Agnieszka Magdalena Rygiel[1], Małgorzata Wojnicka-Stolarz[2], Katarzyna Niepokój[1], Grzegorz Oracz[3], Jerzy Bal[1], Katarzyna Wertheim-Tysarowska[1], Krzysztof Gutkowski[2]

CHRONIC PANCREATITIS IN A PATIENT WITH THE p.Asn34Ser HOMOZYGOUS SPINK1 MUTATION – OWN EXPERIENCE

PRZEWLEKŁE ZAPALENIE TRZUSTKI U PACJENTA Z HOMOZYGOTYCZNĄ MUTACJĄ p.Asn34Ser W GENIE SPINK1 – BADANIA WŁASNE

[1]Department of Medical Genetics, Institute of Mother and Child, Warsaw, Poland
[2]Department of Gastroenterology and Hepatology
with Internal Disease Unit of Specialist District Hospital in Rzeszow, Poland
[3]Department of Gastroenterology, Hepatology and Feeding Disorders,
The Children's Memorial Health Institute, Warsaw, Poland

Abstract

Chronic pancreatitis (CP) is characterized by progressive damage to the exocrine and endocrine cell structures and pancreatic ducts with subsequent fibrosis of the organ. Patients with no apparent etiological factor are classified as having idiopathic CP (ICP). Genetic studies indicate the importance of mutations in the serine protease inhibitor, Kazal type 1 gene (SPINK1) in the pathogenesis of CP. This report describes a case of a 29-year-old Polish-Vietnamese patient with the p.Asn34Ser (p.N34S) homozygous mutation in the SPINK1 gene. The patient was hospitalized due to pain of average intensity in the epigastric area which occurred for the first time in his life. Imaging examination showed the atrophy of the pancreatic parenchyma with the presence of numerous small calcifications and a single calcified lodgement with a diameter of 22 mm in the distal segment of Wirsung 's duct. Clinical interview did not reveal any obvious etiological pancreatitis risk factors implying the causative role of the p.Asn34Ser homozygous mutation of SPINK1 in this case as proven in our investigation.

Key words: chronic pancreatitis, *SPINK1*, homozygous p.Asn34Ser mutation

Streszczenie

Przewlekłe zapalenie trzustki (PZT) stanowi długotrwały proces zapalny, prowadzący do upośledzenia zewnątrz- i wewnątrzwydzielniczej czynności trzustki oraz nieodwracalnych zmian morfologicznych z postępującym włóknieniem tego narządu. Pacjenci, u których nie stwierdzono potencjalnych przyczyn egzo- czy endogennych przewlekłego zapalenia trzustki klasyfikowani są jako przypadki idiopatycznego PZT (IPZT). Badania molekularne wskazują na znaczenie mutacji inhibitora proteazy serynowej, Kazal typ 1 (SPINK1) w patogenezie PZT. Opisujemy przypadek pacjenta o pochodzeniu polsko-wietnamskim z homozygotyczną mutacją p.Asn34Ser (p.N34S) w genie SPINK1. Pacjenta hospitalizowano z powodu bólu w okolicy nadbrzusza o miernym nasileniu, które wystąpiły po raz pierwszy w życiu. Badania obrazowe trzustki wykazały atrofię miąższu trzustki z obecnością licznych drobnych zwapnień oraz uwidoczniły zwapniały złóg o średnicy 22 mm w końcowym odcinku przewodu Wirsunga. W wywiadzie nie stwierdzono potencjalnych czynników ryzyka rozwoju PZT, co sugeruje, że w tym konkretnym przypadku przyczyną choroby jest homozygotyczna mutacja p.Asn34Ser w genie SPINK1, tak jak udowodniliśmy w naszych badaniach.

Słowa kluczowe: przewlekłe zapalenie trzustki, *SPINK1*, homozygotyczna mutacja p.Asn34Ser

A CASE REPORT

A 29 year old male of Polish-Vietnamese origin was admitted to the Department of Gastroenterology and Hepatology of the Specialist District Hospital in Rzeszow, Poland. On admission, the patient suffered from encircled pain of average intensity in the epigastric area which occurred after consuming a high-fat meal. The symptoms appeared for the first time in his life. The patient denied alcohol abuse, use of medications or other drugs. Cholelithiasis was diagnosed in the patient's mother but there was no history of pancreatitis, diabetes mellitus or pancreatic cancer in the family. During admission to the hospital, the patient's condition was good. Physical examination showed no significant deviation of the normal condition except tenderness in the upper abdomen during deep palpation. Laboratory tests revealed increased activity of the lipase (1120 U/L, n: 23-300 U/L) and total cholesterol (220 mg/dl, n: <200 mg/dl). Ultrasonography of the abdomen revealed the presence of lodgements in the pancreatic duct in the head of the pancreas and the widening of Wirsung 's duct all the way long, compressing the remaining pancreatic parenchyma. Examination was extended to abdominal computed tomography (CT), revealing atrophy of the pancreatic parenchyma and the presence of numerous small calcifications, as well as a calcified lodgement with a diameter of 22 mm in the distal segment of Wirsung 's (fig. 1). The CT image corresponded to chronic pancreatitis. After a few days of strict diet, fluid resuscitation, and the application of pain relievers, the pain symptoms reported on admission subsided and the patient achieved a normal lipase activity.

Since clinical interview did not reveal any obvious pancreatitis etiological factors, our patient was qualified for genetic tests. Written consent from the patient and his parents was obtained before the analysis. Evaluation of mutations in the high risk genes associated with CP was performed. Selected regions of *PRSS1*, *SPINK1*, *CTRC* and *CFTR*) were analyzed. DNA was isolated from peripheral leukocytes using Genomic maxi AX isolation kit (A&A Biotechnology, Gdynia, Poland). The DNA was amplified by polymerase chain reaction (PCR) and sequenced using the Sanger method. PCR was performed for selected exons and flanking non-coding regions of the *SPINK1*(exon 3) and *PRSS1* (exons 2 and 3) gene, *CFTR* (ex 4, 7, 9, 10, 11, 13, 17b, 20, 21, 3849+10kbC>T; dele2,3(21kb) and *CTRC* (exons 2, 3 and 7). The following primers for exon 3 of *SPINK1* were used: 5' primer - GAAGAACGTGCCCCAAGAT ; 3' primer - GTTTGCTTTTCTCGGGGTGAG. Primers for the *PRSS1*, *CFTR* and *CTRC* genes are available on request. The fluorochromatograms were analyzed using Mutation Surveyor (Softgenetics) software with NM_002769.2 (*PRSS1*), NM_003122 (*SPINK1*), NM_000492.3 (*CFTR*) and NM_007272 (CTRC) as reference sequences. Identified variants were named according to HGVS (Human genome variation society) recommendations (http://www.hgvs.org).

The homozygous p.Asn34Ser mutation in the *SPINK1* gene was detected. Biparental origin of the mutation was confirmed by genetic testing of the parents. No mutations were found in any other tested genes.

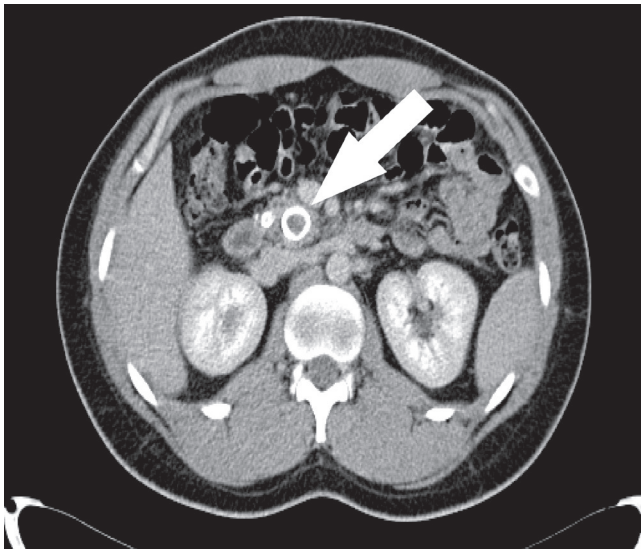

Fig. 1. Computed tomography (CT) of the pancreas. The arrow points to a large, calcified structure with a diameter of 22 mm the in distal segment of Wirsung's duct.

Ryc. 1. Zdjęcie z tomografii komputerowej trzustki. Strzałka wskazuje na dużą, uwapnioną strukturę o średnicy 22 mm w końcowym odcinku przewodu Wirsunga.

DISCUSSION

We report a CP case with the p.Asn34Ser homozygous mutation in the *SPINK1* gene. The absence of obvious pancreatitis etiologic risk factors suggest a causative role of the p.Asn34Ser (p.N34S) homozygous mutation of *SPINK1* in this case.

In many patients CP results as consequence of the interplay between genetic factors (gene mutations) and environmental factors (e.g. alcohol, cigarettes). Other known risk factors include, for example, drug abuse, infectious diseases, as well as anatomic anomalies, autoimmune and metabolic disorders [1]. In approximately one third of all cases with CP, no apparent etiological factor can be found, and these patients are classified as idiopathic CP (ICP) [2]. An increasing number of scientific reports indicate the importance of mutations in the serine protease inhibitor, Kazal type 1, *SPINK1* in the pathogenesis of CP [3, 4]. SPINK1 acts as the first line of defense against prematurely activated trypsinogen by inhibiting approximately 20% of total trypsin activity within the pancreas. A strong association between *SPINK1* and CP was, for the first time, demonstrated by Witt et al in 2000 [5]. Subsequently, other authors reported the p.Asn34Ser mutation in *SPINK1* as a factor associated with idiopathic and hereditary CP [6-8]. The frequency of the *SPINK1* mutations in ICP shows a discrepancy in different reports ranging from 6.4% in French patients [9], 25% in patients from Finland [10] to as high as 40% in patients from the USA and The European Registry of Hereditary Pancreatitis and Familial Pancreatic Cancer (EUROPAC) Recruitment [7]. In the Polish group of

ICP patients, the frequency of the *SPINK1* mutations was reported to be around 29% [11].

The role of *SPINK1* mutations in the development of CP is still a matter of debate. Witt et al. [5] suggested that there is a causative link between the *SPINK1* locus and CP families with the p.Asn34Ser mutation. However, other studies indicated the *SPINK1* mutation associated risk of pancreatitis is low [6, 7]. Modeling and familial clustering suggest that *SPINK1* mutations are disease modifying and by themselves do not cause the disease. This implies that other genetic or environmental factors may be involved in CP development in patients with *SPINK1* mutations. Recently Masson et. al. [12] provided a conservative assessment of the major genetic causes of ICP in a sample of 253 young French ICP patients. It is for the first time that the authors have assessed the pathogenic relevance (causative or contributory) of the detected variants/genotypes in the major pancreatitis genes including *PRSS1*, *CTRC*, *CFTR* and *SPINK1* to their respective carriers. They concluded that *SPINK1* p.Asn34Ser heterozygous mutations contribute to CP, whereas p.Asn34Ser homozygous ones are causative of CP. In general, data combined from this study and other studies performed in Europe and the United States indicate that 2.6-5.5% of CP patients are homozygous for p.Asn34Ser [13, 14]. It is suggested that homozygosity for the p.Asn34Ser doubles the functional effect of the single heterozygous p.Asn34Ser allele and thereby reduces the SPINK1 expression below a threshold level that is sufficient to cause the disease [12]. Importantly, no p.Asn34Ser homozygote has ever been reported in a control population [13-15]. Given that already a 10-15 fold increased risk is conferred by the heterozygous p.Asn34Ser mutation [13, 15] it seems reasonable to classify the homozygous p.Asn34Ser as a causative mutation [12].

It is unclear whether the *SPINK1* mutations have an impact on the clinical course of CP. A study by Gasiorowska et al. [11], failed to reveal any association between mutations and the severity of CP phenotype. Truninger et. al. showed that the ICP course, at least with regard to the development of calcifications and exocrine/endocrine insufficiency, was similar in early onset ICP patients, regardless of heterozygosity in *SPINK1* [16]. In contrast, the study by Creighton et al. [17] indicates that CP patients carrying the p.Asn34Ser mutation presented with a more severe disease compared to those without the *SPINK1* mutation and were predisposed to an earlier age of disease onset.

In conclusion, our patient who was homozygous for the p.Asn34Ser, despite the substantial changes in the structure of the pancreas showed mild symptoms of pancreatitis which did not appear until the age of 29. The absence of anatomical anomalies of the pancreas, and other main environmental and genetic factors points to the causative role of the p.Asn34Ser homozygous mutation in the development of CP in this case. It also confirms that genetic testing of *SPINK1* is important in ICP and may help to establish the cause of the disease. Since the p.Asn34Ser *SPINK1* mutation was shown as a risk factor of pancreatic cancer [18] in the Asian population, it might be reasonable to screen for this mutation in patients with idiopathic CP and especially ones of Asian origin. Besides, the implementation of genetic testing in the closest relatives of *SPINK1*-p.Asn34Ser-associated CP patients can help identify individuals with a risk of developing CP and implement primary prevention as lifestyle modification.

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Address for correspondence:
Agnieszka M. Rygiel
Institute of Mother and Child
Kasprzaka 17a, 01-211 Warsaw, Poland
tel. (00 48) (22) 32-77-177
fax. (00 48) (22) 32-77-200
e-mail: agnieszka.rygiel@imid.med.pl