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Genetics of Chronic Pancreatitis – New Aspects?

Genetyka przewlekłego zapalenia trzustki – Nowe aspekty?

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S u m m a r y

Fifteen years ago the discovery of a mutation of the cationic trypsinogen gene (*PRSS1*) in patients with hereditary chronic pancreatitis supported Hans Chiari's theory that chronic pancreatitis is the result of autodigestion of the pancreas and depicted the fundament for further research in this field. A genetic basis for chronic pancreatitis had to be assumed in a pedigree described by Comfort and Steinberg already in 1952 it needed additional 44 years to identify this first genetic association. Thereafter, research had its focus on proteases and anti-proteases that are assembled in the digestive enzyme cascade, an approach that identified serine protease inhibitor, Kazal type 1 (*SPINK1*) as another pancreatitis gene. Aside the digestive enzyme cascade, the cystic fibrosis transmembrane conductance regulator gene (*CFTR*) that is responsible for Cystic Fibrosis, was investigated by two groups and both found an enrichment of *CFTR* variants in patients with chronic pancreatitis. Support for this investigation came from Cystic Fibrosis, where 1-2% of patients suffer from chronic pancreatitis. New aspects in the field of genetics in chronic pancreatitis emerged in the last five years. A variant of anionic trypsinogen (*PRSS2*) was found to be overrepresented in controls and protects against chronic pancreatitis. Additionally, variants of the calcium sensing receptor (*CASR*) seem to influence the pathogenesis of chronic pancreatitis in *SPINK1* p.N34S carriers. Triplication and duplication of the trypsinogen locus represents a completely new disease causing mechanism that predisposes to chronic pancreatitis by a so-called gene dosage effect. Identification of chymotrypsin C (CTRC) as the enigmatic Enzyme Y described by Rinderknecht over 20 years ago displayed another reasonable candidate gene. Investigations of *CTRC* found low penetrance loss of function variants that diminish secretion and/or activity of CTRC and thereby contribute to the development of the disease. Taken together recent data further support the importance of a balanced digestive enzyme cascade in that trypsin captures a key role. Further research in this field will yield additional associated genes by hypothesis-driven and hypothesis free approaches.

Key words: genetics, chronic pancreatitis, *PRSS1*, *PRSS2*, *SPINK1*, *CFTR, CTRC, CASR*

S t r e s z c z e n i e

Piętnaście lat temu, odkrycie mutacji genu trypsynogenu kationowego (PRSS1) u pacjentów z dziedzicznym przewlekłym zapaleniem trzustki poparło hipotezę Hansa Chiari sugerującą, że przewlekłe zapalenie trzustki jest wynikiem samotrawienia trzustki oraz stworzyło fundament pod przyszłe badania naukowe tego zagadnienia. Podejrzenie istnienia genetycznego podłoża przewlekłego zapalenia trzustki zostało zasugerowane w 1952 roku przez Comfort i Steinberg, lecz dopiero po 44. latach zidentyfikowano pierwsze powiązanie genetyczne w tej chorobie. Następnie, badania koncentrowały się na proteazach i antyproteazach funkcjonujących w kaskadzie enzymów trawiennych, co doprowadziło do zidentyfikowania inhibitora proteaz serynowych (Kazal type 1, SPINK1) jako kolejnego genu związanego z zapaleniem trzustki. Obok kaskady enzymów trawiennych, gen regulatorowy odpowiedzialny za mukowiscydozę (transmembrane conductance regulator gene, CFTR) badany był przez dwie grupy badaczy, które stwierdziły częstsze występowanie mutacji genu CFTR u pacjentów z przewlekłym zapaleniem trzustki. Stymulacją do tych badań była mukowiscydoza, w której 1-2% pacjentów towarzyszy przewlekłe zapalenie trzustki. W ciągu ostatnich pięciu lat pojawiły się nowe aspekty w dziedzinie genetyki przewlekłego zapalenia trzustki. Warianty receptora rozpoznającego wapń (calcium sensing receptor, CASR) wydają się mieć znaczenie w patogenezie przewlekłego zapalenia trzustki u nosicieli SPINK1 p.N34S. Tryplikacja i duplikacja locus genu trypsynogenu jest zupełnie nowym mechanizmem wywołującym chorobę, predysponującym do przewlekłego zapalenia trzustki poprzez tzw. efekt dawkowania genu. Identyfikacja chymotrypsyny C (CTRC) jako zagadkowego Enzymu Y, opisanego przez Rinderknechta ponad 20 lat temu, wskazała kolejny gen kandydujący. Badania CTRC wykazały małą penetrację wariantów z utratą funkcji, które zmniejszają wydzielanie lub aktywność CTRC i przez to przyczyniają się do rozwoju choroby. Podsumowując, wyniki ostatnich badań dodatkowo wspierają znaczenie równowagi w kaskadzie enzymów trawiennych, z trypsyną odgrywającą kluczową rolę. Dalsze badania w tej dziedzinie, stymulowane hipotezami oraz niezwiązane z hipotezami, wyłonią dodatkowe geny skojarzone z przewlekłym zapaleniem trzustki.

Słowa kluczowe: genetyka, przewlekłe zapalenie trzustki, *PRSS1*, *PRSS2*, *SPINK1*, *CFTR*, *CTRC*, *CASR*

INTRODUCTION

Chronic Pancreatitis (CP) is a progressive inflammatory disease of the pancreas leading to permanent impairment of pancreatic exocrine and endocrine function that can result in maldigestion and diabetes mellitus (1). Certainly, one of the most displeasing characteristics of CP are recurring attacks of acute abdominal pain that in some patients pass into a state of chronic pain often hindering patients to participate in a normal working life (2). In the western world the most predominant underlying cause is chronic alcohol abuse. Notably, only about five percent of chronic alcoholics develop CP. Aside chronic alcohol abuse several other factors such as nicotine, hypercalcemia, drugs, trauma and genetic alterations underlie CP. Over a century ago Hans Chiari postulated that chronic pancreatitis is the result of autodigestion of the pancreas, a thesis that is now again supported by genetic studies and functional analyses of genetic variants conducted within the last fifteen years (3). The first pedigree of a family with inherited CP was described in 1952 by Comfort and Steinberg illustrating the influence of genetic alterations that may contribute to the pathogenesis of CP (4). Thereafter, in the end 44 years were necessary until the p.R122H variant of the cationic trypsinogen (*PRSS1*) was identified in patients with hereditary CP (5). This finding was fundamental for a search for further genetic alterations in CP and also highlighted the importance of a balanced digestive enzyme cascade. In the following years research focused on proteases and anti-proteases that participate in this digestive enzyme cascade as well as on the *CFTR* gene whose major genetic alterations are responsible for Cystic Fibrosis. Further, "milder" genetic alterations of CFTR were associated with CP. In this rather compact review our we focus on the latest findings in the field of genetics in CP and former findings like association of *PRSS1*, *CFTR* and *SPINK1* variants are only briefly discussed.

CATIONIC TRYPSINOGEN (*PRSS1*) AND HEREDITARY CHRONIC PANCREATITIS

Hereditary CP, an autosomal dominant disease with a clinical penetrance rate of about 60-70% is a very rare form of chronic pancreatitis. The clinical course is different from that in alcoholic CP, since it encompasses an earlier onset and has a slower progression. However, morphological features and laboratory findings do not differ as well as given treatment opportunities. After the first description of the p.R122H variant several other rare variants were found in the *PRSS1* gene (6 and references within). Most of the variants seem to cause premature trypsinogen activation *in vitro* with the consequence of an overweight of proteases since trypsin is able to activate the digestive enzyme cascade. This ssumption was further supported by transgenic animal models that express R122H mutated trypsinogen, because in these animal models elevated serum levels of lipase and amylase were observed (7, 8). However, definite histological changes resembling CP in humans were only found after repetitive supramaximal stimulations with caerulein. However, taken together, functional data *in vitro* and *in vivo* support the theory that a disarrangement of the balance between trypsin and its inhibitors in favour of more protease activity is responsible for the development of CP. An important question for the clinician as well as for the patient is whether patients with hereditary CP have a higher risk for the development of pancreatic cancer. Compared to patients with alcoholic CP who have a 20-fold increased lifetime risk of pancreatic cancer the lifetime risk is about 50-fold in patients with hereditary CP (9, 10). In our cohort from Leipzig 3 out of 101 patients with hereditary CP (carrier of the p.R122H mutation) developed pancreatic cancer and this corresponds to a rate of about 1 per 1200 person years among carriers of this mutation (11). In conclusion, the risk to develop pancreatic cancer is elevated in patients with hereditary CP, but the relative risk increase is not unambigously defined so far. Since cigarette smoking is a further evident risk factor for the development of pancreatic cancer, patients with hereditary CP should be strongly encouraged not to smoke (12).

CFTR VARIANTS IN CHRONIC PANCREATITIS

The role of *CFTR* variants in CP seems to be the most complex chapter of genetics in CP. This is on the one hand due to the variety of described *CFTR* variants and on the other hand due to the multifaceted functional consequences of some variants that in many cases are poorly understood so far. Moreover, it seems reasonable that *CFTR* variants alone will not lead to the development of CP. However, a rather complex interaction of variants in previously described genes and so far unkown low-risk genes will predispose to CP. Noteworthy, it seems appropriate to characterize patients with two *CFTR* variants without other unequivocal aspects of CF as patients with an "atypical" form of CF or as a *CFTR*-related disorder and this complex issue was profoundly discussed in a recent consensus conference (13).

Cystic fibrosis is an autosomal recessive disorder with an incidence in Caucasians of approximately 1 in 2500 live births. In 1989, *CFTR* was identified as the underlying gene. Since 1-2% of patients with CF suffer from CP it was a logical step to investigate *CFTR* in CP and in 1998 Sharer and colleagues and Cohn and colleagues were able to show an association of *CFTR* variants with CP that was replicated thereafter (14-23). According to their effect *CFTR* variants are divided in five or six classes (I-V/VI) (24, 25). In cystic fibrosis, the most common mutation is F508del, accounting for approximately 66% of all mutated alleles (26). In CP the distribution of *CFTR* variants does not resemble the distribution found in CF. Most of all rare and mild *CFTR* variants (class IV-VI) that are found in CP are only seldomly described in CF or congenital aplasia of the vas deferens (CBAVD). Consequently, it was postulated that compound heterozygous *CFTR* carriers have

a distinct elevated risk for the development of chronic pancreatitis, which is even higher when an additional *SPINK1* variant is present (27, 28).

SPINK1 VARIANTS IN CHRONIC PANCREATITIS

Aside other protease inhibitors, SPINK1 seems to be a specific inactivator of intrapancreatic trypsin activity. According to its high expression in the pancreas *SPINK1* represented a convincing candidate gene. In 2000 an association of *SPINK1* variants with CP was described and the most frequently variant was an amino acid substitution at position 34 from asparagine to serine (30). Carriers of the p.N34S variant were predominantly designated as patients with idiopathic CP in that no other underlying cause for CP could be found. Further studies confirmed the association and even broadened the findings to alcoholic and tropical calcific CP (31-35). Functional investigations with incubation of equimolar quantities of *SPINK1* and trypsin revealed formation of a covalent bond between the catalytic serine residue of trypsin and the lysine carboxyl group of the reactive site of *SPINK1*. After prolonged incubation trypsin activity reappeared. This finding may be explained by the degradation of SPINK1 by trypsin (36). Aside the temporary inhibitor capacity of *SPINK1* it has to be kept in mind that the p.N34S variant is found in 1-2% of controls what implicates the assumption that this variant alone might not explain the pathogenesis of CP. Analysis of recombinant *SPINK1* carrying the N34S mutation showed an unchanged function of N34S *SPINK1* as well as an unchanged trypsin susceptibility indicating that mechanisms other than the conformational change of N34S may underlie the predisposition to CP (37). Above all, it is currently discussed whether p.N34S could display just a marker for another variant (or haplotype) within *SPINK1* or an adjacent gene or even in an distinct regulatory region.

New aspects in Chronic Pancreatitis – A degradation sensitive variant of *PRSS2* protects against Chronic Pancreatitis

Most studies focus on the question whether specific changes of mechanisms predispose to the development of disease entities. The other side of the coin represents potentially protective mechanisms. However, those are rarely investigated. Anionic trypsinogen (*PRSS2*) represents a minor form of trypsinogen that accounts for up to one third of trypsinogen secreted in the pancreatic fluid (38-40). According to the success achieved by candidate gene approaches *PRSS2* was investigated and variant p.G191R was found to be overrepresented in controls to the surprise of the authors (41). In 2466 patients with CP (including 1857 with hereditary or idiopathic CP) and 6459 controls *PRSS2* variant p.G191R was found in 220 controls compared to 32 patients (odds ratio 0.37; $P = 1.1 \times 10^{-8}$). Analysis of recombinantly expressed R191 PRSS2 revealed a complete loss of trypsin activity by introduction of a novel tryptic cleavage site that renders the enzyme hypersensitive to autocatalytic proteolysis. In conclusion, R191 PRSS2 mitigates intrapancreatic trypsin activity and hence protects against CP. The results obtained in the large Caucasian cohort could be confirmed in patients of Japanese descent recently but not in patients originating from India (42-45).

Duplication and Triplication of the Trypsinogen locus – A new mechanism for the development of CP

Ten years after the first description of the p.R122H *PRSS1* mutation a completely new mechanism responsible for the pathogenesis of CP was described in French patients with hereditary CP. In these patients a triplication of an approximately 605 kb fragment of the human trypsinogen locus could be found that was assumed to result in a gain of function of trypsinogen by a so called gene-dosage effect (46). Thereafter, a duplication of the same fragment was additionally found in patients with idiopathic CP from France (47). The duplication and the triplication that seem to be generated in a two-step process are thought to arise from a common founder chromosome. A first duplication block is generated by break-induced serial replication slippage during mitosis and this duplication will provide the sequence homology required to promote non-allelic homologous recombination during meiosis. In a second step the complex triplication allele is generated (48). Summarized, these data reveal new insights in the complex mechanisms underlying the pathogenesis of CP that seem to be of importance in the investigated group of patients with hereditary and idiopathic CP from France. Additionally, these data further support the importance of a well-defined balance between proteases and anti-proteases with trypsin as key player. Taken together all data available on *PRSS1* and *PRSS2* variants it seems rather unlikely that a loss of function variants, like p.A121T, predispose to the development of CP (49, 50).

Chymotrypsinogen C (CTRC) the enigmatic Enzyme Y – A new Pancreatitis Gene

More than twenty years ago Rinderknecht described enzyme Y that was enigmatic until recently Szmola and Sahin-Tóth were able to identify Chymotrypsinogen C (CTRC) to be Enzyme Y (51). CTRC is capable to degrade prematurely activated trypsin at low calcium concentrations and possibly depicts a second line of defence when SPINK1 is not sufficient in inhibiting trypsin activity. Otherwise this trypsin degrading capacity is hindered at high calcium levels that are likewise present in the intestine where physiologically trypsin degradation would not be reasonable. In the case of high calcium levels CTRC is even able to activate trypsinogen, what may be rather useful when trypsinogen is not completely activated within the duodenal lumen. The third most frequent *PRSS1* variant p.A16V is activated by CTRC four times faster than wildtype cationic trypsinogen, a mechanism that explains the importance of CTRC for the balance of the digestive

enzyme cascade and in which way variant p.A16V may lead to the development of CP by enhanced trypsinogen activation (52). These data made *CTRC* an interesting further candidate gene and in 2008 we found that variants of *CTRC* were associated with CP, a finding that was meanwhile replicated by others (53-56).

In our study we investigated 901 German patients with idiopathic and hereditary CP and found predominantly two out of 13 variants, p.R254W and p.K247_R254del, that were significantly overrepresented in patients (30/901, 3.3%) compared to 2804 German controls (21/2804, 0.7%). The variants were recombinantly expressed in HEK293T and AR42J cells and displayed a diminished activity and/or reduced secretion of CTRC. Findings of the German cohort with idiopathic and hereditary CP were replicated in patients with tropical calcific CP from India. In these patients the enrichment of *CTRC* variants (10/71, 14.1%) in contrast to controls (1/84, 1.2%) could be confirmed. Interestingly, variants detected in Indian patients were different from that found in German patients. The most common variant found in patients originating from India was p.A73T that elicits endoplasmatic reticulum stress and thereby contributes to parenchymal damage through acinar cell apoptosis (57). To replicate our findings we investigated 348 patients with alcoholic CP and compared our data to 432 patients with alcoholic liver disease. Overall, 10 of 348 alcoholic CP patients (2.9%) but only 3 of 432 patients (0.7%) with alcoholic liver disease carried a *CTRC* variant verifying the association found in idiopathic, hereditary and tropical calcific CP. These findings demonstrate that loss of function *CTRC* variants predispose to the development of CP by reduction of its protective trypsin degrading ability.

However, genetic and functional data qualify *CTRC* as a low-penetrance gene, since for example p.R254W is found in up to 0.6% of the controls and the reduction of *CTRC* activity is only about 50% of wildtype *CTRC*. Nevertheless, *CTRC* represents another piece within the puzzle of genetic alterations that alone or only in complex interaction with other genetic alterations predispose to CP (fig. 1).

Calcium homeostasis – A new approach?

Calcium homeostasis seems to play a pivotal role for the maintenance of a balanced digestive enzyme cascade. The calcium sensing receptor (*CASR*) is a member of a G-protein coupled receptor superfamily and contributes to calcium homeostasis in pancreatic acinar and ductal cells (58). Because variants of *CASR* were present in patients with familial hypocalciuric hypercalemia (FFH) and some patients with FFH have been associated with CP, *CASR* represents another candidate gene. However, analysis of patients with CP revealed only a modifying effect of *CASR*. In German kindreds only patients with a *CASR* variant in combination with the p.N34S *SPINK1* variant developed CP (59, 60). Analysis of Indian patients with tropical calcific CP supported the latter finding, since *CASR* variation was a potential risk for CP to develop (61). In conclusion, *CASR* variants may contribute to the pathogenesis of CP when present with variants of other genes like *SPINK1*.

Environmental Influences and Genetic Aspects – Complex Interaction

A more than one hundred years old theory is supported by genetic findings of the last decade, however, genetic alterations alone seem not to be sufficient in most cases to explain the pathogenesis of CP. For instance, only about 80% that carry the p.R122H mutation develop CP, representing an incomplete pen-

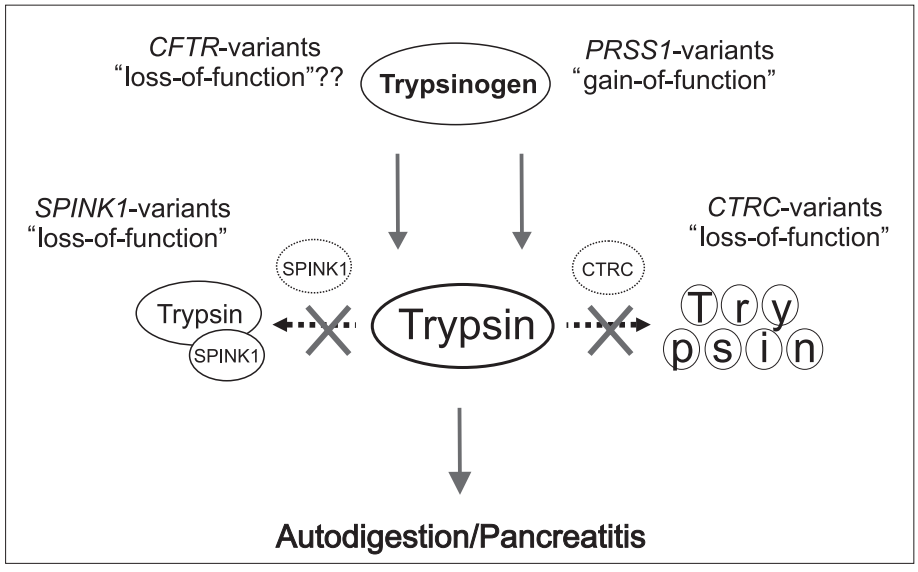

Fig. 1. Model of genetically determined pancreatitis. Gain of function variants of *PRSS1* (cationic trypsinogen), loss of function variants of *SPINK1* (serine protease inhibitor, Kazal type 1) and *CTRC* (Chymotrypsinogen C) predispose to chronic pancreatitis. The functional effect of *CFTR* (cystic fibrosis conductance regulator) variants associated with chronic pancreatitis is poorly understood. (Adapted from Rosendahl et al., *Nature Genetics* 2008).

etrance of this mutation. Environmental factors that contribute to CP are difficult to specify. Alcohol abuse represents an unequivocal risk factor for CP. However, as already mentioned, only about 5% of patients with chronic alcohol abuse will suffer from CP. Patients with a diagnosis of CP should stop smoking, since smoking increases the risk to develop pancreatic cancer and influences the course of CP negatively. In summary, there seems to be a complex interaction between genes and environmental influences and additionally between different genes. In many cases only these complex interactions may explain the pathogenesis of CP (fig. 2).

What is next?

The last fifteen years following the first description of a genetic alteration undoubtedly associated with CP have brought a wide variety of new genetic and functional insights into the pathogenesis of CP. Therefore, it seems reasonable to propose that within the next years several other genetic factors that predispose to CP will be identified. However, all these findings will render the picture of the genetic basis of CP we have today even more complex. Looking at the immense technical developments to identify genetic alterations it must be expected that emerging genetic techniques like deep sequencing will help to find further genes in a hypothesis free approach in hereditary and idiopathic CP. Other hypothesis free techniques like genome wide association studies seem to be more appropriate to find genetic alterations that may be present in alcoholic CP. For sure, a hypothesis based candidate gene approach (or possibly an even intuitive approach) will identify further genes associated with CP.

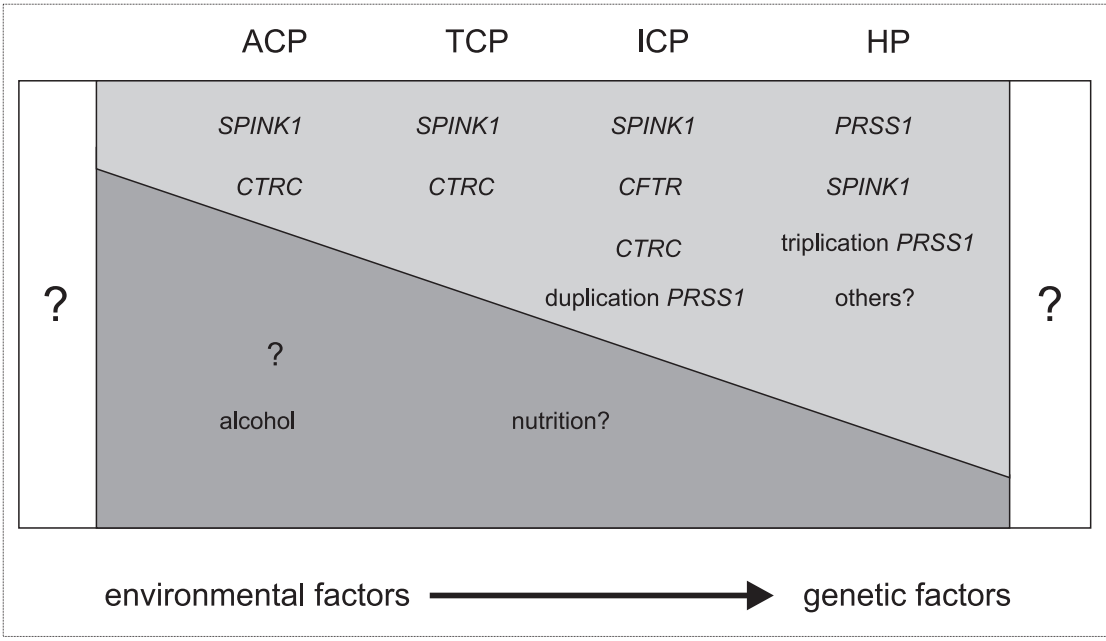

Fig. 2. Diagrammatic illustration of genetic and environmental factors with their suspected influence on the pathogenesis of chronic pancreatitis. Abbreviations: ACP = alcoholic chronic pancreatitis, TCP = tropical calcific chronic pancreatitis, ICP = idiopathic chronic pancreatitis, HCP = hereditary chronic pancreatitis; abbreviations of the genes see within the text. (According to Witt, *Gut* 2003).

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otrzymano/received: 10.01.2011
zaakceptowano/accepted: 22.02.2011

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