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CASE REPORT

A Novel *SPINK1* Gene Mutation, c.206C>T, in a Thai Patient with Chronic Alcoholic Pancreatitis

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ABSTRACT

Context The exact mechanism of alcoholic pancreatitis has not yet been clarified. Recent studies suggest that alcohol represents only a risk factor for developing pancreatic inflammation in genetic or environmental susceptible subjects. In this regard, various genes involving an alcohol-metabolizing pathway or pancreatitis protecting factors have been extensively studied in order to identify genetic predisposition to alcoholic pancreatitis.

Case report A 43-year-old man with a history of heavy alcohol drinking presented with recurrent abdominal pain. Alcoholic pancreatitis was diagnosed and responded well to pancreatic stricture dilatation with stent insertion. Sequencing analysis revealed that he was heterozygous for a novel transition c.206C>T in exon 4 of the *SPINK1* gene, resulting in the substitution of threonine for isoleucine at codon 69 (T69I). Evidence supporting its etiologic role includes the alteration of the polarity of the amino acid change, its revolutionary conservation among mammals and its absence in 100 ethnic-matched control alleles.

Conclusions We identified a novel *SPINK1* mutation, c.206C>T (T69I), in a Thai patient with alcoholic pancreatitis. This extends the total number of confirmed *SPINK1* mutations and polymorphisms to more than 30. It also supports a previous observation that the *SPINK1* gene is a susceptibility locus for alcoholic pancreatitis.

INTRODUCTION

The serine protease inhibitor, Kazal type 1 (SPINK1), is a single polypeptide chain consisting of 79 amino acid residues, including a 23-amino acid signal sequence [1]. It is a member of the trypsin inhibitor family which is believed to act as a first-line defense against premature trypsinogen activation in the pancreas. The gene located on chromosome 5 is approximately 7.5 kb long and consists of 4 exons [2]. SPINK1 was first shown to be associated with chronic pancreatitis [2] and subsequently with many other forms of pancreatitis, including alcoholic pancreatitis. Frequently, the *SPINK1* N34S mutation was reportedly slightly increased in patients with alcoholic pancreatitis as compared to controls [3, 4, 5, 6, 7, 8]. Our aim was to expand our understanding of the spectrum of genetic mutations and provide more evidence of the association between the *SPINK1* gene and chronic alcoholic pancreatitis.

A total of 10 unrelated patients (8 males and 2 females) with chronic alcoholic pancreatitis were enrolled in this cohort study. The mean

age of onset and duration of symptoms was 49.6±3.0 years (range: 42-69 years). Alcohol-related pancreatitis was defined as chronic pancreatitis in a patient without other identifiable causes together with at least one of the following: 1) patient admission of excessive alcohol use as the cause of the disease; 2) patient admission of a history of excessive alcohol intake of at least 80 g/day for males, or 40 g/day for females, for more than 2 years; or 3) other evidence of alcoholism, defined by using a modified TWEAK (Tolerance: T1 number of drinks to feel high, T2-number of drinks one can hold, Worry about drinking, Eye-opener :morning drinking, Amnesia: blackouts, Cut down on drinking: K/C) alcohol screening questionnaire [9]. The diagnosis of chronic pancreatitis was based on two or more of the following findings: presence of a typical history of recurrent pancreatitis, pancreatic calcifications and/or pancreatic ductal irregularities revealed by endoscopic retrograde pancreatography or by magnetic resonance imaging of the pancreas and/or pathological sonographic findings. This study was approved by the local Ethics Committee; written informed consent was obtained from each person included in this study.

Genomic DNA was extracted from the peripheral blood leukocytes using the phenol-chloroform method. The entire coding sequence of the *SPINK1* gene was amplified by PCR using primers and conditions as previously described [2]. The PCR products were treated with ExoSAP-IT (USP Corporation, Cleveland, OH, USA) according to the protocols supplied by the manufacturer and were sent to Macrogen Inc. (Seoul, South Korea) for direct sequencing. The non-synonymous coding variant, c.206C>T, in exon 4 was verified by restriction enzyme *Bsa*BI (Fermantas, Vilnius, Lithuania) and then electrophoresized on 3% agarose gel stained with ethidium bromide. Fifty Thai control individuals were studied by restriction enzyme analysis.

Of the ten patients investigated for chronic alcoholic pancreatitis, one exhibited an alteration in the coding region of *SPINK1*.

CASE REPORT

A 43-year-old Thai man was referred due to recurrent pancreatitis. He had been healthy until at the age of 41 when he developed recurrent upper abdominal pain. Acute pancreatitis was confirmed by elevated serum amylase and lipase levels. One year after the first attack, he had another bout of pancreatitis which was complicated by pseudocyte formation requiring percutaneous drainage. One day before this admission, he developed acute upper abdominal pain radiating to his back. He reported a history of excessive alcohol consumption, averaging 80 g/day, beginning at 21 years of age. He had stopped alcohol consumption 2 years prior to admission. No diarrhea, steatorrhea or weight loss was noted. There was no family history of pancreatitis, pancreatic cancer or diabetes mellitus. Physical examinations revealed a body mass index of 21.2 kg/m^2 and diffuse abdominal tenderness, predominantly in the upper abdominal quadrant. Laboratory findings showed a fasting plasma glucose level of 158 mg/dL (reference range: 70-100 mg/dL), elevated serum amylase (546 U/L; reference range: 28-100 U/L), elevated serum lipase (167 U/L; reference range: 0-50 U/L), but no ketoacidosis. His liver function tests and lipid profiles were normal. An abdominal computerized tomography (CT) scan showed calcification along the pancreatic shadow (Figure 1). Endoscopic retrograde cholangio-

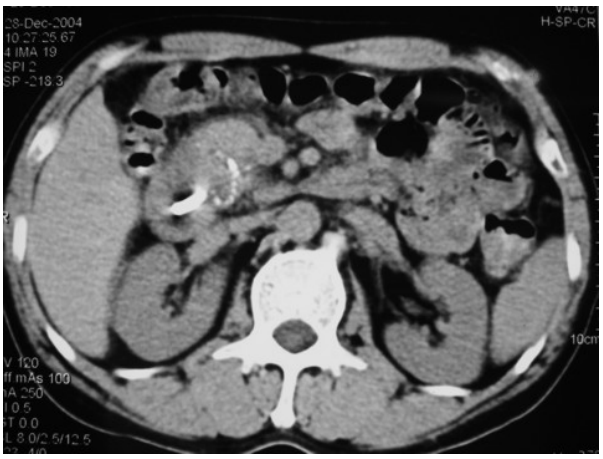

Figure 1. CT of the abdomen. In pre-contrast phase, the CT revealed an enlarged pancreatic head with multiple stipple calcifications. The metallic stent in the common bile duct was also noted.

pancreatography (ERCP) revealed only a prominent pancreatic duct at the distal part. Recurrent pancreatitis and diabetes mellitus were diagnosed and responded well to diet therapy and pancreatic stricture dilatation with stent insertion.

Sequencing analysis revealed that he was heterozygous for a transition c.206C>T in exon 4 of the *SPINK1* gene (Figure 2a), resulting in the substitution of threonine for isoleucine at codon 69 (T69I). The mutation was confirmed by restriction digestion analysis and was not found in 50 healthy Thai subjects (100 alleles). No other sequence variants were found in the patient's *SPINK1* coding regions. His unaffected parents were not available for testing.

DISCUSSION

Up to now, at least 30 mutations and polymorphisms of the *SPINK1* gene have been reported in patients with pancreatitis (Database of Genetic Variants in Patients with Chronic Pancreatitis, University of Leipzig: http://www.uni-leipzig.de/pancreasmutation/db.html). Some of these DNA changes are considered to be disease-causing while others are considered to be disease modifiers. The most frequent mutation site identified as being associated with chronic alcoholic pancreatitis was N34S. In fact, it was associated with various types of pancreatitis and has been hypothesized as a disease-modifying factor due to its presence in normal individuals, with a prevalence of 1-2% [2]. Moreover, there is no clear phenotypic difference between individuals who are homozygous and those who are heterozygous for the N34S mutation. It is believed that an individual who carries this mutation has a lower threshold for the development of pancreatitis from other primary cofactors. Its association with chronic alcoholic pancreatitis was found in several ethnic groups including Caucasians and Asians [2, 3, 4, 5, 6, 7, 8]. Very recently, an association between the *SPINK1* mutation IVS3+2T>A, not N34S, and alcoholic pancreatitis was revealed in Japanese patients [10]. This study also showed that patients with this mutation had an earlier onset of pancreatitis. This prompted us to investigate an association between the *SPINK1* gene and chronic alcoholic pancreatitis in the Thai population.

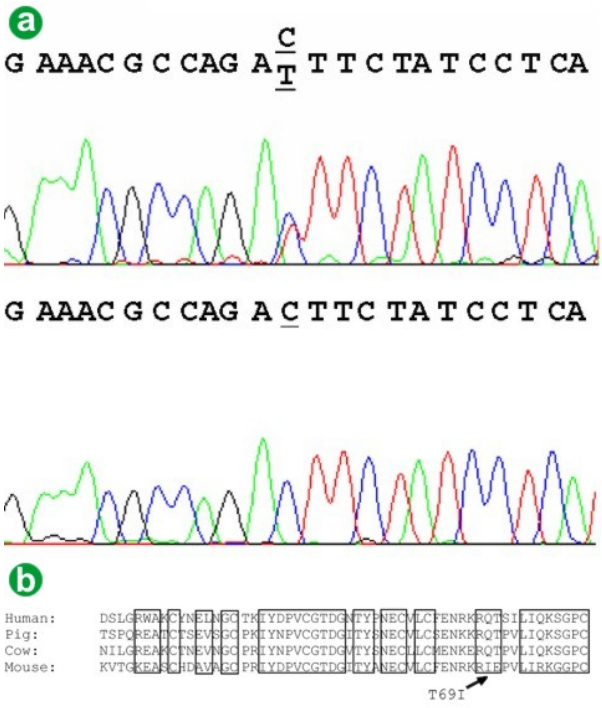

Figure 2. a. The sense sequence of *SPINK1* exon 4 of the proband (upper panel) showing a heterozygous c.206C>T change and of a control showing only CC at nucleotide 206 (lower panel). **b.** Alignment of a SPINK1 protein fragment with a similar domain from different species of mammals indicated on the left. Amino acids which are highly conserved are boxed.

In our cohort study, one patient exhibited an alteration in the coding region of *SPINK1*, a heterozygous c.206C>T in exon 4, expected to have an effect on the SPINK1 protein by changing the residue at position 69 from threonine to isoleucine. It has never been reported previously. Evidence supporting its etiologic role includes the alteration of the polarity of the amino acid change, its revolutionary conservation among mammals (Figure 2b), and its absence in 100 ethnic-matched control alleles. However, the number of patients tested was limited; the role of c.206T>C needs to be clarified in larger populations or by functional studies.

In conclusion, we identified a novel *SPINK1* gene mutation, c.206C>T (T69I), in a Thai patient with chronic alcoholic pancreatitis. This supports a previous observation that the *SPINK1* gene is a susceptibility locus for chronic alcoholic pancreatitis.

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Keywords Pancreatitis, Alcoholic; Pancreatitis, Chronic; Sequence Analysis, DNA; Trypsin Inhibitor, Kazal Pancreatic

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