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TOPIC HIGHLIGHT

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Genetics of acute and chronic pancreatitis: An update

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Abstract

Progress made in identifying the genetic susceptibility underlying acute and chronic pancreatitis has benefitted the clinicians in understanding the pathogenesis of the disease in a better way. The identification of mutations in cationic trypsinogen gene (*PRSS1* gene; functional gain mutations) and serine protease inhibitor kazal type 1 (*SPINK1* gene; functional loss mutations) and other potential susceptibility factors in genes that play an important role in the pancreatic secretory functions or response to inflammation during pancreatic injury has changed the current concepts and understanding of a complex multifactorial disease like pancreatitis. An individual's susceptibility to the disease is governed by genetic factors in combination with environmental factors. Candidate gene and genetic linkage studies have identified polymorphisms in cationic trypsinogen (*PRSS1*), *SPINK1*, cystic fibrosis trans-membrane conductance regulator (*CFTR*), Chymotrypsinogen C (*CTRC*), Cathepsin B (*CTSB*) and calcium sensing receptor (*CASR*). Individuals with polymorphisms in the mentioned genes and other as yet identified genes are at an enhanced risk for the disease. Recently, polymorphisms in genes other than those involved in "intra-pancreatic trypsin regulatory mechanism" namely Claudin-2 (*CLDN2*) and Carboxypeptidase A1 (*CPA1*) gene have also been identified for their association with pancreatitis. With ever growing number of studies trying to identify the genetic susceptibility in the form of single nucleotide polymorphisms, this review is an attempt to compile the available information on the topic.

Key words: Chronic pancreatitis; Acute pancreatitis; Genetic susceptibility; Single nucleotide polymorphisms; Inflammation

Core tip: Pancreatitis is a progressive inflammatory disease. Though the pancreas has adequate protection against environmental and metabolic stress, if the magnitude of this stress exceeds the threshold which the organ can handle, it leads to pathologic effects. Although genetic variables have been identified that affect the function of pancreas, namely polymorphisms in serine protease inhibitor kazal type 1 (*SPINK1*), polymorphisms in cationic trypsinogen (*PRSS1*) and Chymotrypsinogen C (*CTRC*) genes in the acinar cells and cystic fibrosis trans-membrane conductance regulator (*CFTR*), calcium sensing receptor (*CASR* genes) in the ductal cells leading to pancreatitis, off late many genetic factors outside of the "intra-pancreatic trypsin regulatory mechanism" have been identified for their role in pancreatitis. This review is an update on the genetic aspects of acute and chronic pancreatitis.

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INTRODUCTION

Chronic pancreatitis (CP) is a disease associated with

inflammation where the secretory parenchyma of the pancreas is progressively destroyed. There is involvement of several known risk factors and processes such as inflammation, necrosis, apoptosis or duct obstruction despite the heterogeneity in pathogenesis. The process of fibrosis usually leads to progressive worsening in lobular morphology, structure of pancreas, changes in arrangement and composition of the islets and deformation of the large ducts[1]. These conditions lead to diabetes that is due to irreversible morphological and structural changes and exocrine and endocrine dysfunction[2]. The major types of pancreatitis are acute pancreatitis (AP), recurrent acute pancreatitis (RAP) and CP.

In spite of an individual carrying a genetic risk and being subjected to oxidative or metabolic stress, the pancreas is histologically normal in appearance in the pre-acute phase. "First hit" in terms of injury due to excess alcohol consumption, metabolic factors, hyperlipidemia, gallstones and genetic factors leads to AP-which is a sentinel AP event (SAPE)[3]. During this proinflammatory phase, inflammatory related damage occurs due to the infiltration of the pancreas with inflammatory cells. This phase may end through an anti-inflammatory response that is mediated partly by tissue macrophages and is associated with the activation of stellate cells and subsequent proliferation causing fibrosis. However clinical recovery is attained in most of the cases.

If this phase is followed by RAP due to genetic risks namely polymorphisms in serine protease inhibitor kazal type 1 *(SPINK1),* polymorphisms in cationic trypsinogen (*PRSS1*), cystic fibrosis trans-membrane conductance regulator (*CFTR*) genes and other as yet unknown genes, or chronic cell stressors develop like alcohol, smoking, oxidative stress, *etc.*, after the SAPE (second hit), it leads to CP which is due to chronic inflammation and progressive fibrosis. CP may also manifest as a direct result of extensive pancreatic necrosis, duct obstruction in the proximal region directly resulting from severe AP which is independent and without the second hit[4].

Many risk factors that contribute varyingly to pancreatitis have been identified. These include alcohol, metabolic factors, toxins, insecticides, certain medications, viral and bacterial infections, trauma caused by surgery[5]. Growing evidence suggests a substantial contribution of genetic predisposition to pancreatitis. As early as 1950's, genetic studies on pancreatitis suggested that it may be an inherited disease[6]. After this initial description, a mutation inherited in autosomal dominant mode was identified in the cationic trypsinogen gene that is located on 7th chromosome in individuals with hereditary pancreatitis[7,8]. Further to this, a number of other mutations/polymorphisms in genes that have a role in inhibition, regulation or modulation of the pancreatic trypsin activity, secretory function and inflammatory injury respectively were identified. Mutations in the *PRSS1*, *SPINK1*, *CFTR* and polymorphisms in other genes namely the ones regulating the response to inflammation [tumor necrosis factor (TNF), interleukin-1 (IL-1) and IL-10][9] are the major genetic contributors to the development of AP and CP.

A model (two hit model) for the pathogenesis of pancreatitis has been proposed[10], suggesting that "there is a loss of balance between events associated with activation and degradation of active trypsin enzyme leading to the presence of persistent "super-trypsin" with in the acinar cell that is due to mutations or polymorphisms in genes namely *SPINK1,* Cathepsin B (*CTSB*), Chymotrypsinogen C (*CTRC*) and other yet to be identified susceptibility genes. This loss of balance leads to inflammation and these events are the first hits that contribute to the pathogenesis of pancreatitis". The presence of additional genetic and/or environmental risks leading to one or more phenotypes namely fibrosis, stone formation and/or diabetes and these events are the second hit.

AP: DEFINITION, SYMPTOMS AND RISK FACTORS

AP is a syndrome of acute and sudden inflammation of the pancreas. Clinically, it is detected by upper abdominal pain with sudden onset, digestive enzymes namely pancreatic amylase and lipase that are elevated in the serum and/or typical findings like edema, peripancreatic fat stranding, fluid collection on the abdominal imaging studies. The process in AP is initiated by an injury that is acute followed by an inflammatory response (also acute) which is mostly out of proportion and to the extent of tissue injury. The above response is due to premature activation of digestive enzymes in the pancreas that digest the tissue, consequently activating the inflammatory cascade. The immune system may also be cross-activated by the activated pancreatic digestive enzymes. Many risk factors for AP have been identified. The most important of them being duct obstruction by gall stones, parasites, tumors, anatomical abnormalities and endoscopic retrograde cholangio-pancreatography; metabolic factors like hyperlipidemia, hypercalcemia and acidosis; toxins like ethyl alcohol, insecticides, scorpion toxins, medications (azathioprine, NSAIDs, tetracycline, *etc.*); Bacterial and viral infections, trauma caused by blunt or penetrating or surgery apart from genetic susceptibility namely mutations in *PRSS1*, *SPINK1* and *CFTR*[5].

CP: DEFINITION, SYMPTOMS AND RISK FACTORS

CP is a disease associated with inflammation that is progressive and is characterized by three main features. Abdominal pain that is recurrent or persisting at the clinical level, damage of the parenchyma in pancreas with irregular sclerosis and inflammation, accompanied by ductal dilation, strictures or stones at the morphological level and finally a progressive loss of exocrine and endocrine functions at the functional level[11-13]. Based on the etiologies and risk factors, a working classification for CP

Table 1 General genetic information of the genes which confer susceptibility to pancreatitis[1]

Name of the gene	Chromosome	No. of splice variants	Length (bp) of exon region	No. of exons
CTRC	1	4	898	8
CASR	3	4	5009	7
PRSS1	7	6	800	5
CTSB	8	35	3875	10
SPINK1	5	3	542	4
CFTR	7	11	6128	27
CLDN2	X	3	3150	2

[1]Extracted from ENSEMBL. *CTRC*: Chymotrypsin C; *CASR*: Calcium sensing Receptor; *PRSS1*: Trypsinogen Gene; *CTSB*: Cathepsin B; *SPINK1*: Serine protease inhibitor kazal type 1; *CFTR*: Cystic fibrosis transmembrane conductance regulator; *CLDN2*: Claudin 2.

has been elaborated by the American Gastroenterological Association according to its prevalence and mechanism named TIGAR-O classification system (toxic-metabolic, idiopathic, genetic, autoimmune, recurrent and severe AP, obstruction)[14]. The toxic metabolic include alcohol, smoking (tobacco), hyperlipidemia, hypercalcemia, chronic renal failure and certain medications; idiopathic includes early onset, late onset and tropical; mutations in cationic *PRSS1* gene, *CFTR* gene, *SPINK1*, α-1 antitrypsin deficiency and other unidentified genes comprise genetic risk; autoimmune includes isolated autoimmune chronic pancreatitis, autoimmune syndromic CP including Sjogren's syndrome-associated CP, primary biliary cirrhosis-associated CP and inflammatory bowel disease-associated CP. Recurrent and severe AP-associated CP includes post necrotic (severe AP), vascular disease/ischemic and post-irradiation. Obstructible risk factors include sphincter of Oddi disorders, pancreas divisum, duct obstruction (tumor), preampullary duodenal wall cysts and post-traumatic pancreatic duct scars.

GENETIC RISK FACTORS FOR AP AND CP

It has long been suggested that inappropriate activation of trypsinogen in the pancreas is the first and most important step in the development of pancreatitis[15] and all the known genetic susceptibility factors for pancreatitis identified till date can be categorized as members of the intra-pancreatic trypsin regulatory mechanism and were identified employing a candidate-gene approach based on the above mechanism and they include polymorphisms/mutations in genes namely *CTRC*, *CASR*, Trypsinogen gene (*PRSS1, 2* and *3*), Cathepsin B (*CTSB*), *SPINK1/PST1*, *CFTR* gene. General information about the genes is presented in Table 1. A recent study[16] identified an underlying genetic susceptibility in approximately half of idiopathic CP patients, when they screened for mutations in *PRSS1, SPINK1, CTRC* and *CFTR* genes, emphasizing the important role of genetics in CP. A detailed list of different types of polymorphisms identified in these genes till date has been extracted from ENSEMBL and presented in Table 2 and the list of polymorphisms in these genes are also listed in the web site www.pacreasgenetics.org, however only the important polymorphisms/mutations have been discussed in detail in this review.

Trypsinogen (PRSS1, 2 and 3) genes

PRSS1 (cationic trypsinogen), anionic trypsinogen (*PRSS2*) and mesotrypsinogen (*PRSS3*) are the three types of trypsinogen that are expressed by the pancreas to an extent of two-thirds to one-third to less than 5% respectively[17,18]. Eight trypsinogen genes are shown to be located in the beta T-cell receptor locus at 7q35[19]. The *PRSS1* gene that is mapped to the long arm of chromosome 7 encodes the trypsin-1 (TRY-1) protein[8,20]. Important mutations (gain of function namely A16V, N29I, R122H) have been identified in the *PRSS1* gene that are associated with hereditary pancreatitis in Caucasians[21,22], French[23], D162D variant in Chinese[24] however a study from India reported that *PRSS1* gene mutations are not associated with CP[25]. A study from Korea reported that 5.4% of subjects with idiopathic CP and 40% with pancreatitis that is hereditary carried R122H mutation in the *PRSS1* gene and other variants were not reported apart from R122H. None of the 50 controls had the mutation[26]. One important study[27] screened for *PRSS1* mutations in a Belgian patient with sporadic CP and observed a migration pattern that is altered different from the transition (g.133283G > A) in exon 3 of the gene. Subsequent analysis by DNA sequencing revealed a DNA variant that was novel (g.133283-133284GC > AT) also resulting in R122H, however they concluded that in contrast to the change in codon CGC to CAC, codon CGC > CAT strongly suggested an alternative mutational mechanism of gene conversion.

Apart from the polymorphisms and their associations with pancreatitis, studies have also looked in to the copy number variations (CNVs) for their role in pancreatitis. A study[28] identified a duplication and triplication of 605kb segment on chromosome 7q35 in French ICP patients, which increased the copy number of *PRSS1* and 2 genes that code for cationic and anionic trypsinogen. The same study identified a trypsinogen gene that was hybrid with exon 1, 2 from *PRSS2* and exons 3 to 5 from *PRSS1*, which had two gain of function effects namely increase in trypsinogen gene copy number with N29I mutation in it. The 605kb segment duplication was also assessed further in French and Indian patients with idiopathic CP (ICP) and concluded that it was associated with French ICP but not in Indian patients with CP[29], however the CNVs in *PRSS3* were not associated[30].

Serine protease inhibitor Kazal type 1/pancreatic secretory trypsin inhibitor gene

SPINK 1/pancreatic secretory trypsin inhibitor (*PSTI*) is a specific trypsin inhibitor and an acute phase protein which is secreted by the acinar cells[31]. The gene encoding *SPINK1* has 4 exons and 3 introns that is located at

Table 2 Summary[1] of the polymorphisms in genes related to pancreatitis

Name of the gene	Upstream gene variants	Downstream gene variants	Non-coding exon variants	Synonymous variants	Missense variants	Stop gained	Intron variants
CTRC	490	430	102	28	57	5	789
CASR	580	732	129	433	1459	57	4707
PRSS1	1031	1634	431	126	280	6	637
CTSB	5763	11413	621	682	1261	10	18675
SPINK1	366	252	38	8	37	0	236
CFTR	1193	2377	87	447	2533	558	13723
CLDN2	205	171	0	36	78	0	560

[1]Extracted from ENSEMBL Upstream Gene variants: A sequence variant located 5′ of a gene. Downstream gene variants: A sequence variant located 3′ of a gene. Non-coding exon variants: A sequence variant that changes non-coding exon sequence. Synonymous variants: There is no change in the resulting aminoacid. Missense variants: Variant that changes one or more bases, resulting in a different aminoacid but where the length is preserved. Stop gained: Sequence variant whereby at least one base of a codon is changed, resulting in premature stop codon, leading to a shortened transcript. Intron variants: a variant occurring within an intron. *CTRC*: Chymotrypsin C; *CASR*: Calcium sensing Receptor; *PRSS1*: Trypsinogen Gene; *CTSB*: Cathepsin B; *SPINK1*: Serine protease inhibitor kazal type 1; *CFTR*: Cystic fibrosis transmembrane conductance regulator; *CLDN2*: Claudin 2.

5q32 and is approximately 7.5kb long[32]. SPINK1 protein plays a role in the prevention of premature activation of zymogen that is catalyzed by trypsin within the pancreatic duct system or the acinar tissue. A reactive site in the protein serves as a specific target substrate for trypsin[33] and it can inhibit up to 20% of the activity of pancreatic trypsin. It is the first line of defense against auto digestion, thereby protecting the pancreas[9], however inhibition of trypsin by *SPINK1* is temporary as trypsin may target the trypsin-SPINK1 complex and subsequently degrade the inhibitory molecule and restore trypsin activity[34]. *SPINK1* mutations cause a loss of function mutations as against *PRSS1* which generate gain of function mutations. There are several mutations/polymorphisms that are identified till date in the *SPINK1* gene (Table 2), however N34S is the most common missense mutation, that is a substitution of asparagine by serine at codon 34. N34S polymorphism was found in individuals especially without a family history and many studies have confirmed its association in different ethnic groups[25,35-37]. A substantial number of patients (15%-40%) with ICP carry N34S mutation in either heterozygous or homozygous state based on the above studies. The *SPINK1* polymorphisms (N34S) are in complete linkage disequilibrium with other variants that are located in the introns[38]. Other mutations/polymorphisms have also been identified namely a promoter mutation (-215-A and -215 G > T), a mutation in the start codon that destroys the only translational initiation codon of *SPINK1* (2 T-C, Met to Thr; MIT)[39], -53C > T; -41G > A, -2C > A; L14P; D50E; IVS3 + 125C > A; IVS3 + 184T > A; R65Q; R67C which were reported predominantly in single patients or families[35,38,40].

Polymorphisms in *SPINK1* gene are generally associated with loss of function. Although the *SPINK1* N34S polymorphism is associated with pancreatitis, the association is weak with very few individuals with the mutation developing pancreatitis some time during their life time[35,41]. Furthermore there is no difference in the severity of the disease with respect to the heterozygous and homozygous genotypes of SPINK1; there are complex interactions and the effect of the mutation depends on the reduction in the enzyme. Pancreatitis may be initiated in the homozygous N34S state, however the heterozygous genotype may only cause a lowering of the enzyme level and it requires other additional factors (genetic and environmental) to initiate the disease[42]. Therefore in general *SPINK1* polymorphism is hypothesized to be a susceptibility factor for a polygenic complex trait or a disease modifier[3] with polymorphisms in other genes being involved.

Apart from the above polymorphisms, two copy number mutations (deletions) in the SPINK1 gene that were associated with loss of function and encoding pancreatic secretory trypsin inhibitor (*PSTI*) were identified by a study[38]. In a particular family these deletions were co-inherited with a missense mutation (p.L997F) in the *CFTR* gene, suggesting complex interactions between the CNVs and single nucleotide substitutions contributing to the disease phenotype. SPINK1 polymorphisms are common in the general population (approximately 2%) but are shown to be significantly associated with pancreatitis.

Chymotrypsin C gene

CTRC encodes Chymotrypsin C, a digestive enzyme. It is produced by the acinar cells in the pancreas. It is packaged with zymogen granules and is secreted along with other digestive enzymes from the pancreas. Prematurely activated trypsin is destroyed by CTRC by acting on the molecule within the calcium-binding loop in the absence of calcium and therefore is a crucial candidate gene in the pathogenesis of pancreatitis[43]. Many polymorphisms have been identified in this gene till date (Table 2). A study[44] had sequenced all the 8 exons (8.2 kb) of the *CTRC* gene in a total of 621 individuals with idiopathic or hereditary CP and 614 control subjects of German origin and identified that the large majority of the variants were in 2nd, 3rd and 7th exons. Only exons 2, 3 and 7 were sequenced in an additional 280 CP patients and 2075 controls for exons 2 and 3 and 2190 controls for exons 7. Although a number of missense and deletion variants were found they concluded that the two most frequent variants

which were significantly overrepresented in the pancreatitis group as compared to the controls were c.760C > T (p.R254W) and c.738_761del24 (p.K247_R254del) (30/901 (3.3%) affected individuals but only in 21/2804 (0.7%) controls), both of which were located in exon 7. Furthermore, this group also studied 71 and 84 individuals of Indian origin with tropical pancreatitis and controls respectively, and suggested a higher frequency of *CTRC* alterations in this cohort [10/71 (14.1%) in Tropical pancreatitis Vs 1/84 (1.2%) controls] as compared to the German cohort and two relatively frequent variants were found in the Indian cohort namely c.217G > A (p.A73T) missense alteration and the c.190_193del ATTG (p.I64LfsX69) frame shift deletion[44]. Another study from India[45] identified 14 variants in 584 CP patients and 598 normal subjects [71/584 CP patients (12.2%) and 22/598 controls (3.7%)], when all the eight exons and flanking regions of the *CTRC* gene were sequenced. It was p.V235I variant which was common in the Indian CP patients as against the p.K247_R254del variant in the Caucasians. Apart from this variant the study also identified other pathogenic variants namely p.A73T and c.180C > T as significantly associated with Indian CP.

Cathepsin B gene

The human *CTSB* is 25.6kb. It has 12 exons. Several transcript species are known to be produced by alternative splicing[46]. It is hypothesized that chronic pancreatitis is a result of mutations in the *CTSB* gene and they may be involved in premature activation of trypsinogen or inappropriate localization[47]. A study on the CTSB gene polymorphisms and tropic calcific pancreatitis identified significant association of Val26Val polymorphism (allele frequency of 0.48 in patients *vs* 0.30 in controls) with Odds of 2.15 apart from differences in the mutant allele frequencies that are significant at Ser53Gly (allele frequency of 0.10 *vs* 0.04 in patients and controls respectively) and C595T SNPs (allele frequency of 0.12 *vs* 0.20 in patients and controls respectively. Further L26V polymorphism was equally as common in N34S positive and wild type patients suggesting that CTSB is involved independently with the disease. This study suggested that *CTSB* polymorphisms may be associated with pancreatitis more so in the absence of mutations in *PRSS1* gene and N34S *SPINK1* polymorphism proposed to play a disease modifier role[47], however another study failed to associate polymorphisms in this gene with pancreatitis in European cohort (allele frequency of 0.398 in patients and 0.48 in controls)[48].

Calcium-sensing receptor gene

Auto-activation and autolysis are processes in which trypsinogen molecule is activated to trypsin and is also degraded by other trypsin molecules. For the mentioned purpose, two specific cleavage sites exist for potential attack by other trypsin molecules. Lysine 23 (L23) is the first site and arginine 122 (R122) the second. The cleavage of L23 causes trypsinogen activation to trypsin with 8-amino acid trypsinogen activation peptide being released while R122 cleavage causes inactivation of trypsin. The susceptibility of the two sites for an attack is regulated by calcium concentration and concentration dependent occupation of the calcium binding sites[49]. In normal acinar cells low calcium concentrations are prevalent and these low concentrations limit the activation of trypsinogen, thereby promoting inactivation of trypsin by exposing the second site (R122), however calcium hyper stimulation or dysregulation in the acinar cells favors activation of trypsinogen and prevention of trypsin inactivation[50]. Thus regulation of calcium levels (intra-acinar) is critical for preventing trypsinogen activation and pancreatic injury. CASR plays a major and important role in maintaining the calcium homeostasis through its effect on renal tubules and parathyroid gland. A variety of hypercalcemia-associated syndromes are associated with genetic variants in the *CASR* gene[51]. The first of the reports associating *CASR* mutations with CP came from a family study of 5 individuals who were all heterozygous for the N34S *SPINK1* polymorphism. Only two of the 5 heterozygous individuals developed CP and both these individuals presented with a T > C mutation at position 518 in the *CASR* gene, that is a leucine to proline amino acid change in the extracellular domain of the CASR protein[52], suggesting that *CASR* mutations may be a predisposing genetic factor that may increase the susceptibility for CP. Another study[53] that screened for mutations in *SPINK1* and *CASR* gene on a small Indian cohort of 35 patients with Tropical chronic pancreatitis (TCP) and an equal number of controls reported that a combination of mutations in both the genes was seen in 6% of the patients, while 22% had mutation in single gene, suggesting that *CASR* mutations may be a risk for TCP and that risk may be further increased with associated *SPINK1* mutation. A study by Muddana *et al*[54] initially included 115 subjects with pancreatitis and 66 controls. Of the study group, 57 patients and 21 controls were predetermined to carry the N34S *SPINK1* polymorphism. Based on the initial results, the study included an additional 223 patients and 239 controls to analyze the three common non-synonymous SNPs in exon 7 that were found to be significant from the initial study. The *CASR* exon 7 polymorphism (R990G) was significantly (Odds, 2.01 and $P = 0.01$) associated with CP and the association of this SNP was stronger in subjects with moderate to heavy alcohol consumption. This study however did not find any significant associations between the various *CASR* genotypes and *SPINK1* N34S in CP. None of the earlier reported polymorphisms from Germany and India were also detected in this US-based study. All the association studies suggest that recurrent trypsin activation/dysregulated calcium and failed inhibition increase the risk of pancreatitis *via* the intracellular calcium dysregulation.

CFTR gene

The impact of *CFTR* gene continues to be debated, although variants in this gene are strongly associated

with pancreatitis. *CFTR* gene in humans has 27 exons, is located at 7q31 and is 250 kb in length[55]. For the proper functioning of the duct cells in the pancreas and other anion secreting epithelial cells, CFTR anion channel is a critical molecule. CFTR apart from regulating the functions of other channels also conducts both chloride and bicarbonate channels, the opening and closing of which controls the bulk of fluid secretion from the pancreas[50]. The association between idiopathic CP and *CFTR* mutations was demonstrated in 1998[56,57]. More than 1200 mutations have been identified and based on the mechanism by which they disrupt the function; they are classified in to five different groups with group V mutations subsequently being included in group I (as they cause functional alterations in the levels of mRNA)[58]. Class I mutations affects biosynthesis, class Ⅱ mutations affect protein maturation, class Ⅲ affect chloride channel regulation/gating while class Ⅳ mutations affect chloride conductance[59]. An additional class of mutations was proposed by Haardt *et al*[60] as class Ⅵ which included protein stability mutations.

A higher frequency of mutations in the *CFTR* gene was seen in a significant number of patients (30%) with ICP. There was six and two times higher frequency of CFTR mutations and 5T allele respectively in patients[56,57,61]. With few of these mutations there was a reduction in the amount of functional CFTR. The others might be a combination of a severe and a mild mutation or either type of mutations with 5T allele in intron 8 of the gene[9]. There is an increased risk (up to 40 fold) for pancreatitis when individuals are compound heterozygotes[62]. Complete coding sequences of the *CFTR,* P*RSS1* and *SPINK1* genes were analyzed for mutations and it was seen that 25%-30% of the patients with CP carried at least a single mutation in the *CFTR* gene and majority were compound heterozygotes for a *CFTR* mutation or were trans-heterozygotes for *CFTR, PRSS1* and *SPINK1* mutations[62,63]. Furthermore, a combination of two *CFTR* mutations and N34S in *SPINK1* gene increases the risk of pancreatitis by 900 fold[9]. It is clear from these studies that *CFTR* variants are associated with CP, however the mechanisms of the complex interactions of various susceptibility loci has to be understood in a better way.

Proinflammatory cytokine genes

It is already established that the cytokine profile with in the pancreas is different in CP as compared to normal pancreas[64]. A potential factor that could affect the production of proinflammatory cytokines are polymorphisms in these genes. Association studies involving polymorphisms in various cytokine genes have shown varying results in various populations. Various genes namely *TNF-a* (tumor necrosis factor-α), *Monocyte chemoattractant protein-1*, and *IL-8*[65-67] have been studied for their association with pancreatitis.

It is known that *TNF-a* along with *IL-1* is a major early cytokine to mediate the systemic inflammatory response syndrome (SIRS)[68-70]. A study[71] reported the association between *TNF-a* -238 AG but not -308 SNP genotype with organ failure (shock and/or respiratory failure) and in the IL-6 gene the CC genotype at position 174 was associated with biliary etiology of AP. The study included 84 patients with AP (no controls were included) and known polymorphisms in *TNF-a*, interleukin 1 (*IL-1*), IL-1 receptor antagonist (*IL1RN, IL-6* and *IL-10)* were genotyped for etiology associated susceptibility and severity, however other polymorphisms like *TNF-a*-1031, -863 and -857 SNPs were not included in the study. Another study[72] reported a negative association between *TNF-a*-308 and severity of pancreatitis (397 patients and 300 controls with major allele frequency in *TNF* gene being 0.87 for patients with AP and 0.86 for controls) from Finland, however they did not study the *TNF-a*-238 SNP. These results were similar to studies reported from United Kingdom, by[73], who studied 190 and 102 AP patients and controls respectively and Sargen *et al*[74], who studied 135 AP and 107 controls respectively (78.3% and 84.4% for *TNF-a*-308 and 21.7% and 15.6% for *TNF-a*-238 in controls and AP respectively). However, *TNF-a*-308 allele was reported to be associated with severe AP in Hungarian patients[75]. The study included 77 patients (mixed etiology and grouped according to the severity of the disease on the basis of Ranson scores) and 71 controls. Another study[76] associated *TNF-a*-308 allele with shock in patients with severe AP, however suggested that the polymorphism played no part in disease severity or susceptibility. The study included 208 AP cases and 116 ethnicity matched controls. A recent meta-analysis[77] integrated the previous findings on *TNF-a*-308 G > A and -238 G > A alleles and explored whether the polymorphisms were associated with susceptibility and severity to pancreatitis. The study included 1569 pancreatitis cases and 1330 controls from 12 published case-control studies and concluded that polymorphisms in these two sites did not alter the risk of pancreatitis.

Monocyte chemoattractant protein 1 (MCP-1) is a member of the C-C chemokine family. It exerts a strong chemo attractant activity in macrophages, lymphocytes and monocytes[78]. A common polymorphism-2518 A > G alters the expression of the gene with G allele being associated with higher levels of MCP-1 protein which is associated with higher risk of pancreatitis. A study from United States[65] included 77 consecutive patients and 116 controls for the mentioned genotype and concluded that the -2518 genotype is a risk factor for severe AP (12 of 14; 86% with AP *vs* 50 of 116; 43% control subjects) and also suggested that MCP-1 serum levels appear to be an accurate predictor of severity of AP and death when measured early in the course of the disease. Another study from Italy[79] studied 118 AP, 64 ARP, and 142 CP patients and 88 controls and concluded that all patients with pancreatic inflammatory disease had significantly higher serum MCP-1 levels. A study[80] which looked at the relationship between a polymorphism in the *MCP-1* gene (-2518A/G) and AP in the Han population of Suzhou, China suggested an increased risk of AP associ-

ated with G allele [72.4% (113/156) and 76.1% (35/46) in severe AP; 47.1% (113/240)]. However, the 2518A/G polymorphism in the *MCP-1* gene did not significantly alter the susceptibility to CP[81].

Interleukins are proinflammatory cytokines and polymorphisms in these genes have been shown to affect the immune response[82]. A meta-analysis[83] on the interleukin gene polymorphisms which included a total of 10 studies, covering a total of 1220 AP cases and 1351 controls showed evidence for significant association between *IL-8* -251 T/A (rs4073) polymorphism and AP risk, suggesting that *IL-8* -251 A allele was associated with an increased risk of AP. However, there were no significant associations between *IL-1* [IL-1 +3954 C/T (rs1143634) and IL-1 -511 C/T (rs16944)], IL-6 [IL-6 -174 G/C (rs1800795) and *IL-6* -634 C/G (rs1800796)] and *IL-10* [IL-10 -1082 A/G (rs1800896), *IL-10* -819 C/T (rs1800871) and *IL-10* -592 C/A (rs1800872)] gene polymorphisms and AP risk. In summary, the study concluded that the IL-8 -251 T/A polymorphism was associated with an increased risk of AP. In addition, there were no significant associations between IL-1, IL-6 and IL-10 gene polymorphisms and AP risk.

Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine. It is released by macrophages and lymphocytes[84]. It plays an important pathogenic role in AP and a study[85] investigated the role of -173 G > C polymorphism and the (CATT) n repeat microsatellite at position -794 in 164 patients with AP and 197 controls C allele 58/160 [18.1% in AP *vs* 47/097 (11.9%) in controls]. There was no significant difference in the repeat length of the microsatellite marker between patients and controls, however the C allele of the -173 G > C genotype was significantly higher in patients.

Claudin-2 and Carboxypeptidase A1 gene

New susceptibility loci for CP have been identified. The first SNP in the Claudin-2 (*CLDN2*) locus is the outcome of the first and only reported Genome wide association study for pancreatitis done till date, which included 1676 cases and 4507 controls in stage I and 910 cases and 4170 controls in stage Ⅱ. The study identified two SNPs namely one SNP in *PRSS1-PRSS2* locus (allele frequency of 0.576 in controls *vs* 0.634 in pancreatitis) and the other in the Claudin-2 locus (*CLDN2*) (allele frequency of 0.261 in controls *vs* 0.322 in pancreatitis). The SNP in the *PRSS1* locus affects susceptibility by altering the expression of trypsinogen and the SNP in the *CLDN2* is associated with atypical localization of claudin-2 in pancreatic acinar cells. Homozygous or hemizygous genotype (in females and males) confers the greatest risk and the alleles also interact with alcohol consumption to increase the risk of pancreatitis[86]. Another study[87] analyzed variants in Carboxypeptidase A1 (*CPA1*) encoding carboxypeptidase A1, primarily in Germany discover set and in thee replication sets from Europe, India and Japan. *CPA1* variants were associated with non-alcoholic CP with varying levels of significance in the discovery [29/944 (3.1%) of German cases and 5/3,938 (0.1%) controls] as well as all the three replication sets 8/600 (1.3%) of European cases and 9/2,432 (0.4%) controls, 5/230 (2.2%) of Indian cases and 0/264 controls and 5/247 (2.0%) of Japanese cases and 0/341 controls. The study concluded that variants may confer an increased risk of CP and the mechanism may involve endoplasmic reticulum stress that may be induced by misfolding rather than trypsin activity that is elevated.

GENETIC TESTING FOR *PRSS1, SPINK1* AND *CFTR* GENES - WHEN TO ORDER THE TEST?

A valuable diagnostic genetic test to investigate AP and CP has been added ever since a point mutation in the *PRSS1* gene has been identified. Consensus guidelines for ethical molecular genetic testing for hereditary pancreatitis has been proposed[88] which recommends it under the following conditions: (1) Unexplained two or more (recurrent) episodes of documented pain that are separate with hyperamylasemia attack; (2) Idiopathic CP; (3) Family history of pancreatitis [in a parent, sib, child (first degree) and in aunt, uncle or grand parent (second degree)]; (4) A need to exclude significant concern of hereditary pancreatitis in a child with an unexplained episode of documented pancreatitis that required a hospitalization; (5) As part of research protocol that is approved. Genetic testing (*PRSS1* mutations) in children below 16 years is indicated after; (6) Hospitalization that was required in an individual because of an episode of documented pancreatitis of unknown etiology that is severe enough; (7) Pancreatitis of unknown etiology in an individual with two or more documented episodes; (8) A child with an episode of documented pancreatitis, who has a relative with hereditary pancreatitis mutation that is known; (9) Recurrent abdominal pain (unknown etiology) in a child, where there is a distinct clinical possibility of hereditary pancreatitis; and (10) Diagnosis of hereditary pancreatitis as a distinct clinical possibility in an individual with CP of unknown etiology[88].

Currently genetic testing for mutations in SPINK1 or CFTR genes is considered as premature as the identification of mutations in these genes neither convincingly explains the disease in an individual who has been diagnosed with pancreatitis or has the ability to predict the possibility of developing the disease[88-90].

The significance of a positive test result for *PRSS1* genetic testing should be explained clearly to the subjects. Variable clinical course, mode of inheritance and incomplete penetrance are the important aspects apart from others, where counseling needs to be imparted to the patients. Strategies should be discussed to prevent future episodes of AP namely avoiding concomitant risk factors like alcohol, metabolic disturbances and drugs.

Important risk factors namely choledocolithiasis and other obstructive factors that contribute to AP have to be

identified and treated. Therefore patients have to be advised to undergo radiological and endoscopic evaluation to identify the above risks[91]. Furthermore, as these mutations (R122H or N29I) also significantly increase the risk for pancreatic cancer, the patients should be counseled for abstinence from tobacco and smoking[92] and counseling may be imparted and genetic testing ordered for at risk relatives if warranted[3].

CONCLUSION

As emphasized earlier many of the susceptibility loci identified till date have taken the candidate-gene approach and to the best of our knowledge there are no GWAS (Genome wide association studies) which are available apart from the only study which identified *PRSS1* and *CLDN2* polymorphisms recently[86]. Furthermore, a better understanding of the interactions of the etiological factors with susceptibility SNPs will aid in diagnosing and treating the disease at an early stage. There is an urgent need to utilize the advances in genomics namely GWAS and/or exome sequencing on NGS platform to unravel as yet unidentified susceptibility loci for pancreatitis, which is a multifactorial and a complex disease for a better understanding at the molecular level.

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