Rezumat

Polimorfismul NAT2 (arilaminice N-acetil 2), determinant-cheie al variațiilor individuale în capacitatea de acetilare, este suspectat de modificarea riscului de tumori maligne. Ca fumul de tutun şi alte substanțe inhalate, conține o varietate de substraturi NAT2. Relația dintre fenotipul NAT2 şi cancerul pulmonar este obiectul de cercetare intensivă al diferitor studii de caz-control, ale căror rezultate au produs controverse.

Summary

NAT2 (arylamine N-acetyltransferase 2) polymorphism, being a key determinant of individual variations in acetylation capacity, is suspected to modify the risk of carcinogen-related malignancies. As tobacco smoke and other inhaled hazards contain a variety of NAT2 substrates, the relationship between NAT2 phenotype and lung cancer (LC) risk has been a subject of intensive research, however different case-control studies produced controversial data. In the present report, we employed a novel „comparison of extremes" approach, i.e., we compared the distribution of NAT*2* genotypes in lung cancer patients (LC, n = 178) not only to the population controls (healthy donors (HD), n = 364), but also to the subjects with a putative cancer-resistant constitution (elderly tumor-free smokers and non-smokers (ED), n = 351). Frequencies of homozygous rapid, heterozygous rapid and slow acetylators were 6%, 39% and 56% in LC, 8%, 32% and 60% in HD, and 6%, 35% and 59% in ED, respectively). Comparison of the *NAT2* genotype frequencies between affected and non-affected individuals did not reveal any statistical deviations, irrespectively of smoking history, gender, age, or histological type of LC. Adjusted odds ratio for rapid vs. slow acetylators was 1.12 (95% confidence intervals (CI): 0.73 - 1.74) comparing LC vs. HD, and 1.10 (95% CI: 0.74 - 1.62) comparing LC vs. ED. *Similar distribution of NAT2 acetylator genotypes both in tumor-prone and in tumor-resistant groups suggests that, despite the presence of NAT2 carcinogenic substrates in tobacco smoke. NAT2 polymorphism does not play a noticeable role in lung cancer susceptibility.*

POLYMORPHIC VARIATIONS IN APOPTOTIC GENES AND RISK OF LUNG CANCER

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Introduction. Lung cancer (LC) is the leading cause of tumor-related mortality in the world. Smoking plays an utmost role in the etiology of this disease, being responsible for more than 85% of LC cases. Another important factor contributing to LC risk is the genetic background of the individual. Although LC is not a part of highly penetrant single-gene cancer syndromes, normal genetic variations within humans are likely to play an essential role in LC susceptibility. For example, unfavorable combinations of single nucleotide gene polymorphisms (SNPs) of genes involved in the metabolism of tobacco smoke carcinogens have been shown to slightly modify the risk of LC. In addition, overrepresentation of certain DNA repair gene SNPs in LC patients has been described in the literature [1-3].

Another class of polymorphic candidates, which deserves particular attention, consists of genes participating in apoptotic response to DNA damage. It has been proposed that cell failure to execute suicide upon non-repairable alteration of DNA structure may result in accumulation of clones, which contain cancer-driving mutations. Several phenotypic studies demonstrated an association between low apoptotic capacity and elevated cancer risk, but negative findings have been reported as well. There is also a number of reports describing distinct distribution of apoptotic SNPs in LC cancer patients, but systematic studies in this field remain to be done [4-12].

Search for low-penetrance gene-disease interactions is complicated due to weakness of the expected associations. Current demands aimed to improve the efficiency of molecular epidemiological research are focused on the drastic increase of the study size as well as perfect adjustment of cases and controls for all possible confounding parameters. However, this approach certainly has its limits; at the moment, available DNA collections and genotyping technologies allow to obtain truly definite results for no more than a few dozens or hundreds of SNPs. Therefore, alternative strategies for robust sorting of polymorphic candidates

are warranted. We have introduced earlier a non-traditional design for molecular epidemiological study, which is called „comparison of extremes" [13-16]. Instead of performing SNP testing in random sets of cases and controls, we suggested to select for the initial SNP assessment only those subjects, who are characterized by particularly demonstrative characteristics of cancer predisposition or tolerance. For example, at-risk genotypes are likely to accumulate in those patients who acquired LC disease at a relatively young age and/or experienced relatively low carcinogen exposure (e.g., in LC non-smokers or light smokers). In contrast, elderly tumor-free smokers appear to represent an example of LC tolerance and thus may serve as a valuable „supercontrol" for LC association studies. We attempted to use these „extreme" groups of cases and controls for the rapid assessment of the role of apoptotic gene SNPs in LC predisposition.

Materials and methods

Patients and controls.Case-control comparison of SNP frequencies was done by 2-stage approach. Initial analysis of candidate SNPs was intended to compare subjects with highly demonstrative characteristics of LC susceptibility or tolerance. Group of „extreme" LC susceptibility (n = 111) was selected from the total collection of 351 LC samples, and included 17 non-smokers as well as 94 patients characterized by relatively modest smoking history (mean: 30 pack/years; range: 10 - 40) and young age at the disease onset (mean: 54 years; range: 32 - 64). „Supercontrol" group of elderly tumor-free heavy smokers was selected from a total collection of 2791 non-affected subjects based on the highest cigarette consumption and age threshold 75 years, and contained 110 individuals (mean age: 79 years; age range: 75 - 89; mean smoking exposure: 55 pack/years; range: 30 - 126). SNPs demonstrating promising trends upon „comparison of extremes" were subjected to the traditional case-control comparison. 351 LC (mean age: 61 year; range: 30 - 84) included 303 smokers (mean pack/years: 41; range: 10 - 150) and 48 non-smokers. Control group (n = 538; mean age: 61 years; range: 32 - 84) did not include subjects, who were genotyped at the "comparison of extremes" stage, and consisted of 474 smokers (mean pack/years: 22; range: 1 - 111) and 64 non-smokers. Peripheral blood leucocytes were the source of DNA for all the above groups. For the genotyping of Casp8 His302Asp polymorphism, additional 127 DNA samples obtained from archival normal tissues of LC non-smokers (mean age: 61 year; range: 16 - 82) were utilized. All DNA samples described above were obtained from residents of St.-Petersburg (Russia). In addition, we utilized an independent set of cases and controls, who were recruited in another republic of former Soviet Union, Moldova. This collection consisted of 296 LC (mean age: 58 years; range: 22 – 79; 232 smokers (mean pack/years: 43; range: 3 - 132) and 64 non-smokers) and 295 non-affected subjects (mean age: 56 years; range: 22 - 80; 207 smokers (mean pack/years: 25; range: 3 - 130) and 88 non-smokers).

DNA isolation and SNP genotyping. Genomic DNA was extracted from peripheral blood leucocytes by modified salt–chloroform extraction [17]. Briefly, 3 ml of blood were diluted by water up to 10 ml in order to achieve hemolysis, mononuclear cells were pelleted by gentle centrufugation and resuspended in 1 ml of TE solution (10 mM Tris-HCl (pH 8.3), 1 mM EDTA). Plasma membranes were destroyed by the addition of Triton X-100 up to 1%, and the samples were centrifuged again to pellet the nuclei. The pellet was resuspended in the TE solution and incubated with proteinase K (100 μg/ml) at 60°C overnight. Proteins were precipitated by the addition of NaCl up to 1.5 M, and the lysates were subjected to chlorophorm extraction. Then equal volume of isopropanol was added, DNA was picked up by rotating glass stick, washed in 70% ethanol and dissolved in the TE buffer.

Archival samples were processed as described in [18]. 10 μm archival sections were deparaffinized in xylene and boiled for 5 min. in the lysis buffer (10 mM Tris-HCl (pH 8.3); 1 mM EDTA; 0.5% NP-40, 0.5% Tween 20). Proteinase K was added up to 500 μg/ml, and the samples were incubated at 60°C overnight. Finally, the proteinase K was inactivated by boiling for 5 min in the presence of Chelex-100, and the obtained lysates were used for PCR amplification.

The list of 37 coding non-synonymous SNPs in apoptotic genes was adopted from our previous publication [7]. SNPs were genotyped by allele-specific polymerase chain reaction (AS-PCR) [19]. Sequences of the PCR primers are presented in the Table 1. PCR reactions were carried in a 20 μl final volume using iCycler iQ Real Time Detection System (Bio-Rad). Each tube contained 50-100 ng of genomic DNA, 1 μM of each primer, 200 μM deoxynucleotide triphosphates, 1^x PCR buffer, 2,5 mM $MgCl_2$, 0.5^x SYBR Green I, and 1 unit of hot-start *Taq* polymerase („Thermostar", Helicon, Moscow). *Taq* polymerase was activated by 10 min. heating at 95°C. 45 PCR reaction cycles included denaturation at 95°C for 20 s, annealing at 55°C - 67°C for 35 s (see Table 1), and synthesis at 72°C for 35 s. The reliability of allele discrimination was systematically controlled by gel-electrophoresis of PCR fragments.

Statistical analysis. Deviation of genotypes distribution from Hardy-Weinberg equilibrium (HWE) in control subjects was assessed using the chi-square test with one degree of freedom. Primary comparisons of genotype frequencies in cases versus controls were performed by the trend test (*p-trend*) (one degree of

freedom) and the heterogeneity test (*p-het*) (two degrees of freedom). The significance of potential at-risk genotypes was assessed by calculating odds ratios (OR) with 95% confidence intervals (CI).

Results. We have considered for the study 37 coding non-synonymous SNPs in apoptotic genes, which have been validated by populational frequency (SNP NCBI database (http://www.ncbi.nlm.nih.gov/SNP/) and listed in our earlier publication [7]. p53 Arg72Pro polymorphism was excluded from the analysis, because its involvement in LC predisposition has been explicitly tested in previous reports [20]. Successful genotyping assays have been developed for 33 SNPs; we were not successful in analyzing the remaining 3 polymorphisms (Casp1 Gln37Lys, DR3 Gly159Asp, and DR5 Leu32Pro), despite the repetitive attempts to optimize primer sequences and PCR conditions. Another 14 SNPs (Bcl2 Thr43Ala, Bik Pro148Leu, Bcl-x Gly160Val, Casp5 Leu13Phe, Casp5 His152Arg, Casp5 Leu201Val, Casp6 Glu34Ala, Casp6 Lys35Glu, Fas Thr16Ala, Fas Ile122Thr, DR4 Ile33Thr, DR4 His297Asn, TNFR1 Leu75Pro, TRAIL Glu47Asp) demonstrated null frequency in our collection of DNA samples.

Thus, comparison of allele distribution in groups with „extreme" degrees of cancer susceptibility *versus* tolerance was performed for 19 SNPs. Because the involved categories of subjects which appear to have indeed opposite characteristics of LC risk, we assumed that truly meaningful LC-predisposing alleles would demonstrate clearly detectable odds ratios (ORs). If we consider OR = 3 as a reasonable threshold for the comparison of „extremes" [16, 21], and rely on a p value = 0.1, the study would have 99% power to detect at-risk allele with populational frequency 30%, and 90% power for the allele occurring in 10% of the subjects. 4 out of 19 tested SNPs (Casp5 Ala90Thr, Casp5 Val318Leu, Casp8 His302Asp, DR4 Lys441Arg) demonstrated $p < 0.1$ (Table 2). However, Casp5 Ala90Thr polymorphism was excluded from further study because the difference in its distribution between cases and controls was caused purely by the alteration of the Hardy-Weinberg equilibrium (depletion of heterozygotes) in the group of elderly tumor-free smokers; furthermore, we considered our data sets obtained for other categories of subjects (breast cancer patients and female controls; data not shown), and concluded that the Casp5 Ala90Thr variation is an unlikely contributor in LC susceptibility. Subgroup analysis of LC cases according to smoking status and tumor histological type did not reveal additional promising SNPs (data not shown).

Based on the presented above results of the preliminary screening, Leu/Leu-homozygotes for Casp5 Val318Leu (OR = 2.41 (95% CI: 1.02 – 5.70)), His-carriers for Casp8 His302Asp (OR = 2.26 (95% CI: 1.18 - 4.3)) and Arg-carriers for DR4 Lys441Arg (OR = 1.89 (95% CI: 1.06 – 3.38)) polymorphisms were considered as a candidate LC-predisposing genotypes and therefore subjected to the extended analysis. For the LC group, we added to the genotyping set the remaining 240 samples from our total collection of 351 non-selected LC cases, while for the control group we were able to compose an independent set of 538 non-affected subjects. In addition, we involved an additional collection from Moldova consisting of 296 LC patients and 295 controls. The study had 80% power at nominal significance level 0.05 to detect Mantel-Haenszel OR = 1.48, 1.41, and 1.38, respectively, for the mentioned above at-risk genotypes. The results of the case-control comparison are presented in Table 3. Interestingly, all three suspected at-risk genotypes demonstrated OR values above 1 both in Russian and Modovian case-controls series. However, none of statistical tests passed the threshold for significance (Table 4).

The subgroup analysis for LC histological type, gender, age and other disease variables failed to reveal additional associations. However, consideration of smoking status provided suggestive evidence for the association between Casp8 genotype and LC cancer risk in non-smokers. The frequency of His-carriers in the latter category of Russian patients (19/48 (40%)) significantly exceeded the one in the controls (118/538 (22%), $p = 0.006$); furthermore, similar but statistically non-significant trend was noticed in Moldovian subjects (16/64 (25%) versus 63/21 ($p = 0.52$)). Recent evidence indicates that LC in non-smokers may represent entirely distinct disease; therefore one could speculate that this type of neoplasia has authentic determinants of genetic predisposition [22, 23]. In order to validate the obtained correlation, we invoked additional 127 DNA samples from Russian LC non-smokers by utilizing normal tissues from archival paraffin-embedded samples. The frequency of His-carriers in newly genotyped LC non-smokers was identical to one observed in controls, i.e. 22% (28/127), that argued against the above association.

Discussion. This investigation was aimed to analyze the association between coding SNPs in apoptotic genes and LC predisposition. The preliminary sorting of SNP candidates was done by comparing subjects with „extreme" degrees of LC susceptibility *versus* tolerance. The group of LC cases was composed of patients selected on the basis of either absence of smoking history, or light smoking exposure coupled with the young age at the disease onset. It is expected, that truly at-risk alleles would have an evident overrepresentation in this group of patients, although the exact extent of the effect is difficult to estimate. Research on breast cancer (BC) genetic susceptibility has demonstrated that the probability to detect BC-predisposing variant increases several-fold in properly selected categories of patients [21]. However, similar calculations for the

frequencies of LC-associated alleles in „high-risk" *versus* non-selected categories of lung cancer patients are more complicated, due to shortage of well-proven gene-disease interactions and corresponding case-control data sets. On the other hand, LC studies have an unique opportunity of enrichment of control category of subjects. Current evidence indicates that smokers have drastically decreased chances to achieve a reasonably old age while remaining cancer-free [24]. Again, the exact prediction of the degree of the effect is complicated for the time being, due to the impact of concurrent smoking-related illnesses and geographic variations in life expectancy. Nevertheless, one would certainly expect an evident depletion of LC-predisposing at-risk genotypes in a group of elderly tumor-free heavy smokers. Given the above assumptions, OR = 3 and $p < 0.1$ may look as an appropriate threshold at the „comparison of extremes" stage, and the present study was sufficiently powered to detect promising SNP candidates for extended testing.

Three SNPs were pinpointed by the „comparison of extremes" approach (Casp5 Val318Leu, Casp8 His302Asp, and DR4 Lys441Arg). Although none of these associations passed the validation by extended case-control analysis, all candidate at-risk genotypes consistently demonstrated odds ratios above 1 both in Russian and in Moldovian case-control series (Table 4). The biological function of the caspase-5 protein is poorly understood. Caspase-5 is believed to play a role in various aspects of inflammation [25]; in addition, Casp5 gene was repeatedly shown to be a target for mutation in a subset of human cancers [26]. In contrast, the role of caspase-8 in the programmed cell death has been studied with significant level of comprehension. Interestingly, His allele carriers for Casp8 His302Asp were reported to have a reduced risk of breast cancer [27]. DR4 mediates ligand-induced apoptosis; some data indicate that DR4 may be involved in predisposition to tumor development [4, 28].

If we assume that these polymorphisms indeed predispose to LC with OR = 1.2 (Table 4), the validation of this association will require the analysis of 3000 LC cases and 3000 controls. The collections of this size are not yet available in individual cancer institutes, but can be utilized in collaborative multicenter studies [29]. Aside the sample size, another potential limitation of the study is related to the mode of SNP selection. Our investigation relied on the list of coding non-synonymous SNPs in apoptotic genes, which was composed in 2005 [7]. The number of identified SNPs continues to grow on a regular basis, and there are some interesting candidates which could not be considered when the above study was planned. For example, NCBI SNP database (http://www.ncbi.nlm.nih.gov/SNP/) contains some new validated coding polymorphisms, which are characterized by relatively high frequency (> 5%) and therefore deserve attention at the first hand (Boo Arg21Leu (rs2231292), Casp1 His15Arg (rs1042743), Casp7 Glu4Asp (rs11593766), Casp9 Arg176Gly (rs2308949), DcR2 Pro345Thr (rs34622674), DcR2 Ser310Leu (rs1133782), DR3 Gln23Arg (rs35771371), DR5 Val67Ala (rs1047266), DR5 Ala191Val (rs13265018), FasL Val266Leu (rs35178418), XIAP Phe133Ser (rs28382722)). Furthermore, while functional consequences of non-coding gene polymorphisms are less understood relative to coding SNPs, it is beyond the doubt that genotype-phenotype relationships are not limited to amino acid variations. Interestingly, recent advances in genome-wide analysis of cancer associated SNPs led to an identification of several tagging at-risk polymorphisms, including some located in the vicinity to apoptotic genes, but no linked changes in amino acid sequence have been reported yet [10, 30]. Non-coding apoptotic gene SNP candidates have not been considered in the present report. Finally, this study did not consider the role of SNP combinations in determining the disease susceptibility. It is possible that certain SNPs modify cancer predisposition only within particular genetic context, however significantly larger sample size and extensive subgroup analysis are required to reveal such gene-gene interactions.

The existence of multiple cancer subtypes also complicates the search for disease predisposing SNPs. LC may serve as a particularly valuable example of this type of difficulties. Although some investigations discussed specific features of lung cancer in non-smokers since a long time, this subtype of LC was rarely considered as a truly distinct disease. Recent clinical trials on epidermal growth factor receptor (EGFR) inhibitors (gefitinib or erlotinib) led to an identification of previously unknown intragenic mutations in the EGFR gene, which are associated with pronounced tumor response to the treatment. Interestingly, these mutations demonstrate strong preference to occur in non-smoking lung cancer patients, thus gefitinib and erlotinib showed clearly superior therapeutic efficacy for LC in non-smokers *versus* smokers. This unexpected clinical discovery attracted high level of attention to the comparison of molecular profiles of lung tumors in non-smokers *versus* smokers; there is some evidence that tobacco-unrelated LC may develop by entirely distinct mechanisms, and therefore be mediated by a separate group of risk factors [22, 23]. In our study, we deliberately limited the proportion of non-smokers in the „extreme" group of LC cases to the one observed in non-selected LC patients, therefore our approach to the initial sorting of SNPs looks fairly adequate relative to the current knowledge on LC subtypes. However, one cannot exclude the possibility that more profound LC categorization will be required in future molecular epidemiological studies. Within this context, one could make a parallel with the most studied cancer

type, i.e. breast cancer (BC). Recent studies indicate that this disease consists of several molecular variants, and, strikingly, estrogen-dependent BC appears to have distinct risk factors when compared to the hormone-independent disease [30-32].

In conclusion, this study involved 2-stage design for the systematic analysis of coding non-synonymous SNPs in apoptotic genes. Three candidate at-risk genotypes (Leu/Leu-homozygotes for Casp5 Val318Leu polymorphism, His-carriers for Casp8 His302Asp polymorphism, and Arg-carriers for DR4 Lys441Arg polymorphism) were identified while comparing groups with „extreme" degrees of LC susceptibility *versus* tolerance (early-onset light-smoking or non-smoking LC patients *versus* elderly tumor-free heavy smokers). In the validation study, all these candidate genotypes consistently demonstrated OR above 1 in two independent case-control series, however the associations did not achieve the level of statistical significance. Taken together, the obtained data indicate that Casp5, Casp8 and DR4 gene polymorphisms may deserve consideration in large-scale case-control studies of LC risk modifiers.

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Table 1

SNPs and primers for allele-specific PCR

Gene	SNP	Primers		Annealing temperature
Bcl2	Thr43Ala (rs1800477)	allele 1	CCCGGTGCGGGGGC	65°C
		allele 2	CCCGGTGCGGGGGT	
		common	GCGCACGCTGGGAGAA	
Bid	Gly10Ser (rs8190315)	allele 1	TGCACTCATCCCTGAGGCT	67°C
		allele 2	TGCACTCATCCCTGAGGCC	
		common	GCTCTCTGGGACCTGTGAG	
Bik	Pro148Leu (rs11574527)	allele 1	CCCGCTGAGCAGCGGCA	63°C
		allele 2	CCCGCTGAGCAGCGGCG	
		common	GTCACCCGTCTGGCCCC	
Bcl-x	Gly160Val (rs7362890)	allele 1	AGTGAGCCCAGCAGAACCA	65°C
		allele 2	AGTGAGCCCAGCAGAACCC	
		common	AACAATGCAGCAGCCGAGAG	
Casp2	Leu141Val (rs4647297)	allele 1	AGACAATAAAGATGGTCCTG	63°C
		allele 2	AGACAATAAAGATGGTCCTC	
		common	AATTTCCCAACTCTCTTCCT	
Casp5	Leu13Phe (rs3181320)	allele 1	CAGAGTGGATTGGATAACTTC	60°C
		allele 2	CAGAGTGGATTGGATAACTTG	
		common	TGATCCGTATTAGGTACTAGG	
	Ala90Thr (rs507879)	allele 1	CCTTGTCTTCAATTTTGGT	60°C
		allele 2	CCTTGTCTTCAATTTTGGC	
		common	CAGTTAAGATGTTGGAATAC	
	His152Arg (rs3181179)	allele 1	CAGTCTCAGGAATTCTTCAC	65°C
		allele 2	CAGTCTCAGGAATTCTTCAT	
		common	GTCACAGAGGCAGAAACCA	
	Leu201Val (rs3181326)	allele 1	GGGCTCACTATGACATCG	63°C
		allele 2	GGGCTCACTATGACATCC	
		common	TATTAGAAGAATCTGTGTTGC	
	Val318Leu (rs523104)	allele 1	GATGACTGTGAAGAGATGAG	58°C
		allele 2	GATGACTGTGAAGAGATGAC	
		common	AACAAACCTACACGTTCTGC	
Casp6	Glu34Ala (rs11574696)	allele 1	GGTCCATTTTGTACTTTTCTG	60°C
		allele 2	GGTCCATTTTGTACTTTTCTT	
		common	GTTGACCTAGAACTTGGAG	
	Lys35Glu (rs11574697)	allele 1	GAAATGTTTGATCCGGCAG	60°C
		allele 2	GAAATGTTTGATCCGGCAA	
		common	CGGGTAAGATTGTCTCTAT	
Casp7	Glu255Asp (rs2227310)	allele 1	GGAGGAGCACGGAAAAGAC	60°C
		allele 2	GGAGGAGCACGGAAAAGAG	
		common	GAGCATGGAGACCACACAG	
Casp8	His302Asp (rs1045485)	allele 1	TTTGAGATCAAGCCCCACG	60°C
		allele 2	TTTGAGATCAAGCCCCACC	
		common	CAGCAGATGAAGCAGTCCA	

Casp9	Val28Ala (rs1052571)	allele 1	CTCGCGGCTCAGCAGGA	65°C
		allele 2	CTCGCGGCTCAGCAGGG	
		common	GCCATGGACGAAGCGGAT	
	His173Arg (rs2308950)	allele 1	AACAATGTGAACTTCTGCCG	63°C
		allele 2	AACAATGTGAACTTCTGCCA	
		common	CACAGATAGTGAGTGTACCT	
	Arg221Gln (rs1052576)	allele 1	AGCACCGTGGTCCTGCC	65°C
		allele 2	AGCACCGTGGTCCTGCT	
		common	CCAGTCTGCATCTAGACCT	
Casp10	Ile479Leu (rs13006529)	allele 1	GCCCCTGGATGCACTTTCAT	63°C
		allele 2	GCCCCTGGATGCACTTTCAA	
		common	ACCACCGATTCCTGTGCTG	
DR4	Ile33Thr (rs20577)	allele 1	GAGGCAGCCGCGGCCAC	63°C
		allele 2	GAGGCAGCCGCGGCCAT	
		common	GTGCTGTCCCATGGAGGTA	
	Arg141His (rs6557634)	allele 1	AGGATCTCATAGATCAGAACG	60°C
		allele 2	AGGATCTCATAGATCAGAACA	
		common	TACATGGGAGGCAAGCAAAC	
	Thr209Arg (rs4871857)	allele 1	TGGCTGTTGTCTCACCCTC	55°C
		allele 2	TGGCTGTTGTCTCACCCTG	
		common	AGAAGTCCCTGCACCACGA	
	Ala228Glu (rs20576)	allele 1	CGCCCTGGAGTGACATCGA	55°C
		allele 2	CGCCCTGGAGTGACATCGC	
		common	CTGCTGGTCCCTGTCTCCT	
	Lys441Arg (rs2230229)	allele 1	AGGTCCTGAATCTTCTCTT	65°C
		allele 2	AGGTCCTGAATCTTCTCTC	
		common	AATGCTGATGAAATGGGTC	
Fas	Thr16Ala (rs3218619)	allele 1	ACAGGTTCTTACGTCTGTTG	63°C
		allele 2	ACAGGTTCTTACGTCTGTTA	
		common	CAAGTTCTGAGTCTCAACTG	
	Ile122Thr (rs3218614)	allele 1	GAAATAAACTGCACCCGGAC	65°C
		allele 2	GAAATAAACTGCACCCGGAT	
		common	TACTTGGTGCAAGGGTCACA	
FAIM	Thr117Ala (rs641320)	allele 1	GCACCATACGTCCATAGC	65°C
		allele 2	GCACCATACGTCCATAGT	
		common	CTGGTATATCAATCCTTTCCT	
	Ser127Leu (rs13043)	allele 1	ACTTACCGCTGTCTCCA	67°C
		allele 2	ACTTACCGCTGTCTCCG	
		common	CTTTAAATCACAGAATTATAAC	
Survivin	Lys129Glu (rs2071214)	allele 1	GAATTTGAGGAAACTGCGA	63°C
		allele 2	GAATTTGAGGAAACTGCGG	
		common	TGGCACCAGGGAATAAAC	
TNFR1	Leu75Pro (rs4149637)	allele 1	AGTCCGTATCCTGCCCCG	65°C
		allele 2	AGTCCGTATCCTGCCCCA	
		common	GCTCCTTCCTTGTGTTCTCA	
	Gln121Arg (rs4149584)	allele 1	CTTCTTGCACAGTGGACCG	63°C
		allele 2	CTTCTTGCACAGTGGACCA	
		common	AGGAGAGCTGCGCTCACA	
TRAIL	Glu47Asp (rs16845759)	allele 1	GCTGAAGCAGATGCAGGAC	60°C
		allele 2	GCTGAAGCAGATGCAGGAA	
		common	TGACGGAGTTGCCACTTGA	
XIAP	Pro423Gln (rs5956583)	allele 1	AGTCTGACTTGACTCATCTT	60°C
		allele 2	AGTCTGACTTGACTCATCTG	
		common	GCTATACGAATGGGGTTCA	

Table 2

„Comparison of extremes": distribution of apoptotic SNPs in subjects with highly demonstrative characteristics of LC predisposition versus tolerance

SNP		LC (%)	Control (%)	OR (95% CI)	HWE, p value	Heterogeneity test, p value	Trend test, p value
Bid Gly10Ser (G/A)	AA	108 (97.3)	109 (99.1)	1	0.99	0.32	0.32
	AG	3 (2.7)	1 (0.9)	3.03 (0.31-29.6)			
	GG	0 (0.0)	0 (0.0)	-			
Casp2 Leu 141Val (C/G)	GG	102 (91.9)	101(91.8)	1	0.90	0.98	0.98
	CG	9 (8.1)	9 (8.2)	0.99 (0.38-2.60)			
	CC	0 (0.0)	0 (0.0)	-			
Casp5 Ala90Thr (G/A)	AA	29 (26.1)	34 (30.9)	1	0.11	0.07	0.58
	AG	61 (55.0)	44 (40.0)	1.63 (0.87-3.05)			
	GG	21 (18.9)	32 (29.1)	0.77 (0.37-1.61)			
Casp5 Val318Leu (G/C)	GG	48 (43.2)	52 (47.3)	1	0.86	0.09	0.14
	CG	43 (38.7)	49 (44.5)	0.95 (0.54-1.68)			
	CC	20 (18.0)	9 (8.2)	2.41 (1.00-5.80)			
Casp7 Glu255Asp (C/G)	CC	57 (51.4)	57 (51.8)	1	0.99	0.32	0.62
	CG	50 (45.0)	44 (40.0)	1.14 (0.65-1.96)			
	GG	4 (3.6)	9 (8.2)	0.44 (0.12-1.52)			
Casp8 His302Asp (C/G)	GG	77 (69.4)	92 (83.6)	1	0.65	0.03	0.01
	CG	33 (29.7)	18 (16.4)	2.19 (1.44-4.19)			
	CC	1 (0.9)	0 (0.0)	-			
Casp9 Val28Ala (T/C)	TT	40 (36.0)	38 (34.5)	1	0.96	0.51	0.44
	TC	57 (51.4)	52 (47.3)	1.04 (0.58-1.86)			
	CC	14 (12.6)	20 (18.2)	0.67 (0.30-1.50)			
Casp9 His173Arg (A/G)	GG	108 (97.3)	102 (92.7)	1	0.92	0.12	0.12
	AG	3 (2.7)	8 (7.3)	0.35 (0.09-1.37)			
	AA	0 (0.0)	0 (0.0)	-			
Casp9 Arg221Gln (G/A)	GG	39 (35.1)	37 (33.6)	1	0.99	0.75	0.57
	AG	56 (50.5)	53 (48.2)	1.00 (0.56-1.80)			
	AA	16 (14.4)	20 (18.2)	0.76 (0.34-1.68)			
Casp10 Ile479Leu (A/T)	TT	33 (29.7)	43 (39.1)	1	0.13	0.33	0.28
	AT	52 (46.8)	43 (39.1)	1.58 (0.85-2.89)			
	AA	26 (23.4)	24 (21.8)	1.41 (0.69-2.89)			
DR4 Arg141His (G/A)	GG	27 (24.3)	32 (29.1)	1	0.94	0.37	0.99
	AG	64 (57.7)	53 (48.2)	1.43 (0.76-2.68)			
	AA	20 (18.0)	25 (22.7)	0.95 (0.43-2.07)			
DR4 Thr209Arg (C/G)	GG	22 (19.8)	32 (29.1)	1	0.52	0.21	0.39
	CG	61 (55.0)	49 (44.5)	1.81 (0.94-3.51)			
	CC	28 (25.2)	29 (26.4)	1.40 (0.66-2.98)			

DR4 Ala228Glu (C/A)	AA	81 (73.0)	87 (79.1)	1	0.86	0.56	0.29
	AC	27 (24.3)	21 (19.1)	1.38 (0.72-2.63)			
	CC	3 (2.7)	2 (1.8)	1.61 (0.26-9.89)			
DR4 Lys441Arg (A/G)	AA	70 (63.1)	84 (76.4)	1	0.66	0.08	0.06
	AG	38 (34.2)	23 (20.9)	1.98 (1.08-3.64)			
	GG	3 (2.7)	3 (2.7)	1.20 (0.23-6.13)			
Faim Thr117Ala (A/G)	GG	101 (91.0)	106 (96.4)	1	0.98	0.10	0.10
	AG	10 (9.0)	4 (3.6)	2.62 (0.80-8.63)			
	AA	0 (0.0)	0 (0.0)	-			
Faim Ser127Leu (C/T)	AA	109 (98.2)	108 (98.2)	1	0.99	0.99	0.99
	AG	2 (1.8)	2 (1.8)	0.99 (0.14-7.16)			
	GG	0 (0.0)	0 (0.0)	-			
Surv Lys129Glu (A/G)	AA	101 (91.0)	100 (90.9)	1	0.89	0.98	0.98
	AG	10 (9.0)	10 (9.1)	0.99 (0.40-2.48)			
	GG	0 (0.0)	0 (0.0)	-			
TNFR1 Gln121Arg (A/G)	GG	108 (97.3)	103 (93.6)	1	0.94	0.19	0.19
	AG	3 (2.7)	7 (6.4)	0.41 (0.10-1.62)			
	AA	0 (0.0)	0 (0.0)	-			
XIAP Pro423Gln (C/A) (females)	AA	6 (40.0)	55 (38.7)	1	0.18	0.79	0.68
	AC	7 (46.7)	58 (40.8)	1.11 (0.35-3.50)			
	CC	2 (13.3)	29 (20.4)	0.63 (0.12-3.33)			
XIAP Pro423Gln (C/A) (males)	A	53 (55.2)	70 (63.6)	1	-	0.22	0.22
	C	43 (44.8)	40 (36.4)	1.42 (0.81-2.48)			
Total		111 (100)	110 (100)				

XIAP is located on X chromosome; therefore males have only one allele of this gene. For the analysis of XIAP Pro423Gln genotypes in females, additional 142 elderly non-affected females were examined as controls. Three genotypes demonstrated potential association with LC risk, i.e. Leu/Leu-homozygotes for Casp5 Val318Leu (OR = 2.47 (95% CI: 1.07 – 5.69), $p = 0.03$), His-carriers for Casp8 His302Asp (OR = 2.26 (95% CI: 1.18 - 4.31), $p = 0.02$), and Arg-carriers for DR4 Lys441Arg (OR = 1.89 (95% CI: 1.05 – 3.40), $p = 0.03$).

Table 3

Validation study for the candidate SNPs

SNP		**Russia**		**Moldova**	
		LC (%)	**Control (%)**	**LC (%)**	**Control (%)**
Casp5 Val318Leu (G/C)	GG	137 (39.0)	221 (41.1)	103 (34.8)	107 (36.3)
	CG	158 (45.0)	248 (46.1)	134 (45.3)	133 (45.1)
	CC	56 (18.0)	69 (12.8)	59 (19.9)	55 (18.6)
Casp8 His302Asp (C/G)	GG	263 (74.9)	420 (78.1)	226 (76.4)	232 (78.6)
	CG	83 (23.6)	112 (20.8)	67 (22.6)	58 (19.7)
	CC	5 (1.4)	6 (1.1)	3 (1.0)	5 (1.7)
DR4 Lys441Arg (A/G)	AA	250 (71.2)	399 (74.2)	210 (70.9)	222 (75.3)
	AG	89 (25.4)	126 (23.4)	83 (28.0)	70 (23.7)
	GG	12 (3.4)	13 (2.4)	3 (1.0)	3 (1.0)
Total		351 (100)	538 (100)	296 (100)	295 (100)

Table 4

Odds ratios and confidence intervals for candidate at-risk genotypes

SNP	OR (95% CI) and p values		Mantel-Haenszel OR (95% CI) and p values
	Russia	Moldova	
Casp5 Val318Leu , Leu/Leu-genotype	1.31 (0.87 - 1.98), $p = 0.20$	1.11 (0.71 - 1.76), $p = 0.64$	1.22 (0.90 - 1.65), $p = 0.21$
Casp8 His302Asp, His-carriers	1.19 (0.87 - 1.63), $p = 0.28$	1.14 (0.78 - 1.68), $p = 0.51$	1.17 (0.92 - 1.50), $p = 0.21$
DR4 Lys441Arg, Arg-carriers	1.16 (0.86 - 1.57), $p = 0.34$	1.24 (0.87 - 1.80), $p = 0.24$	1.19 (0.95 - 1.51), $p = 0.14$

Rezumat

Apoptoza joacă un rol important în eliminarea celulelor ADN-deteriorate, protejând astfel gazda de la dezvoltarea cancerului. Unele date indică faptul că variațiile normale în secvența de gene apoptotic poate duce la capacitatea de apoptotic suboptimal şi, prin urmare, la creşterea riscului de cancer. Am testat 19 secvențe de codificare SNP apoptotic, în 2 etape studiu epidemiologic molecular. Pentru sortarea preliminară a candidaților SNP, am utlizat o „comparație a extremelor".

Summary

Apoptosis plays a role in the elimination of DNA-damaged cells thus protecting the host from cancer development. Some data indicate that normal variations within the sequence of apoptotic genes may lead to suboptimal apoptotic capacity and therefore increased cancer risk. We tested 19 coding apoptotic gene SNPs in 2-stage molecular epidemiological study. For the preliminary sorting of SNP candidates, we employed a „comparison of extremes" approach, where 111 patients with highly pronounced LC susceptibility (non-smokers or young-onset light smokers) were analyzed against 110 subjects with the evidence for LC tolerance (elderly tumor-free heavy smokers). Three genotypes demonstrated possible association with LC risk (Leu/Leu-homozygotes for Casp5 Val318Leu versus other genotypes: OR = 2.47 (95% CI: 1.07 – 5.69), $p = 0.03$; His-carriers for Casp8 His302Asp: OR = 2.26 (95% CI: 1.18 - 4.31), $p = 0.02$; Arg-carriers for DR4 Lys441Arg: OR = 1.89 (95% CI: 1.05 – 3.40), $p = 0.03$), and therefore were selected for the validation. The extended study included 2 case-control series, namely subjects from Russia (351 LC cases and 538 controls) and Moldova (296 LC cases and 295 controls). Interestingly, all three candidate genotypes consistently demonstrated OR above 1 both in Russian and in Moldovian groups. Although the combined Mantel-Haenszel analysis yet failed to reach statistical significance (OR = 1.22 (95% CI: 0.90 - 1.65), $p = 0.21$; OR = 1.17 (95% CI: 0.92 - 1.50), $p = 0.21$; OR = 1.19 (95% CI: 0.95 - 1.51), $p = 0.14$, respectively), the obtained data indicate that Casp5, Casp8 and DR4 gene polymorphisms may deserve consideration in large-scale case-control studies of LC risk modifiers.

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Sarcomul Kaposi este o tumoare de origine vasculară cu localizare muco-cutanată, cu manifestări clinico-morfologice foarte variabile, care deseori ridică probleme de diagnostic [1]. Această afecțiune tumorală se dezvoltă mai frecvent la persoanele în vârstă cu deficiență imună, deseori la pacienții cu SIDA, transplant de organ, infecție cu herpesvirusul uman tipul 8 (HHV 8) şi alte infecții virale [2]. Tot mai mult se presupune că sarcomul Kaposi este un neoplasm al endoteliului limfovascular infectat cu HHV 8 care reprogramează genele responsabile de reglarea dezvoltării vaselor limfatice [3]. Imunohistochimic, în celulele fusiforme neoplazice, pozitive la markerul limfovascular D2-40, din sarcomul Kaposi se detectează antigenul nuclear latent (LNA-1), asociat la HHV 8 [4].

Conform manifestărilor clinice, sunt descrise [2] patru tipuri principale de sarcom Kaposi: 1) tipul clasic, deosebit de răspândit în Europa, la persoanele în vârstă, care se caracterizează prin leziuni cutanate ale extremităților cu evoluție lentă; 2) tipul african endemic, care afectează copiii şi persoanele tinere, în care leziunile cutanate pot fi asociate cu limfadenopatii generalizate şi progresare rapidă a bolii; 3) tipul imunosupresiv, cel iatrogenic, apărut la pacienții cu transplant de organ, manifestat prin leziuni cutanate localizate sau diseminate şi printr-o evoluție lentă sau progresivă în circa 30% cazuri; 4) tipul epidemic, instalat la bolnavii de SIDA, caracterizat prin leziuni muco-cutanate şi viscerale, deseori, cu o evoluție fulminantă.