Genetic Factors in Pancreatitis

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Abstract

The understanding of pathogenesis of acute and chronic pancreatitis has benefited from the progress made in genetic investigations. The discoveries of the gain of function mutations of cationic trypsinogen gene (PRSS1) and the loss of function mutations of pancreatic secretory trypsin inhibitor (SPINK 1) or other potential defects in genes that regulate pancreatic secretory function or modulate inflammatory response to pancreatic injury has changed our current concepts on the pathogenesis of pancreatitis.

Genetic factors play an important role in the susceptibility to pancreatic injury, severity and evolution of inflammatory process, leading in some cases to chronic inflammation and/or fibrosis. Acute pancreatitis is viewed as an event and chronic pancreatitis as a process, sequentially linked, reflecting a complex interaction between genetic and environmental factors.

Key words

Pancreatitis - PRSS1 mutations - SPINK 1 mutations - CFTR - immune modulating genes

Rezumat

Progresele înregistrate în genetică au condus la o mai bună înțelegere a patogenezei pancreatitei acute și cronice. Descoperirea mutațiilor genei tripsinogenului cationic (PRSS1) și a inhibitorului secretor pancreatic al tripsinei (SPINK 1) sau a altor defecte potențiale ale genelor care reglează funcția secretorie pancreatică sau modulează răspunsul inflamator pancreatic la agresiune a schimbat concepțiile asupra patogenezei pancreatitei.

Factorii genetici joacă un rol important în susceptibilitatea față de agresiunile pancreatice, severitatea și evoluția procesului inflamator, conducând în unele cazuri la inflamații cronice și/sau fibroză. Pancreatita acută este concepută ca un eveniment, în timp ce pancreatita cronică ca un proces, legate secvențial, reflectând o interacțiune complexă între factorii genetici și cei de mediu.

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Introduction

Chronic pancreatitis (CP) is a progressive inflammatory disease, characterized by three main features: *clinically*: recurrent or persisting abdominal pain (but possibly even without pain); *morphologically*: a progressive process which leads to destruction of pancreatic parenchyma with inflammation and irregular sclerosis (focal, segmental or diffuse), accompanied by ductal dilatation, strictures or stones and *functionally*: progressive loss of exocrine and endocrine function (1-3).

The American Gastroenterological Association has elaborated a working classification of risk factors and etiologies of chronic pancreatitis, according to its mechanisms and prevalence: TIGAR-O classification system (**T**oxic-metabolic, **I**diopathic, **G**enetic, **A**utoimmune, **R**ecurrent and severe acute pancreatitis, **O**bstruction) (4).

About 10-30% of patients with chronic pancreatitis, even after ERCP, do not have an apparent underlying cause, and are classified as *idiopathic pancreatitis*. Some of them have a history and a clinical course compatible with hereditary pancreatitis.

In 1952, Comfort and Steinberg, studying some families suggested that CP may be an inherited disease (5). Since the first description, a great deal of research has identified the clinical features of hereditary pancreatitis: the autosomal dominant inheritance pattern, attacks of acute pancreatitis which usually begin in childhood, the variable spectrum of severity of disease (from mild abdominal discomfort to severe episodes of pancreatic necrosis), sequentially development of CP, which follows recurrent attacks of acute pancreatitis and lack of other identifiable aetiologies (6-8).

After the description of an autosomal dominant mutation in the cationic trypsinogen gene on chromosome 7 in kindreds with hereditary pancreatitis (9, 10), the genetic investigations led to the identification of a number of other potential defects in genes that inhibit pancreatic tryptic activity, regulate pancreatic secretory function or modulate the inflammatory injury (11).

There are at least four major genetic factors, which may contribute to the development of pancreatitis (11):

- mutations in the cationic trypsinogen gene (PRSS1);
- mutations in the pancreatic trypsin inhibitor gene (SPINK1);
- mutations in the cystic fibrosis tansmembrane conductance regulator gene (CFTR) and
- polymorphism in other genes, especially genes regulating the inflammatory response (TNF, IL-1, IL-10).

Mutations in the cationic trypsinogene gene (PRSS1)

Human pancreatic juice contains 3 isoforms of trypsinogen, which on the basis of their electrophoretic mobility are named: cationic trypsinogen (PRSS1), anionic trypsinogen (PRSS2) and mesotrypsinogen (12, 13). Cationic trypsinogen is the major isoform and represents about two-thirds of total trypsinogen, with anionic trypsinogen one-third, whereas mesotrypsinogen accounts for less than 5% of trypsinogen.

Genetic studies show that there are 8 trypsinogen genes embedded in the beta T-cell receptor locus or cluster of genes (TCRB), mapping to 7q35, T_1 through T_8 (14). The gene encoding trypsin-1 (TRY1) is referred as serine protease-1 (PRSS1) and is located on the long arm of chromosome 7:7q35 (10, 15).

Trypsin, the major pancreatic protease is a serine protease made by two globular protein domains connected by a single side chain, named autolysis loop, on the surface opposite the active site (16). Activation of trypsinogen into trypsin supposes cleavage of a short exposed peptide chain (trypsinogen activation peptide) by the action of enterokinase or of an other trypsin molecule. The molecule of trypsin contains also a calcium binding pocket situated near the side chain which connects the two globular domains.

The side chain contains an arginine residue at amino acid R122, which is the target for the attack by other molecule. Enzymatic cleavage of the side chain at R122 by the second trypsin produces a rapid inactivation of the first trypsin molecule by autolysis. The autolysis loop is flexible and R122 comes close to the calcium binding pocket and as the concentration of calcium rises, calcium enters the calcium pocket and limits the exposure of R122 to the enzymatic attack by another trypsin (17, 18).

Thus calcium plays a central role not only in trypsin secretion but also in trypsin stabilization, because trypsin is susceptible to rapid autolysis within the acinar cells, where calcium levels are low, but protected from autolysis after active secretion into the pancreatic duct system and duodenum, where calcium levels are high (19).

Acute pancreatitis is an event that develops after intrapancreatic trypsinogen activation, which in turn converts all proteolytic proenzymes to their active forms, resulting in pancreatic autodigestion (20).

In physiological conditions, the exocrine pancreas incorporates redundant protective systems at different levels in order to control the activity of enzymes able to induce parenchymal autodigestion: digestive enzymes are synthetized intracellulary, transported and secreted as inactive proenzymes or zymogens; during their intracellular traffic the proenzymes are contained in the structures isolated by phospholipid membranes and are segregated from lysosomal enzymes (the phenomenon of compartmentalization); acinar cells produce a secretory trypsin inhibitor (SPINK1), which prevents trypsin-catalyzed premature activation of zymogen within pancreas and pancreatic duct; R122 dependent trypsinogen/trypsin autolysis; control of intraacinar cell calcium levels to facilitate autolysis; flushing of zymogens from the ductal system into the intestine in a sodium-bicarbonate rich fluid, produced by the duct cells; the activation of trypsin is produced only at the intestinal level, under the action of enterokinase (16, 21, 22).

Acute pancreatitis develops after the breakdown of the critical protective mechanisms.

In experimental acute pancreatitis, the activation of trypsinogen requires the co-localization of zymogen and cathepsin B (23). In humans, cationic trypsinogen has several peculiarities: it has a higher concentration, has the ability to autoactivate; the activation may occur in very different locations and under different circumstances and cationic trypsin is more resistant to autolysis than anionic trypsinogen, especially in the presence of raised calcium concentration (24, 25, 26).

In families with hereditary pancreatitis over 20 mutations were discovered in the cationic trypsinogen gene (PRSS1) (27, 28, 29), which are *gain of function mutations* and interfere with autolysis and/or cause premature trypsinogen activation (26, 30).

The first and the most common PRSS1 mutation identified was R122H (a substitution of arginine by hystidine at residue 122) in the third exon of cationic trypsinogen (27). This mutation eliminates the initial hydrolysis site, rendering trypsinogen and/or trypsin resistant to autolysis and permanent inactivation. In this way, when trypsinogen is activated to trypsin within the gland in quantities that exceed the inhibitory capacity of SPINK1, and the trypsin remains active by R122H mutation, then trypsin could activate all other proenzymes, initiating pancreatic autodigestion (26).

Another mutation is N29I (a substitution of asparagine by isoleucine) at codon 29 (28, 31). It is hypothesized that N29I substitution alters the conformational structure of cationic trypsinogen, blocking the cleavage site or reducing its ability to bind to the trypsin inhibitor (28).

The finding of identical mutations (R122H and N29I) in Caucasians and Japanese families with hereditary pancreatitis indicated heterogeneity, but not racial specificity in cationic trypsinogen gene mutations in patients with this disease (32).

From the clinical point of view, patients with R122H mutation have a more severe course of disease and an earlier age of onset compared to patients with N29I mutation (32).

Patients with PRSS1, R122H or N29I mutation have hereditary pancreatitis, inherited by autosomal dominant pattern, with 80% phenotypic penetrance (6,7,34). The disease is characterized by: recurrent episodes of pancreatitis beginning usually in childhood, but the age of onset may range from infancy to the third or fourth decade of life or even later; equal gender distribution; a family history of at least two other affected members and lack of other precipitating factors: alcohol, gallstones (8). Half of the subjects with acute pancreatitis develop CP, with calcified stones in the pancreatic duct and pancreatic cancer is increased 50-70 fold over the prevalence in general population, with a 40% cumulative risk by the age of 70 years (16,34).

It is important to emphasize that a different clinical course in subjects with hereditary pancreatitis involves both environmental and genetic modifiers and even identical twins with PRSS1 mutations can be discordant for affected status (an argument for environmental triggers), but similar in course when both are affected (an argument for modifier genes) (16,36).

Another mutation identified was A16V (substitution of alanine by valine at position 16), a mutation placed at the signal peptide cleavage site involved in the intracellular processing of trypsinogen (37). A16V mutation shows a low penetrance and is found in patients without a family history of CP, suggesting that PRSS1 mutations do not follow exclusively an autosomal dominant inheritance pattern. Because of the low penetrance of A16V mutation and the association with a mutation in CFTR gene it is supposed that A16V mutation may function as part of a polygenic process (11).

Several other PRSS1 mutations were described: - 28 del TCC, D22G, K23R, N29T, P36R, G83E, K92N, L104P, R116C, R122C, V123M, C139F, most of them with the exception of R116C and R122C in a single patient or family (38-43). The significance of these mutations remains uncertain.

Recently a new mutation PRSS1: E79K was identified in three European families affected by sporadic or familial pancreatitis. Although E79K mutation introduces a potential new tryptic cleavage site, catalytic activity of E79K trypsin was normal and its inhibition by PSTI was unaffected. The potential mechanism of pancreatitis in subjects with this mutation consists in the fact that E79K trypsin activates anionic trypsinogen (PRSS2) two fold better than wild type cationic trypsin, while the common mutants R122H and N29I have no such effect (44).

The true frequency of PRSS1 gene mutations in the setting of idiopathic pancreatitis is unclear. A study, which reviews data from previous studies, shows that the mean prevalence of trypsinogen gene mutations in 1319 patients with idiopathic pancreatitis was 1.9% with a range of 0.2 to 10% among individual studies and the frequency correlated inversely with the size of the study group (45).

Extensive studies concerning the role of cationic trypsinogen mutations in the development of alcoholic (36, 40, 42, 46) or tropical pancreatitis (47, 48) show that these mutations have generally been found in only 0 to less than 1% of patients in the absence of a suggestive family history.

It is important from the practical point of view to make the distinction between *hereditary pancreatitis* and *familial pancreatitis*.

The term *hereditary pancreatitis* should be restricted to patients with gain of function PRSS1 mutations (49) or unexplained pancreatitis in a subject from a family in which pancreatitis phenotype seems to be inherited in an autosomal dominant pattern (5, 50).

The term *familial pancreatitis* is used for pancreatitis from any cause that occurs in a family with an incidence that is greater than would be expected only by chance (50).

The distinction is important from the prognostic, genetic counselling and family planning perspectives (16).

Indications for genetic testing for PRSS1 mutations

PRSS1 genetic testing is recommended according to consensus report in symptomatic patients in the following circumstances:

- recurrent (two or more separate documented episodes of typical pain with hyperamylasemia) attack of acute pancreatitis for which there is no explanation;
- unexplained (idiopathic) chronic pancreatitis;
- family history of pancreatitis in a first degree (parent, sib, child) or second degree (aunt, uncle, grandparent) relative;
- an unexplained episode of documented pancreatitis occurring in a child that has required hospitalization and where there is significant concern that hereditary pancreatitis should be excluded;
- as part of an approved research protocol (51).

Genetic testing in children under 16 years for PRSS1 mutations is indicated after:

- an episode of documented pancreatitis of unknown aetiology and severe enough to require hospitalization;
- two or more documented episodes of pancreatitis of unknown aetiology;
- an episode of documented pancreatitis occurring in a child where a relative is known to carry a hereditary pancreatitis mutation;
- a child with recurrent abdominal pain of unknown aetiology where the diagnosis of hereditary pancreatitis is a distinct clinical possibility;
- chronic pancreatitis of unknown aetiology, where the diagnosis of hereditary pancreatitis is a distinct clinical possibility (51).

The significance of a positive test should be explained to the subjects who carry the major mutations in order to know the autosomal dominant mode of inheritance, incomplete penetrance, variable clinical course and the strategies to prevent episodes of acute pancreatitis avoiding the concomitant risk factors: alcohol, drugs, metabolic disturbances. Patients with hereditary pancreatitis phenotype should undergo radiological and endoscopic evaluation in order to identify and treat risk factors, mainly choledocolithiasis and other obstructive factors, which may contribute to attacks of acute pancreatitis (34).

Taking into consideration the risk of pancreatic cancer in patients with major PRSS1 mutation (R122H or N29I), the patients should be advised to avoid tobacco smoking (52).

Also, the patient's relative who may be at risk, should receive counselling and possibly genetic testing for family members if warranted (16).

SPINK 1 mutation

The acinar cells secrete a pancreatic secretory trypsin inhibitor (PSTI) also named **S**erine **P**rotease **In**hibitor **K**azal Type **1** (SPINK 1) (53).

The gene encoding SPINK 1 is located on the long arm of chromosome 5: 5q32, is approximately 7.5 Kb long and has 4 exons and 3 introns (54).

The role of SPINK 1 is the prevention of trypsin-catalyzed premature activation of zymogen within the acinar tissue or pancreatic duct system. SPINK 1 has a reactive site that serve as a specific target substrate for trypsin (55). SPINK 1 can inhibit up to 20% of the tryptic activity in pancreas and is the first line of defence in protecting the pancreas against the autodigestion generated by activation of trypsinogen to trypsin within the gland (11). But trypsin inhibition by SPINK 1 is only temporary because the trypsin- SPINK 1 complex may serve as substrate for trypsin, followed by a subsequent degradation of the inhibitory molecule and the restoration of trypsin activity (56).

Contrary to PRSS1 mutations which generate gain of function mutations, SPINK mutations cause a *loss of function mutations*, decreasing protease inhibition (43).

There are several mutations in the SPINK 1 gene, the most common being a missense mutation, consisting of a substitution of asparagine by serine at codon 34:N34S (57, 58, 59). N34S mutation was found especially in patients without a family history of chronic pancreatitis. In a reference study carried out on 96 unrelated children or adolescents with chronic pancreatitis, 18 of them (23%) carried the mutation N34S and 6 were homozygous for the mutation, but N34S mutation was not found in any of the 52 normal control subjects (57). The association between N34S mutation and CP was confirmed in other studies.

It is estimated that 15-40% of patients with *idiopathic pancreatitis* carry N34S mutation on one or on both alleles (58, 59). N34S is in complete linkage disequilibrium with other four intronic sequence variants: IVS1-37T>C, IVS2+286A>G, IVS3-604G>A, IVS-66-65 insTTTT, giving rise to a number of 7 polymorphisms (60).

Moreover, other mutations of SPINK 1 gene were identified: a homozygous promoter mutation (-215G-A and -215 G-T), a mutation at the level of start codon which destroys the only translation initiation codon of SPINK1 (2 T-C, met 1 to thr; M1T) (57) and other additional SPINK 1 mutations, reported in single patients or families: -53C>T; -41G>A; -2C>A; L14P; D50E; IVS3+2 T>C; IVS3+125 C >A; IVS3+184 T>A; R65Q; R67C (57, 58, 60, 61).

It is interesting to note that the SPINK 1 mutations were relatively common in the general population: about 2% and N34S occurred in 0.77% (43, 58).

An association of SPINK1 N34S mutation and *alcoholic pancreatitis* was also found: a heterozygous N34S mutation was identified in 5.8% of alcoholic chronic pancreatitis, but in only 0.8% of healthy control subjects and in 1% of alcoholic control subjects (62), suggesting the role of the combination of exogenous and genetic factors in the predisposition to alcoholic pancreatitis.

The association between SPINK 1 mutations and CP is more evident for *tropical chronic pancreatitis*. One study shows that N34S mutation was found in 20% of the patients with tropical calcific pancreatitis and in 55% of those with fibrocalculous pancreatic diabetes, compared with only 1.3% of control subjects (63, 64).

Although the SPINK1 N34S allele is associated with multiple types of acute and chronic pancreatitis, the association is weak, only < 1% of carriers developing pancreatitis (58, 65).

Moreover, no phenotypic differences between homozygous, heterozygous or compound heterozygous genotypes concerning the age of onset and the severity of pancreatitis were found and the pattern of inheritance was neither autosomal dominant nor recessive (11, 58).

Different SPINK 1 mutations may lead to different inheritance patterns. Because M1T mutation destroys the start codon and results in a null allele, this mutation gives rise to a dominant inheritance (57). The N34S SPINK 1 mutations decrease the SPINK capacity resulting in recessive or complex trait (66). Some authors suggest that SPINK 1 mutations are not sufficient alone to initiate pancreatitis but rather may act as a disease modifier gene or as a susceptibility factor for a polygenic complex trait, lowering the threshold for initiating pancreatitis or contributing to the severity of the disease caused by other genetic defects or environmental factors (16, 43, 58).

The effect of SPINK 1 mutations depends on the rate of pancreatic SPINK reduction. In the homozygous state, N34S mutation may initiate pancreatitis, but in the heterozygous state it may cause only a subliminal lowering of SPINK 1 and requires additional exogenous or endogenous factors to initiate disease (43).

A new possibility of such interaction was identified between SPINK 1 and mutations in the calcium sensing receptor gene. These mutations produce an autosomal dominant disease with nearly 100% penetrance, characterized by

familial hypocalciuric hypercalcaemia. Patients carrying these mutations and N34S SPINK 1 allele mutation have numerous episodes of pancreatitis and can develop CP, a suggestive example of an additional genetic factor required to initiate pancreatitis in patients with heterozygous SPINK 1 N34S mutation (67).

Cystic fibrosis transmembrane conductance regulator (CFTR) mutations

Human CFTR is located on the long arm of chromosome 7: 7q31, spans about 250 Kb and contains 27 exons (68). CFTR belongs to the ATP binding cassette superfamily and encodes a transmembrane protein present at the surface of most epithelial cells, functioning as a cyclic adenosine monophosphate chloride channel (69, 70).

CFTR conducts both chloride and bicarbonate and is essential for normal bicarbonate secretion by pancreatic duct cells, which have the function of a protective mechanism centering on flushing and draining secretion, because the luminal concentrations of calcium is relatively high and the protection of the pancreas by autolysis of trypsin at R122 is no longer possible (22, 43).

More than 1200 CFTR gene polymorphism have been reported which can be divided into six classes, based on the functional consequences of the polymorphisms on channel function:

- class I-III mutations are severe ($CFTR^{sev}$), comprising:
  - class I: defective protein synthesis (R553X, W1282X, 3950 del T);
  - class II: abnormal processing trafficking (del 508, N1303K);
  - class III: defective activation (G551D) and all result in functional loss of CFTR from the epithelial cell surface;
- class IV mutations (R117H, R347P, D1152H) are mild-variable mutations ($CFTR^{m-v}$) and result in reduction but not absence of channel ion conductance;
- class V mutations (3849+10KbC >T) diminish protein synthesis or stability and
- class VI mutations may affect the regulatory function of CFTR on other ion channels (71-73).

The increased frequency of CFTR mutations has been reported in up to 30% of patients with idiopathic chronic pancreatitis (74, 75, 76). The frequency of CFTR mutations in these patients was six times higher than expected, whereas the frequency of the 5 T allele was twice as high. Also, the frequency of CFTR mutations in alcohol related CP was two times higher than expected (74).

Some of these mutations involve a single allele, whereas others are a combination of a severe and a mild mutation or either type of mutation with the 5T allele in intron 8, which reduces the amount of functional CFTR (11).

The studies carried out in patients with idiopathic pancreatitis to assess the risk associated with CFTR mutations revealed that compound heterozygous patients had a 40 fold increased risk for pancreatitis (78).

There are several distinct situations.

1. The most severe genetic risk is associated with $CFTR^{sev}/CFTR^{sev}$ genotype, which determines typical cystic fibrosis and the pancreas histology includes all of the features of chronic pancreatitis.

2. Patients with genotype $CFTR^{sev}/CFTR^{m-v}$ develop *atypical cystic fibrosis* having a residual CFTR function (less than 10% of normal); these patients are at increased risk of pancreatitis (approximatively 80 fold), but most of them do not develop the disease (74, 75, 79). Recurrent acute pancreatitis and CP occur only in the context of another environmental or genetic risk factors.

3. A distinct category is the presence of heterozygous CFTR mutations, which increase the risk of recurrent acute and CP. In this category there are also three possible situations:

- patients with $CFTR^{m-v}/ CFTR^{m-v}$ genotype and atypical cystic fibrosis, associated with mutations that are not included in cystic fibrosis screening panels, which increase the risk of pancreatic injury. These specific mutations appear to disrupt the function of exon 9 and 10 of the CFTR gene product;
- patients with a complex disorder such as polygenic CFTR-SPINK 1 pancreatitis, with heterozygous CFTR and heterozygous SPINK 1 mutations occurring together;
- patients with some specific CFTR variants which may increase the susceptibility to environmental factors, especially to alcohol (16).

Two studies which analyzed the complete CFTR coding sequence and PRSS1 and SPINK 1 mutations in CP showed that 25 to 30% of patients carried at least one CFTR mutations (without 5T allele) and several patients were compound heterozygous for a CFTR mutation or were trans – heterozygous for a CFTR mutation and a mutation in SPINK 1 or PRSS1 (78, 80). The combination of two CFTR mutations and a N34S mutation increased the risk of pancreatitis 900 fold (11).

These studies emphasize the significance of a combination of different mutations in different genes in inherited pancreatitis.

Indications for genetic testing for SPINK 1 and CFTR mutation

Taking into consideration that the identification of heterozygous SPINK 1 or CFTR mutations does not explain the disease in a subject, which has been diagnosed with pancreatitis and does not predict the possibility of developing pancreatitis in an unaffected person, the genetic testing for these mutations is currently considered as premature (51, 81, 82).

Other genes associated with chronic pancreatitis

UDP glucuronosyltransferase (UGT1A7)

UDP glucoronosyltransferases are a family of phase II enzymes involved in metabolism, dezoxification and some

other aspects of cellular defense. Only the UGT1A7 gene is expressed in high levels in the pancreas from the major isoform of this superfamily. The UGT1A7 gene has common single nucleotide polymorphisms which are linked as three polymorphic UGT1A7 alleles: UGT1A7*2, UGT1A7*3 and UGT1A7*4 (83). The UGT1A7*3 haplotype determines a significant decrease in UGT1A7 enzyme activity. Several lines of evidence suggest the implication of this polymorphism in pancreatitis: UGT1A7*3 allele was found in 21% of control population, but in 37% of alcohol pancreatitis (89% of them being smokers); there is a trend toward chronic pancreatitis in non-alcoholic patients with UGT1A7*3 allele that are SPINK1 N34S negative, but not in patients SPINK 1 N34S positive (58).

These findings suggest the role of a common polymorphism in drug metabolizing genes associated with CP, possibly associated with alcohol or tobacco smoking.

Alcohol metabolising genes

Extensive studies have not found the association between alcoholic pancreatitis and mutations in the major alcohol metabolising gene: alcohol dehydrogenase 2, aldehyde dehydrogenase 2, alcohol dehydrogenase 3, cytochrome P450-2E1 (CYP2E1), glutathione S-transferase M1 (GSTM1), GSTT1 (4, 84-87).

Concerning the role of the GST family of genes, the only finding was that the GSTM1 null genotype seems to protect a subset of subjects (women under the age of 50 years) from alcoholic chronic pancreatitis (88).

Polymorphisms for genes involved in the regulation of the inflammatory response

Genetic polymorphisms, that is variations in gene structure found in a population may be important factors in determining the severity of pancreatitis by their effect on the inflammatory response.

Several cytokines such as: IL-1, IL-6, IL-10, TNF-α are involved in modulating inflammation and fibrosis in CP, through actions on the pancreatic stellate cells (89). The only finding was that the TNF-238A promoter polymorphism may be a modifier gene, accelerating CP in patients with underlying trypsinogen mutations (90).

Despite the intensive studies, many of the antinflammatory mediator IL-10 gene polymorphisms have been excluded as playing a major role leading to CP.

Conclusions

The current concepts on pathophysiology of pancreatitis is that acute pancreatitis is an event and CP is a process, and that environmental, genetic and structural factors play a distinct role in this complex phenomenon.

The new acquisitions concerning the role of genetic factors in susceptibility to pancreatitis, the severity and nature of the inflammatory process and the likelihood of various complications, lead to an important conceptual change in the understanding of CP as a complex, multifactorial disorder (22).

In a new conceptual model, CP requires three hits to develop: environmental stressors, recurrent pancreatic injury from trypsinogen activation and an inappropriate immunological response, leading to chronic inflammation and/or fibrosis.

These factors have been systematized into the SAPE model hypothesis (**S**entinel **A**cute **P**ancreatitis **E**vent), a model which organized the factors associated with CP according to a hypothetical pathway that lead from an episode of acute pancreatitis towards CP (16, 92). According to this model, the first episode or sentinel episode of acute pancreatitis initiates the recruitment and activates the immune cells and stellate cells, that have a major role for the development of chronic inflammation and fibrosis.

Genetic factors that are much more common than originally envisioned may play a major role in determining multiple episodes of recurrent pancreatitis: mutations in the cationic trypsinogen gene are capable of initiating pancreatitis, while mutations in SPINK1 and the CFTR gene act in concert with other genes and by a complex interaction with environmental factors. The predominant clinical feature of inherited pancreatitis is a relapsing or recurrent pancreatitis, starting in childhood (16).

Multiple episodes of recurrent pancreatitis may lead in a particular genetic and immunological context to chronic pancreatitis. Thus, acute and chronic pancreatitis seem not to be different nosological entities, but rather different stages of one dynamic disease process.

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