Idiopathic Chronic Pancreatitis: Genetic Predisposition

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1. Introduction

The major causes of chronic pancreatitis (CP) include toxic injury due to alcohol and smoking, hereditary and non-hereditary genetic predisposition, metabolic derangements in the form of hypercalcemia and hypertriglyceridemia, anatomical abnormalities such as pancreas divisum, obstructive pathology such as tumours, and idiopathic. The cause of idiopathic CP has long been unclear. Initial thinking regarding the pathogenesis of idiopathic CP revolved around multiple environmental factors such as diet and toxins but such hypotheses were never proven in well-designed case-control studies (59). During the last decade, attention has dramatically shifted towards underlying genetic susceptibility as a risk factor in developing CP. With regard to alcohol as the cause of CP, it is not known why only a minority of patients who abuse alcohol develop CP, again suggesting genetic predisposition as a significant risk factor. Indeed, intense research has revealed a strong genetic influence in the pathogenesis of CP and has renewed interest in the study of gene-environment interactions, similar to other common polygenic and poorly-understood diseases such as diabetes.

Historical perspective

The role of genetic susceptibility for CP has been considered for over 30 years. Initial efforts were directed towards the association of HLA genes with CP. In 1979, Faucet et al (24) in a study of 90 patients and 523 controls showed that HLA B40 was associated with chronic alcoholic pancreatitis (*P*<0.01). In 1981, Homma et al (37) showed HLA B5 to be associated with chronic idiopathic pancreatitis but not alcoholic pancreatitis. Abnormal class I and class II MHC antigens expression was shown in pancreatic ductular epithelial cells in 57% of patients with CP (mainly alcohol related) along with T lymphocyte infiltration (40). The results of various HLA association studies have been summarized in **Table 1**. The limitations of these studies were – (i) there was no consistency among different studies with many different loci being associated with CP of both alcohol and idiopathic variety, (ii) the sample size was small in most studies although they did show significant differences between cases and controls, and (iii) the basic concept of autoimmunity as an underlying cause for CP was flawed to a large extent. The concept of autoimmune pancreatitis as we understand it today had not been established at the time when these studies were conducted. Nevertheless, these studies did point toward a possible genetic predisposition for developing CP.

We will review various studies dealing with genetic predisposition to different types of CP. There is mounting evidence of strong genetic susceptobility in different types of CP with increasing number of genetic mutations being discovered in patients with CP.

Table 1: Summary of the studies on the association of HLA with CP

Author	Year	n	controls	Gene of interest	p value
Faucet (24)	1979	90	523	HLA B40	<0.01
Homma (37)	1981	46	120	HLA B5	<0.05
Gullo (35)	1982	64	425	HLA B13	<0.02
Wilson (92)	1984			HLA Bw39	<0.02
Forbes (27)	1987	50		HLA Cw5 (alcoholic CP)	<0.05
				A25, Cw1 (idiopathic CP)	<0.05
Anderson (1)	1988	88	344	HLA B21 (alcoholic CP)	<0.01
				HLA A1 (idiopathic CP)	<.002

Hereditary Pancreatitis

Hereditary pancreatitis (HP) is a type of CP that affects multiple members of a family. It is transmitted as an autosomal dominant disease with about 80% penetrance (which could be less for some of the mutations) and variable expressivity. HP offered itself as a perfect model to find out the causal genetic mutation. Indeed, in a landmark study, a mutation in the cationic trypsinogen gene *(PRSS1)* was found in a large kindred of HP with multiple affected members through genetic linkage analysis. Whitcomb et al (90) reported arginine to histidine substitution at residue 122 (p.R122H, p. designates protein coding; originally named R117H in the chymotrypsin numbering system) in the cationic trypsinogen gene on the long arm of chromosome 7 (7q35). Subsequent studies not only confirmed this exciting finding but also revealed many more mutations in the *PRSS1* gene. The Second most common *PRSS1* mutation is p.N29I mutation in exon 2 with a change of isoleucine to asparagine at position 29 (34). Hereditary pancreatitis is covered in another chapter in more detail.

Idiopathic chronic pancreatitis

Idiopathic CP has long been shrouded in mystery as far as its etiopathogenesis is concerned. Different hypotheses such as immune mediated injury and environmental toxins were proposed earlier but were subsequently discarded.

However, there has been tremendous advancement in the field of genetic mutations in the pathogenesis of idiopathic CP leading to almost an explosion of studies on this subject. It is now generally accepted that genetic mutations are the most important risk factor for idiopathic CP. It is thought to be a polygenic disorder with strong environmental influence. Mutations in two important genes i.e. *CFTR* gene and *SPINK1* gene have been strongly implicated in the pathogenesis of idiopathic chronic pancreatitis.

2. *CFTR* gene mutations and idiopathic chronic pancreatitis

In 1998, 2 groups simultaneously showed that *CFTR* gene mutations were significantly associated with idiopathic CP. Sharer et al (79) showed that the frequency of minor *CFTR* mutations was increased 2.5 times in patients with idiopathic CP compared with that in healthy controls in an English population. Similarly, Cohn et al (19) reported that the frequency of minor *CFTR* mutations was 11 times more common in patients with idiopathic CP compared with controls in an American population of predominantly northern European ancestry. These groups introduced the concept of pancreas sufficiency in such patients with minor *CFTR* mutations who have no overt manifestations of cystic fibrosis but with isolated pancreatitis, a

situation akin to absent vas deferens which is also associated with some minor *CFTR* mutations. In contrast, patients with classical *CFTR* mutations have pancreatic insufficiency and atrophy but no pancreatitis. A recent study has confirmed this concept of pancreas sufficiency and pancreatitis. Of 505 Israeli patients with cystic fibrosis, 139 (27.5%) were found to be pancreas sufficient, none of whom harboured the two mutations associated with severe disease; 20 (14.3%) of the 139 patients developed pancreatitis versus none of the 366 pancreatic insufficient patients (5). Subsequently, other groups have also confirmed the observations that indeed minor mutations in the gene are common in patients with idiopathic CP compared with the general populations **(Table 2)**. A study of employing *CFTR* gene sequencing from Germany revealed that the frequency of minor mutations of *CFTR* gene was 2 times more common in patients with idiopathic CP as compared to controls (87). In another large study of 381 patients from USA, 32% (122/381) of patients had 166 mutant *CFTR* alleles, including

12 novel *CFTR* variants: c.4243-20A>G, p.F575Y, p.K598E, p.L1260P, p.G194R, p.F834L, p.S573C, c.2657+17C>T, 621+83 A>G, p.T164S, c.489+25A>G and c.3368-19G>A (42). [The CFTR gene mutations are expressed as c. (coding DNA sequence) ### (position of the last nucleotide in the adjacent exon) +/- ## (postion of the change in the intron) followed by nucleotide change] (66). In a study of Brazilian patients with CP, *CFTR* gene mutations were found to be common in patients with idiopathic CP (6) However, in a study of 92 children with CP or recurrent acute pancreatitis from Poland, no definite association was found with *CFTR* gene mutations (80). The natural history of patients with *CFTR* gene mutation associated pancreatitis has been analyzed in a study which showed that it is characterized by recurrent attacks of pancreatitis over many years leading finally to the development of CP, but that endocrine and exocrine insufficiencies were rare or delayed (29).

Table 2: Summary of the important studies on the association of *CFTR* mutations with idiopathic CP

Author	Year	n	controls	Gene of interest	p value*
Sharer (79)	1998	134	600	CFTR (13.4% vs. 5.3%)	<0.001
				5T allele (10.4%)	0.008
Cohn (19)	1998	27		CFTR (37% cases)	<0.001
Truninger (82)	2001	82		CFTR (21.4%, 4.8 times)	<0.05
Audrezet (4)	2002	39		CFTR (20%)	<0.05
Fujiki (30)	2004	65	121	CFTR (12.3% vs. 3.7%)	<0.05
Weiss (87)	2005	67	60	CFTR (25/134 vs. 11/120)	<0.05
Chang (13)	2007	78	200	CFTR 22/156 vs. 19/400)	0.001
Zoller (94)	2008	133		CFTR (12.7% vs, 3.2% controls)	<0.05
Aoyagi (3)	2009	20	110	5T in intron 8 (20% vs 4.5%)	<0.05

Association of *CFTR* mutations with idiopathic CP in non-Caucasian populations

As cystic fibrosis is generally more common among Caucasians, it is important to find out if *CFTR* mutations/polymorphisms are associated with idiopathic CP in non-Caucasian patients. In a Japanese study of 65 patients with CP, a high association of p.Q1352H (12.3% in CP patients vs. 3.7% in controls) and p.R1453W (6.2%v 3.1%) was found, suggesting an association of *CFTR* variants with CP in Japan where CF is very rare (30). In this study, none of the common CF-causing mutations found in Caucasian populations were detected. Very recently, Nakano et al (63) reported a comprehensive analysis of *CFTR* variants in Japanese patients with CP by aid of next-generation sequencing. They found 10 non-synonymous *CFTR* variants (p.R31C, p.R31H, p.I125T, p.K411E, p.V470M, p.I556V, p.L957fs, p.L1156F, p.Q1352H, and p.R1453W) in patients with idiopathic CP. The frequency of the p.L1156F variant was higher in patients with idiopathic CP than that in controls (10/121 vs. 46/1136, *P*=0.033). A report from Koreas showed that the haplotype containing p.Q1352H showed the strongest association with bronchiectasis and CP (*P*=0.02 and *P*=0.008, respectively) (48). Another study from Japan (45) showed the association of polythymidine tract 5T splicing variants of the intron 9 acceptor splice site [c.1210-12T(5_9)] with CP. In a study from China, the occurrence of abnormal *CFTR* alleles was found to be thrice as frequent in idiopathic CP patients as in controls (22/156 vs. 19/400, *P* <0.0001) (13). 5T allele was associated with early onset of idiopathic CP. The haplotype containing c.125G/c.1001+11C, (TG)12 repeats, p.470M, c.2694T and c.4521G haplotype was associated with an increased risk of idiopathic CP (odds ratio 11.3; 95% confidence interval 2.3-54.6, *P*=0.008) in Chinese patients. In a study of Indian patients with idiopathic CP, we found that minor *CFTR* variants were 5 times more common when compared with healthy controls and six novel variants c.2280G>A, c.2988+35A>T, c.3718-41C>G, c.473G>A, c.1680-99C>T, and c.1392+4G>T) were detected (58).

Mechanism of *CFTR* gene mutation and pancreatitis

The mechanism(s) involved in the causation of pancreatitis in patients with CFTR minor mutations is not known. A study showed that ion channel transport, as measured by sweat chloride, and nasal transepithelial potential difference were variable in patients with pancreatitis having *CFTR* minor mutations but the ion channel measurements became worse with increasing number and severity of *CFTR* mutations (9). Another study showed that there were abnormal ion transport in patients with 2 *CFTR* minor mutations and pancreatitis which suggested that quantitatively the loss in CFTR function lies between that observed in cystic fibrosis patients and in normal carriers (64). A recent study has shown that p.M348V minor *CFTR* mutation resulted in decreased Cl$^-$ and HCO$_3^-$ fluxes across the xenopus oocyst cell suggesting the possibility of similar defect in the pancreas (88). However, whether such a putative defect in the ionic fluxes operates across the acinar cell or the ductal cells is not known and how such an effect leads to the initiation of pancreatitis is also not understood. Whether it result in a different ion concentration in the pancreatic juice within the ducts due to the defect in ductal cells which then that leads to precipitation of proteins and obstruction or does a defect in the acinar cells lead to perturbation in the internal milieu of the acinar cells resulting in disturbance in enzyme activation or secretion is not known. As cystic fibrosis is a disease associated with overt bacterial infections, studies on the role of intestinal flora in the progression of CP are warranted.

In summary, major and minor mutations in the *CFTR* gene are associated with idiopathic CP in both Caucasians and non-Caucasian patients.

3. Serine Protease Inhibitor Kazal Type 1 (*SPINK1*) gene and idiopathic chronic pancreatitis

SPINK1 is an acute phase reactant protein. It is a natural protease inhibitor and inhibits active trypsin within the acinar cells of the pancreas. Thus, it provides protection against a prematurely activated trypsin in the acinar cells. In 2000, three important studies reported significantly higher frequency of the p.N34S mutation in exon 3 of the *SPINK1* gene in patients with idiopathic CP (17, 71, 93). Subsequently, many other studies have reported mutations in the *SPINK1* gene in patients with idiopathic CP from different ethnic origin. Studies from India showed that *SPINK1* gene mutations were quite common in patients with idiopathic (tropical) CP (7, 11). In addition to N34S mutation, another mutation p.P55S in the *SPINK1* gene has also been found to be common in patients with idiopathic CP (23). Other rare variants include p.D50E, p.Y54H, p.R65Q, and p.R67C in the *SPINK1* gene. A recent meta-analysis of all the studies on *SPINK1* mutation in CP published to 2007 has shown that this mutation was detected in 469 of 4,842 patient alleles and in 96 of 9,714 control alleles yielding a pooled odds ratio of 11.00 (95% C.I. 7.59–15.93) based on allelic frequency for all aetiologies of CP (2). The odds ratio was higher for idiopathic CP compared with that for alcoholic CP [14.97 (95% C.I. 9.09–24.67) vs. 4.98 (95% C.I. 3.16–7.85)]. A comprehensive list of the studies included in the meta-analysis is available from this study (2).

In a more recent study of Chinese patients with idiopathic CP from Taiwan, it was shown that *SPINK1* mutation is associated with 32.4% of patients with early onset and 2.1% of late onset CP (15). The most common mutation was the intronic variant IVS3+2T>C (c.194+2>C) and not p.N34S as seen in other studies. The association of the IVS3+2T>C with CP was first reported in Japanese patients with CP (41). This study showed a clear distinction between early onset and late onset CP with regards to genetic mutation suggesting that mutation leads to early onset and more severe form of pancreatitis.

In Korea, *SPINK1* mutations, p.N34S and IVS3+2T>C were identified in 3 and 11 out of 37 patients with idiopathic CP patients, respectively, including one with compound p.N34S/IVS3+2T>C heterozygote. The prevalence of *SPINK1* IVS3+2T>C mutation was 26.8% in patients with idiopathic CP (67).

In a Japanese study, the frequencies of p.N34S and IVS3+2T>C in the *SPINK1* gene were significantly higher in patients with idiopathic CP (10.6% and 11.6%, respectively) than in controls (0.4% and 0%) (54).

The highest frequency of *SPINK1* p.N34S mutation has been reported in Indian patients with idiopathic CP. Two studies reported that *SPINK1* p.N34S mutation was present in 47% and 44% of patients with idiopathic (tropical) CP (7, 11, 58).

Mechanism of *SPINK1* mutation and pancreatitis

The mechanism as to how *SPINK1* p.N34S mutation causes CP is not well understood (16). One study showed that p.N34S mutation was not associated with alternative splicing (55). Two other studies almost simultaneously showed that the common p.N34S and p.P55S polymorphisms involve amino-acid substitutions with similar physicochemical properties but do not cause any significant reduction in terms of SPINK1 mature peptide expression (10, 46). On the other hand, the IVS3+2T>C mutation caused skipping of the entire exon 3, encoding the region in which the trypsin binding site is located. This leads both to production of a mutated protein and lowered expression to 62% of that observed in the healthy control in these CP patients (47). The p.R65Q missense mutation involves the substitution of a positively charged amino acid by a non-charged one and causes an approximately 60% reduction of protein expression (10). Other rare polymorphisms p.G48E, p.D50E, p.Y54H, and

p.R67C, involve charged amino acids and lead to complete or nearly complete loss of SPINK1 expression possibly due to intracellular retention and degradation (46).

In summary, *SPINK1* gene mutations particularly pN34S are asociated with idiopathic CP more commonly in patients with CP from India.

4. Mutations in other genes and idiopathic CP

Since mutations in cationic trypsinogen gene were significantly associated with hereditary pancreatitis, mutations in anionic trypsinogen were also tested in patients with CP. However, it was found that anionic trypsinogen (PRSS2) p.G191R might actually confer protection against CP in Europeans. However, its protective role has been questioned in other populations (52, 81).

Chymotrypsinogen C (CTRC) degrades trypsinogen and its loss-of-function variants have been found in European patients with CP. In Indian and Japanese patients with idiopathic CP, no significant association with *CTRC* variants was found initially (21) but a unique loss-of-function p.R29Q variant has been identified in Japanese patients and significant association has been shown for Indian patients with CP as well (21, 57, 69).

A mutation in the calcium sensing receptor (*CASR*) gene has been suggested to play a role in idiopathic CP in German patients (26). In a US population also, the CASR exon 7 p.R990G polymorphism was significantly associated with CP (OR, 2.01; 95% CI, 1.12-3.59; *P*=0.015) (61). The association between *CASR* p.R990G and CP was stronger in subjects who reported moderate or heavy alcohol consumption (OR, 3.12; 95% CI, 1.14-9.13; *P*=0.018). Also in Indian patients with idiopathic (tropical) CP, an association with the *CASR* gene mutation was observed (62).

Pancreatic stone protein (PSP) was considered to be a major protein component of pancreatic stones in CP. PSP is a secretory stress protein (8). The human PSP or Reg protein is encoded by *reg1*a gene (regenerating gene). However, polymorphisms in *reg1*a gene, including the regulatory variants were not found to be associated with idiopathic (tropical) CP (51).

Angiotensin-converting enzyme (ACE) activity might be related to pancreatic stellate cell activation and pancreatic fibrosis. However, no significant differences were found in the prevalence of the ACE-deletion genotype frequencies when patients with alcoholic (27.5%), nonalcoholic (26.4%), and acute pancreatitis (32.7%) were compared with controls (26.9%) in a recent European study (39).

HFE gene is a major risk factor for hereditary hemochromatosis but whether it might increase susceptibility to CP is not known. No significant differences were found in heterozygosity for p.C282Y and p.H63D among patients with alcoholic (8.0, 21.5%), idiopathic (7.3, 24.5%), or familial (9.8, 23.0%) pancreatitis, or pancreatic adenocarcinoma (5.4, 28.6%) and healthy (6.2, 24.8%) and alcoholic (7.0, 25.0%) controls in a recent study (38).

In a study from Taiwan, polymorphism of the TNF-alpha gene was shown to be a risk factor for CP. The 2863A allele of the TNF-alpha promoter was associated with an increased risk for CP (odds ratio 4.949 (95% CI 2.678–9.035)). In multivariate analysis, 2863A and 21031C were independently associated with higher susceptibility to CP (*P*<0.0001) (12).

5. Genome Wide Association Studies

It was recognized that hypothesis driven investigations might take a long time and still not produce satisfactory results. Thus, in more complex polygenic diseases such as CP, multiple

genes contribute to the pathogenesis through quantitative rather than qualitative change. Thus, a hypothesis independent approach through genome wide association studies (GWAS) was initiated to find genes that influence disease risk through GWAS (36). Applying the GWAS approach, Whitcomb et al identified two loci at *PRSS1-PRSS2* and X-linked *CLDN2* being robustly associated with recurrent acute pancreatitis and alcohol-related CP in subjects of European ancestry (91). Subsequent studies have confirmed the association of these loci with idiopathic CP in patients of different ancestry i.e. Chinese, Europeans, Japanese, and Indians (20, 33, 56, 85). Very recently, another genome wide association study showed a novel association between alcoholic CP and polymorphisms in the genes encoding fucosyltransferase 2 non-secretor status (FUT2 locus rs632111 and rs601338) and blood group B (ABO locus rs8176693) (86).

Is idiopathic chronic pancreatitis a genetic disease?

In a study of 381 patients with CP, 32% had 166 mutant *CFTR* alleles, including 12 novel *CFTR* variants: c.4243-20A>G4375-20 A>G, p.F575Y, p.K598E, p.L1260P, p.G194R, p.F834L, p.S573C, c.2657+17C>T2789 + 17 C>T, 621+83 A>G, p.T164S, c.489+25A>G 621+25 A>G, and c.3368-19G>A3500-19 G>A.. *SPINK1* mutation was seen in 14.5% (55/381) and *PRSS1* mutation was present in 8.1% (31/381) of patients (42). Thus, 49% (185/381) of the patients had one or more mutations. In 242 Indian patients with idiopathic CP, up to 66% of patients had either *SPINK1* or *CFTR* or both mutations (58). These observations lend strong support to the concept that the majority of cases of idiopathic CP have an underlying genetic predisposition. In addition however, there must be environmental influences modulating the overt presentation and phenotype of the disease. Thus, it seems that the term 'idiopathic CP' may no longer be justified and a more meaningful term such a "CP-G " is proposed where 'G' denoted genetic susceptibility.

6. Genetic mutations/polymorphisms and Alcoholic Pancreatitis

The discovery of a variety of gene mutations in idiopathic and hereditary CP it was thought that the same might hold true for alcohol related CP. However unlike idiopathic CP, genetic mutations in the usually suspected genes i.e. *SPINK1*, *PRSS1*, and *CFTR* genes have not been found commonly in the patients having alcoholic CP.

A study in European patients did not find any significant association with any of the 3 genes i.e. *CFTR*, *PRSS1*, and *SPINK1* (70). Both *CFTR* and cationic trypsinogen mutations were not found to be predisposing risk factors for alcohol-related pancreatitis in a study from USA (60). *CFTR* mutations did not seem to play an important role in alcoholic CP (89). Similarly, a study from USA also did not find *SPINK1* p.N34S mutation more commonly in alcoholic CP than in controls (6.3%, vs. 1.1% controls; P>0.05) (76). Studies from other parts of the world also reported similar results. A study from Korea did not find any association of chronic alcoholic pancreatitis with *CFTR* or *SPINK1* gene mutations (49).

Polymorphisms at the known loci in the TNF-alpha, TGF-beta(1), IL-10, IFN-gamma genes which are involved in inflammation were not found to be associated with alcoholic CP (73). It was initially thought that pancreatitis associated protein (PAP) might be involved in the pathogenesis of CP. However, there was no evidence for polymorphism of the PAP gene in patients with alcoholic pancreatitis (43).

Polymorphisms of the genes related to metabolism of the oxidative compounds such as NADPH-quinone oxidoreductase 2 (NQO2), multidrug resistance 1 (MDR1), and lipoprotein lipase (LPL) were analyzed in alcoholic CP. However, no significant difference was found between patients and controls with regard to these genes (53). Similarly, polymorphisms in

other metabolizing enzymes such as glutathione-S-transferase P1 (GSTP1) and manganese-superoxide dismutase (MnSOD), and detoxifying phase II biotransformation enzymes such as the UDP-glucuronosyltransferases have not been found to be associated with the susceptibility to alcoholic CP (68, 83). However, one study did show significant association with UDP-glucuronosyltransferases and CP with an increased risk with the UGT1A7*3 allele (K129-K131-R208) (OR, 1.76; 95% CI, 1.26-2.46; P=0.0009). Moreover, UGT1A7*3 allele was specifically associated with the subgroup of patients with alcoholic pancreatitis, of whom 89% were smokers (OR, 2.24; 95% CI, 1.46-3.43; P = 0.0001) (65).

Polymorphisms in the monocyte chemotactic protein-1 (*MCP-1*) and heat-shock protein 70-2 (HSP70-2) were also not found to be associated with alcoholic CP (50).

Since alcohol is considered as causing toxic injury to pancreas, polymorphisms in the alcohol metabolizing enzymes have been studied as a basis of individual susceptibility to develop pancreatitis. In the alcohol dehydrogenase 1B (*ADH1B*) gene, ADH1B*1 wild-type allele frequency was found to be significantly lower in alcoholic CP compared with alcoholics without CP (35). No significant difference was found between the patient and control groups in the aldehyde dehydrogenase enzyme *ADH2* genotypes. But a significant difference was found between the two groups in the acetaldehyde dehydrogenase enzyme *ALDH2* locus in another study (44). The frequency of the ALDH2*1 wild-type allele was found to be 0.681 and that of the ALDH2*2 allele (p.E504K) was 0.319 in the controls, while these values were 0.935 and 0.065 in the patients, respectively [ALDH2 isoenzyme exists in 2 isoforms 1 and 2 which code for active and inactive subunits respectively. It is expressed as ALDH2*1 or ALDH2*2. A person can be homozygous or heterozygous i.e. ALDH2*1/*1 or ALDH2*1/*2]. Most of the patients (27 of 31) were

ALDH2*1/*1, only four were ALDH2*1/*2, and none of the patients were ALDH2*2/*2. Thus, genetic polymorphism of the ALDH2 gene might influence the risk of developing alcoholic pancreatitis (44). In another study, the frequencies of *ADH3* and *CYP2E1* c1c2 genotypes did not differ among CP patients, alcoholics and healthy controls (84). In a Polish study, ADH2*1, ADH3*1 alleles and ADH2*1/*1, ADH3*1/*1 genotypes were statistically more frequent among the patients with alcoholic CP than among the controls (18). In another study from Australia, alcoholic cirrhosis but not alcoholic CP was associated with ADH3*2/*2 and perhaps with ADH2*1/*1 (28). Thus, there are contradictory and variable reports and the data so far do not suggest any definite association of polymorphisms in either alcohol metabolizing or detoxifying enzymes.

7. Genetic mutations in other types of chronic pancreatitis

Some of the specific causes of CP are related to metabolic derangements or anatomical defects and it is generally believed that these abnormalities are the sole cause for pancreatitis. However, recent studies have brought in the role of genetic predisposition in such patients.

In a study of patients with primary hyperparathyroidism, 4 (16%) of 25 patients with pancreatitis carried the p.N34S mutation in the *SPINK1* gene, while all 50 controls (hyperparathyroidism without pancreatitis) showed no mutation in *SPINK1* or *PRSS1* genes (P < 0.05 vs. controls, P < 0.001 vs. general population) (25). In addition, *CFTR* mutations were present in four patients (P < 0.05 vs. general population), while one patient carried a 5T allele. One patient was transheterozygous (*SPINK1*: p.N34S/*CFTR*: p.R553X). Importantly, the mean serum calcium levels in pancreatitis patients did not differ significantly from the mean of patients without pancreatitis thus questioning the value of serum calcium levels in the causation or initiation

of pancreatitis. The authors concluded that genetic mutations significantly increased the risk of pancreatitis in patients with hyperparathyroidism.

In hypertriglyceridemia (HTG) related CP, Chang et al (14) have shown a higher frequency of *CFTR* gene mutations suggesting that the mechanism of pancreatitis may be related to genetic predisposition. In their study of 126 HTG patients, 13 (10.3%) carried a *CFTR* mutation (all were p.I556V), the *CFTR* gene mutation rate was significantly higher in those with than those without pancreatitis (26.1% (12 of 46) vs. 1.3% (1 of 80); *P* <0.0001). A multivariate analysis of HTG patients indicated that triglycerides, *CFTR* 470Val, and *TNF* promoter 863A were independent risk markers for HTG associated pancreatitis.

There is considerable controversy whether or not pancreas divisum causes recurrent pancreatitis (72). In patients with pancreas divisum presenting with recurrent pancreatitis, a study showed lower nasal transepithelial potential difference suggesting a functional defect in the *CFTR* gene to account for the risk of pancreatitis in pancreas divisum (32). Another case report showed presence of minor *CFTR* mutations in 2 patients with PD presenting with recurrent pancreatitis (22). Another study showed *SPINK1* gene mutations were significantly associated with pancreas divisum associated with pancreatitis compared with controls. *SPINK1* mutations were present in 38% of patients with pancreas divisum and recurrent pancreatitis compared with 2% in healthy controls suggesting that pancreas divisum alone is unlikely to cause pancreatitis and pancreatitis may be a result of both genetic predisposition and anatomical defect, a 2-hit theory (31).

Genetic mutation not found to be associated with CP
Polymorphisms in the tumor necrosis factor (TNF) promoter region and the entire coding region of the corresponding TNF receptor 1 (*TNFR1*) gene

were not associated with hereditary, familial, or idiopathic CP (77).

Functional polymorphisms in the transforming growth factor-beta1 gene, interleukin-10 gene, and in the interferon-gamma gene were not found to be associated with hereditary, familial or sporadic pancreatitis (74).

Mutation in the genes coding for glutathione s-transferases - MGST1, and GSTM3 genes or common deletions in the GSTT1 and GSTM1 genes were also not associated with hereditary pancreatitis (78).

Keratin 8 gene mutation was not found to be associated with either hereditary or idiopathic CP (75).

8. Future prospects

Although there has been significant gain in our understanding of the genetic predisposition in patients with CP, there are equally significant gaps in our knowledge. Thus, currently known genetic mutations are associated with 50-60% of cases in idiopathic CP (42, 58). Furthermore, the causative role of genetic mutation in the initiation and progression of pancreatitis is also not clear. For example, the *SPINK1* p.N34S mutation, which is the commonest mutation reported in patients with CP, does not result in any functional loss of enzyme activity. How this leads to pancreatitis is unknown. Whether it is just a bystander or modifier and not the causal mutation remains to be determined. In alcohol related pancreatitis, it is not known why only <5-10% of alcoholics develop pancreatitis. The genetic predisposition to alcohol related pancreatitis has so far not yielded much information.

The modest effect of common variation, the basis of current GWAS screening technology, on many human diseases as well as related traits is turning interest to studies on rarer variants with larger effects on disease outcome. Thus stringent

selection of clinical phenotypes and prioritization of smaller patient cohorts for direct whole genome sequencing might be the best solution to identify putative causative variants.

9. References

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