next-generation sequencing to detect nucleotide variations among the following 10 genes: cationic trypsinogen protease serine 1 (*PRSS1*), serine protease inhibitor, Kazal type 1 (*SPINK1*), cystic fibrosis transmembrane conductance regulator gene (*CFTR*), chymotrypsin C (*CTRC*), calcium-sensing receptor (*CASR*), cathepsin B (*CTSB*), keratin 8 (*KRT8*), CLAUDIN 2 (*CLDN2*), carboxypeptidase A1 (*CPA1*), and ATPase type 8B member 1 (*ATP8B1*). Mutations were searched against online databases to obtain information on the cause of the diseases. Certain novel mutations were analyzed using the SIFT2 and Polyphen-2 to predict the effect on protein function.

Results There were 45 patients with CP and 10 patients with ARP who harbored 1 or more mutations in these genes; 45 patients had at least 1 mutation related to pancreatitis. Mutations were observed in the *PRSS1*, *SPINK1*, and *CFTR* genes in 17 patients, the *CASR* gene in 5 patients, and the *CTSB*, *CTRC*, and *KRT8* genes in 1 patient. Mutations were not found in the *CLDN*, *CPA1*, or *ATP8B1* genes. We found that mutations in *SPINK1* may increase the risk of pancreatic duct stones (OR, 11.07; $P = .003$). The patients with *CFTR* mutations had a higher level of serum amylase (316.0 U/L vs 92.5 U/L; $P = .026$).

Conclusion Mutations, especially those in *PRSS1*, *SPINK1*, and *CFTR*, accounted for the major etiologies in Chinese children with CP or ARP. Children presenting mutations in the *SPINK1* gene may have a higher risk of developing pancreatic duct stones. *(J Pediatr 2017;191:158-63).*

See editorial, p 10

Advances in genetics have led to the realization that hereditary chronic pancreatitis (CP) with autosomal-dominant inheritance can be caused by mutations, such as mutations of the cationic trypsinogen protease serine 1 (*PRSS1*), serine protease inhibitor, Kazal type 1 (*SPINK1*), cystic fibrosis transmembrane conductance regulator gene (*CFTR*), and chymotrypsin C (*CTRC*).[1-4]

Studies have found that gene mutations can cause CP and are correlated with acute recurrent pancreatitis (ARP). Candidate genes have been continuously discovered, and reports have indicated that variations in the genes calcium-sensing receptor (*CASR*), cathepsin B (*CTSB*), keratin 8 (*KRT8*), CLAUDIN 2 (*CLDN2*), carboxypeptidase A1 (*CPA1*), and ATPase type 8B member 1 (*ATP8B1*) can lead to CP and ARP.[5-10] Similar to the findings of a genetic background for CP and ARP, studies have also found that a fraction of idiopathic CP cases of unknown origin were caused by a gene mutation.[11-13]

Because of the age of onset, several differences are noted between pediatric and adult patients with CP. Traditional risk factors have played a limited role in

ATP8B1	ATPase type 8B member 1
CASR	Calcium-sensing receptor
CFTR	Cystic fibrosis transmembrane conductance regulator gene
CLDN2	CLAUDIN 2
CP	Chronic pancreatitis
CPA1	Carboxypeptidase A1
CTRC	Chymotrypsin C
CTSB	Cathepsin B
HGMD	Human Gene Mutation Database
KRT8	Keratin 8
PRSS1	Cationic trypsinogen protease serine 1
SNV	Single nucleotide variation
SPINK1	Serine protease inhibitor, Kazal type 1

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children with CP, and idiopathic CP is observed at a higher proportion in children (approximately 30%-60%) relative to the adult population.[12,14] Therefore, children are suitable candidates for genetic studies of CP. Because of the differences in ethnic and socioeconomic conditions, climate, culture, and eating habits between China and Western countries, the genetic background of Chinese pediatric patients with CP must be further studied. In this study, we used Illumina MiSeq (Illumina, San Diego, California) to perform next-generation sequencing of these 10 candidate genes of Chinese Han children diagnosed with CP and ARP. We also analyzed the relationship between different gene mutations and clinical features.

Methods

From August 2008 to July 2015, 94 children with chronic abdominal pain were diagnosed with CP, and 14 patients were diagnosed with ARP at the Department of Pediatrics, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine. Informed consent was obtained from the children's parents. In total, 69 patients (CP, 55; ARP, 14) provided signed informed consent and were enrolled in the study.

Considering the "second-hit" theory of CP,[15,16] our study included cases of CP and ARP caused by anatomic abnormalities. The number of CP and ARP cases with different causes were as follows: 52 idiopathic cases (idiopathic CP, 41 cases; ARP, 11 cases), 9 cases with pancreas divisum (all CP), and 8 cases with an anomalous junction of pancreaticobiliary duct (CP, 5 cases; ARP, 3 cases).

The diagnosis of CP and ARP is based on the criteria defined by INSPPIRE (International Study Group of Pediatric Pancreatitis: In Search for a Cure).[17]

Ethics committee approval for the study was granted by the Institutional Review Boards at Ruijin Hospital affiliated with the Shanghai Jiao Tong University School of Medicine.

Genetic Analysis

Peripheral blood samples were collected from the 69 patients with CP or ARP, and genomic DNA was extracted from peripheral blood leukocytes using the FlexiGene DNA Extraction Kit (Qiagen, Hilden, Germany).

We used Oligo 7 to design 131 pairs of primers for the exons of *PRSS1*, *SPINK1*, *CFTR*, *CTRC*, *CASR*, *CTSB*, *KRT8*, *CLDN2*, *CPA1*, and *ATP8B1*(**Table I**; available at www.jpeds.com). A multiplex polymerase chain reaction assay of the coding regions of candidate gene was performed using the Qiagen Multiple Polymerase Chain Reaction Kit (Qiagen, Hilden, Germany). The purified polymerase chain reaction products were sequenced using the Illumina MiSeq next-generation sequencing platform, and then the sequencing results were compared with the information in GenBank. The databases ClinVar (https://www.ncbi.nlm.nih.gov/clinvar), Human Gene Mutation Database (HGMD; http://www.hgmd.cf.ac.uk/ac/index.php), 1000 Genomes (http://phase3browser.1000genomes.org/index.html), and Genetic risk factors in CP (http://www.pancreasgenetics.org/) were used to analyze each single nucleotide variation (SNV) to determine whether it was a disease-causing mutation and identify a functional study of the mutation. For SNVs that were not included in the databases or SNVs with unknown pathogenicity, 2 online tools, SIFT and Polyphen-2, were used to predict the effect of this mutation on the function of the protein.[18] The selected variants were further validated by Sanger sequencing of samples from the pediatric patients and their parents.

Clinical Evaluation

We retrospectively reviewed the following clinical data for each patient based on their medical records: sex; age at onset; degree of abdominal pain; height, weight, and body mass index; family history of pancreatitis; history of trauma; medication history; IgG4 level; serum amylase and lipase levels (on first admission to our hospital); and other measures. The morphologic features of the pancreatic duct were demonstrated by abdominal ultrasound imaging, magnetic resonance cholangiopancreatography, or endoscopic retrograde cholangiopancreatography. A sweat test was not performed in any patients because of the very low incidence of cystic fibrosis in China.

Statistical Analyses

SPSS 13.0 for Windows (SPSS Inc., Newark, DE) was used for the statistical analysis. If the continuous variable showed a normal distribution, then the statistical data were expressed as the mean ± SD. The independent sample *t* test was used to compare the differences between 2 samples, and an ANOVA was used to perform comparisons of multiple samples. Enumeration or grade data that were not normally distributed were expressed as frequencies or medians and IQRs, and the values were compared using the Mann-Whitney test or χ^2 test, respectively. We used a logistic regression to analyze the relative risk factor of pancreatic duct stones and then calculated the OR. $P < .05$ was considered significant.

Results

Among the 69 cases of pancreatitis, 14 (6 boys and 8 girls) were diagnosed with ARP. The mean age of the first episode of pancreatitis was 9.5 ± 3.5 years (range, 3.8-13.9). Another 55 cases (31 boys and 24 girls) were diagnosed with CP. The mean age of the first attack was 8.1 ± 3.9 years (range, 1.7-15.2). Significant differences were not observed between these 2 groups for the age at initial onset, sex composition, etiologic composition, the number of attacks of pancreatitis during past year, height SDS, weight SDS, body mass index SDS, or serum amylase and lipase levels (**Table II**; available at www.jpeds.com).

In this study, 45 patients (65.2%) had 1 or more mutations primarily in the *PRSS1*, *SPINK1*, *CFTR*, *CASR*, and *CTSB* genes. Thirteen patients (18.8%) had more than 1 gene affected (**Table III**). Mutations were detected in 63.6% of the patients with CP (35 cases) and 71.4% of the patients with ARP (10 cases). Of the 52 patients with idiopathic pancreatitis, 37 (71.2%) had mutations, and of the 17 patients with anatomic abnormalities, 8 (47.1%) had related mutations (**Figure**; available at www.jpeds.com).