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GENETIC MUTATIONS IN PATIENTS WITH CHRONIC PANCREATITIS IN UKRAINE

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Summary

The authors conducted a genetic analysis of patients with chronic pancreatitis in Ukraine. It turned out that more than a quarter of patients had mutations of PRSS1, SPINK1, CFTR genes. Peculiarities of combinations of ADH, ALDH, CYP2E1 genes, encoding enzymes involved in ethanol metabolism, are revealed. Obtained data testify to the reasonability of continuing research in this field.

Keywords: *chronic pancreatitis, genetic predisposition, ethanol metabolism.*

Резюме

Генетические мутации у больных хроническим панкреатитом в Украине

Авторы провели генетический анализ у больных хроническим панкреатитом в Украине. Оказалось, что более чем у четверти больных имеют место мутации генов PRSS1, SPINK1, CFTR. Выявлены особенности комбинации генов ADH, ALDH, CYP2E1, кодирующих ферменты, участвующие в метаболизме этанола. Полученные данные свидетельствуют о целесообразности продолжения исследований в этом направлении.

Ключевые слова: *хронический панкреатит, генетическая предрасположенность, метаболизм этанола.*

Introduction

Problem of chronic pancreatitis (CP) is one of the most complex ones in medicine and particularly in gastroenterology. Frequency of CP in the world has doubled in the past 30 years, diagnostics and treatment being difficult for general practitioners. CP represents itself a serious medico-social problem, predominantly able-bodied population suffering from this disease while it leads to the decreasing quality of patients' life, partial or full loss of working capacity [5].

Except main etiological factors of CP (alcohol abuse, biliary pathology), special attention has been recently paid to the genetic predisposition. Since active trypsin has the potential of driving the pancreatic digestive enzyme activation cascade, number of mechanisms are engaged in protection of the pancreas and body from autodigestion. There's an occurrence of breach in the protection of acinar cells due to the genetic mutations [1].

It was defined less than a decade ago that the hereditary pancreatitis was a rare pathology of the pancreas, clinically characterized by recurrent episodes of acute pancreatitis in the form of abdominal pain and dyspeptic syndromes, gradually increasing frequency and severity of relapses, increasing degree of functional (exocrine and/or endocrine) pancreatic insufficiency, burdened familial history, high risk of pancreatic cancer. Now it should be admitted that a wide range of possible associations of geno- and phenotype of hereditary pancreatitis may vary from direct autosomal dominant traits of the disease with nearly complete penetrance (dominant cationic trypsinogen (PRSS1) gene mutation) through mild genetic risk factors without evidence of Mendelian inheritance (pancreatic secretory trypsin inhibitor (SPINK1), cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations) to subtle hereditary disease modifiers that can be identified only in complex studies (genetic mutations in chymotrypsin C, anionic trypsinogen (PRSS2), etc.). Thus, identification of mutations in different genes, particularly in PRSS1, PRSS2, SPINK1, keratin, etc., has changed the understanding of CP pathophysiology, to some extent having determined the significance of influence of environmental factors on the degree of penetrance of hereditary pancreatitis, severity of its clinical symptoms and age of onset [1, 3].

Nowadays alcohol and drug addiction are considered to be the most acute socially significant

problems. Nature of these occurrences may vary; in each case alcoholism and drug addiction can be the consequences of effect of different groups of such factors as social, environmental, and psychological ones. However, heredity, i.e. human genotype, is supposed to be their background, and it can strengthen or weaken the effect of other factors while addictions' development. Predisposing environment includes all the factors contributing to the manifestation of relevant genetic traits [2].

It's crucial to keep in mind that the identification of specific genetic variants cannot be regarded as determination or confirmation of diagnosis, as well as cannot be used for diagnosing of various addictions, it being only an ancillary physician's test, allowing to choose proper method of therapy [3].

Role of genetic mutations defining the enzymes involved in alcohol metabolism is widely discussed. Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) are the key enzymes involved in ethanol metabolism. At the first stage of ADH metabolism converts ethanol to acetaldehyde, then, with the help of ALDH enzyme, turning the acetaldehyde into acetate non-toxic products, CO_2 and water [3].

Different populations are described to have different isoforms of these enzymes: active and inactive ones. ADH activity is determined by aminoacid in position 48 of protein; histidine in such a position is typical for the active form (ADH1B*2), while arginine – for inactive one (ADH1B*1). ALDH activity is determined by aminoacid in position 504: glutamine – active form (ALDH2*1), lysine – inactive form (ALDH2*2). Presence of allele encoding ADH active form (p.48His, c.143A, ADH1B*2) and/or ALDH inactive form (r.504Lys, c.1510A, ALDH2*2) in human genotype leads to increased concentrations of the aldehyde, causing the number of such unpleasant symptoms as nausea, vertigo, hyperemia of the face skin subjectively evaluated as "discomfort in the body", etc., thus leading to the rarer alcohol intake and its intake in smaller quantities [1].

Therefore, presence of alleles c.143A of ADH2 gene and c.1510A of ALDH2 gene in genotype may be considered as a protective factor, but it cannot be an obstacle for the development of alcohol addiction.

Cytochrome 2E1 (CYP2E1) is involved in the metabolism of acetone, benzene, carbon tetrachloride and other carcinogens that are present in tobacco smoke. Enzyme also takes part in the ethanol metabolism. Variant T of RsaI (RsaI c2) polymorphism is characterized by increased transcriptional activity and is associated with alcoholic liver disease, while variant C of PstI (PstI+) polymorphism corresponds to the increased risk of oncological diseases' development. Variant C of DraI polymorphism is also a cancer marker. In Caucasian populations rate of occurrence of variants RsaI c2 and PstI+ makes up 1–3%, occurrence of variant DraI is about 10% [1].

Oxidative stress caused by ethanol seems to be the main mechanism of ethanol lesion of the liver. CYP2E1 enzyme is a producer of hydrogen peroxide and free peroxide and hydroxyl radicals. CYP2E1 is known to be induced by ethanol. Consequently, change of the enzymatic activity or its level in the tissues corresponds to the risk of lesion of different tissues [1, 3].

The aim of study is to analyze the frequency of mutations of different genes upon chronic pancreatitis in Ukraine.

Materials and methods

We examined 68 patients with definite CP, including 52 males and 16 females aged from 17 to 61. All the patients were diagnosed to have CP according to M-ANNHEIM criteria [4]. It means that patients had typical clinical history of CP and one or more of the following additional criteria: pancreatic calcifications, moderate or evident ductal lesions (according to the Cambridge classification), evident and persistent exocrine insufficiency defined as a pancreatic steatorrhea markedly reduced by enzyme supplementation, typical histology of an adequate histological specimen. M-ANNHEIM severity index in our patients was the following: A — 9 (13.2%), B — 25 (36.7%), C — 27 (39.8%), D — 5 (7.4%), E — 2 (2.9%) patients.

According to the results of fecal elastase test by immune-enzyme method, 48 (70.6%) patients had severe, 20 (29.4%) patients had moderate and mild pancreatic insufficiency.

Control group included 80 healthy persons matched with CP patients in age, sex and alcohol consumption, but without CP.

52 patients were diagnosed to have alcoholic CP, 15 patients had idiopathic pancreatitis, 1 patient – familial pancreatitis.

DNA-diagnostics was performed in the department of molecular-genetic studies at Central Research Laboratory on the basis of Donetsk National Medical University. Genomic DNA, isolated from the leukocytes of whole blood with the use of reagent «DNA-express-blood» by «Lytech» (Moscow, Russia), was subjected to analysis for the detection of mutations (polymorphisms). Diagnostic test-systems «SNP-Express» were applied in the study in order to detect such mutations as: mutations of PRSS1, SPINK1, CFTR genes; mutation of «alcoholic

cytochrome» *CYP2E1* -1293G/C (c1/c2), ADH mutation *ADH1B* Arg47His (ADH2*1/ADH2*2), ALDH mutation *ALDH2* Glu487Lys (ALDH2*2) with two pairs of allele-specific primers. Analysis of polymorphic DNA-loci was conducted by means of polymerase chain reaction (PCR) followed by electrophoretic detection. Reaction was performed under the following conditions: initial denaturation at 93°C for 1 min, followed by 35 cycles consisting of denaturation — 93°C, 10 sec; annealing of the primers — 64°C, 10 sec; elongation — 72°C, 20 sec. PCR was performed on the amplifier Gene Amp® PCR System 2400 (Applied Biosystems). Detection of the amplified fragments was carried out by electrophoresis in 3% agarose gel stained with ethidium bromide. Visualization of results was conducted in the ultraviolet transilluminator "TFX-20.M" ("Vilber Lourmat", France). Risk assessment, frequency of genotypes, alleles and confidence intervals were performed with the help of Microsoft Excel. Differences in the frequency of alleles and genotypes between groups were assessed using $\chi 2$ criterion and calculation of risk ratio (RR) with confidence intervals (CI). Differences were considered to be significant upon $p<0.05$. Statistical analysis was performed with the use of the application program "Statistica 6.0".

Example of electrophoregram showing the results of PRSS1 gene restriction is presented on the *fig. 1*.

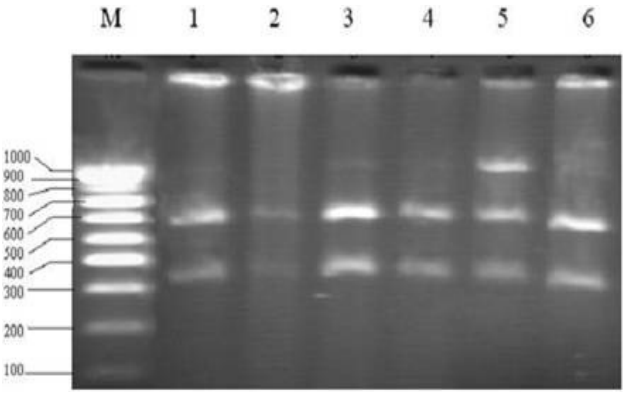

Figure 1. *Electrophoretic detection of amplificated products by PCR restriction analysis in 1.5% agarose gel. Electrophoregram showing the results of PRSS1-gene restriction (№ 5 — Heterozygous to PRSS1 mutation)*

Results and discussion

Different mutations of PRSS1, SPINK1, CFTR genes were detected in 18 (26.5%) patients and only in 4 (5.0%) healthy persons ($p<0,001$).

Spectrum of mutations identified in our patients was following: PRSS1 mutations were detected in 5 (27.8%), PRSS1+SPINK1 — in 1 (5.6%), PRSS1+CFTR — in 2 (11.1%), CFTR — in 4 (22.1%), CFTR+SPINK1 — in 1 (5.6%), SPINK1 — in 5 (27.8%) patients of all the patients with mutations. Only isolated cases of mutations in PRSS1, CFTR and SPINK1 genes were found in healthy persons (*fig. 2*).

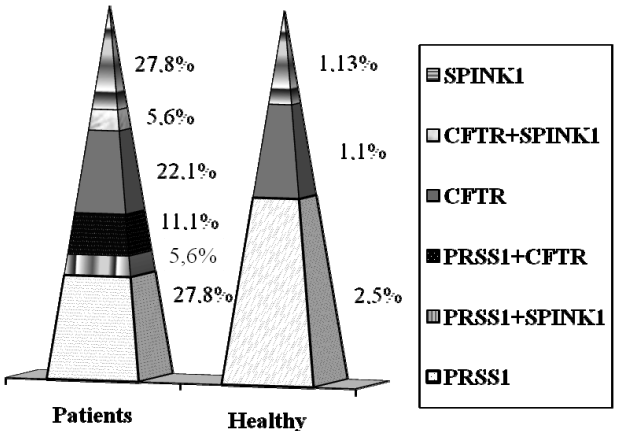

Figure 2. *Spectrum of PRSS1, SPINK1, CFTR genes mutations*

Majority of the patients had a combination of active form of ADH gene (ADH1B*2) and active form of ALDH gene (ALDH2*1) — 38 (55.9%) patients. Combination of low-activity form of ADH gene (ADH1B*1) and active form of ALDH gene (ALDH2*1) was detected in 14 (20.6%) patients. Combination of low-activity form of ADH gene (ADH1B*1) and low-activity form of ALDH gene (ALDH2*2) — in 10 (14.7%) patients. Combination of active form of ADH gene and low-activity form of ALDH gene was the rarest one (in 6 patients — 8.8%).

Distribution of 'alcoholic cytochrome' genotypes -1293G/C CYP2E1 in patients with a combination of active form of ADH gene (ADH1B*2) and active form of ALDH gene (ALDH2*1) corresponded to: G/G — 14.2%, G/C — 47.1%, C/C — 38.7%. Number of patients with allelic variant of G/C and C/C gene CYP2E1 -1293G/C increased in 1.3 and 3.5 times in comparison with control group.

We tried to draw a parallel between mutations and CP manifestations. In patients with PRSS1 gene mutations, indicators of fecal elastase at the level of less than 100 mcg/g, i.e. severe pancreatic insufficiency, were detected in 2.3 times as likely as in patients with ADH gene mutations — in 2.9 times more likely than in other patients.

In patients with SPINK1 mutations, calcification of the pancreas was determined in 2.1 times, and pseudocysts of the pancreas — in 2.5 times more likely than in other patients.

Conclusion

CP in Ukraine is being developed in more than a quarter of patients on the background of genetic predisposition. In our opinion, it's a very high muta-

tion rate. Peculiarities of the combinations of allelic variants of ADH, ALDH and CYP2E1 genes occur upon alcoholic CP. Therefore, it requires further studying and analysis.

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PANCREATITA CRONICĂ ŞI COMPLICAŢIILE EI: TRATAMENT CHIRURGICAL CONTEMPORAN

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Summary

Chronic pancreatitis and its complications: surgical treatment

Our study includes the results of surgical treatment of 412 patients, during the perriod 1990-2012 in Surgical Clinic nr. 2. Operations of choice were following: pancreatojejunoanasthomosis (PJA), CDP, toracoscopic splanchnectomy. In the complicated cases with pancreatic pseudocyst (PP) – cystpancreatojejunoanasthomosis (CPJA) on the loop by Roux external drainage, ultrasound guided puncture of PP, toracoscopic splanchnectomy. PC complicated cases by obstructive jaundice – PJA with cholecysto-or-holedocho-jejunoanasthomosis (CoCJA) on the splitted loop by Roux (Igr.), CPJA with CoCJA on the splitted loop by Roux II gr. Remained operations were not folowed by postoperative lethality.

Keywords: *chronic pancreatitis, pancreatic pseudocyst, pancreatojejunoanasthomosis, cystpancreatojejunoanasthomosis.*

Резюме

Хронический панкреатит и его осложнения: современное хирургическое лечение

В работе представлены результаты хирургического лечения 412 больных хроническим панкреатитом (ХР) и его осложнений. Были применены следующие хирургические вмешательства: панкреатоеюноанастомоз на изолированной петле по Ру, панкреатодуоденальная резекция, торакоскопическая спланхникэктомия. В случае псевдокист поджелудочной железы-кистпанкреатоеюноанастомоз на изолированной петле по Ру, наружное дренирование. При хроническом панкреатите, осложнённом механической желтухой – панкреатоюноанастомоз и холецисто-холедохо) – еюноанастамоз на расщеплённой петле Ру. Ранней послеоперационной летальности не отмечалось.

Ключевые слова: *хронический панкреатит, псевдокиста поджелудочной железы, механическая желтуха.*

Introducere

Conform datelor OMS, pe parcursul ultimilor 20 de ani mortalitatea prin pancreatită cronică (PC) şi complicaţiile ei este în creştere continuă. Frecvenţa PC variază în populaţie de la 0,2% până la 0,6% la 100 000 populaţie, înregistrându-se anual 7–10 cazuri noi de PC. Incidenţa în Europa diferă semnifcativ, se estimează la 8,2 cazuri noi la 100.000 de locuitori/an şi variază de la 1 caz nou de PC la 100 000 de locuitori (Anglia) la 13 (Spania) şi 23 (Elveţia). Prevalenţa PC în Europa este de 26,4 cazuri/an (în Franţa – 15,8 şi în Spania – 18,3 la 100.000 de locuitori). PC şi complicaţiile ei rămân o afecţiune gravă şi imprevizibilă, cu prognostic incert, chiar şi în contextul unor terapii ce se înscriu în linia protocoalelor terapeutice moderne [5].

Material şi metode

Studiul prezintă rezultatele tratamentului chirurgical aplicat la 412 pacienţi cu PC şi complicaţiile ei, desfăşurat în perioada 1992-2012 în Clinica Chirurgie nr. 2. În lotul I au fost incluşi pacienţi cu următoarele forme de PC: indurativă – 21 (15,24%) cazuri, pseudotumoroasă – 26 (29,52%) cazuri, calculoasă – 68 (55,24%) cazuri. Lotul II a inclus cazurile de PC complicate cu pseudochist pancreatic (PP) – 261 (92,6%) cazuri, icter mecanic – 45 (9,0%) cazuri,