

Genotype–phenotype correlation in 9 patients with tropical pancreatitis and identified gene mutations

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Abstract

The etiopathogenesis of tropical chronic pancreatitis (TCP) remains unclear. Malnutrition, dietary toxins like cyanogens in cassava and micronutrient deficiency are proposed factors. The description and characterization of genetic factors in TCP has added a new dimension to the understanding of pathogenesis of the disease. However, there is sparse data on the association of TCP with cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations. We report 8 patients of TCP with CFTR gene mutations, including one with a novel mutation, and describe the clinical profile of these patients. Further prospective genetic studies on the association of CFTR gene mutations are essential in order to unravel the genetic basis of TCP.

Keywords Chronic pancreatitis · Fibrocalculous pancreatic diabetes · SPINK1 gene · Tropical calcific pancreatitis

Introduction

Etiopathogenesis of tropical calcific pancreatitis is poorly understood. Environmental factors, malnutrition, dietary toxins like cyanogenic glycosides and micronutrient deficiency have been proposed as pathogenic factors. In the last few years, data from genetic studies have emerged. A strong association of N34S mutation in the serine protease inhibitor, Kazal type1 (SPINK1) gene¹ with TCP has been

observed. However, phenotypic features in patients with and without SPINK1 mutations, and N34S homozygotes and heterozygotes were comparable. Cationic trypsinogen (PRSS1) gene mutations were not detected in any patient in the same study. It is now considered that SPINK1 gene mutations play only a modifier role. In another study, cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations, including the 5T variant, were detected in 2 of 18 North Indian patients, both were women.² Chymotrypsinogen C (CTRC) variants were found in 10 of 71 (14.1%) Indian subjects with tropical pancreatitis but only 1 of 84 (1.2%) healthy controls (OR=13.6; CI=1.7–109.2; P=0.0028).³ An association with cathepsin B (CTSB) gene polymorphisms has also been described.⁴ Lastly, an association of TCP with calcium-sensing receptor (CASR) gene mutations, including 4 novel mutations, has been reported from our institution.⁵ The genetic aspects of tropical pancreatitis have been extensively reviewed recently.⁶

We report gene mutations in 9 patients (5 women) with TCP in a cohort of 30 patients with chronic pancreatitis.

Methods

Patients were recruited through the Pancreas clinic. Informed consent was obtained from the patients, and the study was approved by the Institutional Ethics Committee. Blood samples from 30 patients (21 men), aged between 8 and 42 years, were tested for mutations. PCR-based mutation screening was used for CFTR and sequence-based analysis⁵ was used for both SPINK1 and CASR mutations.

TCP was diagnosed using previously reported criteria (non-alcoholic CP patients with onset before 30 years age, body mass index (BMI) <18 kg/m² and no other specific etiology).⁷ Alcoholic chronic pancreatitis (ACP) was diagnosed in cases of CP who had consumed ≥80 g/day alcohol for at least 5 years.

Results

Thirty patients with CP (TCP 22, ACP 7, hyperparathyroidism-related CP 1) underwent testing for mutations in above-mentioned 3 genes.

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Nine (41%) of 22 TCP patients had one or more genetic mutations. The remaining (13 TCP, 1 hyperparathyroidism and 7 ACP) patients had no mutations.

Of 9 patients with TCP 3 patients had combination of SPINK1 and CFTR gene mutations, 3 had combination of CASR and CFTR gene mutations, 1 had a combination of SPINK1 and CASR gene mutations, 1 had a solitary CFTR gene mutation, and 1 had mutations in all 3 genes. There were 4 novel CASR gene mutations, one each in 4 patients: exon 3 Pro163 Arg (Gen Bank DQ912403), exon 4 Asp 433 (Gen Bank DQ 912402), exon 4 Ile 427 Ser (Gen Bank DQ 912404), and exon 5 Val 477 Ala (Gen Bank DQ 912405). One patient had a novel CFTR gene mutation – del 1646A (Gen Bank DQ 504435) in addition to previously described exon 10 Gly 470 Asp CFTR gene mutation (Table 1).

Abdominal pain

Eight of 9 patients had abdominal pain as predominant symptom. Three of them had combination of CASR and CFTR gene mutations while another 2 had combination of SPINK1 and CFTR gene mutations. One patient had a combination of SPINK1 and CASR gene mutations while yet another had a solitary CFTR gene mutation. One patient had mutations in all 3 genes.

Four of the 9 patients had history of abdominal pain in childhood; among these patients, 2 had both SPINK1 and CASR gene mutations, 1 had CFTR and CASR gene mutations, while 1 had mutations in all 3 genes.

Diabetes

One patient who had no history of abdominal pain, a 26-year-old man (case 9) with diabetes, had exon 3 N34S SPINK1 gene mutation and also exon 10 Gly 470Asp CFTR gene mutation. However, two other patients (both women) with similar mutations had abdominal pain, and were also diabetic. All 6 patients with diabetes had exon 10 Gly 470Asp CFTR gene mutations. Of these, 3 also had exon 3 N34S SPINK1 gene mutations; while remaining 3 also had various CASR gene mutations. Five of the 6 patients having a history of diabetes in first degree relative(s), had exon 10 Gly 470Asp CFTR gene mutations.

Steatorrhea

All 3 patients (all women) with steatorrhea had exon 10 Gly 470Asp CFTR gene mutations. Of these, 1 had no other mutation while 1 had exon 4 Ile 427 Ser CASR gene mutation, and the third patient had exon 4 Val 477 Ala CASR gene mutation and also additional 1646 del A CFTR mutations.

Complications

Only one patient (23-year-old man) had complication of chronic pancreatitis (pseudocyst) and underwent surgery subsequently for pain. He had combination of SPINK1 and CASR mutations.

Table 1 Genetic and clinical profile of TCP patients with mutations

Sl. no.	Age (years)	Sex	CASR	SPINK	CFTR	Pain	Steatorrhea	DM	Family history of DM	Pain in childhood
1	34	F			Exon10-Gly 470 Asp	+	+		+	
2	39	F		Exon3-N34S	Exon10-Gly 470 Asp	+		+	+	+
3	23	M	Exon5- Val 477 Ala	Exon3-N34S		+			+	+
4	24	M	Exon3-Pro 163 Arg		Exon10-Gly 470 Asp	+		+	+	
5	43	F		Exon3-N34S	Exon10-Gly 470 Asp	+		+	+	
6	13	M	Exon4-Asp 433 His	Exon3-N34S	Exon10-Gly 470 Asp	+				+
7	35	F	Exon4- Ile 427 Ser		Exon10-Gly 470 Asp	+	+	+	+	+
8	27	F	Exon5-Val 477 Ala		Exon10-Gly470Asp, 1646delA	+	+	+		
9	26	M		Exon3-N34S	Exon10-Gly 470 Asp			+		

Cancer

None of these patients had cancer. However, 1 patient, a 14-year-old boy reported that his father had died of pancreatic cancer complicating TCP. This patient had exon 10 Gly 470Asp CFTR gene mutations along with exon 3 N34S SPINK1 gene mutations and exon 4 Asp 433 His CASR gene mutations. None of his relatives had pancreatitis, pancreatic cancer or diabetes. The patient is on close follow up.

None of the 8 patients with CFTR gene mutations had any evidence of sinopulmonary disease. Of 13 patients with TCP who had no mutations, 4 had one or more first degree relatives with chronic pancreatitis. While 12/13 patients had abdominal pain as predominant manifestation, steatorrhea was reported by 4 patients and 3 patients were diabetic. Five patients had one or more first-degree relative(s) with diabetes. The mean age (mean±SD) of these 13 TCP patients was 29.7±15.4 years as compared with 30.5±11.7 years for the 9 patients with mutations (p=ns).

All 7 patients with ACP had abdominal pain as predominant manifestation. Steatorrhea was seen in 3 patients; 5 patients were diabetic while 2 patients had one or more first degree relative(s) with diabetes.

The sole patient with hyperparathyroidism, a 33-year-old woman, had abdominal pain as predominant symptom and also had steatorrhea and diabetes. Her father had chronic pancreatitis with diabetes. She underwent parathyroidectomy, and had symptomatic improvement after surgery.

Discussion

Tropical pancreatitis has traditionally been regarded as an exotic entity. However, this disease has some clinical characteristics as well as genetic attributes like that of idiopathic chronic pancreatitis in the West; this unique aspect of tropical pancreatitis *vis a vis* other forms of CP has been reported recently.⁸ Wide phenotypic variability has been observed in TCP. While majority present with pain, some patients present almost exclusively with diabetes and related complications (FCPD). Furthermore, whether FCPD is distinct from type 1 and type 2 diabetes mellitus has been a controversial issue. Presently, it is widely accepted that FCPD merely represents a form of secondary diabetes. However, Chandak *et al.* have recently observed that type 2 diabetes-associated TCF7L2 variants were observed in FCPD.⁹ A possible interaction between TCF7L2 variants and SPINK1 and CTSB gene mutations was hypothesized to determine onset of diabetes.

CFTR gene mutations were seen in 8/30 (27%) patients. The age of the TCP patients with and without gene mutations was comparable. Diabetes was seen in two-thirds and steatorrhea in one-third of the patients. A female gender-specific effect should be actively considered. In the recently published national collaborative study on chronic pancreatitis, it was observed that female gender appeared to be a risk factor for diabetes.⁷

Sharer *et al.*¹⁰ and Cohn *et al.*¹¹ reported association of mutations in CFTR gene in patients with idiopathic and alcoholic chronic pancreatitis. It has been observed that CFTR mutations, affecting bicarbonate (rather than chloride), secretion are associated with pancreatitis. On the other hand, Quinton¹² has suggested that defective bicarbonate secretion may play a pathogenetic role as important as that of defective chloride secretion. He has hypothesized that bicarbonate is crucial to normal mucin expansion because it forms complexes with cations like Ca²⁺ and H⁺ and removes them. In presence of defective bicarbonate (HCO³⁻) secretion, mucin would tend to remain aggregated, poorly solubilized, and less transportable.

Ahmed *et al.* have demonstrated that the CFTR genotype correlates with pancreatic phenotype in cystic fibrosis and also with quantitative measures of pancreatic acinar and ductal function.¹³ In cystic fibrosis the course of the disease has been seen to vary between patients with identical mutations in the same family. Unlike pancreatic function the course of pulmonary disease largely depends on secondary factors.¹⁴ A similar proposition is likely in TCP wherein diabetes may be a secondary consequence of pancreatitis. However, the influence of environmental factors, or other genetic co-factors, in the development of diabetes, cannot be excluded.

Casals *et al.* have studied the differences in mutational spectrum of CFTR gene in alcoholic and idiopathic chronic pancreatitis.¹⁵ A study from China seems to suggest differences in CFTR gene mutations between ethnic groups.¹⁶

SPINK1 and CASR gene mutations probably exert additional and probably different effects on phenotype. SPINK1 gene mutations induce earlier onset while CASR mutations may contribute to effects produced by CFTR gene mutations. The genetic burden (presence of multiple mutations) may indicate increased risk of complications and cancer risk.

There is paucity of data on the association or prevalence of CFTR mutations with TCP² or even cystic fibrosis¹⁷ in India. This report, though small, lends support to the only other brief report so far, on association of CFTR gene mutations with TCP in India. We also report a novel mutation in the CFTR gene. It is a preliminary exploration into possible relationships between genotype and phenotype in TCP. In addition, the report of a young patient, whose father died of carcinoma pancreas complicating TCP; and has mutations in three different pancreatitis-associated genes, indicates a possible genetic link between tropical pancreatitis and pancreatic cancer. This study does not address the prevalence of CFTR mutations in TCP, which is an area for future prospective studies.

In conclusion, our preliminary observations despite the obvious limitations like small sample size, highlight need for greater attention to CFTR gene. Identification of further candidate genes in TCP preferably by genome wide association studies and characterization of the relative effects of various mutations, may help clarify the reasons behind

phenotypic variability; and ultimately help in better understanding of the etiopathogenesis.

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