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Polymorphism of gene apolipoprotein B in patients with chronic pancreatitis, combined with obesity and diabetes mellitus type 2

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Abstract

The purpose of research: To analyze the Ins / Del - polymorphism of Apo -B (rs17240441) in the structure of patients with chronic pancreatitis (CP), with comorbidity with type 2 diabetes mellitus and obesity.

Materials and methods: The study of 90 participants who were divided into 2 groups. The first group consisted of 49 patients with CP in combination with obesity and type 2 diabetes, the second - almost 41 healthy individuals (control group).

Genomic DNA was isolated from peripheral blood using the test - systems "innu PREP Blood DNA Mini Kit (Germany). To determine the polymorphic variants of the gene ApoB ins / del using the PCR method. The distribution of genotypes and allele frequency was assessed χ^2 test, according to the law of distribution of genotypes Hardy - Weinberg.

Results and discussion: Analysis of the multiplicative model of inheritance revealed that I- allele is more common than "minor" D- allele at 40, 82% ($\chi^2 = 32, 65, p < 0,001$) in the experimental group and 21, 96% ($\chi^2 = 7, 90, p = 0,005$) in the control. In total, the surveyed population of 180 selected alleles dominated "wild" I - allele of the mutant to 32, 22% ($p < 0,001$). The distribution of genotypes in the cohort studied law consistent with Hardy - Weinberg ($\chi^2 = 0, 25, p = 0, 62$). Analysis of the overall co-dominant inheritance pattern, showed a significant advantage relative frequency InsIns- and InsDel-genotypes of the gene Apo-B over DelDel-genotype as that of persons investigated and the control groups.

Conclusions: 1. Thus, the deletion of the functional portion of the DNA of the gene Apo-B (id. Rs17240441) in the homozygous state in the second pair 2r23-24 chromosomes found in the studied population of North Bukovina in 13, 33% of cases: among patients with CP, type 2 diabetes and obesity - to 10,20%, among control group - in 17,07% of cases ($p > 0,05$). 2. InsIns-gene genotype Apo-B is found in every second patient HP, type 2 diabetes with obesity (51, 02%), which is 12, 0% more than in the control group ($\chi^2 = 3, 95, p = 0, 047$).

Keywords: chronic pancreatitis, diabetes mellitus type 2, the gene Apo-B genotype

1. Introduction

Dislipidemia is an important verified risk factor of ischemic heart disease (IHD), obesity, metabolic syndrome (MS), myocardial infarction (MI), arterial hypertension (AH), etc. [1, 7]. Investigations of the last years have shown, that side by side with hypercholesterinemia, a series of other disorders of the lipid spectrum are also risk factors of IHD and atherosclerosis. An increase of the level of triglyceride (TG), leptin, apolipoprotein $-\beta$ (apo- β) and a low level of lipoprotein cholesterol of high density (ChS LPHD), blood adiponectin, etc., may be to the point. [2, 8]. Peculiarity is that the risk factors strengthen the effect of each other, increasing probability of atherosclerosis development for several times. If the presence of separate diabetes mellitus (DM) type 2, or AH increases solitary risk of MI development in men aged 40-65years by 2,5 times, then in case of their combination the risk increases by 8 times, and at concomitant disorders of the spectrum of the blood lipoproteins the risk increases by 19 times [10]. The results of investigations of the last years are evidence that clinically apparent atherosclerosis in more than 50% of cases is observed in persons without hypercholesterinemia and severe IHD clinical course and even MI development are typical for hemodynamically insignificant stenosis of the venous arteries. Furthermore, coronary heart disease (CHD) occurs more frequently in asians and afroamericans, despite low indices of general cholesterol (GChs), lipoprotein cholesterol of low and very low density (ChS LPLD, ChS LPVLD). The afore said testifies to the necessity of the revision of classical conceptions about atherosclerosis pathogenesis and its clinical manifestations, and induces to draw attention to inflammatory component of adipose tissue influence, oxidative stress, insulinoreistance (IR), genetic factors, etc. [6].

Discrepancy of the results in different populations, ethnic groups, racial distinctions show the necessity of the search of the new predictors of the development of dislipodemias, atherosclerosis, vascular catastrophes etc. including the genetic ones. As apo-β lipoprotein (Apo-β) (intestinal – chylomicrons, Apo-B-β-48; hepatic-including ChS LPVLD, Apo-β-100) is a protein product of the majority of atherogenic lipids, it plays a significant role in TG and GCh transporting, therefore, polymorphism of the given gene is of great interest for clinical medicine [11].

Some dozens of DNA types of Apo-B gene polymorphism are known. Gene is located in the second pair of chromosomes 2p23-24, contains 29 axons and 28 introns. One of the clinically significant gene Apo-B mutations is insertion-deletion polymorphism (Ins/Del) of the signal peptide, situated in the 5-pointed zone of the particular gene [3]. ApoB (Ins/Del) polymorphism (rs17240441), produces the difference in three aminoacids in the signal peptide, is associated with GChs content when fasting [5], TG [9], ChS LPHD, ChS LPLD (A.Kallel, M.Feki,Karani Santhanakrishnan Vimalaswaran, 2015), and also with cardiovascular catastrophes [4].

Thus, the data obtained about association of Ins/Del polymorphism gene Apo-B with lipid metabolism, atherosclerosis in different populations and ethnic groups are discrepant and require further studying. But peculiarities of expression of the given mutation depend upon totality of the genetic, ecological, cultural and social-economical factors, which are defined by the style of the diet and life as a whole. Ins/Del-polymorphism gene Apo-B studies in case of pathology of inner organs were not conducted in Ukraine at the moment of the beginning of this investigation. The aim of our research was to carry out analysis of Ins/Del-polymorphism gene Apo-B (rs17240441) in the structure of patients suffering from chronic pancreatitis (ChP) in comorbidity with DM type2 and obesity.

2. Material and methods of the research

90 persons, divided into 2 groups, took part in the investigation. The first group consisted of 49 patients with chronic pancreatitis (ChP) in combination with obesity and diabetes mellitus type2 (DM type2), 41 apparently healthy persons constituted the second group (control group). Middle age of the patients was 49, 3±0,87.

Genomic DNA was discharged from the peripheral blood for molecular-genetic investigation by means of commercial test-system “innu PREP Blood DNA Mini Kit” (Analytik Jena, Germany) using centrifuge filters. Modified protocols with oligonucleotide primers [1] with application of PCR method were used to determine polymorphous variants of gene Apo-B ins/del (rs 17240441).The investigated gene areas were amplified be means of specific primers (“Metabion”,

Germany). Succession of primers (5’-3’) – F: 5’ is ACCGGCCCTGGGGCCCGCCA-3’, R:5’ – CAGCTGGCGATGGACCCGCCGA – 3’.

Specific fragment of gene Apo-B ins/del was amplified applying commercial set DreamTag Green PCR Master Mix (firm “Thermo Scientific”, USA).

Common working amplified mixture was prepared to make fragment DNA of gene Apo B (ins/del), carried into tubes in 22μl, and then added DNA. Tubes with ready amplified mixture were put into Amplificator FlexCycler BU (Analytic Jena, Germany).

The condition of the amplified fragments was analyzed in 4% agarous gel (agarose firm “Cleaver Scientific”, Great Britain), with addition of bromine ethid, marker of the molecular weight GreneRuler 50 bp DNA Ladder (“Thermo Scientific”, USA) and subsequent visualization by means of computer programmer Vitran. The data obtained were visualized in Transilluminato.

The results of IIIIP were taken into consideration depending on the length of the available amplified DNA fragments – 93 p.n.or 84п.н. Polymorphous variant of gene ApoB – ins/ins was registered at availability of amplified DNA fragment in 93 p.n. or 84 p.n. - Ins/del, but at the fragment length of 84 p.n. - Del/del.

Statistical processing of the results was carried out by means of computer program Microsoft Excel using standard methods of variation statistics. Genotype distribution and allele frequency were evaluated by χ² test, including the accordance of genotype distribution with Hardy-Weinberg law.

3. The results of the research and their discussion

The analysis of the multiplicative model inheritance has shown that relative frequency I – allele and D-allele of gene Apo B in patients with ChP, combined with DM2, obesity and in persons of the control group were not reliably different. However, I – allele was observed more often than “minor” D-allele 40, 82% (χ²=32,65, p<0,001) in the group under study and 21, 96% (χ²=7,90, p=0,005) in the control one. In all, “wild” I-allele prevailed over the mutant 3, 22% (p<0,001) in 180 isolated alleles of the studied population.

Distribution conformity of genotypes Ins/Del of polymorphism of gene Apo-B with Hardy-Weinberg law in the control group was verified by means of test xu- quadrant with 1 freedom extent without use of Yets’ correction, but the difference in genotype distribution in the group of control and sick persons –by means of test χ-quadrant with 2 degrees (table 1). Distribution of genotypes in cohort of the inspected persons corresponded to Hardy-Weinberg law (χ²=0, 25, p=0, 62), without reliable difference between expected and actual heterozygosity in both groups of observation.

Table 1: Heterozygosity indices of Ins/Del polymorphism gene Apo-B in patients with chronic pancreatitis, combined with obesity and diabetes mellitus type 2

Groups	Alleles, n (%)		P _I	P _D	H _O	H _E	F	χ ²	P
	I	D							
Group under study, n=98	69 (70,41)	29 (29,59)	0,70	0,29	0,39	0,42	0,07	<1,0	>0,05
Control group, n=82	50 (60,98)	32 (39,02)	0,61	0,39	0,44	0,48	0,08	<1,0	>0,05
In all, n=180	119 (66,11)	61 (33,89)	0,66	0,34	0,41	0,45	0,08	<1,0	>0,05

Notes: 1.P – relative frequency of I allele; P- relative frequency of D-allele. 2. H – actual heterozygosity; H – expected heterozygosity; F – coefficient of inbreeding. 3. χ²p – criterion of correctness of “zero” hypothesis between actual

and expected heterozygosity. 4. n (%) – absolute quantity (per cent) of observation.

The analysis of the general co-dominant model of inheritance has shown a reliable advantage of the of relative frequency of

InsIns and InsDel-genotypes of gene Apo-B over DelDel-genotype both in persons of the group under study and control group: among patients – in 5 and 3,80 times ($\chi^2 = 26,67$ and $\chi^2 = 16,33$, $p < 0,001$), among the inspected persons of the control group – in 2,28 and 2,57 times ($\chi^2 = 7,04$, $p = 0,008$ and $\chi^2 = 9,68$, $p = 0,002$) accordingly, without considerable distinctions in frequency of intermediate genotype and wild homozygotes ($p < 0,05$). Information criterion Aikaike (AIC) for the particular model is 17.16 with probability $\chi^2 = 1.64$, $p = 0,44$. Relative frequency of InsIns- genotype of Apo-B gene in patients with ChP, DM2 with obesity exceeded such frequency in apparently healthy 12, 0% ($\chi^2 = 3,95$, $p = 0,047$). Reliable distinctions in frequency of other genotypes were not established between groups of observation. Possible models of ChP investigation in combination of DM2 and obesity (dominant, recessive, overdominant and additive) taking into account Ins/Del polymorphism of gene Apo-B, from which recessive model appeared to be efficient at information criterion Aikaike 15, 89 have been analyzed by means of logistic regression. The data obtained testify to that Chp at comorbidity with DM2 and obesity in population is inherited as a recessive sign, which can realize itself only under certain “favourable” conditions.

4. Conclusions

Thus, deletion of the functional DNA area of gene Apo-B (id.: rs17240441) in homozygous state in the second pair of chromosomes 2p23-24 occurs in the examined population of the inhabitants of Northern Bukovina in 13.33% of cases: among patients with ChP, DM type2 and obesity – in 10,20%, among apparently healthy persons – in 17,07% of cases ($p < 0,05$). As to the character of allele distribution Ins/Del polymorphism of gene Apo-B wild Ins-allele prevails over Del-allele: in the sick persons – 40,82% [OR= 5,66, 95%, CI= 3,06 – 10,45, $p < 0,001$], in the control – 21,96% [OR=2,44, 95%, CI=1,30 – 4,57, $p = 0,005$], without reliable changes of inbreeding coefficient, in general does not upset the expected population balance Hardy-Weinberg and determines uniformity of alleles distribution in the examined population.

2. InsIns- genotype of gene Apo-B occurs in every second patient suffering from ChP, DM2 with obesity (51, 02%), that 12% more often than in the control group ($\chi^2 = 3,95$, $p = 0,047$). Distribution frequency of genotypes of gene Apo-B corresponds to the same for European population including allele distribution (PI = 0, 61 – 0, 70, $p > 0,05$) and PDel = 0,29– 0,34 against PDel = 0,30 – 0,39, $p > 0,05$). Inheritance model analysis has shown that ChP in combination with DM2 and obesity is inherited as recessive sign (criterion Aikaike 15, 89).

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