Genetic determination of pancreatitis

Determinacja genetyczna zapaleń trzustki

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Abstract

Pancreatitis is a complex disease with varied aetiology and clinical course. Genetic factors, in combination with environmental factors, may play a considerable role in the development of pancreatitis. Genetic studies may help in understanding the transition from acute pancreatitis to recurrent acute pancreatitis and progression to the chronic state. Various groups of genetic mutations may play a role in the pathogenesis of pancreatitis. Mutations in the cationic trypsinogen gene (PRSS1), anionic trypsinogen (PRSS2), the pancreatic secretory trypsin inhibitor gene (SPINK1), cystic fibrosis transmembrane conductance regulator gene (CFTR), chymotrypsinogen gene (CTRC), calcium-sensing receptor gene (CASR), and the protein claudin-2 (CLDN2) were found in different types of pancreatitis. The presented study demonstrates the role of the best recognised genetic mutations in the development of acute and chronic pancreatitis.

Summing up: Identification of patients with pathogenic genetic variants may change the approach to the factors related with lifestyle, such as alcohol consumption and cigarette smoking, and prevent or delay the occurrence of pancreatitis.

Streszczenie

Zapalenie trzustki jest złożoną chorobą o różnej etiologii i przebiegu klinicznym. Czynniki genetyczne w połączeniu z czynnikami środowiskowymi odgrywają znaczącą rolę w rozwoju zapalenia trzustki. Badania genetyczne pomagają zrozumieć przejście ostrego zapalenia trzustki do nawrotowego ostrego zapalenia trzustki i progresję do stanu przewlekłego. W patogenezie zapalenia trzustki mogą odgrywać rolę różne grupy mutacji genetycznych: w genie kodującym kationowy trypsynogen (cationic trypsinogen/serine protease1 gen, PRSS1), w genie trzustkowego inhibitora wydzielania trypsyny (serine protease inhibitor, Kazal Type I, SPINK1), w genie kodującym białko tworzące kanał chlorkowy (cystic fibrosis transmembrane conductance regulator, CFTR), w genie chymotrypsyny C (chymotrypsin C, CTRC), w genie receptora rozpoznającego wapń (calcium-sensing receptor gene, CASR), w genie kodującym białko klaudynę 2 (protein claudin-2, CLDN2). W pracy przedstawiono udział najlepiej poznanych mutacji genetycznych w rozwoju ostrego i przewlekłego zapalenia trzustki.

Podsumowanie: Identyfikacja osób z mutacjami w genach znanych jako patogenne dla zapalenia trzustki może zmienić podejście do czynników związanych ze stylem życia, takich jak picie alkoholu i palenie papierosów, i zapobiec lub opóźnić wystąpienie zapalenia trzustki.

Introduction

Inflammatory conditions of the pancreas and pancreatic cancer are complex diseases with varied aetiology and clinical course, but they are mutually connected. Acute pancreatitis may be the first symptom of pancreatic cancer, and the risk was nine-times higher in patients in whom chronic pancreatitis developed in the future [1]. It was confirmed that pancreatic cancer frequently develops in the group of patients with CP [2–4]. Alcohol dominates among the causes of acute, as well as chronic pancreatitis [5–8]; nevertheless, in some patients the aetiology remains unknown [2, 6, 9, 10]. This proves the existence of other environmental and genetic factors that should be taken into consideration [11]. The advancement in the field of genetics and medical technologies that has been observed in recent years provides new opportunities for early evaluation of the risk factors of pancreatitis and pancreatic cancer. The discovery that genetic factors in combination with various environmental factors may be responsible for contracting inflamma-
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ber of hospitalisations of patients with a severe form of AP in 2010 to 5462 in 2016 [20]. High incidence rates are registered in Spain 72.5/100,000 [21], Finland 73.4/100,000, and Great Britain 56.5/100,000 population [9]. The main aetiological factors of AP are gallstones and alcohol, responsible for 60–75% of the causes of the disease [22]. In Polish studies, cholelithiasis was diagnosed in 30.1% of patients hospitalised due to AP, especially females at an older age, and alcohol-related cause in 24.1% of patients, mainly the young. It should be presumed that in a large group, 41.1% of unrecognised causes of the disease were also alcohol and gallstones [10, 23]. Recurrent AP occurred in nearly 15% of patients [24]. Alcohol consumption and impaired bile flow as a result of gallstones most frequently start a cascade of metabolic disorders, which ultimately lead to mild, moderate, or severe form of acute pancreatitis. Studies of the relationship between known aetiological factors of AP, such as alcohol and gallstones, and genetic factors also seem to be interesting. Despite common knowledge concerning the relationship between AP and alcohol consumption, the mechanism of this relationship remains unknown. Only 3% of those who consume large amounts of alcohol will develop AP. According to Yadav, 30–35% of patients with AP will develop RAP [25]. Acute episodes of alcohol-related AP lead to irreversible changes in the pancreas in the form of acute pancreatitis in approximately 15–20% of patients [25, 26]. Therefore, the majority of epidemiological studies pertaining to the effect of alcohol on diseases of the pancreas refer to AP [11].

Patients with gallstones and genetic susceptibility to AP are an especially interesting group. According to Mouzner [13], it seems justifiable to consider a delay in the performance of cholecystectomy in the group of patients with indications for genetic testing using the NGS technology until obtaining the results of genotyping. Also, the guidelines by the Polish Pancreatic Club recommend the performance of genetic tests (PRSS1, CFTR, SPINK1) in young patients with a family history of recurrent AP [19]. The data obtained may help to make rational clinical decisions with consideration of the risk factors.

The results of studies provide sufficient evidence that AP may progress to a chronic process with changeable speed, according to the frequency of recurrent acute episodes, severity of the course of the disease, and the type of immune response [13, 27]. The risk factors playing a special role in the progression of AP to CP are alcohol consumption and tobacco smoking, as well as various genetic variants [12, 28]. Many individuals exposed to risk factors, incurring genetic ones, will never contract AP [13].

Some studies focus on an attempt to establish in what way genetic risk affects the clinical course of AP. One of the potential risk factors of transition from mild to severe AP may be the production of pro-inflammatory cytokines, which depends on gene polymorphisms [29, 30]. The studies focused on genetic factors causing an increase in IL-8 during the inflammatory process. IL-8 plays a key role in the development of acute respiratory failure by activation of neutrophils, which is of basic importance for severe pancreatitis [30]. However, the results of these studies are unequivocal. In one of the studies, heterozygous

Acute and chronic pancreatitis

Pancreatitis may take an acute or chronic form. Chronic pancreatitis is the problem defined as an inflammatory condition of the pancreas diagnosed based on the presence of two out of three diagnostic criteria: acute abdominal pain, at least a three-fold elevation of serum amylase or lipase in the blood, and changes in imaging findings characteristic of acute pancreatitis [18, 19]. Poland is among the countries with the highest incidence rates [10]. Annually, more than 21,000 hospitalisations due to acute pancreatitis are registered by the Polish National Health Fund. The number of hospitalisations of patients with a severe form of AP is systematically increasing, from 3481 in 2010 to 5462 in 2016 [20]. High incidence rates are registered in Spain 72.5/100,000 [21], Finland 73.4/100,000, and Great Britain 56.5/100,000 population [9]. The main aetiological factors of AP are gallstones and alcohol, responsible for 60–75% of the causes of the disease [22]. In Polish studies, cholelithiasis was diagnosed in 30.1% of patients hospitalised due to AP, especially females at
variants of IL-8 A/T mutation were more frequent in patients with severe AP, whereas a normal TT genotype was more often observed in patients with mild AP [29]. In turn, studies of IL-8 251A/T gene polymorphism in Chinese and Turkish populations did not show any significant correlations with the severity of the course of AP [30, 31]. The researchers concluded that IL-8-251 gene polymorphism may be related with ethnic differences, which exert an effect on the results of studies [31].

Chronic pancreatitis takes a course as a progressive destruction of the pancreatic parenchyma, leading to its atrophy and fibrosis. Exocrine and endocrine pancreatic insufficiency develops in the course of CP [2]. In European countries, the incidence remains within 6-7/100,000 population [32]. A known classification of the causes of CP is the TIGARO system: T – toxic-metabolic, I – idiopathic, G – genetic, A – autoimmune, R – recurrent and severe acute pancreatitis, O – obstructive [33]. Alcohol dominates among various causes of both acute and chronic pancreatitis [5–8]; however, in some patients the aetiology remains unknown [2]. In addition, in 10–25% of patients with CP the risk factors are unclear and are qualified as idiopathic pancreatitis (ICP) [34]. Recent studies suggest the presence of a relationship between tobacco smoking and the development of CP and pancreatic cancer [26, 35–39]. The development of CP is proportional to the dose and duration of alcohol consumption [40]. Although it has long been known that alcohol is the main cause of pancreatitis, only about 5–10% of individuals who consume large amounts of alcohol suffer from diseases of the pancreas [26, 40, 41]. This proves the existence of other environmental and genetic factors, which should be taken into consideration [11, 42, 43]. A family occurrence of chronic pancreatitis was described as early as in the 1950s [44]; however, the discovery of the mutation in the cationic trypsinogen gene/serine protease 1 gene (PRSS1 R122H) was a breakthrough in understanding the aetiology of this disease [34]. During subsequent years, studies focused on proteases and anti-proteases, as well as the CFTR gene, the mutation in which is responsible for cystic fibrosis. The use of possibilities to investigate the whole genome (NAPS) indicated that polymorphisms at the trypsin locus (PRSS1 rs10273639) and Claudin 2 locus (CLDN2-RIPPLY1-MORC4 locus rs7057398 and rs12688220) cause an increased risk of alcohol-related pancreatitis [45].

Hereditary pancreatitis, which usually begins with recurrent episodes of acute pancreatitis in childhood or at a young age, is rarely diagnosed [46–48]. HP is defined as the occurrence of pancreatitis in two or more first-degree relatives, or three or more second-degree relatives in two or more generations with recurrent acute pancreatitis and/or chronic pancreatitis, for which no predisposing factors are identified [48]. It is an autosomal dominant inherited disease with approximately 80% penetrance for PRSS1 [49].

**Genetic risk factors of acute and chronic pancreatitis**

**PRSS1**

The pancreas produces trypsinogen in the form of a pro-enzyme in three similar isoforms. Isoforms are encoded by separate genes: PRSS1 (cationic trypsinogen gene), PRSS2 (anionic trypsinogen gene), and PRSS3 (mesotrypsinogen) [27, 50, 51]. Cationic trypsinogen gene represents 2/3 of total trypsinogen in the pancreatic juice [50]. The PRSS1 located on the long arm of chromosome 7 (7q35) encodes cationic trypsinogen. Mutations may be located in various regions of the gene and result in a single amino acid substitution in the cationic trypsinogen. The changed pro-enzyme is more easily auto-activated inside pancreatic follicular cells. As a result of increased activity of proteolytic pancreatic enzymes self-digestion of the organ occurs. The mutations most frequently detected in the cationic trypsinogen gene (PRSS1) in patients with hereditary pancreatitis (HP) are: R122H [34], N29I [52], and A16V [53]. Pancreatitis in patients with R122H mutation is the result of impairment of the mechanism of Arg 122-Val123 autolytic peptide bond in trypsin, protecting the pancreas against premature activation of trypsin [34]. Approximately 70% of families with HP possess this mutation [47]. The p.R122H mutation increases the risk of recurrence of acute pancreatitis and chronic pancreatitis [54].

The N29I mutation is the second most common mutation (approximately 20%) [47]. An asparagine-isoleucine replacement at position 29 of cationic trypsinogen results in an increase in resistance to autolytic processes [55]. The A16V mutation is the third most frequent PRSS1 mutation with changeable penetration, most often in the group of patients with idiopathic pancreatitis [47, 56]. The results of studies conducted by Nemoda [51] in 2006 confirmed that the A16V mutant protein, contrary to R122H and N29I, does not itself increase auto-activation of cationic trypsinogen; however, the stimulation of auto-activation by chymotrypsin C plays a role in chronic pancreatitis related with A16V mutation [51].

To date, nearly 30 PRSS1 mutations have been identified that have been considered to be pathogenic [57]. The up-to-date list of investigated mutations and their clinical importance is available on the website: Genetic Risk Factors in Chronic Pancreatitis: http://www.pancreasgenetics.org.

Although PRSS1 mutations are related with HP, they may also be found with changeable frequency of occurrence (0–19%) in idiopathic chronic pancreatitis [58–60]. However, no relationship was confirmed
with alcohol-related AP [61]. No PRSS1 mutations were found in any patient with hereditary, idiopathic, or alcohol-related AP, nor in the control group among patients from India [15]. Studies of PRSS1 mutation in the Polish population confirmed the relationship with CP. In the study conducted by Gąsiorowska et al. [60], PRSS1 mutations were detected in 33% of Polish patients with alcohol-related CP, and in 21.4% of patients with ICP, significantly more frequently compared to the control group (p < 0.001). Koziel et al. did not detect PRSS1 mutation in any patient with AP, nor in the healthy controls [16]. The study published in 2017, which covered 136 children with CP (including 61 children with idiopathic CP), reported the occurrence of PRSS1 mutation in 8% of patients [62]. Oracz et al. [63] showed that the Polish paediatric HP population has a different distribution of PRSS1 mutations compared to populations from other countries, with p.R122H and p.R122C mutations being the most prevalent. Furthermore, it was also demonstrated that HP children with PRSS1 mutations show significantly more severe clinical course of the disease than children without PRSS1 mutation, despite the same age of CP onset. The most frequently mutated gene in Polish CP children was SPINK1 (combined frequency 25.4%) followed by PRSS1 (13.1%) and CFTR (7.7%) [64].

**PRSS2**

Anionic trypsinogen (PRSS2) constitutes approximately 30–40% [51]. The PRSS2 mutation is the factor protecting the pancreas against the development of inflammation by decreased PRSS1 expression [54]. Also, a lower risk of the development of RAP and CP was confirmed [54]. In patients with pancreatitis and the genotype PRSS1-PRSS2 other causes of the disease should be sought [13].

Total relative risk of the development of pancreatitis in this group of patients is estimated at 69 [65]. Although there exists a strong relationship between chronic pancreatitis and pancreatic cancer, within 20 years only about five per cent of patients with chronic pancreatitis will develop pancreatic cancer [65]. It has been proven that pancreatic cancer develops frequently in the group of patients with CP [2–4].

In PRSS1 hybrid alleles such as PRSS1-PRSS2 [66] and PRSS1-PRSS3P2 [67] generated by gene conversion events, although rare, are causative mutations in chronic pancreatitis.

**SPINK1**

The pancreatic secretory trypsin inhibitor (PSTI). The role of SPINK1 protein consists of the prevention of premature activation of zymogen, and may inhibit up to 20% of pancreatic trypsin activity, thus protecting the pancreas against self-digestion [68]. To date, nearly 30 various types of SPINK1 mutations have been reported. The complete list is available on the website: http://www.pancreasgenetics.org/. The most frequently observed SPINK1 mutations are N34S and P55S, occurring mainly in Europe and the USA. Many studies provide evidence for the relationship between N34S mutation and chronic pancreatitis of varied aetiology, with a frequency of approximately 9.7% [15, 56, 69–71]. The mechanism combining SPINK1 p.N34S mutation with pancreatitis remains unexplained [71]. The SPINK1 mutations are rarely related with HP and may, in this case, increase the risk of contracting the disease by 23% [72]. Witt et al. [69] suggested an autosomal recessive inheritance pattern. Chandak et al. [15] found, in a large group, the presence of N34S mutation in 73% of patients with HP, in 26.8% with alcohol-related AP, and in 32.5% of patients with idiopathic AP [15]. The researchers suggested a dominant inheritance pattern with low level of penetration [15]. The SPINK1 mutations are more frequently inherited in a heterozygous form, and contracting of pancreatitis depends on the presence of other genetic mutations and/or environmental factors [72]. The p.N34S mutation is strongly related with RAP; however, its effect on the risk of occurrence of the first episode of AP is under discussion [73–75]. In a study on Polish patients with AP, SPINK1 mutation was significantly more often observed, compared to the control group (6.3% vs. 3.2%; p < 0.05) [16]. The SPINK1 mutation was related with a more severe clinical course of AP (p = 0.03), but not with the cause of contracting the disease [16]. Gąsiorowska, in a group of 14 patients with ICP, discovered four patients (28.6%) with SPINK1 mutation (p < 0.05). No relationship was found with alcohol-related CP, clinical course of the disease, and complications [60].

**CLDN2**

Claudin-2 protein forms low-resistance, cation-selective ions, and water channels between endothelial cells [54]. It was confirmed on animal models that it may be expressed during stress in the acinar cells [76]. Genetic variants in the Claudin-2 locus (CLDN2) do not increase the risk of AP and RAP, but are strongly related with an increased risk of progression of RAP to CP [13, 54]. The total odds ratio for patients with alcohol-related AP and CLDN2 mutation was higher, compared to non-alcoholic AP [54].

**CFTR**

CFTR protein participates in the transport of sodium, chlorides, and carbohydrates in the epithelial cells of the respiratory system, sweat glands, and the pancreas [72]. The CFTR mutation (cystic fibrosis transmembrane conductance regulator gene) leads to cystic fibrosis, which may cause changes in the respi-
ratory, reproductive, gastrointestinal, and endocrine systems. To date, more than 1600 CFTR mutations have been recognised [77]. More than 90% of patients with cystic fibrosis experience exocrine pancreatic insufficiency, while more than 50% experience diabetes. Approximately 1.5% of patients with cystic fibrosis contract pancreatitis [72]. This concerns patients with a milder phenotype of cystic fibrosis (molecular classes III, IV, or V), who develop chronic pancreatitis and pancreatic insufficiency considerably later than other patients with cystic fibrosis [78]. The risk of contracting the disease is higher when concomitant with other mutations, e.g. SPINK1 or CTRC [78]. A strong relationship between CFTR mutations and chronic pancreatitis was first recognised and described in 1998 by Cohn et al. [79], and Sharer et al. [80]. LaRusch et al. [81] described nine CFTR mutations (CFTR R74Q, R75Q, R117H, R170H, L967S, I997F, D1152H, S1235R, and D1270N) considered as mild, which do not cause cystic fibrosis but do increase risk of chronic pancreatitis. Schneider et al. [78] observed three times more clearly occurrence of the CFTR variant p.R75Q in patients with pancreatitis, compared to the healthy controls. The risk of the disease increases in the case of the presence of other CFTR and SPINK1 variants [78]. Rosendahl et al., who studied a group of 660 patients with idiopathic and hereditary CP and 1758 controls, did not confirm the relationship between CFTR and CP, and concluded that CFTR exerts a small effect on the development of CP [82]. Also, in our previously published study no relationship was found between CFTR mutation and acute pancreatitis [16]. In the above-mentioned study of Polish children with CP, CFTR mutation was identified in 18% of patients with CP. In two cases (1.5%) the CFTR mutation occurred in combination with mutation in the SPINK1 or PRSS1 gene [62].

**CTRC**

CTRC protein encodes the enzyme chymotrypsin C, which is produced by the pancreatic follicular cells [68]. Chymotrypsin C promotes the degradation of trypsin by selective breaking of the bond between leucine at position 31, and glutamine at position 82, and subsequently by breaking the bond between arginine at position 122 and valine at position 123. Prematurely activated trypsin may be destroyed by CTRC through the action in the particle bonded with calcium [83]. The CTRC mutations may predispose for CP by ineffective degradation of trypsinogen/trypsin, ineffective activation of carboxypeptidase, and induction of the endoplasmic reticulum [82]. The degradation of trypsinogen with participation of CTRC takes place after the exhaustion of the protective mechanisms dependent on SPINK1 [84]. Pathogenic CTRC variants (p.A73T, p.V235I, p.R254W, and p.K247_R254del) were found in patients with CP, as well as healthy controls; therefore, they should be considered as risk factors [85]. The CTRC mutations occur in approximately 4% of patients with CP. Masson et al. [86] observed the presence of rare CTRC variants in 12% of patients with ICP. The majority of variants of heterozygous CTRC increase the risk of the disease by 5–10 times. A recently published Polish study also indicates that CTRC mutations, including c.180TT (p.Gly60Gly), are important risk factors for CP [62]. It was found that the c.493+51C>A variant is insufficiently represented in CP. The results suggest a protective role of this variant in the development of CP [62]. In our study of patients with AP published in 2017, three CTRC mutations were detected in exon 7: p.V235I (c.703 G>A), p.K247_R254del, p.R254W, known to be responsible for CP, and p.1259W, the importance of which has not been sufficiently recognised [87]. The frequent CTRC variant c.180T may exert an effect on the modification of the progression from RAP to CP, especially in patients with CFTR or SPINK1 variant, who consume alcohol and are tobacco smokers [88]. The presence of the SPINK1 and CTRC mutations causes a high risk of contracting pancreatitis and may be responsible for a more severe course of acute pancreatitis [84, 87].

**Conclusions**

In order to establish the ultimate cause of pancreatitis, in some cases, it is necessary to take a comprehensive family history and perform molecular genetic tests. In recent years, many genetic factors have been identified, which, while modified by environmental factors, may be of importance in the development of pancreatitis, or exert an effect on the clinical course of the disease. Simultaneously, it should be emphasised that the presence of a single mutation does not determine the contraction of the disease. Whole genome sequencing (NGS) provides new opportunities with respect to the detection of changes and decreases the cost of genetic tests. Although, until today, routine population studies are not recommended, this should not lead to a lack of interest on the part of attending physicians in the referral of patients for genetic tests in justified cases. At present, according to the recommendations by the Polish Pancreatic Club, all patients with a positive history of hereditary pancreatitis should have genetic tests performed. The identification of patients with pathogenic genetic variants may change the approach to the factors related with lifestyle, such as alcohol consumption and cigarette smoking, and prevent or delay the occurrence of pancreatitis.

**Conflict of interest**

The authors declare no conflict of interest.
References


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