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TOPIC HIGHLIGHT

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Genetic and phenotypic heterogeneity in tropical calcific pancreatitis

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Abstract

Tropical calcific pancreatitis (TCP) is a form of chronic non-alcoholic pancreatitis initially reported in the developing parts of the tropical world. The clinical phenotype of TCP has undergone marked changes since its first description in 1968. The disease is now seen in relatively older people with less severe symptoms. In addition, there are varying reports on the proportion of cases presenting with imaging abnormalities like calcification, ductal dilation, and glandular atrophy. Significant progress has also been made in understanding the etiopathology of TCP. The role of malnutrition and cassava toxicity in its pathogenesis is disproven and few studies have focused on the role of micronutrient deficiency and oxidative stress in the etiopathogenesis of TCP. Emerging evidence support an important role for genetic risk factors in TCP. Several studies have shown that, rather than mutations in trypsinogens, variants in serine protease inhibitor kazal type 1, cathepsin B, chymotrypsin C, cystic fibrosis transmembrane regulator, and carboxypeptidase A1, predict risk of TCP. These studies also provided evidence of mutational heterogeneity between TCP and chronic pancreatitis in Western populations. The current review summarizes recent advances that have implications in the understanding of the pathophysiology and thus, heterogeneity in genotype-phenotype correlations in TCP.

Key words: Chronic pancreatitis; Tropical calcific pancreatitis; Fibrocalculous pancreatic diabetes; Clinical phenotype; Genetic risk factors

Core tip: Tropical calcific pancreatitis (TCP) is a form of chronic pancreatitis of unknown etiology. The phenotype of TCP is quite heterogeneous and has undergone a marked change over the last few decades, such that only a small fraction of such cases represent classical TCP. Several studies have shown the important role of genetic factors in the pathophysiology of TCP and provide evidence of genetic and mutational heterogeneity, compared with that in Western countries. Hence, it is important to understand the genotype-phenotype correlation in TCP. This review summarizes recent developments that provide some understanding of genotypic and phenotypic heterogeneity in this enigmatic disease.

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INTRODUCTION

Pancreas is a heterocrine gland that is comprised of an

exocrine portion constituted by ductal cells and pancreatic acinar cells whose secretion is responsible for digestion of food, whereas the endocrine component is mainly involved in the maintenance of glucose homeostasis[1]. Pancreatic acinar cells synthesize, store and secrete the enzymes required for digestion of nutrients. Trypsin(ogen) is the key enzyme as it converts many proteolytic proenzymes into their active forms in the duodenum. Human pancreatic juice contains three isoforms of trypsinogen. On the basis of relative electrophoretic mobility, they are commonly referred to as cationic trypsinogen (*PRSS1*), anionic trypsinogen (*PRSS2*), and mesotrypsinogen (*PRSS3*). Cationic trypsinogen represents approximately two-thirds of total trypsinogen, while anionic trypsinogen makes up about one-third. Mesotrypsinogen is a minor form, accounting for less than 1% of trypsinogens or 0.5% of pancreatic juice proteins. It is thought that about 5% of trypsinogen is activated within the normal pancreas. However, there are several molecular and cellular mechanisms that have evolved to protect the pancreas from enzymes that are activated in the cell, especially proteases which could otherwise lead to auto-digestion of the pancreas leading to pancreatitis. These include compartmentalization of digestive enzymes in vacuoles like zymogen granules and lysosomes, the presence of pancreatic secretory trypsin inhibitor (PSTI)/serine protease inhibitor kazal type I (*SPINK1*) to antagonize the intrapancreatically activated trypsinogen, and maintenance of suboptimal intracellular pH and Ca^{2+} levels. In 1896, Chiari[1] proposed that intracellular activation of digestive enzymes, especially trypsinogens within the pancreas leads to pancreatitis[2].

Chronic pancreatitis (CP, OMIM 167800) is a progressive inflammatory disease of the pancreas that leads to persistent and irreversible morphological changes such as parenchymal fibrosis and calcification, cysts, necrosis and development of pancreatic stones. Continuous tissue destruction and remodeling often results in loss of exocrine and/or endocrine function. This manifests as two major clinical symptoms: upper abdominal pain and maldigestion. The prevalence of CP varies worldwide from 5-12/100000 individuals per year in United States to as high as 114-200/100000 individuals per year in southern India[3,4]. The TIGAR-O classification system first proposed in 2001 identifies 6 major etiologies for CP[5]. These include: toxic-metabolic factors, such as alcoholism, hypercalcemia, hyperlipidemia; idiopathic causes; genetic predisposition; an autoimmune response; recurrent and severe acute pancreatitis; and obstructive CP associated with pancreas divisum, sphincter of Oddi disorders, and others. Alcohol is the most common risk factor for CP reported in the West and some parts of Asia[4,6,7]. Certain proportions of CP patients do not possess any of the identified risk factors and thus are classified as having idiopathic CP (ICP). In addition, mutations in various candidate genes such as *SPINK1*, cationic and anionic trypsinogens (*PRSS1* and *PRSS2*), cystic fibrosis transmembrane regulator (*CFTR*), chymotrypsin C (*CTRC*) and carboxypeptidase A1 (*CPA1*) have been reported with some variability in different parts of the world[8,9]. Historically, the form of CP that is prevalent in India is known as tropical calcific pancreatitis (TCP, OMIM 608189), described as a disease with "pain in childhood, diabetes in puberty, and death at the prime of life"[10]. Due to wide heterogeneity in symptoms at presentation, a large number of terminologies such as chronic calcific pancreatitis, fibro-calcific pancreatitis, chronic calcified pancreatitis, fibrocalculous pancreatitis and tropical pancreatitis have been used to describe this enigmatic entity. This has made us wonder whether it is the same disease with different names or different diseases with the same name. In this review, we summarize the recent developments and current status of the disease, make an attempt to explain the peculiarities and similarities with CP of other etiologies, and advance hypotheses to explain this dichotomy and its implications.

PHENOTYPE IN TROPICAL CALCIFIC PANCREATITIS

Although Zuidema first described TCP in young diabetics in Indonesia with fibrosis and calcification of the pancreas during the 1950s[11], it was the report by Geevarghese from Kerala in South West India describing young, malnourished patients with a cyanotic hue of the lips, bilaterally enlarged parotid gland, pot belly, and sometimes pedal edema, that caught the attention of the scientific community[10]. The clinical phenotype of TCP seems to have changed considerably since its first description. Recent reports have questioned the existence of classical TCP and instead used the terminology of ICP or simply CP[12]. Although there are clear-cut WHO-defined diagnostic criteria for TCP and fibrocalculous pancreatic diabetes (FCPD), the nomenclature is quite old[13]. Recent studies have defined the disease based on distinctive yet arbitrary features such as; onset at less than 30 years of age, a body mass index (BMI) less than 18 kg/m^2, absence of any other cause of pancreatitis, and presence of diabetes[12,14]. Based on these criteria, only 4%-6% of patients could be classified as having TCP in the study by Balakrishnan *et al*[14]. However, as indicated earlier[15], many of these points are debatable. Firstly, the use of BMI as a diagnostic criterion for classification of CP is contentious as a BMI value < 18.5 kg/m^2 suggests underweight status which may not be the same as malnourished. In addition, malnutrition has been shown to be an effect rather than a cause of CP. Furthermore, the abovementioned studies have sub-grouped ICP into early-onset ($\leqslant$ 30 years) and late-onset (> 30 years) and there are divided opinions over the proposal that those belonging to the early-onset category might resemble TCP[16,17].

In the following sections, we highlight the changes in the clinical profile of TCP observed over the last few decades. Several studies have also compared the features of TCP with alcoholic CP (ACP) that is more common

in Western countries.

Age of onset

The phenotype of TCP was initially described in children and in adolescents[10]. However, subsequent studies have reported a late age of onset of the disease. For instance, Balakrishnan *et al*[18] compared clinical characteristics of 220 TCP patients studied in the 1980s with another recent cohort of 244 patients, and found that both age at onset and age at presentation was nearly a decade later in the recent study than in the previous cohort. Similar observations have been made in other studies irrespective of the geographical location (North/South India)[14,16,17]. In addition, comparative studies of TCP/ICP patients with ACP patients reported a significantly earlier age of onset in the former[14,16,18].

Pain

Irrespective of the disease etiology, abdominal pain is the most predominant presenting complaint in the majority of CP patients. The characteristic pain is moderate-to-severe in intensity, begins in the epigastrium, and often radiates to the back. It may be relieved by sitting forward or leaning forward, by assuming the knee-chest position on one side, or by squatting and clasping the knees to the chest. Pain may increase after a meal and is often nocturnal. Quite frequently, it is associated with nausea and vomiting and occasionally with jaundice.

Macroscopic features of the pancreas

There seems to be heterogeneity in studies reporting the proportion of CP patients developing calcification. While studies from southern India observed calcification in more than 90% of patients[17,19], reports from northern India suggest it to range from 40%-80%[12,16,20]. Imaging studies using endoscopic retrograde cholagiopancreatography (ERCP) and ultrasonography have shown striking differences in the radiological appearance of TCP and ACP. While TCP is characterized by the presence of large, discrete, dense calculi that are usually intraductal, patients with ACP have typically small speckled parenchymal calculi with irregular, indefinite margins. The first ERCP study by Balakrishnan *et al*[21] in TCP reported that calcific TCP displays a greater degree of ductal derangement compared with the non-calcific variety. The study also noted that the changes in TCP were far more pronounced than those described in ACP. Another study observed that the degree of ductal dilation, pancreatic calculi, and pancreatic atrophy is higher in TCP compared with other forms of pancreatitis[22]. Similar observations have been made in a recent prospective nationwide study where calculi (60%-70%), a dilated pancreatic duct (55%-65%), and atrophy of the gland (30%-40%) were major imaging abnormalities[14].

Diabetes

Development of diabetes is known to be one of the common end points of TCP. Earlier, various terminologies such as pancreatic diabetes, pancreatogenous diabetes and tropical pancreatic diabetes were proposed for this form of diabetes. In order to maintain uniformity, the WHO Study Group proposed the use of the term FCPD in reference to diabetes secondary to disease of the exocrine pancreas[23]. One of the characteristic clinical features of FCPD is that, despite the requirement for insulin to control diabetes, the patients rarely become ketotic on withdrawal of insulin. This is attributed mainly to partial preservation of beta cell function as indicated by C-peptide studies[24,25]. Histopathology and immunohistochemistry of the pancreas in FCPD subjects shows atrophy of the pancreatic exocrine tissue and a decrease in the number of islets in some cases and hyperplasia in others[26]. Nesidioblastosis is also seen in some patients. Overall, there is a decrease in insulin positivity in islets that directly correlates with serum C-peptide levels and inversely with the duration of diabetes[26]. The series of patients reported by Geevarghese were actually cases of FCPD[10]. These patients were young (majority < 40 years of age at onset), poor, and extremely emaciated, which emphasized the presence of protein energy malnutrition. However, a recent study reported a decline in the proportion of FCPD cases, from 1.6% of all diabetic patients registered annually during the early 1990s to 0.2% during the period 2006-2010, while the prevalence of diabetes secondary to ACP has remained the same[27]. Additionally, there has been a significant increase in the BMI of FCPD subjects from 19.4 ± 3.6 kg/m^2 during 1991-1995 to 21.2 ± 3.8 kg/m^2 during 2006-2010 ($P < 0.001$)[27]. Also, a progressive increase in the age at diagnosis of FCPD patients has been observed during this period of study, while there was a decrease in age at diagnosis for diabetes secondary to ACP[27]. The prevalence of microvascular complications such as retinopathy, neuropathy, nephropathy, or microalbuminuria observed in FCPD is similar to that in type 2 diabetes[28].

Pancreatic cancer

The lifetime risk of developing pancreatic cancer in CP patients is reported to be around 4%[29]. Several retrospective and prospective studies to date report an increased association between TCP and occurrence of pancreatic cancer[30,31]. In the study by Ramesh *et al*[31], 22 out of 266 TCP patients (8.3%) presenting over an 8-year period had pancreatic adenocarcinoma. Factors associated with high risk for cancer were age > 40 years, short duration of symptoms, weight loss, presence of a mass on ultrasound, and ductal block on ERCP. These patients showed distinct features, such as younger mean age of onset, presence of calculi, and a higher incidence of diabetes compared to those with *de novo* pancreatic cancer. Chari *et al*[30] reported an increased risk of pancreatic cancer in TCP patients compared with the background pancreatic cancer rate (RR = 100.0, 95%CI: 37.0-218.0). A recent study also reported the frequency of malignancy in TCP patients to be around 4%[14]. The risk is thought to be generally higher in TCP compared with ACP.

ETIOPATHOGENESIS OF TCP

Since the first report of TCP[10], several factors have been proposed to be involved in its pathogenesis. The major hypotheses have revolved around malnutrition, cassava/cyanogen toxicity, oxidative stress and trace element deficiency, and familial and genetic factors.

Malnutrition

In earlier reports, TCP was primarily observed in poor, malnourished individuals, which led to the proposition that malnutrition might be an important causal factor for TCP[32]. However, recent observations have questioned this hypothesis. Recent prospective observational and case-control studies have reported that only 20%-25% TCP patients were undernourished before onset, and the majority of patients lost weight only after disease onset[33,34]. It has been argued that malnutrition thus could be the effect rather than the cause since TCP and consequent malabsorption could itself lead to severe weight loss[34]. Although data are convincing that malnutrition is not causally related to the etiopathogenesis of TCP, it is possible that malnutrition modulates the phenotype of the disease. High carbohydrate and low protein diets have also been shown to result in ductal changes, with mucoid metaplasia and parenchymal atrophy in an animal model in the bonnet monkey[35]. However, pancreatic changes were rather different from those typically seen in TCP, and the animals predominantly developed vascular and cardiac changes-features not observed in TCP patients[35].

Cassava/cyanogen toxicity

In several parts of the world, cassava (tapioca, *Manihot esculenta*) is consumed as a staple food by poor people. It is known to contain cyanogenic glycosides such as linamarin and lotaustralin, whose detoxification in the body requires sulfur. Malnourished individuals are deficient in sulfur-containing amino acids such as methionine and cysteine. Since cassava was a staple diet in Kerala, it gained the status of a co-trigger as a logical extension of the nutritional hypothesis. The cassava hypothesis has been discarded because: (1) cassava consumption was not found to be a risk factor in case-control studies including one from Kerala[36]; (2) patients with TCP have been reported from areas where cassava is not consumed[4,14]; and (3) long-term cassava consumption did not produce diabetes or pancreatitis in a rat model[37].

Micronutrient deficiency and oxidative stress

Multiple micronutrient deficiency is common in CP and likely to be related to its pathogenesis through its influence on oxidative stress. A study from Kerala has shown enhanced lipid peroxidation and decreased antioxidant status both in TCP and ACP[38]. The authors have further extended their earlier observation that zinc deficiency may also have a significant role to play in CP[39]. Zinc deficiency may occur as a result of pancreatic exocrine insufficiency. Moreover, zincuria has also been observed in most cases with pancreatic insufficiency[39,40].

Alcohol, smoking and other environmental toxins

Although data are variable, an increasing trend has been observed in the occurrence of ACP cases in India. Reports from southern India indicate a rise in cases of ACP from 2% during the 1980s to 33% over the last decade[17,18], whereas studies from northern India report a near equal (30%-40%) prevalence of ACP or predominance of TCP/ICP[4,12,16]. Even in the cases of TCP/ICP, a large proportion of individuals are alcohol drinkers. A majority of alcohol drinkers have also been reported to be smokers which further increases the risk of TCP[17,18]. Additionally, xenobiotic stress has also been implicated in the etiopathogenesis of TCP[41].

Familial aggregation and genetic factors

In one of the earliest studies, in 98 family members comprising 24 parents, 57 siblings and 17 offspring of TCP probands, familial aggregation was seen in 8% of TCP patients[42]. In some families, there was evidence of vertical transmission of TCP from parents to the offspring, while in others, horizontal distribution of the disease was observed. This suggests, but does not necessarily prove, a hereditary etiology for TCP since several family members could be exposed to the same toxic and/or other environmental factors. This led to the speculation that genes could be involved in the pathogenesis of TCP. However, it was only after the identification of cationic trypsinogen (*PRSS1,* OMIM 276000) as risk factor in Western populations that researchers focused on the role of genetic variants in TCP[43,44].

GENETIC HETEROGENEITY IN TCP

Trypsinogen(s)

As early as 1896, it was hypothesized that pancreatitis results from premature trypsinogen activation within the pancreas[2]. In 1996, independent familial linkage analysis studies mapped the gene locus on chromosome 7q35 and demonstrated its association with hereditary pancreatitis (HP)[45,46]. Subsequent fine mapping studies identified the c.365G>A (p.R122H) mutation in *PRSS1* to be associated with HP[43]. Since then, a large number of variants in *PRSS1* have been identified in CP patients[47]. Of these, p.R122H, p.N29I and p.A16V are most commonly reported, and their causality in CP through diverse mechanisms has been proven beyond doubt[48]. None of the mutations in *PRSS1* that have been reported to be associated with HP, and CP in Western populations has been found in TCP patients[49,50]. Mutations in anionic trypsinogen (*PRSS2*) were hypothesized to cause the disease by a mechanism similar to that of *PRSS1*. Earlier studies by various groups did not find association of any polymorphism of *PRSS2* with ICP and TCP patients[51-53]. The protective role of the p.G191R *PRSS2* mutation, identified in Europeans, has also been not replicated in Indians[54]. In addition, no copy number variation muta-

tion in *PRSS1/PRSS2* was found in TCP patients, suggesting that trypsinogen gene mutations do not play an important role in the pathogenesis of TCP in the Indian population[55].

Serine protease inhibitor Kazal type 1

PSTI encoded by *SPINK1* (OMIM 167790) is also synthesized in acinar cells of the exocrine pancreas. Because of its ability to trap up to 20% of the potential trypsin activity, SPINK1 has long been thought to constitute one of the defense mechanisms against prematurely activated trypsin within the pancreas. Identification of *SPINK1* as a susceptibility gene for CP[44] was followed by several reports confirming its association worldwide with various forms of pancreatitis. To date, more than 40 variants in *SPINK1* have been identified. The most commonly associated variant c.101G>A (p.N34S) has shown a strong association with TCP as well[49,50]. Similar associations of varying strength have been reported by several studies establishing *SPINK1* as a strong candidate for contributing to the pathogenesis of TCP[56-59]. Overall, these studies assessed 351 TCP patients and 973 controls. The high-risk haplotype around p.N34S was detected in 168 of 702 patient alleles and in 44 of 1946 control alleles. The pooled OR calculated using the random-effect model was 19.15 (95%CI: 8.83-41.56)[60]. However, no genotype-phenotype correlation has been found in patients carrying the p.N34S *SPINK1* mutation in homozygous or heterozygous state, and a wide variability has been reported in the pattern of inheritance. Hence, in contrast to the causal nature of *PRSS1* mutations, *SPINK1* has been accorded the role of disease modifier. A more recent study assessed the role of *SPINK1* promoter variants in the pathogenesis of CP[61]. A rare loss-of-function variant c.-142T>C that leads to disruption of the HNF1 binding site and hence reduced *SPINK1* expression was identified exclusively in TCP patients[61]. Another rare variant, c.-215G>T, also identified only in FCPD patients did not affect *SPINK1* expression. These results suggest that p.N34S *SPINK1* continues to be the strongest risk predictor for TCP.

Cathepsin B

Human cathepsin B (*CTSB*, OMIM 116810) is a 339 amino acid long thiol protease belonging to the peptidase C1 family. It is primarily localized to lysosomes and is involved in intracellular degradation and turnover of proteins. Nearly 30 years ago, it was speculated that lysosomal enzymes might play a role in the pathophysiology of pancreatitis[62]. This was because an earlier study had shown that at least one lysosomal hydrolase, cathepsin B, was capable of activating trypsinogen[63], which has been supported by several subsequent studies[64,65]. Additionally, it has been shown that supramaximal stimulation causes redistribution of lysosomal enzymes leading to their co-localization with digestive enzyme zymogens within intra-acinar cytoplasmic vacuoles[66]. These observations made *CTSB* an interesting candidate gene. Indeed, a study involving 306 TCP patients and 330 controls reported that polymorphism p.L26V *CTSB* was associated with TCP (OR = 2.09, 95%CI: 1.55-2.81; $P = 0.013$)[67]. In addition to the p.L26V variant, the polymorphism p.S53G also had a significantly different distribution in p.N34S SPINK1 carriers and non-carriers. These variants, which lie in the pro-peptide region of CTSB, were proposed to lead to mis-localization of cathepsin B to zymogen granules, thus causing premature activation of trypsinogen. However, a recent study conducted in a moderate sample size of 150 cases and 150 controls from North India failed to replicate this association[68]. Surprisingly, the mutant allele frequency of 0.33% in controls reported in the study is far lower than that of 30% reported earlier[67], which raises doubts on the veracity of the results

Chymotrypsin C

The human chymotrypsin C gene (*CTRC*; OMIM 601405) encodes a 268 amino acid long serine protease (a member of the peptidase S1 family) that is secreted from the pancreas and has a chymotrypsin-like protease activity. Recent studies have demonstrated that auto-activation of trypsinogens in humans is proteolytically regulated by CTRC through two independent and seemingly conflicting mechanisms. On one hand, CTRC stimulates the autoactivation of cationic trypsinogen by cleavage at the Phe18-Asp19 peptide bond[69], while on the other hand, it promotes degradation of all human trypsin and trypsinogen isoforms with high specificity by cleaving the Leu81-Glu82 in the calcium binding loop[70]. The act of activation and degradation of trypsinogen by CTRC is regulated by prevailing Ca^{2+} concentrations[70]. Since the intra-acinar activation of cationic trypsinogen is thought to be the primary cause of pancreatitis, impairment of the CTRC-dependent regulation of auto-activation of trypsinogen increases the risk of intra-pancreatic trypsinogen activation and consequent pancreatitis in humans. Two initial studies investigated the role of *CTRC* variants in patients of Indian origin and found them to be associated with TCP[71,72]. However, both studies focused on a specific region of the gene in a small number of subjects. Subsequently, a comprehensive study that screened the whole *CTRC* gene in a large, ethnically matched case-control CP cohort, including TCP, observed significant over-representation of rare *CTRC* variants in CP patients compared with normal individuals[73]. Non-synonymous variants, c.217G>A (p.A73T) and c.703G>A (p.V235I), were the major risk predictors, in comparison to the c.738_761del24 (p.K247_R254del) and c.760C>T (p.R254W) variants that were the predominant mutations in European CP patients. While p.A73T exhibits its pathogenicity by eliciting endoplasmic reticulum stress[74], p.V235I is known to reduce activity and secretion of the protein[75]. In addition, a synonymous variant, c.180C>T [p.(=)], was also found to be significantly associated with CP (OR = 9.89, 95%CI: 2.95-33.18; $P = 5.9 \times 10^{-6}$). Interestingly, the spectrum of *CTRC* mutations identified in TCP patients was similar in all three studies, but entirely different from that observed in Western CP patients.

Based upon the biochemical activities of CTRC and the functional properties of *CTRC* variants, three mutually non-exclusive models for explaining the role of *CTRC* variants in predisposing to CP were put forward[76]: (1) impaired trypsinogen and/or trypsin degradation; (2) induction of endoplasmic reticulum stress; and (3) impaired activation of A-type carboxypeptidases.

Carboxypeptidase A1

The last hypothesis as mentioned above is of special interest because, based on functional evidence, a recent study proposed that CTRC is a physiological co-activator of pro-carboxypeptidase A1 (proCPA1) and pro-carboxypeptidase A2 (proCPA2)[77]. After trypsinogens, proCPA1 is the second largest component of pancreatic juice, contributing more than 10% of the total protein. Indeed, genetic and functional data from a recent study has established the global role of *CPA1* variants in the pathogenesis of CP, including TCP[9]. However, there was evidence of heterogeneity in the spectrum of mutations identified in different populations. In the individuals of Indian origin, three non-synonymous variants (p.D32H, p.R169H, and p.Y308H) were novel and present exclusively in patients, while the frequency of p.A208T was comparable between cases and controls. Apparent activities of p.D32H, p.R169H and p.Y308H were 79%, 24%, and 3%, respectively, of the wild protein, whereas their respective relative secretion levels were 75%, 23%, and 17%, respectively, of the native protein. This confirms the earlier notion that the mutational spectrum in various CP-associated genes is different in TCP than in other types of CP in the Western world.

Cystic fibrosis transmembrane regulator

The *CFTR* gene encodes a member of the ATP-binding cassette transporter superfamily. In the pancreatic duct, CFTR couples functionally to the anion exchangers to generate bicarbonate secretion for alkalinizing the duodenal lumen[78]. Abnormal *CFTR* genotypes are strongly associated with cystic fibrosis (CF)[79]. Considering the fact that patients with CF occasionally suffer from pancreatitis; pancreatic pathology in CP and cystic fibrosis shows intraductal plugging; also, CP is a known cause of false positive sweat tests, and two studies in 1998 simultaneously reported an association between *CFTR* mutations and CP[80,81]. Only two studies have investigated the role of *CFTR* variants in TCP. In the study by Bhatia and colleagues, all 27 *CFTR* coding exons were analyzed in 18 Indian TCP patients[82]. Two patients (11%) showed a *CFTR* variant: one subject was homozygous for the 5T allele and the other heterozygous for p.R1070Q, which is presumed to be a mild missense variant. The overall frequency of *CFTR* alterations was 0.083 (3/36), which was far lower than that observed in white Caucasian subjects with CP (range: 0.20-0.24). In a more recent study, mutations in 19 of 27 exons of the *CFTR* were analyzed in 100 TCP patients and 100 healthy controls[12]. A total of 21 severe and mild *CFTR* variants (including six novel variants) were detected in 50% of patients compared with two different variants in 10% of controls ($P < 0.0001$). Of these, 27 patients were trans-heterozygous for *CFTR* variants and the p.N34S *SPINK1* mutation[12].

Pancreatic stone protein

An important feature of TCP is the high incidence of pancreatic calcification and stone formation. Human Reg protein is encoded by the pancreatic stone protein (*reg1a*) gene (regenerating gene) as a 166 amino acid pre-proprotein with a 22-residue long signal sequence, and is highly represented in the human pancreatic secretions. It was first isolated as a major protein component of pancreatic stones in patients with ACP and hence called pancreatic stone protein. It was suggested that it could promote the nucleation of calcite crystals or prevent pancreatic lithiasis by inhibiting calcite crystal nucleation and growth in the pancreatic juice[83]. With suggestions that it might help in preventing the harmful activation of protease precursors in the pancreatic juice, it was speculated that mutations in this gene could lead to pancreatitis and calcification. However, no association with TCP could be established even on screening of all exons of the *Reg1a* gene (OMIM 167770)[84,85]. As the protein is known to be downregulated in TCP patients, a recent study screened the gene, including the putative promoter and intronic regions, but did not find a significant association with TCP[86].

Glycoprotein 2

The glycoprotein 2 (*GP2*) gene is specifically expressed in the pancreatic acinar cells and represents the major component (about 40%) of the total zymogen granule (ZG) membrane protein[87]. During the secretory process, GP2 is cleaved from the membrane by phosphatidylinositol-specific phospholipase C and is secreted into the duct lumen along with other digestive zymogens. In addition, a soluble form of GP2 is also present in the content of ZGs. Given the fact that intra-ductal plug formation is one of the early events in the development of CP and GP2 is found to be a major component of these plugs[88], it was hypothesized that variations in *GP2* may potentially affect the risk of duct obstruction and CP. Mutational screening of exons 3 and 9 of the *GP2* gene in TCP patients identified two variants of which the variant c.1275A>G showed a disease predisposing effect[89]. A recent study has demonstrated that in the presence of this variant, the ratio of full-length transcript:total transcript is much lower than that derived from the wild type[90]. This is because the c.1275A>G variant significantly reduces the rate of exon 9 inclusion compared with the wild-type sequence[90]. It results in substitution of the last 116 amino acids by 15 new amino acids. These changes may lead to structural alterations and hence compromise the function of the protein[90].

Transcription factor 7-like 2

Progression of TCP to diabetes, also known as FCPD, occurs in a majority of TCP patients. However, the nature of diabetes associated with pancreatitis is contro-

versial since it shows features of both type 1 (T1D) and type 2 (T2D) diabetes. A recent study from our group hypothesized that the type and mechanism of diabetes in FCPD patients can be understood by investigating a known genetic susceptibility factor for T1D or T2D[91]. In this study, T2D-associated polymorphisms in transcription factor 7-like protein 2 (TCF7L2, OMIM 602228) were screened in TCP and FCPD patients. Although no independent association with FCPD was identified, data suggested that polymorphisms in *TCF7L2* might interact with *SPINK1* and *CTSB* mutations and cause FCPD[91].

Calcium sensing receptor

Experimental evidence suggests that intracellular and extracellular calcium levels play an important role in the initiation of protease activation within the pancreas. The function of calcium sensing receptor (CASR) is to sense small differences in the circulating calcium levels. Mutations involving *CASR* (OMIM 601199) have been proposed to increase the risk of CP, since high intracellular levels of calcium activate trypsinogen within the acinar cells. A combination of *CASR* and *SPINK1* gene mutations has also been proposed to predispose to ICP. Another study conducted in India pertaining to TCP patients identified four novel mutations (p.P163R, p.I427S, p.D433H, p.V477A) in *CASR*[92]. A combination of both the p.N34S *SPINK1* mutation and *CASR* mutations was seen in approximately 6% (2/35) of the patients, while 22% (6/35) harbored a single mutation[92]. However, the drawback of this study was that a limited number of patients and controls were screened.

CONCLUSION

The clinical phenotype of TCP has changed over the years. The disease which was common in young adults and adolescents is now reported to occur in relatively older people with both the age at onset and age at presentation being nearly a decade later now than in the previous studies. In addition, the presentation of the disease has become more heterogeneous. Only a fraction of cases now satisfy the criteria of classical TCP[12,14]. This change has been attributed to socioeconomic, dietary, and lifestyle changes over the past 20-30 years. Instead, there have been rising trends in alcohol consumption and smoking habits in young Indians. As a result ACP now comprises nearly one-third of CP patients in India[17,18]. It has been reported that classical TCP in India now presents as ICP whose phenotype is somewhat similar to that reported from other countries. The results from candidate gene studies established that several genetic components are involved in the pathophysiology of TCP, and there is evidence of genetic and mutational heterogeneity between TCP and CP in Western populations. These components work both *via* trypsin-dependent as well as trypsin-independent pathways. Overall, these observations point to the fact that TCP is indeed a complex multifactorial disease and in-depth studies are needed to dissect the role of individual factors and their interaction in the pathophysiology of the disease.

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