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Hereditary Pancreatitis Associated With the N29T Mutation of the *PRSS1* Gene in a Brazilian Family: A Case-Control Study

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Abstract: Hereditary pancreatitis (HP) is an autosomal-dominant disease with incomplete penetrance manifesting as early-onset chronic relapsing pancreatitis. A mutation in the *PRSS1* gene is present in greater than 70% of HP kindreds and leads to a gain-of-function characterized by the increased autocatalytic conversion of trypsinogen to active trypsin, promoting autodigestion and damage to acinar cells. Other genetic defects observed in the pathogenic mechanism of pancreatitis include mutations in the genes encoding *SPINK1*, *CTRC*, and *CPA1*. There are few reports of HP in Latin America, and no families have been investigated in Brazil. A case-control observational study was conducted at Clementino Fraga Filho University Hospital in Brazil. Patients with suspected HP and healthy controls were enrolled in this study, and a detailed questionnaire was administered to patients with HP. *PRSS1* and *SPINK1* genes were analyzed by DNA sequencing, and a family that fit the HP diagnostic criteria was identified. The neutral polymorphism c.88-352A > G in the *SPINK1* gene was found to be prevalent in the individuals studied, but no important alterations were found in this gene. Ten out of 16 individuals in this family carried the N29T mutation in the *PRSS1* gene, with 2 clinically unaffected mutation carriers. The median age of HP onset was 6 years. Pancreatic exocrine failure occurred in 6 patients, 5 of whom also had diabetes mellitus. Surgical procedures were performed on 3 affected members, and no cases of pancreatic cancer have been reported thus far. This study identified the first *PRSS1* gene mutation in a Brazilian family with HP.

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Abbreviations: CPA1 = carboxypeptidase A1, CTRC = chymotrypsin C, EDTA = ethylene diamine tetraacetic acid, EUROPAC = European Registry of Hereditary Pancreatitis and Familial Pancreatic Cancer, HP = hereditary pancreatitis, PCR = polymerase chain reaction, PRSS2 = anionic trypsinogen, PRSS1 = cationic trypsinogen, SPINK1 = serine protease inhibitor Kazal type 1.

INTRODUCTION

Hereditary pancreatitis (HP) is an autosomal-dominant disease with variable expression and an estimated penetrance of 80%. HP presents clinically as recurrent acute pancreatitis with an unusually early onset, progression to fibrosis, and chronic pancreatitis, and it carries a high risk of pancreatic adenocarcinoma beginning in the fifth decade of life.[1] The primary manifestations of HP include abdominal pain, maldigestion due to pancreatic exocrine dysfunction, and diabetes mellitus due to islet cell damage.[2]

Comfort and Steinberg published the first description of HP in 1952.[3] However, the first associated genetic mutation, R122H, was identified in 1996 by Whitcomb et al, in the cationic trypsinogen (*PRSS1*) gene.[4] Mutations in the *PRSS1* gene (including R122H, N29I, and A16V) are responsible for greater than 70% of mutations in HP kindreds, according to various national series.[5–7]

The primary mechanism by which mutations in the *PRSS1* gene cause HP is an increase in the autocatalytic conversion of trypsinogen to active trypsin, leading to autodigestion and damage to the acinar cells, which result in ductal and interstitial injury.[8] Increased autoactivation is associated with the R122H and N29I mutations. Additionally, R122H results in the increased stability of trypsin through the elimination of an essential autolytic cleavage site in trypsin, thereby rendering the protease resistant to inactivation through autolysis.[9]

Additional genetic defects are involved in the pathogenic mechanism of pancreatitis and present a more heterogeneous genetic pattern. The serine protease inhibitor Kazal type 1 (*SPINK1*) acts as a pancreatic secretory trypsin inhibitor, and a mutation in the *SPINK1* gene is associated with chronic pancreatitis with a recessive inheritance pattern.[10] Chymotrypsin C (*CTRC*) degrades all human trypsin and trypsinogen isoforms, and CTRC mutants are poorly secreted in pancreatic acinar cells, accompanied by the loss of the second line of defence against the premature activation of trypsinogen isoforms.[11] Additionally, loss of function of carboxypeptidase A1 (*CPA1*) variants is strongly associated with chronic pancreatitis due to endoplasmic reticulum stress.[12] Carboxypeptidases are metalloproteases that hydrolyse C-terminal peptide bonds in dietary polypeptide chains.[13] *CPA1* is the second most abundant

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protein in pancreatic juices after trypsinogen, contributing to approximately 16% of the total protein content.[14]

Genetic mutations that result in HP have been frequently reported (www.pancreasgenetics.org) in Europe, North America, and Asia.[5–7,15,16] In contrast, reports from Latin America are scarce. Although 1 Mexican study described the *SPINK1* N34S mutation and 2 new mutations in the *PRSS1* gene, these results require further functional studies to define their relevant pathogenic features.[17] In addition, a Venezuelan kindred report studied the *PRSS1* R122H mutation,[18] and a Brazilian study reported the *PRSS1* E79K mutation in 1 patient with alcohol-related chronic pancreatitis and 1 healthy control.[19] However, there is no available information regarding the role and characteristics of HP-related genetic mutations in Brazil. Therefore, the aim of the present study was to identify whether HP in Brazilian patients can be caused by mutations in either the *PRSS1* or *SPINK1* genes.

METHODS

Patients

Patients with suspected HP were recruited at the Hospital of the Federal University of Rio de Janeiro (UFRJ) for 24 months starting in January of 2012. The diagnosis of HP in a family was based on disease occurrence in 2 first-degree relatives or 3 or more second-degree relatives, in 2 or more generations with recurrent acute pancreatitis and/or chronic pancreatitis, for which no predisposing factors were identified according to the European Registry of Hereditary Pancreatitis and Familial Pancreatic Cancer (EUROPAC) trial.[5] A detailed questionnaire was administered to HP patients to assess their dates of birth; dates when the event began; diagnoses of exocrine pancreatic failure and/or diabetes mellitus and requirements for pancreatic enzyme supplements and oral hypoglycaemic drugs and/or insulin, respectively; if they had undergone pancreatic resection and the type of surgery, either a resection for pain or a nonresectional procedure for the complications of chronic pancreatitis; presence of pancreatic cancer; and information regarding the consumption of alcohol and/or tobacco. For the control group, unrelated individuals were selected from among medical students and hospital staff. None of the controls showed any symptoms of pancreatitis.

The study was conducted at the Clementino Fraga Filho University Hospital (HUCFF) of the Federal University of Rio de Janeiro, Brazil. The local ethics committee approved the study protocol (169 11-CEP), in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Patient and control assent, informed parental consent for underage subjects (<18 years old), and approval of the hospital ethics committee were obtained before initiating the studies.

Mutation Screening

Ethylene diamine tetraacetic acid (EDTA) blood samples were collected, and genomic DNA was extracted from blood leucocytes using Wizard Genomic DNA Purification (Promega, Madison, WI). All blood specimens were processed at the Institute of Biophysics Carlos Chagas Filho. Exons 2 and 3 of the *PRSS1* gene and exon 3 of the *SPINK1* gene were amplified by polymerase chain reaction (PCR).[4,20,21] Cycling conditions consisted of an initial 4 min denaturation step at 95°C; 36 cycles of 30 s denaturation at 95°C, 30 s of annealing at 58°C for exon 2 of the *PRSS1* gene, at 54°C for exon 3 of the *PRSS1* gene and at 60°C for exon 3 of the *SPINK1* gene, and 1 min of primer extension at 72°C; and a final extension for 7 min after the final cycle. The PCR products were purified using a GFX PCR DNA and Gel Band Purification Kit (GE Healthcare, Little Chalfont, UK) and then assessed for quality by ethidium bromide-stained agarose gel electrophoresis and for quantity using a nanophotometer (Implen, München, Germany). DNA sequences were analyzed by sequencing both strands of the products using an ABI 3100 automated DNA sequencer, with the same sense and antisense primers used for PCR.

RESULTS

A family with 16 members who fit the diagnostic criteria of HP and 24 healthy controls were enrolled (Figure 1). Ten out of 16 (62.5%) individuals in the family were affected by a mutation in exon 2 of the *PRSS1* gene. This mutation was a heterozygous A to C substitution at nucleotide position c.86, which resulted in an asparagine-to-threonine substitution at

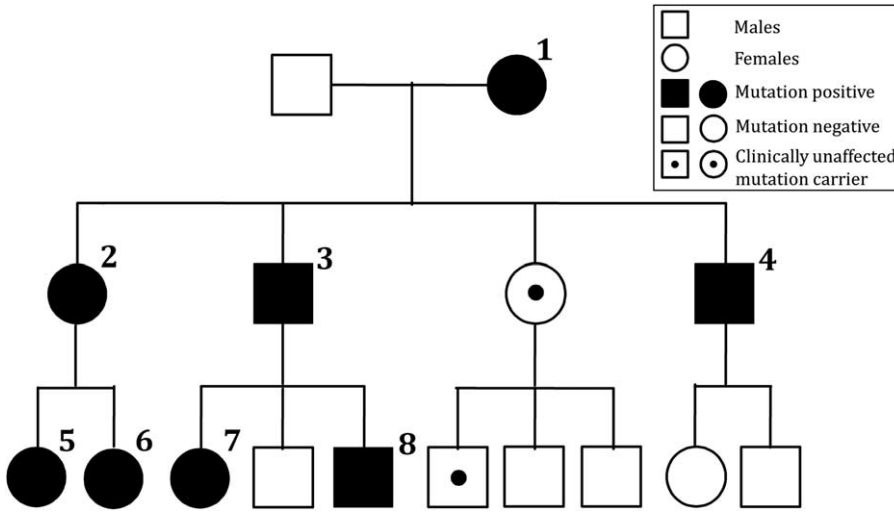

FIGURE 1. Pedigree of the reported family demonstrating the autosomal-dominant inheritance pattern of HP.

codon 29 (Figure 2). All 8 symptomatic patients and 2 clinically unaffected relatives exhibited a heterozygous N29T mutation. Six relatives and the control group had no identified mutations. The clinical characteristics of the affected individuals with mutations are presented in Table 1. Moreover, 3 subjects among the 8 symptomatic patients consumed alcohol, and 1 subject was a former smoker.

One neutral polymorphism in intron 2 of the *SPINK1* gene (c.88−352A > G) was observed in all members of the HP-affected family (13 homozygous and 3 heterozygous), as well as in the majority of the controls (7 homozygous and 11 heterozygotes); 6 controls displayed no alterations. No alterations were found in exon 3 of the *PRSS1* gene in the individuals studied.

DISCUSSION

This report describes the first HP-affected Brazilian family with a mutation in the *PRSS1* gene. Asparagine substitution at position 29 of *PRSS1* is frequently observed in HP, although the asparagine is substituted with isoleucine in most cases (the N29I mutation), which is the second most frequent mutation associated with HP.[6] The N29T missense mutation is rare, with only 5 cases reported.[5,22–25] Nevertheless, this mutation plays a significant role in the pathogenesis of HP, as functional studies show that the N29T mutation results in a gain-of-function that leads to an increased rate of autoactivation and increased trypsin stability, while the N29I mutation increases only the propensity for autoactivation (Figure 3).[26]

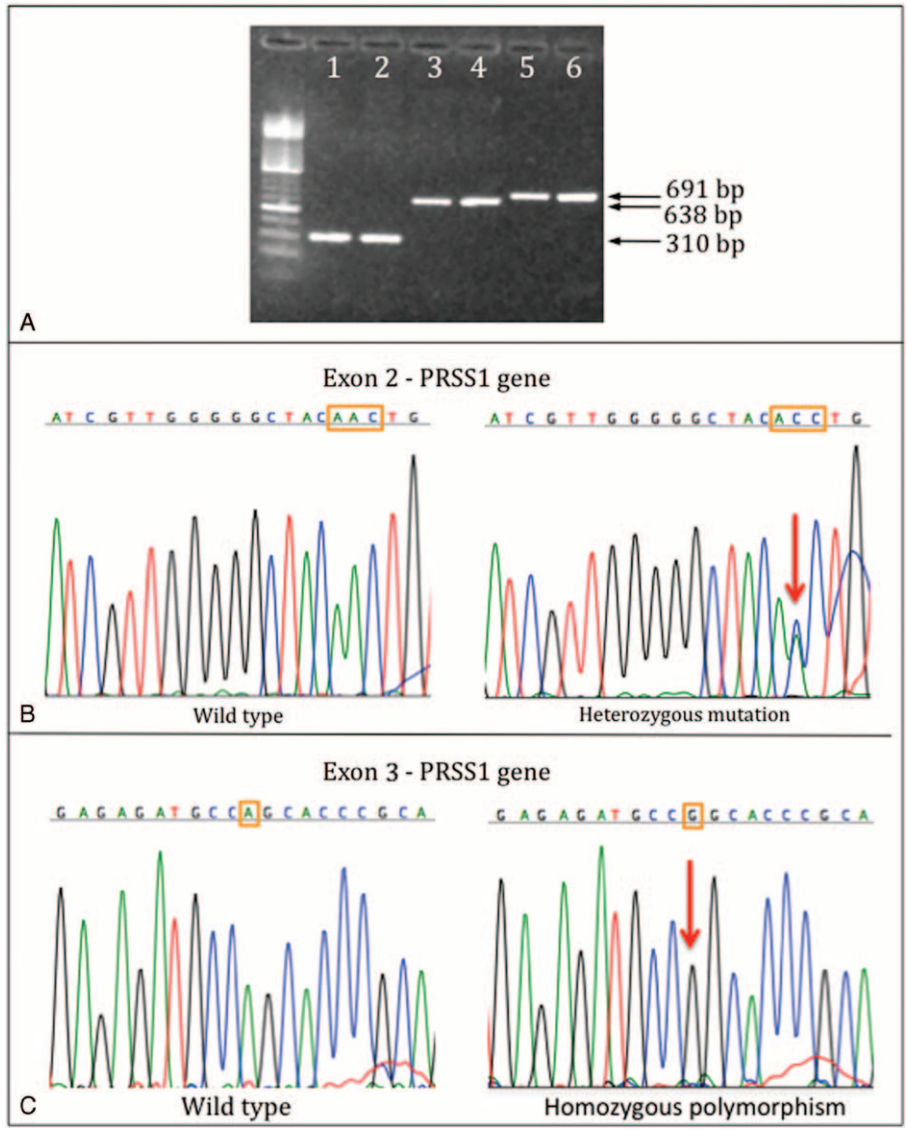

FIGURE 2. A, Agarose gel electrophoresis of DNA against a 100 bp DNA ladder (Promega) following PCR amplification. Lanes 1 and 2, exon 2 of *PRSS1* with a length of 310 bp; Lanes 3 and 4, exon 3 of *PRSS1* with a length of 638 bp; Lanes 5 and 6, exon 3 of *SPINK1* with a length of 691 bp. DNA sequences in affected and unaffected individuals. B, DNA sequence electropherogram of exon 2 of *PRSS1* showing the wild-type sequence and the heterozygous A > C substitution (arrow) at nucleotide position c.86 that replaces asparagine with threonine at codon 29 (box) in the affected individual. C, DNA sequence electropherogram of intron 2 of *SPINK1* showing the wild-type sequence at position c.88-352 (box) and the homozygous A > G polymorphism (arrow).

TABLE 1. Clinical Characteristics of the Affected Patients With the N29T Mutation

Patient	Age of Onset (Yr)	Duration of the Disease (Yr)	Symptom at Onset	Imaging Findings	Surgery (Type)	Diabetes/Steatorrhea	Pancreatic Cancer
1	8	53	Pain	Calcifications Main pancreatic duct changes	No	Yes/Yes	No
2	5	36	Pain	Calcification	No	Yes/Yes	No
3	14	25	Pain	Calcifications Main pancreatic duct changes	No	Yes/Yes	No
4	8	29	Pain	Calcification	No	No/No	No
5	2	16	Acute pancreatitis	Small focus of calcification	Yes (distal pancreatectomy + splenectomy	Yes/Yes	No
6	4	14	Acute pancreatitis	Small focus of calcification	Yes (pseudocyst drainage)	Yes/Yes	No
7	7	9	Acute pancreatitis	Calcifications Main pancreatic duct changes	Yes (Pancreaticojejunostomy)	No/Yes	No
8	2	1	Acute pancreatitis	None	No	No/No	No

The pedigree reported here showed an autosomal-dominant inheritance pattern of HP, in which 8 out of 10 individuals carrying the N29T mutation developed pancreatitis. Thus, the penetrance of the N29T mutation in this family was 80%, which is the same penetrance rate as the most frequently described R122H and N29I mutations.[1,2,27]

It remains unclear why some family members with HP-related mutations do not develop pancreatitis. In particular, 2 members of the present family represent the first reports of clinically unaffected carriers of the N29T mutation in the *PRSS1* gene.

The incomplete penetrance between the members is determinated by genetic, epigenetic, and/or environmental factors, but the mechanism is unknown. One plausible explanation for the incomplete penetrance of the *PRSS1* gene mutation is the presence of mutations that protect against the development of proteolytic pancreatic injury. One such mutation has been described in codon 191 (G191R) of the anionic trypsinogen (*PRRS2*) gene, which was overrepresented in control subjects compared with HP patients.[28]

In accordance with published cohorts, there does not seem to be a gender predilection in the development of HP secondary to the N29T mutation in this family.[5–7] However, the age of symptomatic onset of pancreatitis was lower than those previously described, with a median age of 6 years and 2 subjects showing disease onset at 2 years. In the EUROPAC trial, in which data were collected from 418 HP patients from 14 European countries, the median age of onset was 12 years.[5]

In this study, the most frequent clinical symptom was pancreatic pain, which was experienced by the older members of the family, likely due to a lack of access to appropriate management. Indeed, at the time of diagnosis, these members already showed signs of chronic pancreatitis. In the younger family members, the disease manifested with acute pancreatitis without morphologic or functional alterations suggestive of chronic pancreatitis.

Exocrine and endocrine pancreatic insufficiency occurred in 6 out of 8 (75%) and 5 out of 8 (62.5%) of the affected members of the studied family, respectively. All patients with diabetes mellitus required insulin therapy, following a lack of response to oral hypoglycaemic drugs administered over a short treatment period, and these patients also had high rates of hypoglycaemia due to a deficiency in glucagon secretion compatible with type 3c diabetes mellitus.[29] In contrast, in published cohorts, the rates of malabsorption and diabetes mellitus were 38% and 32%, respectively.[5,6]

Surgical procedures were performed on 3 out of 8 (37.5%) affected family members, of whom 2 underwent pancreatic resection for refractory pain and abdominal complications and 1 received a pancreatic drainage procedure. These findings are similar to those from a French cohort of 200 HP patients in which 40% underwent an interventional procedure.[6] No cases of pancreatic cancer were reported in our study, likely because the majority of patients studied had disease duration of less than 30 years (with only 1 member with a disease duration of >50 years), as disease duration is a risk factor for pancreatic cancer in HP.[5,30]

Finally, the presence of the c.88-352 A> G polymorphism in intron 2 of the *SPINK1* gene was prevalent in this study, affecting 85% of the subjects (all patients and 75% of the controls), which suggests its role as a nonpathogenic component.[31]

In summary, this study contributes to the characterization of the genetic features of HP with the first description of a

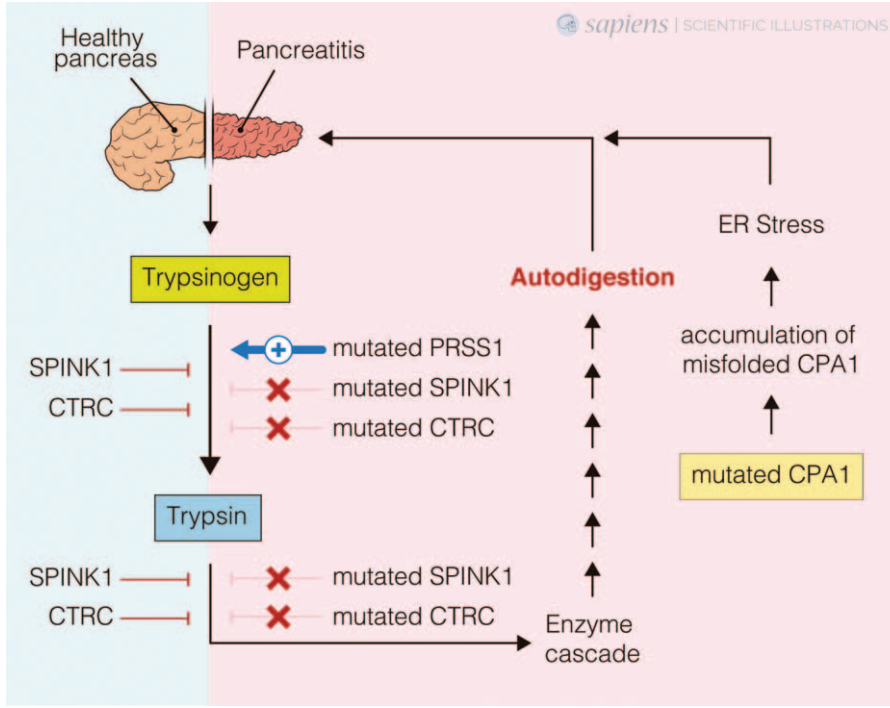

FIGURE 3. Schematic mechanism underlying mutations associated with pancreatitis. The *PRSS1* mutation (blue arrow) leads to a gain-of-function with an increased conversion of trypsinogen to trypsin and increased trypsin stability. The *SPINK1* and *CTRC* mutations (red line) lead to an imbalance of proteases and antiproteases with the respective losses of first and second-line defences against the activation of trypsinogen and the enzymatic cascade that leads to autodigestion and pancreatitis. Mutations in *CPA1* generate misfolded proteins in the endoplasmic reticulum (ER), leading to ER stress and the development of pancreatitis.

PRSS1 gene mutation in a Brazilian family. Furthermore, our study defines the clinical characteristics of affected patients, thereby supporting the clinical management of patients with precise diagnoses and subsequent genetic counselling. National surveys will be required to establish the prevalence of HP in Brazil and Latin America.

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