Genetic aspects of chronic pancreatitis: insights into aetiopathogenesis and clinical implications[1]

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Summary

The recent genetic discoveries in CP support the hypothesis that inappropriate intrapancreatic activation of zymogens by trypsin results in autodigestion and pancreatitis. Two different protective mechanisms prevent activation of the pancreatic digestive enzyme cascade. First, SPINK1 inhibits up to 20% of potential trypsin activity and, second, trypsin itself activates trypsin-like enzymes readily degrading trypsinogen and other zymogens. Pancreatitis may therefore be the result of an imbalance between proteases and their inhibitors within the pancreatic parenchyma. The discovery of PRSS1 mutations in families with CP was the first breakthrough in the understanding of the underlying genetic mechanisms. Enhanced trypsinogen activation may be the common initiating step in pancreatitis caused by these mutations. The discovery of SPINK1 mutations underlines the importance of the protease inhibitor system in the pathogenesis of CP. Thus, gain-of-function in the cationic trypsinogen resulting in an enhanced autoactivation, or loss-of-function mutations in SPINK1 leading to decreased inhibitory capacity, may similarly disturb the delicate intrapancreatic balance of proteases and their inhibitors.

The recent findings of SPINK1, CFTR, and PRSS1 mutations in CP patients *without* a family history have challenged the concept of idiopathic CP as a non-genetic disorder and the differentiation between HP and ICP. There is a clear mode of autosomal dominant inheritance for some mutations (R122H, N29I, possibly M1T), whereas the inheritance pattern (autosomal recessive, complex, or modifying) of other mutations (A16V, N34S) is controverted or unknown. The lack of mutations in the above-mentioned genes in many patients suggests that CP may also be caused by genetic alterations in yet unidentified genes. Evaluation of CP patients without an obvious predisposing factor, e.g. alcohol abuse, should include genetic testing even in the absence of a family history of pancreatitis.

Finally, identification of further disease-causing genes will create a better understanding of pathogenesis and may help to develop specific preventive and therapeutic strategies.

Key words: chronic pancreatitis; trypsin; SPINK1

Introduction

Chronic pancreatitis (CP) is an inflammatory disease causing structural and functional damage resulting in exocrine and endocrine deficits. The most common aetiological factor in industrialised countries is long-term alcohol abuse, but in 10–30% of cases the cause remains unknown. An aetiologically based classification of CP is important, since the aetiology has a major impact on the course and outcome of the disease. The pathogenetic mechanisms of CP are poorly understood and it is not known whether a common factor exists for different types of CP. Recent genetic discoveries have added much to our understanding of CP. This report will focus on the genes involved, the proposed pathophysiology, and future perspectives.

Chronic pancreatitis

Chronic pancreatitis (CP) covers a broad spectrum of clinical and morphological features. Pain of varying severity and duration is a leading clinical symptom in up to 90% of patients [1]. Chronologically, CP is usually manifested in the early stage by recurrent episodes of acute pancreatitis (AP) and eventually evolves over years into a late painless stage dominated by progressive pancreatic dysfunction and/or pancreatic calcification [1]. However, there is also a subgroup of primarily painless CP with initial clinical manifestations typical of end-stage CP [1]. Morphologically, prominent features of early-stage CP are necrosis, distinct interlobular fibrosis and pseudocysts, whereas in late-stage CP acinar destruction, severe widespread (intralobular) fibrosis, variable pancreatic duct lesions and ductal stones are characteristic findings [2].

The reported incidence of CP in industrialised countries has been estimated at 3.5–10 per 100'000 inhabitants [3]. Therapy, which is not further discussed here, usually consists of pain management, enzyme replacement, and management of diabetes mellitus and obstructive complications. Alcoholic CP is associated with a mortality rate approaching 50% within 20–25 years due to malnutrition, severe infections, diabetes, other CP-associated complications, and alcohol- and nicotine-related diseases [4].

The diagnostic gold standard for early-stage CP is adequate (surgical) biopsy, which is rarely available. Since the primary lesions of early-stage CP are usually focal, fine-needle biopsies tend to yield false-negative results. Furthermore, the overlap of clinical and morphological features between acute and chronic pancreatitis renders early diagnosis of CP difficult. The diagnostic accuracy of modern imaging techniques in early-stage CP, ie ERCP, ultrasound, endoscopic ultrasound, CT or MRI has not yet been validated against histopathology as the gold standard [3]. In clinical practice, therefore, definite diagnosis of CP should be deferred until the disease has reached an advanced stage in which diagnosis is possible with high accuracy based on the presence of the typical markers of CP, including pancreatic calcifications and/or persistent pancreatic dysfunction [5]. This "wait-and-watch" approach is not generally accepted but reflects the present imperfect state of the art due to the lack of appropriate and generally accepted criteria of "early" or "mild" CP.

Aetiology

Important for the initial classification of recurrent AP is proper assessment of the aetiology. In up to 80% of cases AP is caused by gallstones or alcohol abuse [6]. The impact of aetiology on disease course in recurrent AP is evident, since pancreatitis caused by gallstones, unlike alcoholic pancreatitis, virtually never progresses to CP. The interval between the onset of alcoholic AP and definite alcoholic CP is 4.8–5.5 years [5]. Thus, it is likely that a large percentage of alcoholic AP evolves into alcoholic CP with prolonged follow-up.

A large amount of data on the various putative predisposing (aetiological) factors and on the impact of aetiology on the natural course of CP has accumulated up to the present. Major predisposing (aetiological) risk factors for the development of CP may be categorised according to the "TIGAR-O" system, namely (1) toxic-metabolic; (2) idiopathic; (3) genetic; (4) "autoimmune"; (5) recurrent and severe AP; and (6) obstructive [3]. In industrialised countries the most common aetiological factor in CP is long-term alcohol abuse, whereas in 10–30% of cases there is no apparent underlying cause and these cases are classified as idiopathic CP (ICP) [7]. In our own series, CP was caused by alcohol abuse in 70%, was idiopathic in 24%, and had rare causes in 6% [8].

Pathogenesis

The absence of effective preventive and therapeutic strategies in CP reflects the lack of understanding of the pathogenetic mechanisms. It is unknown whether common factors exist in different types of CP [9]. One difficulty is the lack of animal models which mimic the human form of the disease. Numerous animal models, ie pancreatic duct obstruction, secretagogue hyperstimulation, diet-induced models etc, have been developed to define the pathogenetic mechanisms in various forms of AP and CP [10]. However, when successful therapeutic agents observed in animal models were administered to humans, treatment failure usually occurred. Steinberg et al. reported an 81% improvement in survival in 25 animal studies of AP, whereas in 13 human studies using the same therapeutic agents only 7.7% of patients showed a positive outcome as to survival [11]. It is believed that the experimental methods used to induce AP in animal models do not mimic the pathogenetic mechanisms of human disease. The recent discovery of genes involved in CP provides new insights and working models for CP.

Several pathogenetic concepts have been proposed with regard to the primary site of dysfunction leading to CP, ie, duct cells and acinar cells. According to the "protein-plug hypothesis" introduced by Sarles in 1963, it has been suggested that the primary step is a ductal lesion [12, 13]. Plugs formed by precipitation of protein within intrapancreatic ducts, which may later calcify, perpetuate inflammation and cause upstream acinar atrophy and fibrosis. An alternative hypothesis, originally proposed by Comfort et al. in alcoholic CP and now referred to as the "necrosis fibrosis concept", suggests that repeated attacks of pancreatitis lead to CP [2, 14]. Data supporting this

hypothesis derive primarily from pathological studies and from a recent long-term prospective clinicomorphological study by Ammann et. al. [15]. Additional pathogenetic concepts have been debated, such as sphincter of Oddi dysfunction, direct injury from ethanol or one of its metabolites ("toxic-metabolic hypothesis"), the influence of ischaemia, and the potential role of antioxidants [16, 17].

The relationship between AP and CP is controverted. Under the Marseilles definition it is assumed that AP and CP are two distinct entities and that AP virtually never progresses to CP [13]. According to this concept, AP in alcoholics is regarded as a flare-up on the basis of pre-existent subclinical CP. However, there is evidence that patients with frequent and severe attacks of alcoholic AP show more rapid progression to alcoholic CP and alcohol cessation has been reported to slow down disease progression [18, 19]. Taken together, these observations suggest that in at least a subset of patients progression from AP to CP occurs [5]. The relationship between AP and CP is of great importance since it has a major impact on pathogenetic and therapeutic strategies.

The hypothesis that pancreatitis results from pancreatic autodigestion was first reported more than 100 years ago [20]. Inappropriate activation of pancreatic proenzymes and especially trypsinogen has been thought to play a key role in this process [21]. There is experimental evidence that trace amounts of trypsin, the most abundant protease synthesised by the pancreas, may become physiologically activated within the pancreatic parenchyma. Obviously, the activation of digestive enzymes within the pancreatic parenchyma would be deleterious and lead to autodigestion and AP. Today multiple mechanisms are known to prevent premature uncontrolled activation of the digestive enzyme cascade within the pancreas and to protect the organ from self-destruction [22, 23]. These include (i) synthesis of digestive enzymes as inactive proenzymes (zymogens); (ii) compartmentalisation of proenzymes from other subcellular components within distinct membrane-bound compartments (zymogen granules) to prevent their contact with vital cytosolic structures; (iii) activation of the proenzymes occurring outside the pancreas by the intestinal brush-border enteropeptidase (this initial activating enzyme hydrolyses trypsinogen to form active trypsin, which subsequently catalyses the conversion of all other proenzymes to their active form); and (iv) synthesis of pancreatic trypsin inhibitors such as serine protease inhibitor, Kazal type 1 (SPINK1). SPINK1, also known as pancreatic secretory trypsin inhibitor (PSTI), reversibly inhibits up to 20% of potentially available intrapancreatic trypsin activity by forming a covalent bond between the catalytic serine residue of the enzyme and the reactive site of SPINK1 (figure 1a). If trypsin activity exceeds the PSTI inhibitory potential, a second line of defense is presumed by the ability of trypsin and trypsin-like enzymes such as mesotrypsin to hydrolyse trypsin and other proteases, resulting in a loss of structural integrity and inactivation [23]

Figure 1

Model of chronic pancreatitis.
a. Condition in the normal pancreas: trypsin resulting from autoactivation of trypsinogen within the pancreatic parenchyma is inhibited by SPINK1 and in the second line by mesotrypsin or trypsin. This defence mechanism prevents activation of the pancreatic enzyme cascade within the pancreas and autodigestion.
b. Condition in chronic pancreatitis: mutations in PRSS1 or in SPINK1 lead to an imbalance of proteases and their inhibitors within the pancreatic parenchyma, resulting in an inappropriate and premature conversion of pancreatic zymogens to active enzymes with autodigestion and inflammation. Mutations in CFTR may disturb this balance by intrapancreatic acidification or by defective apical trafficking of zymogen granules, and thus facilitate intrapancreatic activation of digestive enzymes. Dark boxes represent products of mutated genes. Modified from references 39 and 65. AP, activation peptide.

Figure 1a

normal pancreas

Figure 1b

chronic pancreatitis
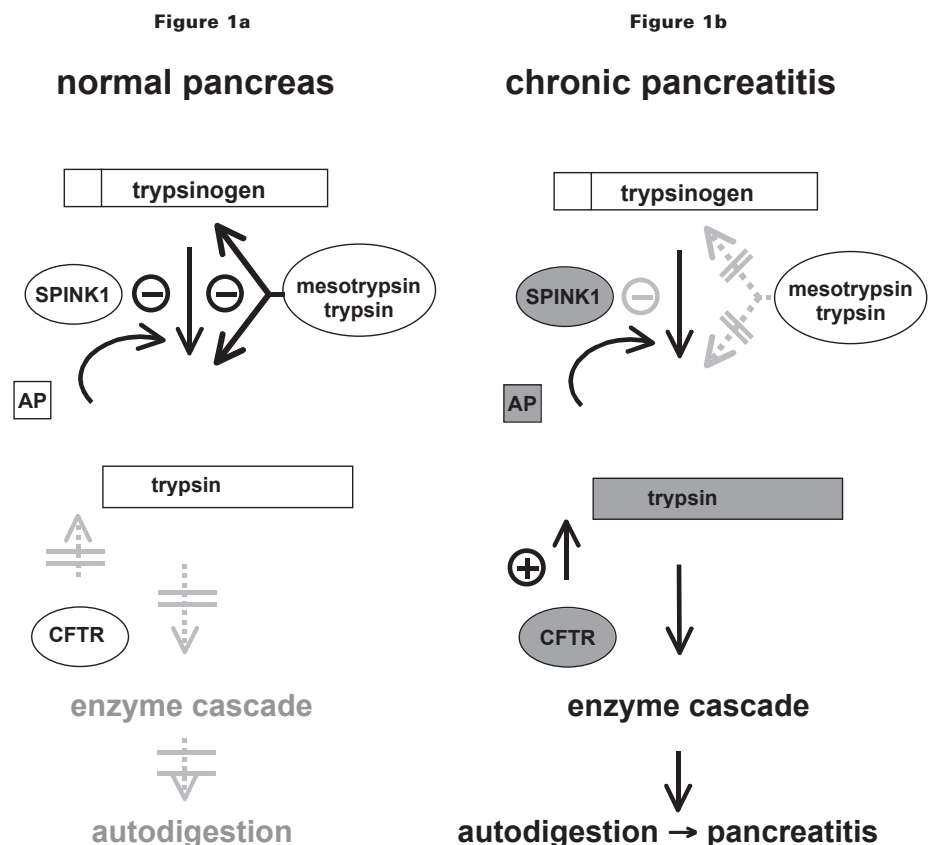
(figure 1a). The multitude of defence mechanisms known so far must be considered in order to understand the relatively rare occurrence of pancreatitis in humans. It appears possible that additional protective mechanisms will be detected. For example, two pancreatic (non-enzymatic) soluble secretory proteins (PAP, pancreatitis-associated protein; PSP, pancreatic stone protein) present in the ductular system precipitate into insoluble fibrils upon trypsin activation [24]. Protection against the potentially damaging activity of trypsin in the duct system is assumed and needs further investigation. This background is essential in discussing the potential mechanisms whereby the recently discovered mutations of the cationic trypsinogen (PRSS1) gene or SPINK1 gene may cause AP and CP.

Hereditary pancreatitis

Hereditary pancreatitis (HP), first described by Comfort and Steinberg in 1952, is a rare form of CP involving at least two or more members of a family [25, 26]. The clinical, laboratory and pathological features are indistinguishable from other forms of pancreatitis. In the literature, HP is referred to as an autosomal dominant disorder with 80% penetrance and variable expressivity [27]. Attacks of pancreatitis usually begin in early childhood but may occur in the second decade or start as late as the sixth decade of life in some cases [27]. Recurrent acute attacks vary from mild abdominal discomfort to severe life-threatening episodes. Progression to late-stage CP with all its complications is observed in many cases [28]. Although the increased risk of pancreatic cancer in patients with alcoholic CP is controverted, strong evidence for this association has been noted in HP. In a study comprising 246 patients with HP, the estimated cumulative risk of pancreatic cancer to age 70 was 40%, but was approximately 70% in patients with a paternal pattern of inheritance [29].

Idiopathic pancreatitis

The distinct differences in the natural history of different types of CP have not always been properly appreciated. For example, it has been shown that ICP comprises two subgroups on the basis of different clinical presentation and natural history [30–32]. Late-onset ICP is characterised by a painless course in up to 70% of patients and presents in the sixth to seventh decade with steatorrhoea and/or diabetes, whereas in early-onset ICP onset of disease is observed in the first three decades. Clinically, early-onset ICP closely mimics HP with recurrent AP in childhood or young adult life [28]. The long-term course of early-onset ICP is, like HP but in contrast to alcoholic CP, characterised by a markedly slower progression to advanced CP [30, 32]. In a clinical comparison of early-onset ICP and HP, the symptoms were similar in both groups but HP presented at a younger age (mean 7 *vs* 12 years) and was accompanied by more frequent complications with the need for surgery [28].

Genetics in chronic pancreatitis

As mentioned above, autosomal dominant inheritance was recognised by Comfort et al. in some families with CP as early as 1952 [25]. Early-onset ICP closely mimics the clinical pattern of HP, suggesting a genetic basis. A genetic background has also been postulated for alcoholic CP based on early reports of familial clustering of the disease and the fact that only 5–10% of alcoholics develop alcoholic CP [33, 34]. Recent developments in molecular genetics have contributed significantly to our understanding of the cause and mechanisms of AP and CP.

Cationic trypsinogen gene (PRSS1)

Using microsatellite linkage analysis, the first HP locus was mapped in 1996 to the long arm of chromosome 7 (7q35) [35–37]. Coincidentally, eight trypsinogen genes were identified and sequenced within this locus as a result of the human genome project and served as candidate genes [38]. Mutational screening analyses identified a single G to A transition in exon 3 of the PRSS1 gene, resulting in a arginine (R) (CGC) to histidine (H) (CAC) substitution at amino acid residue 122 according to the nomenclature system for human gene mutations [39]. This R122H mutation was observed in all affected individuals of 5 HP families but not in 140 unrelated controls.

A second mutation in the PRSS1 gene was discovered in two families with HP without the R122H mutation [40, 41]. In these kindreds a single point mutation in exon 2, an A to T transversion, was identified which resulted in an asparagine (N)(AAC) to isoleucine (I)(ATC) amino acid substitution at residue 29. The N29I mutation results in a clinical syndrome similar to the R122H mutation, although the average age of disease onset is slightly delayed and the disease course less severe. These two mutations have been found in HP families worldwide with the R122H mutation being the most frequent [41–45].

In a study of 44 paediatric patients with CP, a C to T transition in exon 2, leading to an exchange of alanine by valine at codon 16 (A16V), was detected in 4 unrelated patients [46] (figure 2). Three of these patients had no family history of CP although the mutation was inherited in all cases by one parent. Only one of seven first-degree relatives with the A16V mutation was affected, indicating low penetrance of this mutation in contrast to the above-mentioned R122H and N29I mutations.

In the ensuing years, several other PRSS1 mutations (D22G, K23R, -28delTCC) of uncertain significance were detected in single small kindreds [47, 48]. However, a consistent finding in all published series is that several HP patients did not have any PRSS1 gene mutation, suggesting genetic heterogeneity in HP [49]. In a group of Swiss CP patients, 3 clinically HP families were investigated for the R122H, N29I, and A16V mutation. R122H was identified in all affected individuals from 2 HP families but no PRSS1 mutation was detected in two brothers of a third family [45]. No mutations have been identified in other pancreatic digestive enzyme genes, including anionic trypsinogen and mesotrypsinogen, and in the pancreatitis-associated protein in HP families without PRSS1 mutations [50, 51]. Recently a preliminary report suggested a new HP gene on chromosome 12, but this observation needs further confirmation [52].

A still unanswered question is the incomplete disease penetrance of approximately 80% in classical HP [27]. This issue is of great importance since the answer to this problem may provide strategies for rendering PRSS1 mutations harmless. Based on the recently described association of mutations of the cystic fibrosis transmembrane conductance regulator gene (CFTR) with CP (see below), we tested HP patients of our series for CFTR mutations [45]. Analysis of the 31 most common CFTR gene mutations, however, did not reveal any mutation in these patients. The results of a recent study comparing the penetrance and age of onset between monozygotic twins with HP, affected siblings with HP and a comparison group matched for PRSS1-mutation, sex, and age confirmed the 80% penetrance of the pancreatitis phenotype in HP [53]. However, the observation of discordant phenotypic expression between twin pairs suggests that inherited modifier genes or shared environmental factors cannot be the only determinants of penetrance.

The involvement of PRSS1 gene mutations in ICP has been analysed in only a few studies. In our series of Swiss CP patients, 2 out of 16 initially classified clinically as early-onset ICP were shown to have a PRSS1 gene mutation (R122H, A16V) while the remainder tested negative [45]. In another study, no PRSS1 mutations were detected in 20 ICP patients [54]. Witt et al. described the A16V mutation in 3 out of 30 paediatric patients without a family history of pancreatitis [46]. In a very recent mutational screening study of the PRSS1 gene comprising 221 patients with ICP, additional missense mutations were identified in several individual patients [55]. Thus, currently available data suggest that the two most common PRSS1 gene mutations in HP (R122H, N29I) are rarely found in ICP and other mutations of this gene only in a subset of this type of CP. The two subgroups of ICP have not been analysed separately except in our study.

As noted, probably a large percentage of alcoholic AP will progress to alcoholic CP if the follow-up period is long enough [5]. However, there is wide variation in individual susceptibility to alcohol, since only 5–10% of alcoholics develop pancreatitis [33]. At present, predisposing factors are unknown and the impact of genetic factors is unclear. The PRSS1 gene has been analysed in several studies comprising patients with alcoholic CP and no mutations were identified in this type of CP [43, 45, 56]. Nevertheless, some familial clustering of alcoholic CP has been noted previously [34]. In our series we have observed 3 families in which 2 brothers had alcoholic CP but none of them had a PRSS1 gene mutation (R122H, N29I, A16V) [45].

The involvement of PRSS1 gene mutations (R122H) in tropical pancreatitis has been excluded in one study [57]. In addition, no PRSS1 mutations were found in patients with CP due to rare causes including radiation, hyperlipidaemia, hyperparathyroidism, inflammatory bowel disease or haemolytic uraemic syndrome [45] (unpublished data).

Mechanisms of disease with PRSS1 mutations

A mechanistic hypothesis was proposed for the R122H mutation after considering the location of this mutation within the trypsin molecule by x-ray crystallography [39]. Because the R122H mutation, located on the side chain connecting the two

Figure 2

Different techniques for analysis of PRSS1 mutations, ie A16V.
a. Direct DNA fluorescence sequencing (left, heterozygote; right, wild-type).
b. Restriction fragment length polymorphism (RFLP) analysis using Fnu4H I.
c. Single strand conformation polymorphism (SSCP) analysis.
d. Melting curve analysis using fluorescence energy transfer (FRET) probes.
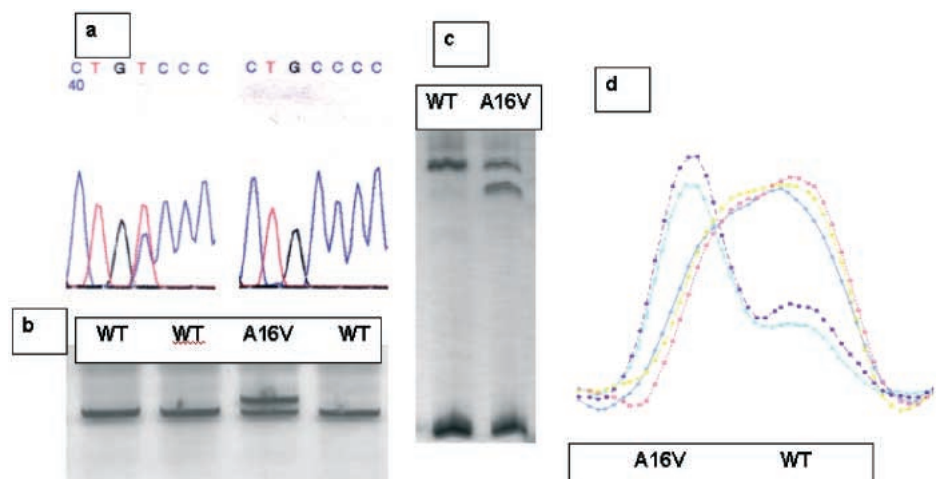
globular domains of cationic trypsinogen, is away from both the substrate binding site, the catalytic site, the SPINK1 binding site, and the trypsinogen activation peptide (TAP) cleavage site, it is unlikely that this mutation would affect trypsin function, SPINK1 inhibition, or trypsinogen activation.

The primary hydrolytic site of trypsin by trypsin itself or other trypsin-like proteases is known to be arginine at position R122 [58–60]. Thus, the R122H mutation would eliminate the primary autolysis site, thereby rendering the mutant trypsin resistant to autolysis and permanent inactivation (Figure 1b). In fact it has been shown that substitution of residue 122 with other amino acids, especially histidine in rat trypsinogen, displays increased enzyme stability [61, 62]. Furthermore, protection of this site by a monoclonal antibody against human cationic trypsinogen resulted in increased enzyme activity [63]. However, a recent study has indicated that increased autoactivation, resulting in a pathological accumulation of trypsin within the pancreatic parenchyma, is presumably the common initiating pathogenetic step [64]. Site-directed mutagenesis of recombinant human cationic trypsinogen revealed that both of the two studied mutations (R122H, N29I) significantly enhanced autoactivation in vitro. In addition, R122H but not N29I inhibited autolysis of the enzyme [64].

No experimental data exist on the functional consequences of A16V. This mutation results in an amino acid substitution that renders cationic trypsinogen identical to functional premesotrypsinogen at this location. Since A16 is the signal peptide cleavage site, it has been hypothesised that the A16V mutation may disrupt intracellular transport of pretrypsinogen (Figure 1b) [46]. On the other hand, as residue A16 is the first amino acid of the activation peptide, it may be speculated that, due to a conformational change of the activation peptide, enhanced autoactivation of trypsinogen results in facilitated cleavage.

Two other mutations in the small 8-amino acid activation peptide, D22G and K23R, have been described for which functional studies were done [47, 48]. Since tryptic digestion of synthetic dodecapeptides of the N-terminal part of PRSS1 corresponds to the wild-type, the D22G and the K23R mutation showed an increased hydrolysis rate of the two mutated peptides, indicating that these mutations facilitate cleavage of the activation peptide and thus trypsinogen autoactivation [48].

Taken together, the pathogenetic events following PRSS1 mutations, especially for the non-R122H mutations, remain largely speculative with but little functional data so far available to support the proposed underlying mechanisms. Nevertheless, clinical studies and the identification of PRSS1 gene mutations provide important insights into the pathogenesis of CP. First, the prominent role of trypsin-induced autodigestive necrosis in AP is emphasised. Second, the progression of AP to CP in HP strongly supports the "necrosis-fibrosis hypothesis" originally reported for alcoholic CP. Def-

inite conclusions have to rely on functional analyses and recombinant human cationic trypsinogen systems will be particularly helpful. In addition, studies are needed to prove whether the observations in HP are also correct in other types of CP.

Serine protease inhibitor, Kazal type 1 gene (SPINK1)

Increased trypsin activity is believed to play an early and important role in the development of pancreatitis [21]. Pancreatitis may therefore be the result of an imbalance of proteases and their inhibitors (Figure 1b). The involvement of altered defence mechanisms may have therapeutic implications for the development of strategies to prevent or control pancreatitis.

Recently, an association of mutations in the SPINK1 gene in CP was demonstrated [65]. A SPINK1 mutation was detected in 22 out of 96 paediatric patients with CP. In 18 patients (19%), an A to G transition resulting in substitution of asparagine by serine at codon 34 in exon 3 was found (N34S). Six patients (6%) were homozygous for this mutation. No phenotypic differences between heterozygous and homozygous N34S patients were observed. Compound heterozygosity of the N34S patients as well as gross deletions or insertions were ruled out by analysing the complete intronic sequences after long-range PCR. The high frequency of N34S in CP has been confirmed by others: in 57 ICP patients, 7 homozygotes (12%) and 16 heterozygotes (28%) were found [66]. In contrast, a French group initially failed to find an association between SPINK1 and CP when investigating 14 HP families and 30 unrelated CP patients without a family history [67]. However, after reinvestigation of their patients this group also described an association [68]. In a study of Swiss patients with early-onset ICP, the N34S mutation was identified in 43% compared to 1% in healthy controls [90].

N34S is chiefly found in patients without a family history of CP, ie 25–40% of ICP patients carry N34S in one or both alleles [65–67]. Approximately 1% of the general population is heterozygous for N34S [69]. It is not known why the vast majority of N34S carriers do not suffer from CP. It may be speculated that only the combination of the N34S mutation with other genetic defects or environmental factors results in CP. However, in two of the above-mentioned studies, approximately 10% of ICP patients were homozygous for N34S [65, 66]. According to the N34S carrier frequency of approximately 1%, the expected frequency of N34S homozygotes is 1:40'000. Assuming an estimated ICP prevalence of about 1:16000, the disease penetrance in N34S homozygotes would therefore be at least 25% [70].

Additional SPINK1 sequence variants have been described but their relevance remains to be determined. In one family with multiple affected members a heterozygous mutation (M1T) disrupting the start codon was detected [65]. This mutation was found in the index patient, his unaf-

fected father, and in the affected grandfather. Moreover, the deceased great-grandfather suffered from CP. The M1T mutation was not found in the great-grandfather's wife, a fact which suggests that the great-grandfather carried the mutation – unless his son developed it de novo. This pedigree thus suggests a dominant inheritance pattern with high penetrance.

Another mutation affecting the T at position 2 of the splice donor site, which is highly conserved in eukaryotes (IVS3+2 T >C), was detected in several CP patients. This mutation was first described in one patient with a family history [65]. Others found this mutation in 3 out of 112 CP patients but not in healthy controls [66]. Several other SPINK1 mutations have been described in a few patients.

The significance and inheritance pattern of SPINK1 mutations has been differently interpreted by different groups. One group concluded that SPINK1 mutations act as gene modifiers and are incapable as single factors of initiating pancreatitis by either an autosomal dominant or recessive mechanism [71]. In our opinion CP may be caused by SPINK1 mutations in an autosomal recessive, a multigenetic, and an autosomal dominant inheritance pattern [72]. An autosomal recessive disease mechanism is supported by the high frequency of N34S homozygotes with a penetrance of at least 25%, as discussed earlier. Autosomal recessive disorders with incomplete penetrance are well known, e.g. alpha 1-antitrypsin deficiency [73]. The family described above, in which a start codon mutation segregates with the disease, illustrates an autosomal dominant inheritance pattern of SPINK1 mutations [65]. The inheritance pattern in patients with CP may be influenced by the functional consequences of the underlying SPINK1 mutation [72]. For M1T it can be hypothesised that this mutation destroys the single ATG initiation codon and suppresses one allele, resulting in a dominant disease expression. On the other hand, SPINK1 inhibitory capacity may be reduced to a lesser extent by the N34S mutation, resulting in a recessive or a more complex trait.

The association of SPINK1 mutations and alcoholic CP has been analysed in only one study [69]. The N34S mutation was detected in 16 out of 274 patients (5.8%), a significant increase compared to alcoholics without CP (1/98, 1%) and healthy controls (4/540, 0.8%). This finding suggests that the SPINK1 N34S mutation is a genetic risk factor for alcoholic CP.

Mechanisms of disease with SPINK1 mutations

Interestingly, N34S is in complete linkage disequilibrium with four other intronic sequence variants: IVS1-37 T >C, IVS2+268 A >G, IVS3-604 G >A, and IVS3-66-65insTTTT [65]. This finding indicates that N34S is an evolutionary ancient mutation which arose a long time ago. So far, no experimental studies have elucidated the functional consequences of N34S. This mutation

located near the reactive lysine-isoleucine site (K41–I42) of SPINK1 may result in decreased inhibitory capacity [74]. However, the asparagine at position 34 is not fully conserved between various species. Alternatively, N34S may affect protein processing, resulting in premature degradation of the inhibitor molecule within the endoplasmatic reticulum known from other inherited diseases such as cystic fibrosis (CF) or alpha 1-antitrypsin deficiency. On the other hand, N34S may only be an innocent bystander and one of the four accompanying intronic variants may represent the pathogenetic mutation. Functional studies should clarify this issue in the near future.

Cystic fibrosis transmembrane conductance regulator (CFTR)

CF, an autosomal recessive inherited disease with multiorgan involvement, is the most common inherited disease of the pancreas [75]. CF is caused by mutations of the CFTR gene which encodes a cyclic adenosine monophosphate (cAMP)-sensitive chloride channel present in several epithelia such as in the lung, biliary tract, pancreas, and vas deferens [75]. Several findings have led to speculation that CFTR may play a role in pancreatic disease other than CF: pancreatic ductal plugging is found in CP similar to the plugging observed in CF, and abnormal sweat electrolyte values have been reported in CP as well as in CF [76, 77].

In 1998, two simultaneous articles reported association of CP and CFTR gene mutations [78, 79]. Additional studies subsequently confirmed a CFTR mutation rate increased approximately 4–6-fold above the expected 5% carrier frequency observed in Caucasian populations [54, 80, 81]. J. Cohn et al. found an even stronger (80-fold) association for compound heterozygous genotypes in ICP patients [79].

About 15% of CF patients have adequate levels of exocrine pancreatic enzymes and are classified as pancreatic-sufficient (PS) [75]. Clinically overt acute pancreatitis is found in only 1–2% of CF patients [82]. A higher percentage of patients with PS are susceptible to recurrent pancreatitis, probably because they retain sufficient residual pancreatic tissue to promote an active inflammatory response [83]. Genotype-phenotype studies indicate that genotypes causing severe loss of CFTR function (<2% residual function) were linked to pancreatic insufficiency (PI), whereas genotypes causing a milder loss of CFTR function (about 5% residual function) were linked to PS [84]. In further studies it was recognised that genotypes resulting in an approximately 10% reduction in CFTR function may cause congenital bilateral absence of the vas deferens (CBAVD) alone. Thus, disease manifestation depends on the amount of preserved CFTR function and there appears to be a tissue-specific threshold. CBAVD is chiefly caused by compound heterozygote phenotypes consisting of one severe and one mild mutation, including the 5T allele. As noted, a stronger associ-

ation was observed for ICP with a compound heterozygous state compared to CFTR mutation carrier frequency [79]. More than 1000 CFTR gene mutations are now known [85]. Commercially available tests, however, chiefly detect severe mutations causing classical CF. It may be speculated that the combination of two mild or of one mild and one severe mutation predisposes to CP, and that many patients tested by standard assays may carry another less frequent mutation on the second allele. Hence future studies with comprehensive CFTR gene testing will need to determine whether more rare mutations and compound heterozygous states will be detected in patients with ICP. Unfortunately, very limited data are available to support the functional consequences of this concept. In one study, CFTR function was impaired in the nasal epithelium of three ICP patients with two abnormal copies of the CFTR gene [79].

CFTR function may be decreased in pancreatic duct cells, at any rate in some ICP patients. This implicates defective ductal bicarbonate secretion as an early event in this type of CP. Thus the pathogenetic mechanisms in ICP associated with CFTR mutations may differ from other types of CP, especially HP, in which the acinar cell is thought to be the primary defective site. It has been suggested that pancreatic dysfunction in CF is a result of a decreased pH in the ductal and acinar lumen [86]. This lowering of pH may lead to defective solubilisation of proteins, defective apical trafficking of zymogen granules or enhanced autoactivation of trypsinogen (Figure 1b).

Several studies have investigated the association of CFTR mutations in patients with alcoholic CP. Sharer et al. reported a non-significantly increased frequency of 8.5% CFTR mutations in 71 patients [78]. In addition, the incidence was not increased in several other studies on this type of CP [43, 81, 87]. In contrast, a CFTR gene mutation was detected in 5 out of 49 patients with alcoholic CP from the Swiss CP patient series, 2.3 times the expected frequency (p <0.05) [88].

Genetic testing

Genetic studies of patients with CP lead to discovery of the major mutations associated with "classical" HP, and there is growing evidence that ICP, at least in the early-onset form, is a genetic disorder as well. Availability of genetic testing soon raises the question of the indications for clinical testing. Potential benefits (diagnosis of the underlying aetiology, early diagnosis to reduce medical evaluations, information on the risk to relatives, and prevention by change of lifestyle), risks (adverse psychological and social effects, especially in presymptomatic carriers) and limitations (lack of a cure for CP, inconclusive results because not all genes and mutations have been recognised) must be discussed by the patients, their families, and physicians [89]. Because there is wide variation in disease expression and incomplete penetrance of genes involved in CP, a positive result in a pre-symptomatic person is not determinant for future disease. In addition, the age at onset and the severity of pancreatitis cannot be reliably predicted. Unless an autosomal dominant acting mutation has already been identified in a family, a negative test result does not eliminate the risk of developing a genetic form of CP. No specific therapy exists for the prevention or treatment of CP. Furthermore, no efficient screening test exists at present for early diagnosis of pancreatic cancer in high-risk groups like classical HP. Genetic testing requires an understanding of all these elements and appropriate counselling.

Little literature is available regarding the indications for genetic testing of genes involved in CP [3, 89]. In our opinion, clinical genetic testing of PRSS1 and SPINK1 mutations should be performed in pancreatitis patients with a family history of CP and in patients without a family history of CP after carefully ruling out other aetiologies (alcohol abuse, metabolic disorders etc). Furthermore, genetic analysis must be considered in patients with CP and a family history of pancreatic cancer.

There is considerable ethical debate regarding presymptomatic testing. Because of the above-mentioned test limitations and the highly variable conditions with respect to age and severity, presymptomatic genetic testing should follow only after extensive pretest counselling and must thus be performed on an individual basis. In our view prenatal diagnosis should be eschewed.

In adult patients with CP, genetic testing for CFTR gene mutations should be performed only in a research context. However, children with cystic fibrosis may suffer from recurrent attacks of pancreatitis without the clinical symptoms of malabsorption or significant lung disease. Sweat chloride measurement should therefore be performed in children with recurrent pancreatitis, and CFTR mutations should be tested in all cases with elevated or borderline sweat chloride concentrations.

At present there are only very limited or no data on the occurrence of PRSS1 or SPINK1 mutations in patients with alcohol-related or other forms of CP. Future studies will need to clarify whether clinical testing is appropriate in these patients. Since genetic analyses are expensive and time-consuming, clinical genetic testing should not be performed in patients with CP caused by known predisposing factors such as alcohol abuse or in patients with AP without a family history of CP.

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