

## PRSS1 and SPINK1 mutations in idiopathic chronic and recurrent acute pancreatitis

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### Abstract

**AIM:** To identify gene mutations in *PRSS1* and *SPINK1* in individuals with early onset idiopathic chronic or recurrent acute pancreatitis.

**METHODS:** The cationic trypsinogen gene (*PRSS1*; exons 2 and 3) and the serine protease inhibitor Kazal 1 gene (*SPINK1*; exon 3) were selectively amplified and sequenced from blood samples of 19 patients admitted to the Pancreas Clinic at our institution with chronic pancreatitis and/or idiopathic recurrent acute pancreatitis that were diagnosed or with onset before age 35. Fifty healthy volunteers served as controls. Whole blood samples were collected and gene specific sequences were amplified by polymerase chain reaction (PCR). All PCR products were subsequently sequenced in order to identify the presence of any mutations.

**RESULTS:** Nineteen patients with pancreatitis (14 males; median age 24 years, range 15-48 years) were included in this study, of which five showed the presence of gene mutations. Direct sequencing results indicated the presence of two previously unidentified mutations in exon 2 of *PRSS1* (V39E and N42S) in two patients with recurrent acute pancreatitis. Two cases had the N34S *SPINK1* mutation. Analysis of the relatives of one patient homozygous for this mutation showed that five of the six family members carried the N34S *SPINK1* mutation. Of these members, three were healthy heterozygous carriers and two were homozygotes (one sibling had diabetes, the other was healthy). Another patient was heterozygous for a novel *SPINK1* mutation located on exon 3 (V46D). All members from this patient's family had normal genotypes, indicating that it was a *de novo* mutation. No mutations in either gene were present in the control subjects.

**CONCLUSION:** Two novel *PRSS1* mutations and one novel *SPINK1* mutation were identified in Mexican patients with early onset idiopathic recurrent acute pancreatitis.

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**Key words:** Cationic trypsinogen; *SPINK1*; *PRSS1*; Chronic pancreatitis; Recurrent acute pancreatitis; Hereditary pancreatitis

**Core tip:** Chronic and recurrent idiopathic pancreatitis has been associated with mutations in genes responsible for the synthesis of pancreatic proteases (*PRSS1*) and protease inhibitors (*SPINK1*). The distribution of these mutations varies among countries, but has not been examined in detail in Latin American countries. This study examined *PRSS1* and *SPINK1* in 19 Mexican subjects with chronic pancreatitis and/or idiopathic recurrent acute pancreatitis and identified two novel *PRSS1* mutations and one novel *SPINK1* mutation.

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## INTRODUCTION

Chronic pancreatitis (CP) is a progressive inflammatory disease that leads to fibrosis and different degrees of exocrine and/or endocrine insufficiency<sup>[1]</sup>. There are many factors contributing to disease development, including alcohol use<sup>[2]</sup>, though some cases do not present any known risk factors and are classified as idiopathic. Hereditary pancreatitis is diagnosed in the case of a positive family history<sup>[3]</sup>. As early as 1952, the observation that CP clustered in certain families suggested a genetic component. However, identification of such genetic factors did not occur until 1996, when mutations in the cationic trypsinogen gene (*PRSS1*) were discovered in families with hereditary CP and in some cases of idiopathic CP<sup>[4,5]</sup>. Later, mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*)<sup>[6,7]</sup>, trypsin inhibitor (*SPINK1*), and chymotrypsinogen C (*CTRC*) genes were described in both idiopathic CP and alcoholic CP<sup>[8,9]</sup>.

*PRSS1* mutations have been linked with hereditary pancreatitis, whereas mutations in the *SPINK1* gene have been associated with pancreatitis of different etiologies<sup>[10]</sup>. However, mutations in *SPINK1* are not always sufficient to induce pancreatitis, and additional pancreatitis-associated factors must be present in order to express the disease. For example, the commonly observed N34S mutation in *SPINK1* by itself has no apparent functional effect<sup>[11,12]</sup>. *CTRC* mutations have been identified in patients with idiopathic CP and hereditary pancreatitis<sup>[13,14]</sup>, as well as in subjects with alcoholic CP. The effect of these genes on pancreatitis likely results from an imbalance between normal mechanisms of protease activation and inhibition and pancreatic fluid composition<sup>[15,16]</sup>. Functional analysis of several identified gene mutations has shown that they result either in a gain of trypsin function (*PRSS1*)<sup>[17,18]</sup>, loss or decreased protein expression or function (*SPINK1* D50E and Y54H)<sup>[19,20]</sup>, and/or altered ductal secretion (*CFTR* mutations)<sup>[21]</sup>.

The distribution of these identified mutations varies among countries<sup>[22-25]</sup>; although, reports from Latin America are scarce, with only information from Brazil available<sup>[26,27]</sup>. Furthermore, there is no available information about the role and characteristics of CP-related genetic mutations in Mexico, a population characterized by a broad genetic admixture<sup>[28]</sup>. However, a previous study by our group found that a large proportion of CP cases in Mexico are idiopathic<sup>[29]</sup>. Therefore, the aim of the present study was to identify mutations in the *PRSS1* and *SPINK1* genes in Mexican subjects with early onset idiopathic CP or idiopathic recurrent acute pancreatitis (IRAP).

## MATERIALS AND METHODS

Subjects with CP and/or IRAP that were diagnosed or with onset before age 35 were prospectively and retrospectively enrolled in the study. For retrospective enrollment, the outpatient and inpatient database from the Pancreas Clinic at our institution was searched, and all eligible subjects were contacted by telephone. For prospective enrollment, all consecutive patients seen at our institution either as inpatients or at the outpatient Pancreas Clinic for CP or IRAP were included. Informed consent was obtained from the patients and 50 healthy volunteers who agreed to participate, and 20 cc of whole blood samples were then collected by peripheral vein puncture. Blood was stored at -70 °C for subsequent DNA extraction. This study was approved by the Institutional Review Board of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán.

### Definitions

Acute pancreatitis was defined by the presence of two of the following: typical abdominal pain, three-fold elevation of serum pancreatic enzymes (amylase and/or lipase), and imaging evidence of acute pancreatitis. CP was considered if patients had any of the following predetermined criteria: histologic evidence (when available), imaging evidence from endoscopic retrograde cholangiopancreatography and/or magnetic resonance cholangiopancreatography with definitive evidence of CP according to the Cambridge classification, presence of pancreatic calcifications on computed tomography scan, plain abdominal X-rays, five or more CP-related findings on endoscopic ultrasound, and definitive pancreatic exocrine insufficiency according to a pancreolauryl test. IRAP was defined as the presence of two or more attacks of documented acute pancreatitis with no evident etiology after a thorough work-up and without imaging evidence of CP.

### DNA extraction and gene- and exon-specific amplification

Whole blood (20 cc) was collected in K2EDTA BD Vacutainer tubes (Beckton Dickinson and Company, Franklin Lakes, NJ, United States). All blood specimens were processed at the Genomic Medicine Unit at the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán. Genomic DNA was extracted using a standard salt precipitation protocol and assessed for quality and quantity by agarose gel electrophoresis. Exons 2 and 3 of the *PRSS1* gene were amplified using primers and PCR amplification protocols previously reported by Nishimori *et al.*<sup>[30]</sup> and Pho-Iam *et al.*<sup>[31]</sup>, respectively. Exon 3 of the *SPINK1* gene was amplified using primers and polymerase chain reaction (PCR) amplification protocols as previously reported by Witt *et al.*<sup>[8]</sup>.

### DNA sequencing

The PCR products from all samples were purified using a QIAquick PCR Purification Kit (Qiagen, Venlo, Limburg, The Netherlands) according to the manufacturer's proto-

**Table 1** Clinical characteristics of subjects with early onset chronic pancreatitis and/or idiopathic recurrent acute pancreatitis

Patient case No.	Sex	Age, in years	Age at symptom onset or diagnosis, in years	Clinical presentation	Mutation
1	Female	45	8	IRAP, pancreatic calcifications at age 15, Puestow procedure at age 22	V39E <i>PRSS1</i>
6	Male	26	20	IRAP, pancreatic calcifications at age 22	N42S <i>PRSS1</i>
8	Female	26	26	IRAP, pancreatic calcifications and dilated main pancreatic duct	N34S <i>SPINK1</i>
15	Male	20	16	IRAP, pancreatic calcifications	V46D <i>SPINK1</i>
19	Male	15	12	Abdominal pain, jaundice, pancreatic calcifications	N34S <i>SPINK1</i>

IRAP: Idiopathic recurrent acute pancreatitis.

col. Gene sequencing was performed using the Applied Biosystems Inc. (ABI) Prism BigDye Terminator Ready Reaction Cycle Sequencing Kit and the ABI Prism DNA Sequencer (model PE; ABI of Thermo Fisher Scientific, Waltham, MA, United States). All PCR products were sequenced in both directions using the same primers that were employed for the PCR amplification.

## RESULTS

Nineteen subjects with either early onset CP or IRAP were identified and agreed to participate (14 males; median age 24 years, range 15–48 years) with a total of five instances of *PRSS1/SPINK1* gene mutations (Table 1). Two mutations in *PRSS1* were found: V39E in case 1 and N42S in case 6, both of which were in exon 2. The N34S *SPINK1* mutation was identified in cases 8 and 19. The family of one of these patients, who was homozygous for the N34S mutation and had early onset chronic calcifying pancreatitis, was also examined, and six of the 14 additional members agreed to participate and provide blood samples for sequencing. Although no *PRSS1* mutations were found in these family members, five of the six carried the same N34S *SPINK1* mutation. Of these, both parents and one sibling were healthy and heterozygous for this mutation, whereas two siblings were homozygous (one was healthy and the other had developed diabetes at an early age). The other studied sibling had a normal genotype and was otherwise healthy. The fifth mutation was found in case 15, who was a heterozygote for a new *SPINK1* mutation located on exon 3 (V46D). The family members of this patient were also studied, though no *PRSS1* mutations were found, and the V46D or other *SPINK1* mutations were not present. Moreover, no *PRSS1* or *SPINK1* mutations were identified in samples from the 50 healthy controls.

## DISCUSSION

The results of this study identified two previously undocumented *PRSS1* mutations. The first mutation, N42S, represents the substitution of one polar hydrophilic amino acid with another similar one. However, the second novel mutation identified, V39E, represents the substitution of a non-polar hydrophobic amino acid to a negatively charged polar one, which could induce a conformational change in the final synthesized molecule. Although

functional studies are needed to elucidate the effect of these mutations on protein structure, expression and/or secretion as well as their contribution to the pathogenic mechanisms of pancreatic injury, the results from the current study suggest that mutations in the *PRSS1* gene are sufficient to induce pancreatic disease. Indeed, *PRSS1* gene mutations have been directly implicated in the pathophysiology of hereditary and idiopathic CP by producing an autolysis-resistant trypsin and/or facilitating auto-activation<sup>[17,18]</sup>. However, the presence and contribution of mutations in other exons, genes or environmental factors remains unclear and should not be ruled out.

Recent reports from India<sup>[22]</sup> and Japan<sup>[23]</sup> indicated that *SPINK1* mutations confer strong genetic susceptibility to developing CP, but alone do not cause the disease. Some *SPINK1* mutations alter peptide expression or binding affinity, though the disease-causing biochemical defect of the N34S mutation remains unknown<sup>[11,12,19,20]</sup>. In the current study, one case with the *SPINK1* N34S mutation had apparently unaffected family members, two healthy siblings who were homozygous for the same mutation, and another sibling and both parents who were heterozygous. Thus, *SPINK1* mutations may require other associated genetic and/or environmental risk factors in order to promote pathogenicity. It is possible that these mutations impact the phenotypic presentation of the disease, with patients developing CP at earlier ages<sup>[8,9,12]</sup>, as seen in this early onset CP population.

The sequencing results of this study identified a novel *SPINK1* mutation (V46D) in a patient with established calcific CP and no other evident predisposing factors. Computational simulations could indicate if this mutation likely aborts *SPINK1* protein synthesis, in contrast to other previously described mutations that reduce the enzymatic activity<sup>[32]</sup>. It appeared to be a *de novo* mutation, as none of the family members had it nor did they present any clinical manifestations of pancreatitis, though no paternity tests were run. In addition, neither the family members nor the affected individual had prior history of exposure to pancreatic disease-related risk factors. As the presence of mutations in other exons or genes remains unknown, a direct causal effect of this new mutation needs to be validated.

In agreement with previous studies worldwide<sup>[24,25]</sup>, the current study provides further support that the frequency, nature and type of mutations vary among populations. This is the first Mexican study to explore the genetics of

early onset idiopathic CP in Hispanics. Although still a minority in the United States<sup>[33]</sup> and European countries, the Hispanic population has shown a steady and continuous growth rate, and thus the results provide valuable information to health care workers responsible for the medical attention of such minorities. The main limitations of this study include the small sample population, incomplete sequencing of the entire *PRSS1* and *SPINK1* genes, and absence of testing for *CFTR* and *CTRC* mutations. However, the findings of this study are consistent with previous reports and identify new pancreatitis-related mutations.

## CONCLUSION

Mexican subjects with idiopathic CP and IRAP present similar mutations in the *PRSS1* and *SPINK1* genes as reported in other populations. This study identified three novel mutations, two in *PRSS1* and one in *SPINK1*, which may be unique to the Mexican population. The novel V46D *SPINK1* mutation may play a direct causal role of pancreatitis, though this finding needs to be validated by future functional studies.

## COMMENTS

### Background

Early onset chronic pancreatitis and idiopathic recurrent acute pancreatitis in the absence of any other established risk factors might result from genetic mutations. Gene mutations that have been linked with pancreatitis result in gain of function or inability to inhibit trypsin, or alteration in secretory mechanisms of the pancreatic ductal cells. Hereditary pancreatitis is an autosomal dominant condition characterized by recurrent attacks of acute pancreatitis in childhood or adolescence which progresses to the development of chronic pancreatitis at early ages. The first reported associated mutation was identified in the cationic trypsinogen gene (*PRSS1*) on chromosome 7. Additional mutations that may contribute are found in the serine protease inhibitor Kazal type 1 (*SPINK1*), the cystic fibrosis transmembrane conductance regulator gene (*CFTR*), and the chymotrypsinogen C (*CTRC*) gene. Mutations in these latter genes are seen in forms of pancreatitis that are initially classified as idiopathic chronic or idiopathic acute pancreatitis, although *PRSS1* mutations have also been seen in non-hereditary cases. These mutations may have an additive effect, increasing individual susceptibility to pancreatitis.

### Research frontiers

Prior reports indicate that new mutations do occur across populations. Due to the genetic heterogeneity, screening for known and new mutations and characterizing them in each population is worthwhile.

### Innovations and breakthroughs

This report identifies three new mutations, one in *SPINK1* and two in *PRSS1*, which are associated with chronic pancreatitis and may be unique to the Mexican population. These data suggest that there are wide genetic and population heterogeneities of the disease.

### Applications

Chronic pancreatitis increases the risk of pancreatic cancer, and hereditary pancreatitis has an estimated cumulative risk of pancreatic cancer near 40%. Although there are no specific treatment recommendations in patients carrying pancreas-related mutations, identification of such could benefit genetic counseling, which is not used for other forms of pancreatitis, and result in the implementation of individualized and specific screening strategies for pancreatic cancer as well as lifestyle recommendations and modifications. In addition, the identification of these gene mutations will decrease the incidence and prevalence of idiopathic pancreatitis.

### Terminology

Cationic trypsinogen, encoded by the *PRSS1* gene, represents 60% of the

trypsinogen secreted by pancreatic acinar cells. Trypsinogen is then converted to trypsin by enterokinase within the duodenum, which then activates the digestive enzyme cascade. Pancreatic secretory trypsin inhibitor, or serine protease inhibitor Kazal type 1, is a protein encoded by the *SPINK1* gene that competitively binds to and inactivates trypsin.

### Peer review

The present study provides new information concerning genetic contributors to chronic pancreatitis in the Mexican population, which has been largely unstudied to date. Patients and relatives were sampled to allow for direct sequencing to promote an understanding of the impact of the occurrence of identified mutations in the development of pancreatitis. The inclusion criteria were restricted to the defined characteristics of an uncommon disease, allowing for the selection of patients most likely to have relevant genetic mutations.

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