

ORIGINAL ARTICLE

## A Study of SPINK 1 Mutation and Other Clinical Correlates in Idiopathic Chronic Pancreatitis

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### ABSTRACT

**Aim** Chronic pancreatitis is labelled as idiopathic when no identifiable factors are found. The identifications of genetic mutations associated with pancreatitis have provided opportunities for identifying patients at risk for idiopathic pancreatitis. The aim of this study was to study the demographic, clinical profile and assess the prevalence of genetic mutation (SPINK 1) in idiopathic chronic pancreatitis. **Material and methods** The study included prospective analysis of patients with idiopathic chronic pancreatitis at a tertiary care hospital in INDIA. Blood was collected for deoxyribonucleic acid isolation and genotyping of mutations in SPINK1 was performed using the high-resolution melting method. Mutations were genotyped using allele-specific amplification polymerase chain reaction. **Results** 33 patients were included with mean age of 31.75±13.07 years. 22 (67%) of patients were males, 11 (33%) were females. Mean age of patients with SPINK positive was 31.96±9.25 yrs. The mean duration of illness was 31.33±19.89 months. Of the 33 patients 12 (36%) were positive for SPINK-1 mutation. Most of the patients' positive for the SPINK-1 mutation had a younger age of onset. SPINK1 mutation patients in idiopathic chronic pancreatitis showed 100% parenchymal calcification by computed tomography. **Conclusions** The prevalence of SPINK1 mutation in idiopathic chronic pancreatitis was found to be 36.36%. SPINK1 mutation patients have more frequent episodes of pancreatitis and parenchymal calcification on computed tomography. The clinical profile of idiopathic chronic pancreatitis is different from what has been reported in the past.

### INTRODUCTION

Chronic pancreatitis (CP) is an inflammatory condition of the pancreas seen as irreversible morphological changes that results in exocrine or endocrine deficiency [1, 2]. In clinical practice evaluation fails to detect the cause of pancreatitis in nearly 10–30% of the patients, and these patients are labeled as idiopathic CP [3]. Many studies have been conducted on acute and CP, but only few have focused on idiopathic CP. Idiopathic CP was earlier thought to be seen only in certain parts of India [4]. During the last few years, many cases of idiopathic CP have been reported from western world and almost all states in India [5].

The SPINK1 is also known as pancreatic secretory Trypsin inhibitors located on chromosome 5. It is a 56 amino acid peptide that inhibits Trypsin by physically blocking the active site [6, 7]. Provides the first line defense against premature Trypsinogen activation within pancreas, because it is capable of inhibiting about 20% of Trypsin activity by competitively blocking the active site of Trypsin. In 2000, the role of SPINK 1 mutation in chronic pancreatitis emerged. The most frequent mutation in (SPINK1) gene exon 3 results in asparagine to serine amino acid change (N34 S) which leads to decreased trypsin inhibitory capacity. SPINK 1 (N34S), mutation are relatively common seen in 2% of general population.

Mutations in cationic trypsinogen gene (PRSS1), SPINK1 gene, cystic fibrosis transmembrane conductance regular gene (CFTR) and Cathepsin B gene have been studied in acute recurrent and CP [8]. Genetic mutations may be the cause of pancreatitis in patients whom etiology is not found [9]. Idiopathic pancreatitis represents a complex disease process resulting from an interaction of genetic mutations and environmental factors. Recent research has shown complex interactions like gene – gene, gene – environment in the pathogenesis of pancreatitis [10]. Mutations in SPINK1 gene, is associated with ICP but its prevalence may

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**Abbreviations** DNA Deoxyribonucleic acid; ICP Idiopathic chronic pancreatitis; PCR Polymerase chain reaction

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be under determined. Hence a prospective study on ICP and prevalence of SPINK 1 mutation was studied.

## MATERIALS AND METHODS

The study was a prospective analysis of patients with idiopathic CP during the period October 2010 to December 2011. It was conducted at the Department of Gastroenterology PSG Institute of Medical Sciences and Research (PSG IMSR) in collaboration with the Centre for Molecular Medicine and Treatment. The study protocol was approved by the Institutional Human Ethics Committee prior to the start of the study.

Patients underwent routine hematological and biochemical tests in addition to radiological imagings like Ultrasound and Computed tomography. Complications of CP like diabetes mellitus, steatorrhea, bile duct obstruction, and pseudocyst were diagnosed as per standard criteria either biochemically or imaging.

### Inclusion Criteria for Idiopathic Chronic pancreatitis

1. Documented episodes of typical pancreatic type of abdominal pain
2. Amylase or lipase greater than 3 times the upper limit of normal
3. Features of pancreatitis on imaging studies (ultrasound / CT abdomen)
4. No identifiable cause or risk factors

### Exclusion Criteria

Patients with identifiable cause and risk factors for acute and recurrent pancreatitis, malignancy, retroviral infection, Psychiatric illness, hereditary and known genetic diseases.

**Genetic analysis:** It involved DNA extraction, PCR to amplify SPINK gene, RFLP using PST I restriction enzyme and Poly Acrylamide Gel Electrophoresis (PAGE) of the digested product.

### DNA Extraction Procedure

Blood sample 300  $\mu$ L + 1 volume of cell lysis buffer + 3 volume of sterile MilliQ water mixed up in 2 mL Eppendorf tubes which were then incubated on the ice 4°C for 10 minutes. The samples centrifuged at 4°C for 20 minutes at 4000 rpm. The supernatant discarded and again 150 $\mu$ l of same cell lysis buffer added in every sample and 480  $\mu$ L of autoclaved H<sub>2</sub>O also added. The sample then centrifuged at 4°C for 20 minutes at 4000 rpm. Then again the supernatant was discarded. Then 720  $\mu$ L of nucleic acid lysis buffer, along with add 15  $\mu$ L of RNase added to the eppendorf tube. The samples were vortex mixed and incubated for 10 -15 minutes at 37°C. 30  $\mu$ L of 10% SDS and 30  $\mu$ L of Proteinase K then added. The sample was incubated in water bath or heat block at 55°C for 2-3 hours. Equal volume of phenol: chloroform (1:1) added and centrifuged at 15800rpm for 5 minutes. The supernatant of the samples (aqueous layer) transferred to the new tube.

## PCR amplification of SPINK gene

### 1. Primers

**Forward primer** SPINKF: TTCTGTTTAATTCCATTTT-TAGGCCAAATGCTGCA

**Reverse primer** SPINKR: GGCTTTTATCATACAAGT-GACTTCT

### 2. DNTPs (Himedia)

### 3. Taq DNA polymerase (colorless Taq Genei)

### 4. Taq Buffer A (Genei)

### 5. DNA Sample Optimised concentration: 0.05 $\mu$ g

### 6. Milliq water

### Identification of Genotypes

The genotypes were determined based on the expected product size.

A/A Genotype (Asn 34 Asn) - 320bp

G/G Genotype (Ser 34 Ser) - 286bp

A/G Genotype (Asn 34 Ser) - 320,286 and 34

Peripheral blood samples were drawn from patients and collected in EDTA tubes. DNA was isolated using Micro AX Blood Gravity Kit (A&A Biotechnology, Gdańsk, Poland). Quality and concentration of isolated DNA was measured by Nano Drop 2000 (Thermo Scientific, TK Biotech, Poland). Genotyping was performed using the following molecular biology techniques: allele-specific PCR (ASA-PCR) and high-resolution melting (HRM)-PCR.

**Statistical analysis:** Descriptive statistics were used to summarize the variables. All data were analyzed using the statistical package SPSS (version 10.0).

## RESULTS

33 patients were included with mean age of 31.75 $\pm$ 13.07 years with youngest being 9 yrs and oldest being 69 yrs. 22 (67%) of patients were males, 11 (33%) were females. Mean age of patients with Spink positive was 31.96 $\pm$ 9.25 yrs. The mean duration of illness was 31.33 $\pm$ 19.89 months. Mean fasting sugar level was 112.57 $\pm$ 12.53 mg/dL. Of the 33 patients 12 (36%) were positive for SPINK-1 mutation. Mutant genotype was seen in the age group of 11-20 years. 6 patients had heterogeneous genotype in the age group of 21-30, 2 each in the age group of 31-40 and 41-50 (**Figure 1**). Most of the patients' positive for the SPINK-1 mutation had a younger age of onset. Nearly 93.34% of patients had pain as their clinical symptoms. The clinical manifestations are given in **Table 1**. Nearly 26/33 (78.78%) of patients had no evidence of malnutrition as evidenced by BMI>18.5. 25/33 (73.75%) patients had ductal dilation and around 29/33 (87.87%) had parenchymal calcification (**Table 2**). All the patients with SPINK positive had 100% parenchymal calcification. Spink mutation is given in **Figure 2**.

Most of the patients in both the group were below 30 yrs. Male preponderance was seen. 36.36% of patients

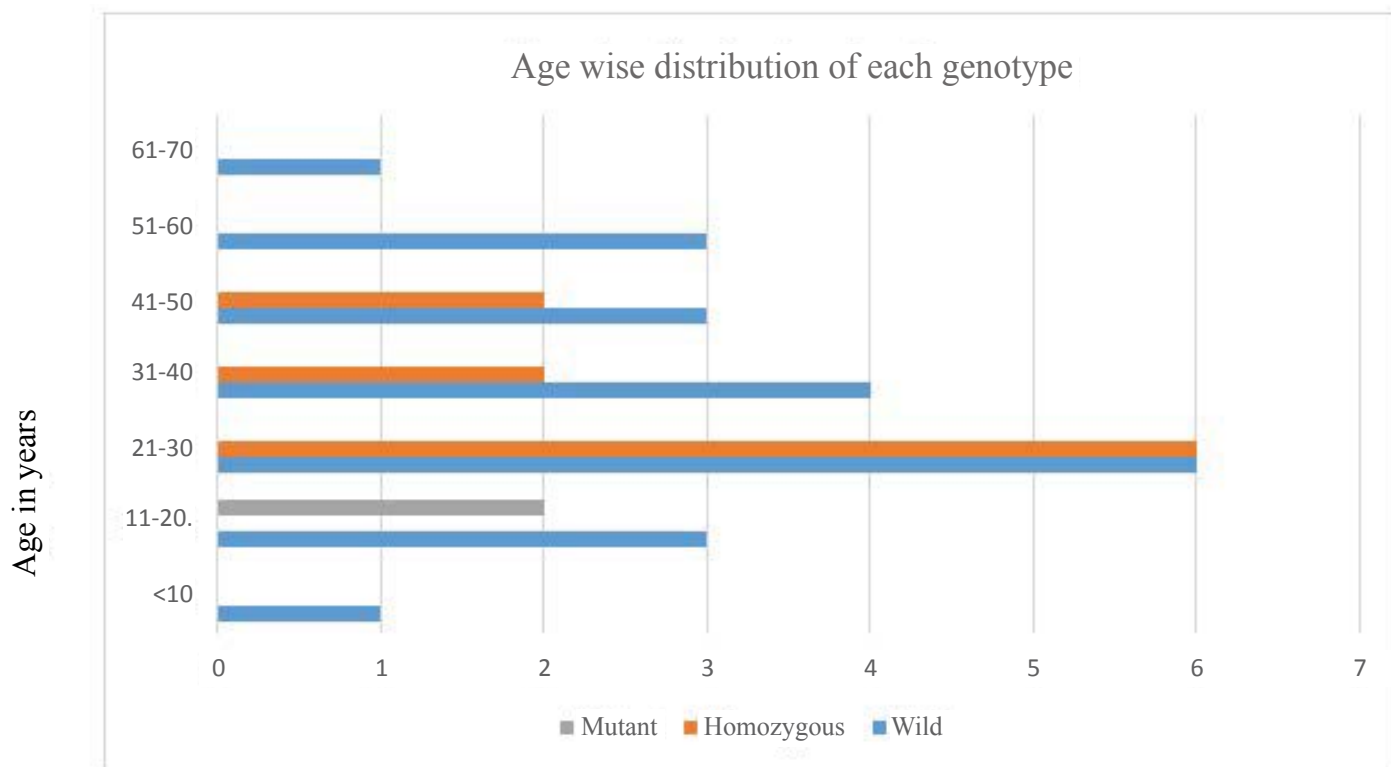


Figure 1. Age wise distribution of ICP patients with genotype.

Table 1. Clinical features of ICP patients.

Clinical Features	No. of Patients N = 33
Pain	31 (93.94%)
Weight Loss	8 (24.24%)
Diabetes	11 (33.33%)
Steatorrhea	5 (15.15%)
Bile Duct Stricture	2 (6.06%)
Pseudocyst	2 (6.06%)
Jaundice	2 (6.06%)

Table 2. CT findings.

CT Findings	No. of Patients (N = 33)	Spink positive (N=12)
Ductal Dilation	25	10
Parenchymal Calcification	29	12
Ductal Calculi	7	2
Atrophy Pancreas	21	9

with idiopathic CP were positive for SPINK1 mutation. Pain was predominant symptom and diabetes was seen only in 11 (33.33 %).

## DISCUSSION

The mean age of ICP was 31.75±13.07 years which resembles the age group given in other studies. Balakrishnan *et al.* showed mean age of patients was 30 years [11]. Data from Layer *et al.* from United States had mean age of 19 yrs. [12]. Kandula *et al.* showed idiopathic CP occurred among children and adolescents [13]. The mean age of patients from northern India in a survey was 36.7 years and study from New Delhi showed majority of patients were younger [14, 15].

Majority of the patients in our study were male (67%). Data from prospective nationwide study from India

showed male preponderance [16]. Study by Balakrishnan *et al.* showed male to female ratio of 2.7:1 [11]. A study from Delhi and Lucknow showed majority of their patients with tropical pancreatitis were males [17, 18]. In our study mean duration of symptoms at the time of presentation was 31 months. The study from Delhi reported mean duration of 48 months while Shallu Midha *et al.* showed in their study mean duration was 27 months [17, 18].

Pain was the common presentation in our study which was similar to other studies by Layer *et al.* [12], Balakrishnan *et al.* [11], Midha *et al.* [18] reported 97% presented with pain which was similar to our data. Diabetes was reported in 33% of patients in our study which is different from study conducted by Geeverghese [19] and Tandon *et al.* who in their study showed upto 90% of patients having diabetes [12]. The study by Balakrishnan *et al.* also showed higher incidents of diabetes upto 70% [16]. The study from Lucknow reported diabetes in 26% of patients with idiopathic CP. Midha *et al.* also reported 27% patients having diabetes in chronic idiopathic pancreatitis [18].

In our study symptomatic steatorrhea was seen around 15% while Midha *et al.* reported frequency of 5% steatorrhea in their study [18] and data from New Delhi also showed around 5% of patients with steatorrhea [17]. The present study showed 26 (78%) of patients with BMI>18.5 and 9 (22%) with BMI<18.5. This is in contrast to older studies from Kerala which showed high incidence malnutrition. Midha *et al.* and Narendranathan showed lack of association of malnutrition and Cassava consumption in their study [19, 20]. In 1988 study by Balakrishnan implicates malnutrition in pathogenesis of

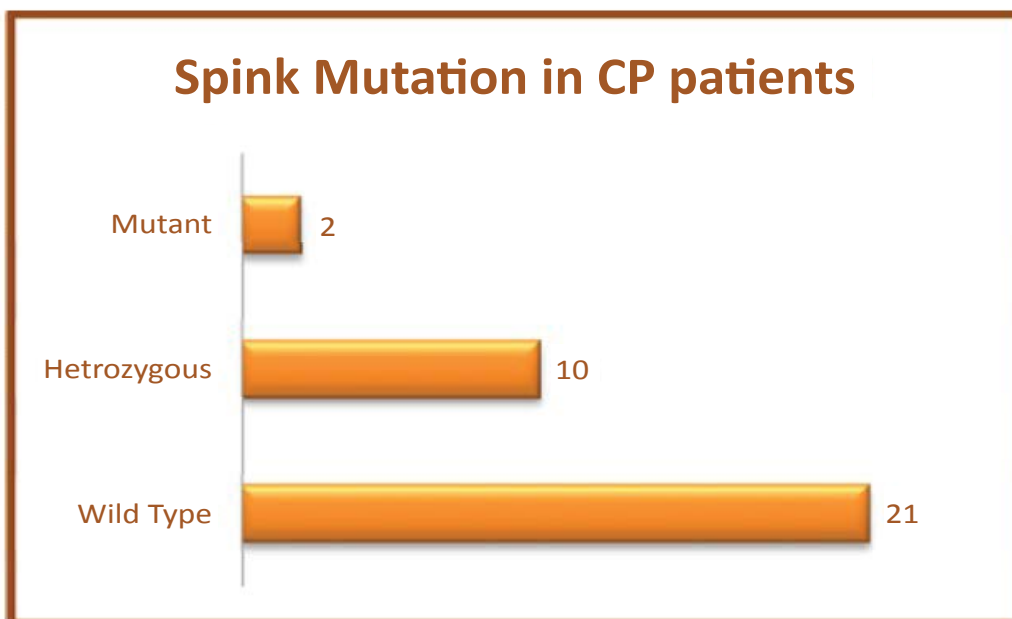


Figure 2. Spink mutation in ICP patients.

tropical calcific pancreatitis [16]. The study from lucknow and delhi showed mean BMI of 19 kg/m<sup>2</sup> and 20.2 kg/m<sup>2</sup> [17].

Ultrasound and CT findings included dilated pancreatic duct, calculi, atrophy. CT was more sensitive in identifying ductal dilatation and calcification. Study from all India institute of medical science showed usefulness ultrasonographic evaluation of calcific pancreatitis [15]. Sensitivity of identifying ductal dilatation and calcification is less than CT abdomen or MRI. In our study 87.87% had parenchymal calcification 75% had ductal dilation and only 21% had ductal calculi. The study from Lucknow reported 57% of their patients with tropical pancreatitis had calcification. Khuroo *et al.* reported 96% of patients with tropical calcific pancreatitis had pancreatic ductal calculi [21]. All the 12 patients with SPINK mutation had 12(100%) parenchymal calcification and 9 (75%) atrophy of pancreas on CT.

The etiology of idiopathic pancreatitis is not well known. Recent studies have implicated SPINK 1 and CFTR gene in idiopathic CP. Study by Pfutzer *et al.* [22], Sundaresan *et al.* [23] have shown strong association of tropical calcific pancreatitis with SPINK 1 Our study showed 36.36% of patients with SPINK mutation .The study of Bhatia *et al.* showed SPINK 1 mutation was found in 40% of patients with tropical idiopathic CP in India. In a study from Bangladesh Schneider *et al.* showed there was difference in SPINK1 mutation between patients with tropical calcific pancreatitis with and without diabetes. An Italian study by Macarena Gomez - showed association of SPINK 1 and CFTR gene mutation in idiopathic pancreatitis [24]. In addition to SPINK1, CFTR gene mutations have been found in patients with CP than controls. The data from AIIMS showed 42% of patients had SPINK mutation and 9% CFTR mutation in patients with idiopathic CP. Studies from south India showed SPINK gene mutations were common in patients with idiopathic chronic patients.

Thus genetic mutations seen to play as important role in the pathogenesis of idiopathic CP. Recent study have shown role of chymotrypsin C gene mutation in idiopathic CP which lands support to the genetic theory of etiopathogenesis of idiopathic CP. The present study shows phenotypic and genetic similarities between idiopathic CP in India and in other countries [25, 26].

Our study reveals that spink mutation is strongly associated with more number of acute episodes in RAP and parenchymal calcification in CP. It may be useful to do functional studies of SPINK 1 mutation in cell cultures to understand the pathophysiology of the disease status more completely. Clinical profile of idiopathic chronic pancreatitis is different from what has been reported previously. Genetic testing and screening may be proposed to have role in diagnosis, prediction of clinical features and severity in future.

### Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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