HHS Public Access

Author manuscript

Curr Opin Gastroenterol. Author manuscript; available in PMC 2022 November 01.

Published in final edited form as:

Curr Opin Gastroenterol. 2018 September ; 34(5): 322–329. doi:10.1097/MOG.0000000000000461.

Chronic pancreatitis: an update on genetic risk factors

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Abstract

Purpose of review—Genetic mutations in genes within and outside of the trypsin-dependent pathologic pathway have been found to be associated with chronic pancreatitis. This review highlights recent developments.

Recent findings—*CTRB1-CTRB2* has been identified as a new risk locus for chronic pancreatitis and the disease mechanism may involve trypsin degradation. Misfolding mutations in *PRSS1*, *CPA1*, and *CEL*, as well as environmental stress factors like tobacco and alcohol can trigger endoplasmic reticulum stress (ER-Stress).

Summary—Protein misfolding as well as enzyme activity changes due to altered autoactivation, intracellular degradation, or enzyme inhibition represent the most important pathological mechanisms of chronic pancreatitis to date. Analysis of composite risk patterns by next-generation sequencing will help elucidate complex gene interactions and identify new potential therapeutic targets.

Keywords

chronic pancreatitis; enzymatic activity; genetic risk; inherited disease; protein misfolding

INTRODUCTION

Pancreatitis, an inflammatory disorder of the pancreas not usually caused by infectious agents, is now regarded as a continuum between an acute and a chronic disease phenotype that overlap (in the form of recurrent pancreatitis) in up to 30% of patients. Its causes are manifold ranging from immoderate alcohol consumption, to gallstones passing through the papilla and triggering transient pancreatic duct obstruction [1], to exposure to cigarette smoke [2], a multitude of pharmaceutical drugs [3] and vascular pathology [4], or as part of an autoimmune disorder [5,6]. On the cellular level, it is now believed that the disease process begins in pancreatic acinar (exocrine) cells and involves the premature activation of digestive proteases, most prominently trypsin. Whether trypsin is activated by lysosomal hydrolases [7,8], or autoactivates has remained a matter of debate [9,10], but

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Conflicts of interest

There are no conflicts of interest.

its intracellular activation makes the century-old concept of pancreatitis as an autodigestive disorder plausible. More recent studies have focused on the role of inflammatory cells in the disease's onset [11,12], which appear to operate even without the need of prior trypsin activation [13] or can be driven directly by trypsin, without its prior activation in acinar cells [14]. The first genetic factor identified for pancreatitis was, indeed, trypsin. The discovery in 1996 that a p.R122H mutation in cationic trypsinogen gene (*PRSS1*) is strongly associated with an autosomal dominant variety of pancreatitis [15] must be regarded as the most convincing evidence for the protease-activation-autodigestion hypothesis of pancreatitis. As of today, most inherited risk factors of pancreatitis involve the digestive protease/antiprotease system. Whether this is due to the prevalent candidate-gene-approach still used by most investigators as opposed to hypothesis-free genome-wide association studies (GWAS) or next-generation sequencing (NGS) studies, or the preeminence of proteolytic over inflammatory disease onset mechanisms remains to be seen.

THE TRYPSIN ACTIVITY PATHWAY

The first identified p.R122H mutation deletes a fail-safe mechanism for intracellular trypsin degradation and associates with autosomal dominant forms of familial or hereditary pancreatitis [15]. The identification of further dominant acting *PRSS1* mutations N29I or A16V suggested a disease mechanism of pancreatitis that is related to trypsin-activity. Enhanced or prolonged trypsin activity seems the common denominator of this pathway and the promoting effects of mutations in pancreatitis risk genes *PRSS1*, serine protease inhibitor Kazal type 1 (*SPINK1*), and chymotrypsin C (*CTRC*) and have recently been reviewed [16]. Their pathogenic potential relates to trypsinogen autoactivation (*PRSS1*), reduced protective intracellular trypsin degradation (*CTRC*), or impaired trypsin inhibition (*SPINK1*). Trypsinogen synthesis, storage, and secretion in the acinar cells are paralleled by coexpression and transport of the serine protease inhibitor SPINK1, sufficient to inhibit 2–13% of activated or secreted trypsinogen. When SPINK1 becomes depleted a second fail-safe mechanism leads to the activation of Chymotrypsinogen C, which itself is a protective intracellular trypsin degrading enzyme and in concert with trypsin results in irreversible trypsinogen degradation.

CHYMOTRYPSIN C

CTRC selectively cleaves the Leu^{81}–Glu^{82} peptide bond within the Ca^{2+}-binding loop, and further degradation is then achieved through tryptic cleavage of the Arg^{122}–Val^{123} peptide bond [17]. In 2008, the association of CTRC variants with chronic pancreatitis risk has been demonstrated in two independent European studies [18,19]. Two alterations, p.R254W and a microdeletion p.K247_R254del, were significantly overrepresented in idiopathic, hereditary, alcoholic, and tropical pancreatitis. Mutations were present in 3.3% of affected individuals but only 0.7% of controls (odds ratio (OR) = 4.6). Loss-of-function variants in *CTRC* predispose to pancreatitis by diminishing its protective trypsin-degrading activity. LaRusch *et al.* [20] evaluated the occurrence of CTRC variants in individuals with recurrent acute pancreatitis (RAP), chronic pancreatitis, and controls from the North American Pancreatitis Study II cohort. Their results confirmed previously reported rare pathogenic p.A73T, p.R254W, and p.K247_R254del variants and p.G60G (c.180 C>T;

rs497078). Compared with controls (minor allele frequency (MAF) = 10.8%), p.G60G was associated with chronic pancreatitis (MAF = 16.8%, $P < 0.00001$) but not RAP (MAF = 11.9% P = NS). Trend test indicated codominant risk for chronic pancreatitis (CT:OR = 1.36, TT: OR = 3.98). The common CTRC variant c.180T was more frequent with concurrent pathogenic CFTR variants and/or SPINK1 variants and with alcoholic vs. nonalcoholic chronic pancreatitis. The authors suggest that CTRC p.G60G may act as disease modifier that triggers the progression from recurrent acute pancreatitis to chronic pancreatitis, especially in combination with *CFTR* or *SPINK1* variants, alcohol, or smoking.

SERINE PROTEASE INHIBITOR KAZAL TYPE 1

Evidence for a pathogenic role of loss of function *SPINK1* mutations comes from association studies that identified the c.194+2T>C variant in cohorts of white and Asian chronic pancreatitis patients. The mutation locates within the splice site in intron 3 and results in markedly reduced SPINK1 mRNA expression. In the United States and Europe, the variant has been found at carrier frequencies of about 3% in patients, but rarely in controls. Higher carrier frequencies have been reported for the c.101A>G (p.N34S) mutation, which according to a recent meta-analysis is present in 9.7% of patients and 1% of controls (OR = 11) [21]. The *SPINK1* c.101A>G variant represents the most frequently identified genetic risk factor for idiopathic chronic pancreatitis (ICP). Four intronic alterations associate with the N34S variant, but the causal variation associated with this risk haplotype remains elusive. Férec *et al.* tried to resolve this enigma and searched for variants in strong linkage disequilibrium with the p.N34S mutation. They identified another SNP (rs142703147:C>A), which disrupts a PTF1L-binding site located ~4-kb upstream of the *SPINK1* promoter [22■]. Analysis of 548 French ICP patients and 562 matched controls showed that rs142703147 is in perfect linkage disequilibrium with the p.N34S mutation in the French population ($r^2 = 1$), but not in two additionally screened cohorts of Chinese and Indian ICP patients (Chinese, $r^2 = 0.80$; Indian, $r^2 = 0.59$). SNP rs142703147 may only be one part of the solution to the N34S puzzle. It seems the N34S enigma awaits further strategies to unravel its role, as the inhibitory capacity of this most common *SPINK1* variant is unimpaired [23].

CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR

For a number of years, it has been known that mutations in the cystic fibrosis (CF) transmembrane conductance regulator (*CFTR*) gene, an ATP-binding cassette transporter-type protein localized to the apical plasma membrane of epithelial cells increase the risk of developing pancreatitis [24–26]. The CFTR protein functions as a cyclic AMP-regulated anion channel and helps in maintaining the luminal hydration of secretory ducts. Anions can move across the open channel along their electrochemical potential gradient, resulting in fluid and/or electrolyte secretion. The exocrine pancreas and, in men, the wolffian ducts are most susceptible to loss of function mutations of this channel and are affected earliest. CF is an autosomal recessive syndrome which is usually caused by compound heterozygosity of two *CFTR* mutations that effectively eliminate chloride conductance. The relative permeability of the CFTR channel for chloride and bicarbonate anions may be dynamically regulated [27], suggesting that point mutations in the channel or in the

permeability pore may affect the anion permeation properties of CFTR. LaRusch *et al.* [28] screened pancreatitis cases for candidate $CFTR^{BD}$ mutations variants and identified nine *CFTR* variants (R74Q, R75Q, R117H, R170H, L967S, L997F, D1152H, S1235R, and D1270N) that were not associated with typical CF but with pancreatitis (OR 1.5, $P = 0.002$). Although the $CFTR^{BD}$ variants are scattered throughout the genetic sequence, they share a specific functional defect in responding to WNK1-SPAK activation with increased bicarbonate permeability. It appears that this mechanism is critical for pancreatic duct and acinar cells and other bicarbonate-secreting cells that utilize CFTR as the primary anion channel.

ENDOPLASMIC RETICULUM STRESS

Another trypsin-independent disease mechanism seems to involve endoplasmic reticulum stress (ER-Stress) caused by mutation-induced protein misfolding of digestive enzymes. Details of this alternative pathologic pathway were recently reviewed [29■■]. The article by Kereszturi *et al.* was the first to demonstrate greatly reduced secretion of PRSS1 mutants p.R116C and p.C139S from transfected HEK293 cells. An accumulation of misfolded proteins within the endoplasmic reticulum leads to the initiation of inflammatory signals and activation of unfolded protein-response pathways which aim at relieving the ER-stress burden and limiting the proteotoxicity of accumulated protein aggregates by proteosomal degradation. For PRSS1 mutants p.R116C, p.C139S, and p.L104P, an activation of ER-Stress-markers XBP-1 and BIP was observed, but other PRSS1 mutants like p.R122H, p.R122C, p.N29I, or p.A16V did not trigger the ER-stress response [30]. PRSS1 mutations p.D100H, p.C139F, P.K92N, p.S124F, and p.G208A showed a diminished trypsinogen secretion that may impart a weaker risk of inducing ER-stress. PRSS1 misfolding variants are rare in comparison with the trypsin activity-altering mutations and have been found primarily in sporadic idiopathic cases of pancreatitis.

CARBOXY PEPTIDASE A1

In 2013, Witt *et al.* [31] published a candidate gene approach in which they sequenced all 10 *CPA1* exons and analyzed association of variants with early-onset pancreatitis. Functionally impaired variants were present in 3.1% of German patients and in 0.1% of controls (OR = 24.9; $P = 1.5 \times 10^{-16}$). They identified 31 missense variants, one nonsense variant, one frame-shift variant, and one splice-site variant. Three of these variants were significantly enriched in patients. Strikingly, 17 of these variants had a more than 80% reduced activity in conditioned medium after activation with trypsin and CTRC. In patients, these loss of function variants were enriched, especially in early onset cases (before 10 years) and the apparent activity-loss was in 13 of the 17 variants due to a misfolding phenotype, comparable with what had previously been found in the misfolding PRSS1 variants. CPA1 misfolding variants with a diminished secretion (≥80%) induce endoplamic reticulum stress and are strong causative risk factors for early-onset chronic pancreatitis. Significantly, and in contrast with the high number of trans-heterozygotes for *SPINK1* and *CTRC* and/or *CFTR* variants, only limited interaction of these variants with *CPA1* variants was found. The pathogenic impact of misfolding mutations may be directly related to their intracellular retention and their specific propensity of being disposed of by proteosomal degradation

via Endoplasmic-reticulum-associated protein degradation pathways. The clinical relevance of misfolding PRSS1 and CPA1 mutants may not be comparable with that of a dominant R122H and N29I mutation, still they significantly increase the risk for chronic pancreatitis and associate with inherited chronic pancreatitis.

CARBOXYLESTER LIPASE

The disease mechanism of misfolding-induced ER-stress has also recently been discussed in a pancreatitis risk gene outside of the protease-antiprotease axis. A nonallelic homologous recombination between the carboxylester lipase gene *CEL* and *CELP*, its adjacent pseudogene, was identified at a five-fold increased prevalence in patients with ICP (3.7% cases and 0.7% controls; OR = 5.2) [32]. Expression of the hybrid CEL-HYB in cellular models showed reduced lipolytic activity, impaired secretion, prominent intracellular accumulation, and induced autophagy. *CEL* is a remarkable gene due to its very polymorphic nature and rare *CEL* mutations can cause an autosomal dominant syndrome of maturity-onset diabetes of the young (MODY) and exocrine pancreatic dysfunction, denoted MODY8. In a recent replication study in Asian cohorts, Zou *et al.* [33] made the surprising discovery that all Asian CEL-HYB alleles exhibited a premature stop codon in exon 10, and this new allele was therefore designated CEL-HYB2. Surprisingly, CEL-HYB2 carrier frequencies among cases and controls were identical (1.7%) in a combined patient cohort from China, Japan, and India. Transfection experiments in HEK293 cells indicate that the CEL-HYB2 transcript is less stable than that originating from the original CEL-HYB1. Molven *et al.* [34■] suggested that the different C-terminal endings are responsible for the specific disease risk of the *CEL* variants. With MODY8, the frame-shift mutation in the VNTR creates a long protein tail with a strong tendency to form aggregates. In CEL-HYB1, the modified C-terminus is considerably shorter and has less severe influences on protein function and cellular secretion. The lack of CEL-HYB1 in Asians suggests that it is an ethnic-specific risk allele for chronic pancreatitis, whereas the presence of the CEL-HYB2 allele is not associated with an increased pancreatitis risk in the Asian population.

ENVIRONMENTAL FACTORS PRODUCING ENDOPLASMIC RETICULUM STRESS

Significantly, ER-stress is also a consequence of environmental risk factors such as alcohol or tobacco consumption. Alcohol feeding of mice induces upregulation of spliced X-box binding protein 1. In a recent study by Lugea *et al.* [35], acinar cells showed a strong activation of protein kinase R-like ER kinase and transcription factor CCAAT-enhancer-binding protein homologous protein following treatment with ethanol and cigarette smoke. It seems that cigarette smoke initiates features of pancreatitis in ethanol-sensitized acinar cells by induction of ER-stress pathways that promote acinar cell death. Pancreatitis is a known to be multifactorial disease, so it seems plausible that mutation-derived misfolded proteins as well as environmental risk factors like smoking and alcohol both contribute to increased pancreatitis risk by synergistically triggering the ER-stress level of acinar cells.

GENOME-WIDE ASSOCIATION STUDIES

Hypothesis-free approaches to identify genetic risk factors have been successfully employed for a variety of inflammatory disease including Crohn's disease [36] or *Helicobacter pylori* colonization [37]. A first GWAS for pancreatitis by Whitcomb *et al.* [38] revealed an association of common variants in the *CLDN2-MORC4* and the *PRSS1-PRSS2* loci with alcoholic and nonalcoholic chronic pancreatitis. The protective *PRSS1* promoter variant reduces transcription, leading to lower intrapancreatic trypsinogen levels and fits into the trypsin activity concept of pancreatitis. Neighboring genes within the *CLND2* locus include *MORC4*, *RIPPLY1*, and *TBC1D8B*, but RIPPLY1 and TBC1D8B are not expressed in the pancreas, and *MORC4* expression is not related to pancreatitis. Claudin-2 represents the most attractive candidate, because it serves as a highly regulated tight junction protein forming low-resistance, cation-selective ion and water channels between endothelial cells. The CLAUDIN 2 (*CLDN2*) risk allele is associated with atypical localization of claudin-2 in pancreatic acinar cells. Experimental studies have previously characterized the role of intact intercellular connections in pancreatitis [11].

A replication GWAS was performed in a large European cohort in which association was strongest in the alcoholic chronic pancreatitis group [39]. Similar observations also followed in Japanese and Indian chronic pancreatitis cohorts [40,41]. Giri *et al.* [42] analyzed the association with chronic pancreatitis in Indian patients by genotyping 1807 unrelated Indians of Indo-European ethnicity, including 519 patients with chronic pancreatitis and 1288 controls. The study confirmed a significant association of two variants in *CLDN2* (rs4409525; rs12008279) and two variants in *MORC4* (rs12688220; rs6622126) in Indian patients with chronic pancreatitis. One variant in the gene *MORC4* (rs12688220) showed significant interaction with alcohol consumption, suggesting gene–environment interaction.

The European consortium also identified *CTRB1-CTRB2* as a new risk locus for alcoholic and nonalcoholic chronic pancreatitis [43■■]. This locus contains two highly similar chymotrypsin genes transcribed in opposite directions. The association within the *CTRB1-CTRB2* locus was linked to a 16.6-kb inversion that altered the CTRB1/CTRB2 expression ratio. The major risk allele is associated with higher relative CTRB1 expression, whereas heterozygous carriers with one minor allele express higher levels of CTRB2. In autoactivation experiments, trypsin levels were more prominently degraded by CTRB2. The inversion switches the signal-peptides of CTRB1 and CTRB2, but at the protein level, the secreted mature proenzymes remain unchanged.

GLYCOSYLATION

Notably, the *CTRB1*/*CTRB2* locus had previously been identified by a population-based GWAS from North Germany to be associated with elevated serum lipase activities in asymptomatic individuals, but a replication in pancreatitis cohorts was not possible [44]. This study further reported that Fucosyltransferase 2 nonsecretor status and blood group B represent genetic cofactors for the development of alcoholic as well as ICP. The role of the ABO blood group in chronic pancreatitis had previously been investigated by Whitcomb *et al.* in the NAPS2 cohort who reported that A, B, and AB blood groups were not associated

with a greater probability of developing chronic pancreatitis, but may decrease the risk of chronic pancreatitis in individuals who are very heavy drinkers [45]. Kirsten *et al.* also analyzed the above-mentioned *FUT2* and *ABO* SNPs regarding association with chronic pancreatitis in a German cohort of 1458 cases and 5133 controls [46]. Their analysis revealed significant interaction effects of rs632111 in *FUT2* with alcohol consumption and for rs8176693 in ABO for the subgroup of alcohol-dependent individuals but could not replicate the findings by Weiss *et al.* Whether the exclusion of alcohol consumers from their control group influenced the prevalence of ABO blood group and FUT nonsecretors in their study remained unclear [47].

ABO blood types vary considerably between populations ranging from 8% for blood type B in Norway to 33% in Kashmir. Identical ethnic backgrounds between cases and controls is therefore the key to a successful analysis of the ABO locus in pancreatitis, just as it was in pancreatic cancer in which blood type non-O is now recognized as a common genetic risk trait [48]. The first replication for blood type B as a pancreatitis risk allele came from a study investigating drug-induced (Azathioprine) pancreatitis in patients with inflammatory bowel disease [49].

HYPERTRIGLYCERIDEMIA

The increase in obesity worldwide is associated with an elevated incidence of metabolic diseases and hyperlipidemia, characterized by serum hypertriglyceridemia, has become a prominent cause of acute pancreatitis which contributes to 1–10% of patients with AP. Dyslipidemias can have a strong genetic component; therefore, Chen *et al.* performed targeted NGS of 11 hypertriglyceridemic acute pancreatitis patients to identify genetic mutations associated with hypertriglyceridemia, including apolipoprotein A-V (*APOA5)*, *APOC2*, *APOC3* and *APOE*, *BLK*, lipoprotein lipase (*LPL*), *GPIHBP1*, and *LMF1* [50■]. Two patients with compound heterozygous mutations in *APOA5* and two patients with a homozygous p.C14F variant in *GPIHBP1* were detected. One had a homogeneous p.R176C mutation in *APOE* which was also found in heterozygosity in two other patients. Limitations of the study are the lack of a control cohort, but the findings suggest that genetic mutations in *APOA5*, *GPIHBP1*, and *APOE* genes may have contributed to the occurrence of hypertriglyceridemia in patients with AP.

Another study by De Castro-Orós *et al.* also aimed at identifying rare dysfunctional mutations in genes encoding regulators of LPL function in patients with familial and nonfamilial primary hypertriglyceridemic (HTG). They sequenced promoters, exons, and exon–intron boundaries of *LPL*, *APOA5*, *LMF1*, and *GPIHBP1* in 118 patients with severe primary HTG and 53 normo-lipidemic controls [51]. Variant functionality was analyzed using predictive software and functional assays for mutations in regulatory regions. The study identified 29 rare variants, 10 of which had not been previously described. Overall, 20 (17.0%) of the patients carried at least one allele with a rare pathogenic variant in *LPL*, *APOA5*, *LMF1*, or *GPIHBP1*. The presence of a rare pathogenic variant was not associated with increased lipid concentrations, a family history of HTG, clinical diagnosis, or previous pancreatitis. Less than 20% of individuals with triglycerides more than 500 mg/dl and no major secondary cause for HTG may carry a rare pathogenic mutation in *LPL*, *APOA5*,

LMF1, or *GPIHBP1*. The results support the concept that most severe HTG result from an interaction of environmental factors with minor risk alleles that have little effect on HTG. Only in a minority of cases, major genetic defects seem involved in triglyceride metabolism. Although it had previously been assumed that only lipid levels more than 1000 mg/dl confer a pancreatitis risk it has recently become evident that even mild serum triglyceride increases the probability of developing pancreatitis [52].

GENETIC TESTING IN CHILDREN

The majority of children with chronic pancreatitis report prior RAP. In comparison with genetic association studies in adults, RAP and chronic pancreatitis in children are more often associated with genetic mutations with reported rates of between 36 and 73%. In a recent article, Grabarczyk *et al*. analyzed *CTRC* mutations in a cohort of 136 Polish children. They found 4.6 and 5.3% carriers of p.R254W and p.K247_R254del mutations, respectively, indicating a significantly associated chronic pancreatitis risk in pediatric patients (OR = 19.1 and OR = 5.5) [53]. They also identified 22% homozygous carriers of the p.Gly60Gly variant (OR 23). *CTRC* variants, including homozygous c.180T-alleles, are strong chronic pancreatitis risk factors in pediatric patients.

A multinational cross-sectional study of children with pancreatitis has been initiated by the INSPPIRE consortium to characterize and identify risk factors associated with RAP and chronic pancreatitis in childhood. A recent report from this study analyzed unique features or disease course differences associated with age at disease onset [54]. Early-onset disease (before the age of 6) was significantly associated with mutations in *PRSS1*, *CTRC*, and a family history of acute or chronic pancreatitis. Later onset disease (between 6 and 18 years) was more likely to present with hypertriglyceridemia, ulcerative colitis, autoimmune diseases, or medication use. A total of 71% of patients in the early-onset group were found to carry at least one pancreatitis-associated gene mutation (*PRSS1*, *CFTR*, *SPINK1*, *CTRC*). On the contrary, *CPA1* mutations were not evaluated by Giefer *et al*., but these mutations had previously been demonstrated by Witt *et al*. [31] to associate with early onset sporadic chronic pancreatitis. According to limited screening panels, the rate of associated mutations may presently be underestimated in this patient population. A NGS approach was performed by Xiao in a cohort of 69 Chinese children to detect nucleotide variations in *PRSS1*, *SPINK1*, *CFTR*, *CTRC*, *CPA1*, calcium-sensing receptor (*CASR*), cathepsin B (*CTSB*), keratin 8, *CLDN2*, and ATPase type 8B member 1 [55■]. They found 65% to carry at least one mutation, but 19% carried more than one mutation. Concurrent mutations were found in carriers of PRSS1 and CFTR or SPINK *mutations*, two with both CFTR and SPINK1, others with CTSB and CASR. The study by Xiao is one of the most comprehensive genetic testing studies to date which allows the identification of composite risk patterns and may provide insight into still obscure pathomechanisms of pancreatitis. The study also illustrates how difficult the interpretation of composite variant patterns in the absence of comprehensive functional data and corresponding clinical phenotype can be.

The question whether a genetic predisposition exists for the progression from RAP to chronic pancreatitis was analyzed by Palermo *et al*. [56] in a comprehensive genetic analysis of *PRSS1*, *CFTR*, *SPINK1*, and *CTRC* in children. A total of 60% of individuals were

tested positive for at least one mutation, and *CFTR* variants were more likely in those with chronic pancreatitis compared with RAP (63 and 24%, $P = 0.01$). Kumar *et al.* [57] had reported from the INSPPIRE cohort that RAP was more common in Hispanics, chronic pancreatitis in non-Hispanics. They observed that *PRSS1* or *SPINK1* mutations were more frequently found in chronic pancreatitis compared with RAP ($P < 0.0001$ and <0.05, respectively). Whether ethnicity and mutations in *CFTR*, *PRSS1*, or *SPINK1* genes influence the development of chronic pancreatitis in patients with early onset disease will have to be confirmed in larger studies.

In a Japanese study, Saito *et al.* recently analyzed mutations in the *PRSS1*, *SPINK1*, *CTRC*, and *CPA1* genes in 128 Japanese children with RAP or chronic pancreatitis. A total of 39% had at least one mutation and 30% had a family history of pancreatitis [58]. The study confirms previous observations that carriers of a dominant PRSS1-R122H mutation rarely have other detectable mutations and that the penetrance of this trypsin mutation is not affected by additional *SPINK1* mutations [59]. In contrast, 51.6% of carriers of *SPINK1*, *CTRC*, or *CPA1* mutations were homozygous or heterozygous for a second mutation. Pediatric patients with idiopathic acute recurrent pancreatitis and chronic pancreatitis represent the patient group in which genetic analysis is highly recommended and useful for identifying the cause of pancreatitis.

CONCLUSION

Our current understanding of pancreatic patho-physiology has been dramatically improved by results from genetic association studies and the biochemical analysis of the intrapancreatic trypsin-dependent pathways. *PRSS1*, *SPINK1*, and *CTRC* represent the major risk genes found associated with recurrent and chronic pancreatitis and hereditary pancreatitis diagnostic screening is focused mainly on genes related to trypsin activity, its inhibition and its degradation. Although *CFTR* has early been recognized as one additional risk gene outside the protease-antiprotease axis, protein misfolding and ER-stress represent another pathogenic concept how genetic mutations may increase the pancreatitis risk. Smoking and alcohol may synergize with the pancreatitis risk of misfolding mutations on the level of ER-stress. A better understanding of its molecular regulation mechanisms will help elucidate the complex interaction of genetic and environmental risk and may provide new therapeutic targets. New technical developments, like massive screening technologies (NGS), will allow the identification of so far unknown or unrelated regulation pathways und further develop our understanding of pancreatic pathophysiology.

Acknowledgements

Work of F.U.W. and M.M.L. are supported by grants from Deutsche Krebshilfe/Dr Mildred-Scheel-Stiftung (109102), the Deutsche Forschungsgemeinschaft (DFG GRK 1947/A3), the Federal Ministry of Education and Research (BMBF GANI-MED 03IS2061A, 0314107, 01ZZ9603, 01ZZ0103, 01ZZ0403, 03ZIK012, FKZ: 01EK1511A), the European Union (EU-FP-7: EPC-TM), V-630-S-150-2012/132/133, ESF/14-BM-A55-0045/16, TBI-V-242-VBW-084, and TBI-V-1-245-VBW-085. M.E.S. is supported by NIH/NIDDK T32DK108733.

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■■ of outstanding interest

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KEY POINTS

- To date, most inherited risk factors of pancreatitis involve the digestive protease/antiprotease system.
- Genetic mutations that cause protein misfolding can be associated with intracellular retention and induction of ER-Stress.
- Genetic and environmental risk factors, including tobacco and alcohol, may synergize on the ER-stress level.
- Genome-wide association studies and next-generation sequencing analyses confirm composite risk patterns of pancreatitis and are powerful tools to identify still unknown pathomechanisms of pancreatitis.