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CHOLESTEROL ESTER TRANSFER PROTEIN GENE POLYMORPHISM IN MEN WITH CORONARY ATHEROSCLEROSIS

Astrakova K.S.*, Shakhtshneider E.V., Ragino Y.I., Chernjavski A.M., Kashtanova E.V., Polonskaya Y.V., Voevoda M.I.
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Key words: coronary atherosclerosis, hypercholesterolemia, CETP, TaqIB, HDL-C

Motivation and Aim: HDL-C is believed to be a protective factor against coronary artery disease (CAD), and the inverse relationship between plasma HDL-C and the incidence of CAD is well established. The aim was to analyze TaqIB polymorphism (rs708272) of CETP gene in groups of men with coronary atherosclerosis and in men of population of Siberia.

Methods and Algorithms: Two groups of men with coronary atherosclerosis were involved into our research. The first group consisted of 84 patients 45-65 (57±1,2) y. o. Cases were collected 2007-2010 years. The second group consisted of 29 patients 50-72 (63±8,9) y. o. Cases were collected 2012-2014 years. Patients of both groups had been living in the West-Siberian region; clinical diagnosis of coronary atherosclerosis were evaluated by percutaneous coronary angiography. All of patients were individuals free of ACS and had stable exertional angina classes II-IV. Cases were collected from E. N. Meshalkin Institute of Circulation Pathology, Novosibirsk, Russia. Also 130 healthy controls 45-69 (55±6,9) y. o. examined during HAPIEE research were carried out. Blood samples were drawn for measurement of serum levels of total cholesterol, triglycerides, HDL-C, LDL-C after a 12-hour overnight fast. Serum levels of TC (mmol/L), triglycerides (mmol/L), HDL-C (mmol/L) and LDL-C (mmol/L) were determined by colorimetric enzymatic assays. TaqIB polymorphism (intron 1, +279G/A) of CETP gene was analyzed by standard method (primers: 5′-ccctc-ctgac-ctcgc-cttca-a-3′ и 5′-gcaac-ccctg-acttt-ggcca-tag-3′).

Results: The frequency of TaqIB different alleles was 62,5%, 58,6%, 53,5% for B1 and 37,5%, 41,1%, 46,5% for B2 (the first, the second and the control group, respectively), (p=0.08). B2B2 genotype was associated with high HDL-C level and low TC, LDL-C, atherogenic index levels ($p<0.05$).

Conclusion: There is statistic significant reduction of B2 frequency in men with coronary atherosclerosis. Statistic significant association between TaqIB polymorphism of CETP gene and HDL-C level was revealed.

Availability: There is an opportunity to examine relatives of patients with severe hypercholesterolemia for preclinical atherosclerosis diagnostics and development of new targets of hypocholesterolemia therapy.

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POSSIBLE ASSOCIATION BETWEEN THE *TRPV1* GENE rs222747 POLYMORPHISM AND PRIMARY OPEN ANGLE GLAUCOMA IN WESTERN SIBERIA PATIENTS

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Motivation and Aim: Primary open angle glaucoma (POAG) is a chronic neurodegenerative disease caused by degeneration of axons of the retinal ganglion cells (RGCs) and subsequent neuron death. The disease can occur at any age, but individuals over 40 are the most susceptible to it. POAG is frequently associated with raised intraocular pressure (IOP), which contributes to RGC death. Capsaicin-sensitive vanilloid subunit 1 (*TRPV1*) is a member of the transient receptor potential (TRP) family of cation-selective ion channels. Its activation occurs in response to any stimulus including the change in hydrostatic pressure during glaucoma. Expression of TRPV1 channel was detected in RGCs. It was shown that elevated intraocular pressure in mice with glaucoma activates TRPV1 channel, which depending on the conditions can induce apoptosis of RGCs through an increased intracellular calcium level or initiate protective cascade. This study was based on estimation of the contribution of polymorphisms in the transient receptor potential cation channel (subfamily V, member 1) (*TRPV1*) gene to disease development. The nonsynonymous polymorphism rs222747 (Met315Ile) (exon 5, G/C) in ankyrin repeat domains was associated with increased protein expression and elevated channel activity. We assumed that the *TRPV1* gene rs222747 polymorphism can be potentially involved in pathogenesis of POAG. The aim of this study was to estimate a possible association between the *TRPV1* gene rs222747 (Met315Ile) (exon 5, G/C) polymorphism and primary open angle glaucoma in Western Siberia patients.

Methods and Algorithms: We analyzed samples from patients with primary open angle glaucoma (252 subjects, average age 69.3 ± 8.9 years) and a control group of individuals without the disease (290 subjects, average age 75.5 ± 8.4 years). Genetic testing was performed using Real-Time PCR System (Applied Biosystems) according to manufacturer's protocol.

Results: We did not find statistically significant differences between patients with POAG and control subjects ($p < 0.05$) in genotype distributions and allelic frequencies of the *TRPV1* gene rs222747 (Met315Ile) (exon 5, G/C) polymorphism.

Conclusion: Our data shows lack of association between the *TRPV1* gene rs222747 (Met315Ile) (exon 5, G/C) polymorphism and primary open angle glaucoma in Western Siberia patients. Possibly, small sample size in groups causes the result obtained. Therefore, the present findings need to be confirmed by larger investigations.

MITOCHONDRIAL DYSFUNCTIONS IN ANIMAL MODEL OF SPORADIC ALZHEIMER'S DISEASE

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Key words: sporadic Alzheimer's disease, mitochondrial dysfunction, β-amyloid

Motivation and Aim: Neurodegenerative Alzheimer's disease (AD) is the one of leading causes of death in elderly humans. AD is late-onset, age-dependent neurodegeneration, characterized by the progressive decline of memory, cognitive functions, changes in behavior and personality. Mitochondria play special fundamental role in the aging, AD pathology, and β-amyloid induced oxidative damage. Mitochondrial dysfunctions accompanied by oxidative stress could be initiates and contributes to the development and progression of the AD disease. We have observed mitochondrial abnormalities in AD brain tissue, and focused on how mitochondrial may be mediate neurodysfunction and neurodegeneration in the AD brain.

Methods and Algorithms: Several studies have suggested that Aβ-induced neurotoxicity in AD could be mediated by oxidative stress and an altered function of respiratory chain associated with oxidative damage These findings have displayed in various human and rodent AD cellular models as well as in triple-transgenic AD mice expressing tau and APP. We investigated a role of mitochondria in AD pathology using sporadic mouse model of AD. Olfactory bulbectomy in mice seems to be a promising and appropriate as a model of nontransgenic, sporadic AD to study neurodegeneration.

Results: Six weeks after bilateral olfactory bulbectomy (OBX) mice displayed prominent impairment in spatial memory when tested in the Morris's water maze. It was shown that β-amyloid was increased in extracts of the neocortex and hippocampus and its level was significantly higher in the OBX animals than in sham-operated mice. The obtained data suggest that bilateral olfactory bulbectomy initiates in the mouse brain pathological processes similar to sporadic AD in location, biochemistry and behavioral manifestation. At the same time mitochondria isolated from the neocortex and hippocampus of OBX mice displayed impairments in respiratory chain (RC) functions, including decline in the mitochondrial respiratory rate and decreased membrane potential, low value of mitochondrial respiration control ratio, reduced activity of the cytochrome c oxidase (COX, complex IV) and increased activity of NADH:quinone oxidoreductase (complex I). We have also established that mitochondrial dysfunctions strictly correlate with the accumulation of soluble β-amyloid into mitochondria from the neocortex and hippocampus of OBX animals. The detected complex IV inhibition could lead to further instability or alterations in the electron flux, and even increased ROS production because of the backup of reduced complexes upstream in the RC.

Conclusion and Availability: We have found mitochondrial energy metabolism impairments in neocortex and hippocampus in sporadic type AD. There was direct link between activity of RC complexes and soluble β-amyloid accumulation in mitochondria. New potential drugs able to modulate mitochondrial dynamics and dysfunction could be propose as potential therapeutic drugs that could help mitigating neurodegenerative Alzheimer's disease.

STUDYING OF GENES REGULATING IMMUNE RESPONSE AT PULMONARY TUBERCULOSIS IN YAKUTS

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Key words: immune response; tuberculosis; polymorphisms; associative analysis

Motivation and Aim: Pulmonary tuberculosis (TB) is one of the most dangerous infections. The important role in TB pathogenesis plays the ability of host organism to give an adequate immune response against *M. tuberculosis*, which strongly defined by different genetic factors. In present study we analyzed associations of 10 SNPs: *INFG* (rs2069705), *INFGR2* (rs17880053) *MCP1* (rs1024611), *PIAS3* (rs12756687), *PIASY* (rs3760903), *TBX21* (rs11652969), *STAT5B* (rs16967593), *IL4RA* (1805010), *CD209* (rs1544767), *SOCS5* (rs6737848) in Yakut's TB patients.

Methods and Algorithms: DNA samples from unrelated TB patients (n=163) were collected in Yakutsk Scientific Center. Control group samples were collected from healthy unrelated individuals of Ust-Aldan region (Republic of Sakha (Yakutia)) (n=135). For comparison of alleles and genotypes frequencies between different groups the Pearson χ^2 criterion with Yates correction for continuity and Fisher's exact test were used.

Results: While checking Hardy-Weinberg equilibrium accordance we found the deviation for two loci in control group (*INFGR2*, *SOCS5*) and for two – in patients (*MCP1, PIASY*). We believe, that in control group this deviation can be explained by the peculiarities of this population structure. A comparison between two studied groups (patients and control) showed a significant difference between alleles frequencies. We found that allele G of rs17880053**INFGR2* is deleterious (OR = 1.56 (0.95% CI: 1.10-2.20), χ^2 = 6.47, p = 0.011) at homo- and heterozygous carriers. The homozygous deletion genotype is protective for TB: OR = 0.39 (0.95% CI: 0.21-0.71), χ^2 = 10.08, p = 0.002. For rs3760903* *PIASY* there is deleterious effect for homo- and heterozygous carriage of G allele (OR = 1.67 (0.95% CI: 1.03-2.71), χ^2 = 4.44, p = 0.035). Rare genotype AA of *STAT5B* (rs16967593) observed twice as often in patients compared with control, however, there was statistically significant effect only for allele A: OR = 1.46 (0.95% CI: 1.02-2.09), χ^2 = 4.33, p = 0.038.

Conclusion: Thus, our study shows the importance of contribution of polymorphic variants rs17880053 (*INFGR2*), rs3760903 (*PIASY*) and rs16967593 (*STAT5B*) in the development of pulmonary tuberculosis in Yakut's for the first time.

CHARACTERISTICS OF TYPE 1 DIABETES SUSCEPTIBILITY REGIONS

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Diseases like diabetes, neurodegenerative diseases and cancers are called "complex diseases" because their instigation includes a combination of multiple genetic, environmental and life style factors.

To study the genetic factors, scientists have traditionally focused on finding single point mutations (SNPs) in the genes of those suffering from the disease. However, the role of disturbed gene regulation rather than disrupted protein coding is increasingly recognized. Yet, even if regulatory aspects are acknowledged, just the identification of associated SNPs, whether they occur in genes or in regulatory modules, by means of a GWAS is just one step in unravelling the genomic aetiology of complex diseases.

To build a broader picture, we suggest characterising areas that are known to confer susceptibility to a particular complex disease by features that capture their distinctive genomic entirety on a higher level of organisation than mere location. What sets certain susceptibility regions apart from others in terms of overall genomic composition and does such a classification render more insight in the causation of a complex disease?

Using Type 1 Diabetes (T1D) as an example, we analysed a set of genomic variables in order to typify the susceptibility regions connected with this disease. The aim was to find out if particular regions differ strikingly in genomic content from others and if so what structural properties are responsible for this classification and whether it is i) associated with a distinction in reputed functional features and ii) reflected in the concomitance of other autoimmune diseases.

Genomic coordinates of T1D susceptibility regions, the genes and SNPs as well as the markers for other auto-immune diseases they contain were collected from T1Dbase.org. Other data were obtained from the Ensembl genome browser and include the coordinates of gene transcripts, lengths of exonic-, intronic, UTR- and intergenic sequences. Following normalisation for the size of the susceptibility regions, the feature values were subjected to hierarchical cluster analysis.

The analysis revealed two main clusters of susceptibility regions and hence suggests the existence of two types of genomic areas that are differently involved in the occurrence of T1D and associated auto-immune diseases. The first cluster consists of regions that contain large sequences of intronic and regulatory DNA. Especially these susceptibility regions are also loci for many other autoimmune diseases. The second cluster, which includes the Human Leukocyte Antigen locus, is made up of two sub-clusters. The first comprises short, gene dense regions with high SNP counts. These regions are also rich in SNPs occurring in experimentally verified transcription factor binding sites and – like cluster 1 - are loci for many co-occurring auto-immune diseases. The second sub-cluster cluster had no particular outstanding attributes and only a small number of its regions are loci for any of the 17 co-occurring auto-immune diseases.

THE USE OF 3D-CHROMATIN STRUCTURE DATA TO PREDICT NOVEL GENES ASSOCIATED WITH THE DEVELOPMENT OF DEPRESSION

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Key words: 3D-chromatin structure, depression, ChIA-PET

Motivation and Aim: Long-range chromatin interactions are increasingly being recognized as an important mechanism to regulate many genes. RNAPII-associated chromatin multigene complexes include promoter-promoter interactions, and a genetic mutation at one particular promoter might also propagate to other promoters and hence could lead to pleiotropic consequences depending on the interaction network. Thus, genes interacting with those already associated with a certain disease may also be involved in the development of pathology. The aim of this work was to identify new genes possibly involved in the development of depression according to their co-localization in a multigene complex with genes already known to be related to depression.

Methods and Algorithms: To explore the promoter-associated chromatin interactions, the results of RNA polymerase II chromatin interaction analysis with paired-end tagging (ChIA-PET) approach were taken from [1]. Chromatin interactions between promoters (±1000bp from start transcription) were identified in several cell types, requiring at least 3 contacts between them. Resulting list of multigene complexes was overlapped with a subset of genes shown to be involved in depressive disorder. The latter gene subset was collected from HuGE Navigator web-resource based on published data, and DepGene list of candidate genes for depression [2] with evidence obtained by gene prioritization approach from various data sources.

Results: Using our approach we obtained a list of 20 genes whose promoters are co-localized in a multigene complex with genes related to depression. We hypothesize that these genes may also be associated with the depressive disorder. Functional enrichment analysis showed that the genes are associated with such biological processes as transmembrane transport, response to abiotic stimulus etc. The sample also contains a portion of genes with an unknown function or yet to be fully annotated, while their activity may be tightly associated with the development of depression. In order to confirm possible involvement of the selected genes in the emergence of depressive disorder, the sample was compared with published data on differential gene expression resulting from depression. The analysis has shown coordinated changes in the expression of genes belonging to a particular unit of chromatin interactions, which makes a hypothesis of their relationship with depression more reliable.

Conclusion: Combining the data on chromatin interactions with information about known gene associations with a particular disease provides a new strategy to identify novel candidate genes that may be involved in the disease development.

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THE STUDY OF BIOLOGICAL ORIGIN OF RARE CO-EXISTENCE OF ATOPIC ASTHMA AND TUBERCULOSIS

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Key words: asthma, tuberculosis, dystropy, associative networks, genes

The concept of syntropy/dystropy addresses a phenomenon of frequent/rare co-existence of diseases. Co-development of atopic dermatitis, allergic rhinitis, and bronchial asthma (BA) in one individual is an example of syntropy. Rare co-existence of tuberculosis (TB) and BA is an example of dystropy. We hypothesize that syntropic/dystropic relationships between diseases are caused by shared proteins/genes that promote or suspend simultaneous development of syntropic/dystropic diseases, respectively.

Using BA and TB as an example of dystropy, we set out to establish shared and specific proteins associated with these diseases.

Two associative protein networks of BA and TB were reconstructed using the ANDCell software. Then we identified 247 specific proteins of BA and 22 specific proteins of TB, and 19 shared proteins associated with both BA and TB. The identified shared proteins have been classified for Gene Ontologies using BiNGO plug-in of the Cytoscape platform. We found 1356 biological processes and 48 molecular functions associated with these proteins which were many related immune response, such as immune cells interaction and proliferation and differentiation of T-lymphocytes.

Among the shared proteins identified in the study, *SPP1* gene previously has not been studied for association with TB; *CXCL10, TNFRSF1B* genes have not been for association with BA; and *CD4, CD79A* genes have not been studied for association with any of the two diseases in "case-control" studies. For these genes we identified SNPs with a MAF of at least 10% in Caucasians located in 5'UTR or exons. Selected SNPs at 5'UTR were evaluated for possible effects on the efficiency of the transcription factors binding to the promoter region. Missense-mutation SNPs at the exons were chosen. The finally selected SNPs were genotyped in cases of BA, TB, and healthy control Russian individuals from the city of Tomsk to analyze the contribution of the polymorphisms into the diseases pathogenesis.

Conclusion: The results of our study revealed a number of genes important for the development of both BA and TB. These genes are mainly involved into the regulation of immune response. The analysis of the associations between polymorphisms of these genes and BA and TB will provide a significant insight into understanding the reasons for dystropic relationships between the diseases.

A NOVEL APPROACH TO FUNCTIONAL SNP DISCOVERY FROM GENOME-WIDE DATA REVEALS NEW VARIANTS, ASSOCIATED WITH COLON CANCER RISK

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Keywords: colon cancer risk, prediction, SNP, ChIP-seq, ChIA-PET, RNAseq

Motivation and Aim: In recent years, high-throughput studies of cancer genomes have generated many notable discoveries; however, the known risk alterations correspond to a small fraction of the estimated cancer heritability in every case. The hope is that combining data from different sources, one could dramatically improve the reliability of genetic marker prediction. In the present study we applied an original systematic approach to search for functional SNPs, associated with colorectal cancer, from genome-wide research data, accumulated to date.

Methods and Algorithms: The data of more than 600 genome-wide studies (samples of 23 types of colon tumors or cell lines derived) were used. Raw NGS data of ChIP-seq experiments (Illumina) were aligned on NCBI36 (hg19) reference human genome sequence with Bowtie2 and processed to minimize the effect of duplicated reads, sequencing and alignment errors. OTFR genome regions (Overlap Transcription Factor Regions, Bryzgalov et al, 2013), enriched with binding sites for transcription factors, were chosen for analysis. In the case of sufficient coating (≥20X), possible allelic variations in the positions, containing SNPs, were determined for each sample and subsequently analyzed. First than, we assessed the frequency of 18746 previously annotated SNPs in the analyzed samples. Further analysis proceeded with non-annotated 3336 SNPs, found at least 3 times in a sample, among which we identified 883 SNPs, found in heterozygote in at least one case. Among those 883 SNPs, we identified 30, which were associated with changes in the surrounding chromatin; and 268, which were found in the heterozygote in HCT116 cell line, for which RNAseq bias study is possible. Then we calculated the cumulative incidence of discovered SNP in the analyzed samples, selecting SNPs, with frequency, differed by more than three times from the average. 14 SNPs showed greater frequency and 13 SNPs – lower frequency, as compared to dsSNP138; all with potential relevance with cancer risk. 10346 SNPs, possibly rare variants, were discovered for first time, with unknown population frequency. Next, bias CHIPseq analysis was performed for SNPs, discovered in the heterozygote in at least one sample. 3 SNPs among 27 (13+14) mentioned above and 91 - among newely detected, showed bias precipitation of different allelic variants. Next, 10 SNPs were found heterozygous in HCT116 cell line, with possible impact on gene expression. Then we selected genes whose promoters are in contact with the regions containing 10 selected SNPs. For that purpose the matrix of contacts was prepared for regions of SNP localization and promoter regions, according to Pol2-ChIA-PET data for HCT116 cell line (ENCODE). Also we carried out the bias expression analysis for 71 genes, selected for their promoter region containing SNPs (+ 1000 bp) or contacting with the region of SNP localization (p-value < $10e^{-6}$), with the use of SNPs in the coding regions. Differential expression was detected for 6 genes with more than 2-fold change.

Conclusion: Understanding how known and currently missing genetic alterations can contribute to the initiation and progression of the specific cancer is not only an important fundamental task, but is essential for practical medicine. Resuming, we discovered some new functional variants, that might simplify colon cancer risk prediction.

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BIOPHYSICAL PRINCIPLES GUIDING NUCLEOSOME POSITIONING *IN VIVO*

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Motivation and Aim: Cells continuously adjust their transcriptional outputs in response to cellular and metabolic cues. Eukaryotic transcription, however, is interfaced with chromatin. Nucleosome is a basic building block for chromatin comprising 147 bp of DNA tightly wrapped around a core histone octamer. A major consequence of the packaging of genomic DNA into chromatin is that nucleosomes can impede access of DNA-binding proteins to their sites. To counter this, eukaryotic cells evolved complex ATP-dependent chromatin remodelling machineries (remodelers).

Results: The prevailing view in the field is that ATP-dependent chromatin remodelers affect nucleosome structure and positioning by disrupting contacts between the histone octamer and DNA, which results in nucleosome sliding, eviction, or histone replacement. Remodelers are highly abundant in cells, and our previous work has shown that they are capable of affecting nucleosome positions globally [1]. Surprisingly, we found that in *Drosophila* S2 cells there are no enrichment of remodelers at promoters of either active or silenced genes. Nonetheless, both inter-nucleosome spacing and positioning downstream of transcription start sites (TSS) are affected. To resolve this paradox, we turned into biophysical models, which show that histone concentration and turnover rates directly affect inter-nucleosome spacing [2]. Thus, we hypothesize that in addition to reposinioning nucleosomes via specific interactions, remodelers modulate nucleosome spacing over genic regions through direct or indirect regulation of histone gene expression levels, which is substantiated by our results.

Conclusion: These results lead to a paradigm-changing idea: that in addition to directly targeting a fraction of genomic nucleosomes, chromatin remodelers change nucleosome positions and occupancies globally simply by modulating availability of histone proteins and their turnover rates.

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SYSTEMIC SHIFTS IN MICRO RNA LANDSCAPE AS A DIAGNOSTIC AND PREDICTION TOOL

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Key words: NGS, miRNA-Seq, attractor state, cancer genomics

Motivation and Aim: The new high-throughput next-generation sequencing (NGS) technologies greatly changed the research approaches in many fields, including cancer genomics. The high sensitivity and specificity of NGS significantly facilitates studies of miRNAs, small non-coding RNA molecules that regulate expression of many protein-coding genes. With NGS, both the abundance profiling of miRNAs in the sample, and the discovery of novel miRNA species is achieved. By comparing miRNA expression profiles between samples, global features of gene expression regulation landscape can be extracted, and used to infer the physiologically relevant information. In this study, we aimed to test our assumption that holistic distance-based measures calculated using miRNA profiles outperform the coding gene expression based distances in differentiating various pathophysiological states.

Methods and Algorithms: The study dataset included NGS-based gene expression profiles of 14 breast carcinoma samples available at TCGA Research Network. Raw miRNA-Seq data were processed by in-house Perl scripts to collapse identical reads and filter the unusable reads. The reads were aligned by Novoalign and the 3' adaptor sequences were trimmed. The reads alignments were then sorted according to their genomic coordinates and cross-referenced to the known locations of precursor microRNAs in miRBase. Next, the read frequencies were accounted to quantify the gene expression levels, then converted into the holistic profile distance values and plotted for visualization. Based on the count matrix, centers for normal and cancer attractors were derived as arithmetic means for each feature. Pearson's based distances were calculated for miRNA profiles individual each data point to both the normal and the cancer center, allowing for comparison and clustering.

Results: The distance plots and miRNA-Seq profiles of invasive breast carcinoma (BRCA) and normal samples showed substantially separation, while the separation based on coding gene expression distances were significant, but pronounced to lesser degree. All normal samples were clustered close to the attractor that defines the normal samples space (Normal Center, NC) In contrast, cancer samples clustered away from NC but at various distances to cancer attractor (Cancer Center, or CC). For all normal samples, the distance to CC was greater than the distance to NC (see Figure). Additionally, we observed distance-based clustering of histologically distinct tumors, thus, suggesting that the holistic miRNA landscapes may, indeed, define subattractors that reflect histological classification of human tumors.

Conclusion: Using holistic distance-based measures, we demonstrated that miRNA profiles are positioned as compact regulatory landscapes upstream of commonly used gene expression profiles. miRNA-based holistic distances may have superior performance as diagnostic tools for differentiating various pathophysiological states.

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ONCOGENESIS MODEL BASED ON THE GENOME STRUCTURE OF MULTICELLULAR ORGANISMS

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Key words: oncogenesis, genome structure, junk DNA

As a rough approximation, the genome structure of a multicellular organism represents an oriented binary tree, which nodes correspond to single-type logic elements ("loop steps of a genome") and arcs indicate the transfer of control between the "steps". For every cell of an organism there is its own "step" that takes control from the "step" of a maternal cell, initiates the execution of a "cell program" determining the cell development and division, and then transfers the control to the "steps" of daughter cells.

The "cell program" represents a graph, in which structural and controlling genes are enabled in a certain order. Each type of specialized cells has its own "cell program", and the whole set of these programs forms a second in volume structure of the genome of a multicellular organism.

In the case of terminal tree nodes, the activated cell programs represent apoptotic programs. In the case of previous nodes, one arc transfers the control to the terminal node, whereas the second arc forms a loop, i.e., transfers the control to itself. Thus, one daughter cell is subjected to the apoptosis, whereas the second one replaces the maternal cell.

The model is based on the hypothesis that the transfer of control between different "steps" is carried out via short RNAs. Being the product of SINE (short interspersed elements), these short RNAs, which, along with transcription factors of a Pol III polymerase, form nucleoproteins, are able to initiate the transcription of other SINE elements. At the same time, complimentary sites of the RNA and promoter of the activated gene can be quite extensive, and even small failure in the complementarity is able to prevent the initiation of the transcription.

SINE mutations are able to arise in both promoter region and the region encoding RNA sites responsible for the initiation of transcription of other noncoding genes. In both cases the control can be transferred to wrong "loop steps of the genome" that will result in various developmental abnormalities.

For example, the modification of SINE responsible for the transfer of the control to the top node of a genome tree can result in the formation of two loops. Therefore, the program of a maternal cell will be initiated in both daughter cells, so the process of uncontrolled cell division will grow exponentially resulting in the appearance of a tumor.

The cycling (transfer of the control to the previous "steps") can occur not only in tree leaves, but also in any arbitrary region. A multiple repeated execution of the same genome tree fragments provides various tumor types differing in their growth rate and external manifestations.

Mutations of the SINE promoter can result in the situation, when the promoter site responsible for the initiation of transcription becomes complementary to RNA, which is not intended for this SINE. If such RNA is transcribed by the above-located "step" of the same genome branch, then two or more "steps" (and, therefore, several various cell programs) are simultaneously activated in the cell in addition to the cycling process. In this case, tumor-forming cells significantly differ in their pathologies

Though the transformation of tumor cells into malignant cells represent a complex multistage process including many mutations, the formation of any malignant tumor is based on the cycling of any genome tree fragment. Therefore, the revealing of a mutation causing the cycling and the neutralization of noncoding genes or their products, which are responsible for such cycling, represents a cardinal way to prevent cancer.

This model was created by the author.

PRACTICAL APPROACH FOR DNA EXTRACTION OF *LINGUATULA SERRATA* NYMPHS: AN ANALYTICAL METHOD

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Key words: DNA extraction, PCI, *Linguatula serrata*

Motivation and Aim: Isolation of high quality genomic DNA is one of the most important steps in molecular biology studies related to food borne parasites. Usually, various protocols are used for different tissues but so far, there is no common, simple and cost benefit procedure for genomic DNA extraction of *Linguatula serrata* larvae as a Pentastomida endangering food safety. One of the procedures used in the other studies is using commercial kits that are very expensive especially for developing countries which have this health problem. This study investigated a simple and cost-benefit method to extract genomic DNA form *L. serrata*.

Methods and Algorithms: In this study, after collection of larvae from sheep, washing was done with phosphate buffer saline. The samples were grinded and incubated in lysis buffer at 56єC for an overnight. The precipitation was done in absolute ethanol. Extracted DNA was analyzed using agarose gel electrophoresis and spectrophotometery.

Results: Results indicated that the mean concentration of extracted DNA was 59.3±2.84 ng/µl, and the mean ratio of A(260)/ (280) were 1.6±0.3. It seems that the efficacy of this modified extraction method for *L. serrata* is appropriate.

Conclusion and Availability: In conclusion, this simple, cost-effective, fast and easy to use method could replace in expensive commercial kits at molecular laboratories for DNA extraction of Pentastomida and some other tissues like that. So, application of this analytical method could be useful to improve safety of food especially liver and other meat products.

RELATIONSHIP BETWEEN ANXIETY AND DEPRESSION IN THE CHRONIC SOCIAL DEFEAT STRESS MODEL: PHARMACOLOGICAL RESEARCH

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Key words: depression, anxiety, mixed anxiety/depression disorder, comorbidity, diazepam, clomipramine, interleukin-2

Motivation and Aim: Chronic social defeat stress in daily agonistic interactions leads to the development of mixed anxiety/depression state in male mice. Our studies have demonstrated a similarity in etiology, symptomatology, sensitivity to the drugs used for treatment and a similarity in neurochemical brain alterations in mice and humans as the disease progresses. Previous experiments showed that development of anxiety precedes and promotes to development of depression. However, interaction between depressive and anxious symptoms at the stage of formed pathology remains unclear. Therefore the aim of this study was to examine the effect of chronic treatment by drugs with different mechanism of action on the psychoemotional state with an emphasis on anxiety and depression-like states in mice.

Methods: The method for screening of psychotropic drugs effects under simulated clinical conditions [1] was used. The high level anxiety or mixed anxiety/depression conditions were generated in male mice by exposure to chronic social defeat stress in daily agonistic interactions. The mice were treated chronically with diazepam (1,5 mg/kg/day, i/p., Polfa) or saline on the background of continuing agonistic interactions (preventive treatment) to reveal the protective effects of the drug under social defeat stress. In another experiment, diazepam or clomipramine (40 mg/kg/day, i/p., SIGMA) or ronkoleukin, human interleukin-2 (5000 unit of activity/kg/day, i/p) or saline were chronically injected to mice after a 20-day period of confrontations during the period of relative rest, after the interruption of agonistic interactions (therapeutic treatment). The pharmacological treatment was carried out during two weeks after which the animals were studied in the partition, elevated plus-maze and Porsolt tests to estimate the levels of communicativeness as a behavioral reaction to a conspecific, anxiety and depressiveness, respectively.

Results: Therapeutic diazepam treatment of animals with high level of anxiety without signs of depression-like behavior induced expressed anxiolytic effect. At the same time preventive diazepam treatment produced no effect on the communicativeness and weak protective anxiolytic and pro-depressive effects. No therapeutic effects of diazepam have been observed in the mice with mixed anxiety/depression conditions. Chronic treatment with the antidepressant clomipramine decreased communicativeness, produced the anxiogenic effect and decreased exploratory activity, as well as exhibited an antidepressant effect. Ronkoleukin decreased communicativeness, increased anxiety level and decreased depressiveness.

Conclusion: Independent and multidirectional changes of anxiety and depression-like conditions in the mixed anxiety/depression state under the different drugs suggest independent mechanisms of development of these psychopathologies at least in our experimental paradigm. Therefore, this psychoemotional disorder presumably requires different pharmacological corrections.

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STUDY OF GENES CAUSING ATYPICAL FAMILIAL MYCOBACTERIOSIS IN TOMSK TUBERCULOSIS PATIENTS

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Key words: atypical familial mycobacteriosis; nucleic acids sequencing; polymorphisms

Motivation and Aim: Syndrome of atypical familial mycobacteriosis (MIM 209950) is of great interest in genetics of susceptibility to tuberculosis (TB). Mutations of 6 genes (*IL12B*, *IL12RB1*, *IFNGR1*, *IFNGR2*, *STAT1*, *NEMO*) play a crucial role in its development. As a result, healthy for other infections individuals, have an increased susceptibility to non-pathogenic mycobacteria and more deleterious *M. tuberculosis*. The aim of this study is to assess the prevalence of mutations in atypical familial mycobacteriosis genes at patients with different forms of TB and healthy control in Russians.

Methods and Algorithms: For RFLP analysis was used DNA of primary (n=61), secondary TB patients (n=270) and healthy individuals (n=279) from Tomsk region. The search for rare variants of studied genes was carried out by Sanger's method in 10 children suffered from aggressive forms of primary TB and complications of BGC-vaccination. Analysis of association was done by using the {{http://ihg.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl.}}

Results: First, we carried out the screening in 76 patients with the most severe TB. For this purpose according to the literature it was chosen 12 most common mutations of the causing genes: *IL12RB1* - Gln32Ter, Gln376Ter, Arg213Trp; *IFNGR1* - Ile87Thr, 4-bp Del, NT818; 1-bp Del, NT818; *IFNGR2* - 2-bp Del, 278AG, Thr168Asn, 663Del27; *STAT1* - Leu706Ser, Gln463His, Glu320Gln. The results of the genotyping revealed no mutations in the studied samples. Direct sequencing of intron-exon regions of these genes did not reveal any mutations causing atypical familial mycobacteriosis; however, 9 previously described SNPs were identified: *IL12RB1* - rs11575934, rs401502, rs12461312, rs17882555, rs3746190; *IFNGR1* - rs2234711, rs7749390, rs17181457; *STAT1* - rs2066797. Then they were genotyped in groups of patients and healthy individuals. As a result, we found a statistically significant difference in the frequencies of alleles and genotypes rs2066797 * STAT1 and rs2234711 * IFNGR1 ($p < 0.05$) in patients with secondary TB in comparing with control group.

Conclusion: Polymorphisms of genes STAT1 and IFNGR1 are associated with susceptibility to secondary TB in Tomsk individuals. An association between the rs2066797 variant of the STAT1 gene and TB was found for the first time.

ROLE OF MITOCHONDRIAL DNA POLYMORPHISM IN GENETIC PREDISPOSITION TO ATHEROSCLEROSIS AND ITS ENDOPHENOTYPES

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Key words: mitochondrial DNA, genetic polymorphism, atherosclerosis

Motivation and Aim: Mitochondrial DNA (mtDNA) is highly variable in human populations, and there are some evidences for the involvement of mtDNA polymorphism in susceptibility to common diseases, including cardiovascular diseases. However, the results of the association studies are controversial. The aim of the present study was to investigate contribution of common mtDNA polymorphisms to the coronary atherosclerosis and concomitant phenotypes, in the sample of patients with acute coronary syndrome.

Methods: For the present study, we used the registry of patients with acute coronary syndrome which is maintained in the Institute of Complex Problems of Cardiovascular Diseases. MtDNA was genotyped in the sample of 418 patients and 189 controls (inhabitants of Kemerovo city), including hypervariable segment 1 sequencing, designation of haplogroups on the base of sequence data, and confirming them by restriction. Association analysis included comparing the haplogroup frequencies in the samples and in sub-samples with concomitant diseases, as well as study of mtDNA polymorphism association with body mass index, lipid spectrum and other important characteristics.

Results: Comparison of haplogroup frequencies has revealed higher frequency of JT phylogenetic cluster in the patients vs controls (0.1794 vs 0.1111, p=0.0438). Further analysis has shown some associations of mtDNA polymorphism with concomitant phenotypes. For example, haplogroup T was more frequent in obese patients, comparatively to non-obese (0.1270 vs 0.0558, p=0.0243). Haplogroup HV0 was associated with acute coronary syndrome in non-diabetic patients vs controls (0.0818 vs 0.0317, p=0.0392); 16519C variant was associated with higher body mass index in women (31.3 in 16519C group vs 28.6 in 16519T group, p=0.043).

Conclusion: Mitochondrial DNA polymorphism contributes to some phenotypes associated with coronary atherosclerosis. The effects are small, and it seems that common polymorphisms in mtDNA do not play major role in atherosclerosis susceptibility. However, these associations emphasize importance of detailed analysis of phenotypes in the study of genetics of cardiovascular continuum.

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MOLECULAR GENETIC PECULIARITIES OF FIBROGENESIS IN DIFFERENT PATHOLOGICAL TRAITS IN HUMANS

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A sample of ethic Russians, N=941, living in Siberia region (Tomsk and Kemerovo) was studied. Ischemic heart disease patients (IHD), all with myocardial infarction, N=156, were divided into two subgroups: "isolated" IHD which had no other concomitant disorders such as arterial hypertension, diabetes militias (DM), obesity or dyslipidemia (60 patients in total), and patients with several comorbidities of the cardiovascular continuum (CVC) – "Syntropy" group where IHD, arterial hypertension, and DM type 2 simultaneously coexisted, N=96. Another groups under study included patients with DM type 1, N=285 and chronic viral hepatitis C (CVHC), N=200. Control sample was a population sample from the city of Tomsk, N=300. Criteria for fibrosis processes for cardiovascular diseases were IM, for hepatic disease – stages of fibrosis, for renal disease – diabetic nephropathy. Genotyping of 58 polymorphic variants was conducted by mass-spectrometry on Sequenom MassARRAY® device.

Case-control study had shown that the majority of associated with diseases genetic variants belong to the genes, which take part in extracellular matrix formation and collagen metabolism - *ADAMDEC1* (rs3765124; rs10087305; *MMP1* (rs514921), *MMP3* (rs679620), *ITGA4* (rs1143674), *ITGB5* (rs1007856), and *COL1A1* (rs2075555). Besides this, associations were detected with lipid metabolism genes: *APOA2* (rs5082) and *LDLR* (rs2738446), cell cycle regulation: *MTAP* (rs7023329) and *CDKN2B* (rs1333049), endothelial cells adhesion process: *KIAA1462* (rs3739998), reparation: *LIG1* (rs20579), membrane channels formation: *AQP2* (rs2878771). Thus, CVHC predisposition was related to *ADAMDEC1*, *MMP1*, *LIG1*, and *KIAA1462*. DM type 1 associated genes were *ADAMDEC1*, *MMP3*, *ITGA4*, *ITGB5* and *LIG1*. Variants in *ADAMDEC1*, *MMP3*, *ITGA4*, *ITGB5*, *COL1A1*, *LIG1*, *PARP1*, *DDX5*, and *NUP155* associated with diabetic nephropathy. "Isolated" IHD and "Syntropy" group differed in the spectrum of associated genetic variants. "Isolated" IHD associated with *ADAMDEC1*, *COL1A1*, *AQP2*, *LIG1*, *KIAA1462.* Syntropy associated with *APOA2*, *LDLR*, *MTAP*, *CDKN2B*, and *KIAA1462*. Therefore, genes involved in fibrogenesis play and important role in predisposition to various diseases in humans. Significant role is played by *ADAMDEC1,* which participates in extracellular matrix formation. This gene was associated with CVHC, stages of hepatic fibrosis, DM type 1, diabetic nephropathy, and isolated IHD. Processes, which regulate DNA reparation and recombination influence diseases development as DNA-ligase I (*LIG1*) associated with CVHC, moderate hepatic fibrosis, DM type 1, diabetic nephropathy and isolated IHD. Cardiovascular continuum Syntropy is a separate state, which is genetically different, as the genetic variants spectrum is not shared with other pathologic states investigated in this research.

XPD, XRCC1, OGG1 AND ERCC6 POLYMORPHISMS AND HUMAN LIFESPAN

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Motivation and Aim: Aging is accompanied by accumulation of DNA damage due to gradually declining DNA repair capacity in cells [1]. Base and nucleotide excision DNA repair pathways remove DNA damage preventing age-related diseases. Supposedly, normal functioning DNA repair systems might facilitate longevity. Based on this background, the objective of the present study was to determine *XPD* Asp312Asn, *XRCC1* Arg399Gln, *OGG1* Ser326Cys and *ERCC6* Met1097Val polymorphisms in Belarusian population to elucidate their possible association with individual lifespan.

Methods: DNA samples from 370 subjects aged from 31 to 94 years were genotyped using a PCR-RFLP method. The frequencies of genotypes and alleles were compared in the total cohort and the subgroup over 80 years using the χ^2 test. Correlation analysis was applied to reveal the relationship between gene polymorphisms and age in subgroups over 70 and 80 years.

Results: The study group was represented by predominantly Belarusians and other Eastern Slaves, and comprised 69% of men and 31% of women with average (m ± SD) age 64.4 ± 13.5 years. The genotype distributions of DNA repair genes in this group were in agreement with the Hardy-Weinberg equilibrium, and the minor allele frequencies were in the range of those in Caucasians in contrast to Asians. The frequencies of genotypes/alleles of analyzed genes were rather similar between a total cohort and the subgroup over 80 years. The previous study of gene-gene interaction in the limited sample (164 individuals) showed no differences between age alternative subgroups, except for frequencies of combinations of homozygous wild type *XPD* 312 and *XRCC1* 399 genotypes or *XPD* 312, *XRCC1* 399 and *OGG1* 326 genotypes, which occurred more frequently in the subgroup over 80 years [2]. Subsequently, the correlation analysis in the subgroup of 135 subjects, aged from 71 to 94 years, demonstrated a significant relationship between frequencies of several combinations of homozygous wild type genotypes and age. This relationship was more evident in the subgroup of 70 subjects over 80 years: $r = 0.7$ ($p = 0.006$) for the Asp/Asp Arg/Arg combination; $r = 0.65$ ($p = 0.022$) for the Met/Met Asp/Asp combination; $r = 0.51$ (0.037) for the Met/Met Asp/Asp Arg/Arg combination.

Conclusions: The data demonstrated increasing frequencies of certain combinations of homozygous wild type genotypes in the elderly confirming the supposition that normal functioning of error-free DNA repair systems during the life may favor the longevity. Among studied combinations, interactions between Asp/Asp Arg/Arg genotypes, Met/Met Asp/Asp genotypes and Met/Met Asp/Asp Arg/Arg genotypes seemed to influence human lifespan.

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EPIGENETIC MECHANISMS OF MEMORY FORMATION: THE ROLE OF THE HISTONE ACETYLATION AND METHYLATION IN AVERSIVE LEARNING

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Key words: epigenetics, histone acetylation and methylation, memory, learning, mollusks

Motivation and Aim: The goal of our investigations is to study the role of the epigenetic processes during memory formation. Learning model - food aversion reflex formation in mollusk *Helix*. The main approach - comparative investigations of histones methylation and acetylation in animals with normal capabilities for learning and in animals with dysfunction of serotoninergic system or damaged mechanisms of the long-term memory formation. To the current knowledge histones acetylation only activates gene expression, while methylation can both activate and repress it.

Results: By means of western-blot analysis we showed that during food aversion reflex formation there is a significant induction of histone H3 acetylation, and Histone H3 methylation on activating site (Lysine 4) and on inhibitory site (Lysine 9). At the same time we found that the most important role in induction of these epigenetic processes belongs to modulatory mediator serotonin, which mediates the action of the nociceptive unconditioned stimulus, as well as mediates activity of the MAP kinases ERK and p38. Disturbance of the serotoninergic system via injection methiothepin (nonselective antagonist of the serotonin receptors) prevents formation of the aversive reflex in *Helix* and leads to decrease of the histone H3 acetylation and histone H3 methylation. At the same time incubation of CNS with serotonin, leads to induction of these epigenetic processes. Induction of histones methylation on the inhibitory sites also can reflect an active influence of habituation processes, which play an important role in CNS functioning. Our data obtained on the juvenile snails, support the important role of the epigenetic processes during long-term memory formation. Incapability of juvenile animals for long-term forms of plasticity of avoidance reflexes is connected to immaturity of serotoninergic system. We showed, that in juvenile snails upon learning, in contrast to adults, there is no activation of histone H3 acetylation, and there is no methylation on the inhibitory sites. Thus we suggest that in juvenile snails there is not only immaturity of the serotoninergic system, but also habituation processes are not developed (late development during ontogenesis). We managed to stimulate formation of the long-term memory formation in juvenile snails by the induction of acetylation, via injection of the histone deacetylase inhibitors NB. Moreover we reversed the capability for long-term memory formation in adult animals, in which memory dysfunction was achieved by the treatment of the neurotoxin 5,7-DOT or methiothepin via injection deacetylase inhibitors NB and TSA. Thus our model can be perspective for the screening of the compounds that can increase long-term memory formation upon epigenetic regulation. Currently there are global investigations on the possible implications of HDACs inhibitors for cure/improvement of different disorders, including Huntington disease, Parkinson disease, Alzheimer, ischemic insults, depression and Schizophrenia. There are optimistic predictions also for the use of HDACs inhibitors for the improvement of the mental issues during aging.

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EVALUATION OF DIFFERENTIAL miRNA EXPRESSION AFTER PERMANENT FOCAL ISCHEMIA IN RAT BRAIN USING REAL-TIME qRT-PCR AND NGS

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Key words: miRNA expression, focal ischemia, real-time qRT-PCR, NGC

Motivation & Aim: Among non-coding RNA, microRNAs are the most intensely studied and their significance has been proven for diverse cellular functions. Despite the numerous reports on miRNAs action in cerebral ischemia the role of the latter in molecular mechanisms of pathology development still remain not completely explored. Determination of miRNAs role in the brain's response to ischemia may be essential for understanding the mechanisms of the disease development as well as for identification of new diagnostic markers of brain damage. The aim of the study is to evaluate miRNA expression profiling at ischemic brain injury using real-time qRT-PCR and next-generation sequencing technology (NGs).

Materials & Algorithms: MiRNA expression profiles were analyzed by real-time qRT-PCR using Custom miScript Primer Assay and miScript SYBR Green PCR Kit (Qiagen) in brain samples of 12 adult male rats subjected to permanent focal cerebral ischemia induced by photothrombosis. MiRNA expression fold changes were determined in ischemic penumbra vs. contralateral cortical region at different post-ischemic periods (24h and 48h). Sequencing was performed using Illumina MiSeq according to provided protocols.

Results: Based on a functional role of stroke-associated gene-targets we selected 45 miRNAs and validated them by real-time qRT-PCR. We found minor differences in expression level (1,5-2,0-fold) of chosen miRNAs: let-7f-5p, miR-27a-5p, 300-5p were down-regulated and miR-30c-2-3p, 376b-5p, 99a-3p were up-regulated. In order to get full representation of miRNA expression profile, we sequenced two samples at 24h point (ischemic and control). In total, 483 known miRNAs were identified, among them 70 miRNAs were found to be differentially expressed (1,5-4.5-fold). qRT-PCR data were in a good agreement with sequencing data, for instance, expression level fold change of miR-99a-3p was 1,86 and 1,41 according to qRT-PCR and NGS, respectively, as well as expression level fold change of miR-300-5p was 0,61 and 0,62, respectively. According to miRWalk and PANTHER databases we found 140 validated gene-targets for miRNAs with expression level fold change more than 2: down-regulated miR-1298, 3587, 218a-1-3p, 183-5p, 145-3p, 375-3p and up-regulated miR-301a-5p, 344b-1-3p,503-5p, 19b-3p, 92a-3p, 6325, 370-3p, 19a-3p, 146b-3p, 423-3p, 667-5p, 6318, 188-5p, 181a-2-3p, 21-3p, 582-3p, 532-5p which are implicated in apoptosis, adhesion, inflammation, immune response, transcription and translation regulation, lipid and protein intracellular transport, exocytosis and synaptic transmission.

Conclusion: Comparative analysis showed that the use of NGS is necessary to assess the entire spectrum of miRNAs differentially expressed in brain tissue in response to ischemia and to identify among them valid markers of ischemic brain damage.

CCR5 AND CXCR4 CORECEPTOR PROFILE IN RESISTANT HIV EXPOSED BUT SERONEGATIVE INDIVIDUALS OF NIGERIAN ORIGIN

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Key words: coreceptor, CCR5, CXCR4, HIV/AIDS, mutation, serodiscordant, nigeria

Motivation and Aim: A mutant allele of CCR5 membrane coreceptor with 32-base-pair deletion has been found in the highly-exposed group of seronegative individuals who are naturally resistant to HIV infection in European populations. This mutation presumably does not occur in African populations hence the high prevalence of HIV in the region. The study therefore, seeks to investigate the HIV-membrane coreceptor expression profile in HIV resistant but serodiscordant-heterosexual partners of Nigeria origin.

Methods: Thirty-four partners (serodiscordant-seronegative{SSN}and serodiscordant-seropositive {SSP}) and 15 seronegative-healthy individuals (SNH) were recruited for the study. HIV was confirmed using immunocomb-II. Flow cytometer was used to measure CD4, CD3, CD8, CCR5 and CXCR4 cell expressions. NucliSens magnetic extraction method based on Boom chemistry was used for HIV-mRNA extraction while real-time quantification was done by Nucleic Acid based amplification and detection assay (NASBA). NCBI protein Blast was used to search for sequence similarity algorithms and scores allocated using PAM matrix.

Results: We noted a significantly increased T-cell ratio in SSN group by 40% on comparison with SSP. HIV-mRNA was not detected in SSN and SNH but was highly expressed in the SSP group (9400±700). Expression profile of the co-receptors showed that SSN's CCR5 (800±45) and CXCR4 (756±80) decreased non-significantly ($P<0.05$) by 7.5% and 9% respectively when compared with SSP. Similarly, expression of CXCR4 (876±65) and CCR5 (900±152) in SSP increased slightly over SSH. SSP, SSH and SSN groups did not show any significant difference in their coreceptor expression patterns while scores were 93% similar.

Conclusion: Since cytokine-mediated increase in binding of HIV to cells is related to increased expression of CCR5 and CXCR4 coreceptors, our results preliminarily indicates that the HIV-coreceptor mutation may not be a factor in conferment of resistance to HIV-seronegative individuals hence the mechanism of HIV membrane fusion needs to be re-examined.

THE COMPELLATION OF HUMAN GENES CONTROLLING FEEDING BEHAVIOR OR ASSOCIATED WITH BODY MASS INDEX AND ITS FUNCTIONAL AND GENOMIC CHARACTERISTICS

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Key words: genes controlling feeding behavior, functional annotation, genomic location, RVIS

Motivation and Aim: The goals of this study were to create the list of genes controlling feeding behavior (FB) and to reveal functional and genomic characteristics of these genes. This knowledge may give a deeper view of molecular-genetic basis of FB abnormalities and may be useful for designing new pharmacological approaches for the treatment of human diseases.

Methods and Algorithms: Data were collected from scientific publications, Entrez Gene, OMIM, UCSC Genome Browser Database, and were analyzed using DAVID. We also used Residual Variation Intolerance Score (RVIS) values for the whole-genome set of human genes calculated by Petrovski et al. [1].

Results: The compellation of genes controlling FB includes 424 human genes. Among them 82 genes were collected from scientific publications as involved in regulation of FB in humans (or in mice or rats). 73 genes have OMIM-annotated allelic variants associated with FB abnormalities (hyperphagia, anorexia) or obesity. 260 genes have OMIM evidences for involvement in FB regulation, but have no data on allelic variants associated with FB abnormalities or obesity. 48 genes have evidences from GWAS meta-analysis: these genes are associated with elevated body mass index at the genome-wide significance level ($P<5.0\times10^{-8}$).

According to DAVID functional annotation basing on GO molecular function vocabulary, considerable portions of genes from the compellation encoded proteins with receptor activity (24.8%), receptor binding activity (23.9%) and transcription regulator activity (15%). Enrichment analyses were performed with respect to Gene Ontology categories and KEGG or REACTOME or BIOCARTA pathways. In total, 39 GO categories with 20 or more genes yielded a p-value of less than 10^{-3} for enrichment (in all these cases the Fold Enrichment was no less than 3). Inspection of pathways whose genes were overrepresented in the compellation identified 15 pathways from KEGG, 7 pathways from REACTOME and 8 pathways from BIOCARTA. In all cases, the fold enrichment exceeded two and p-value was below 10^{-2}.

The genomic locations of human genes controlling FB were analyzed. In total, 17 genomic regions containing 3 genes within 1 Mb and 13 genomic regions containing 4 genes within 2 Mb were revealed.

It was found that genes controlling FB have lower RVIS values than genes from the whole genome dataset [1] demonstrating their decreased tolerance to functional genetic variation and reflecting the impact of purifying selection on these genes.

Acknowledgements: This work was supported by the Government of Russian Federation grant no. 14.B25.31.0033 (to E. I. Rogaev).

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ASSOCIATION OF THE GENETIC MARKERS FOR MYOCARDIAL INFARCTION WITH SUDDEN CARDIAC DEATH IN THE RUSSIAN POPULATION

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Key words: sudden cardiac death, myocardial infarction, single nucleotide polymorphism

Motivation and Aim: Numerous current genome-wide association and case-control studies deal with the genetic predisposition for IHD and myocardial infarction. However, the studies on the associations of gene polymorphisms and mutations with SCD are still rather few and almost absent for the Russian population. Recent genome-wide studies have detected the association of some single nucleotide polymorphisms (SNPs) with an increased risk of IHD and, in particular, myocardial infarction. Later, the hypothesis on involvement of these SNPs in development of atherosclerosis, IHD, and myocardial infarction was tested in case-control studies with various ethnic, gender, and age cohorts. According to ICD-10, SCD does not include the death of myocardial infarction; however, the clinical picture and the mechanism of fatal outcome development in sudden cardiac death and myocardial infarction are rather similar. We assumed that the SNPs associated with the development of myocardial infarction might also contribute to SCD development. So the aim of this work is investigate the association of rs17465637 gene *MIAF3* (1q41), rs1376251 gene *TAS2R50* (12p13), rs4804611 gene *ZNF627* (19p13), rs619203 gene *ROS1* (6q22), rs1333049 gene *IFNE* (9p21), rs10757278 gene *CDKN2A* (9p21), rs2549513 (16q23), rs499818 (6p24) associated with myocardial infarction available from the international genome-wide studies with SCD in a case study of a Russian population.

Methods and Algorithms: A sample of SCD cases (n = 285) was formed using the WHO criteria; the control sample (n = 421) was selected according to sex and age.

Results: No statistically significant differences in the genotype and allelic frequencies of rs17465637, rs2549513, rs1376251, rs4804611, and rs619203 polymorphisms between SCD cases and control were detectable. Genotypes CC of rs1333049 and GG of rs10757278 are associated with an increased SCD risk in men (p=0.019, OR=1.7, 95%CI 1.1-2.8; p=0.011, OR=1.8, 95%CI 1.2-2.8, respectively). Genotype AG of rs499818 is associated with an increased SCD risk in the women over 50 years old (p=0.009, OR=2.4, 95%CI 1.3-4.6).

Conclusion: The association of polymorphisms rs17465637, rs2549513, rs1376251, rs499818, rs4804611, rs619203, rs1333049, and rs10757278 with SCD in a Russian population was studied for the first time. Polymorphisms rs1333049 and rs10757278 are associated with SCD in men and rs499818 in the women aged over 50 years.

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MODELING GENETIC INFLUENCES ON TWO DISEASES THAT ARE UNUSUALLY RARE IN CO-OCCURRENCE: BRONCHIAL ASTHMA AND PULMONARY TUBERCULOSIS

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Key words: differentially expressed genes, gene networks, comorbidity, bronchial asthma, pulmonary tuberculosis

Bronchial asthma (BA) is a common allergic disease, and pulmonary tuberculosis (TB) is a common infectious disease, are both serious health issues worldwide and both show evidence of genetic susceptibility with some underlying genes being shared by the diseases. According to epidemiological observations, a co-occurrence (comorbidity) of BA and TB is extremely rare, and we set out to establish the biological context for shared and specific genes associated with these diseases to reveal possible causes of this phenomenon.

We analyzed the epidemiological relationships between BA and TB, such as prevalence and co-occurrence, using PubMed search and Phenotypic Disease Network (HuDiNe). Then we carried out the analysis of differentially expressed genes using datasets for gene expression in peripheral blood T-cells from patients with severe asthma vs control (GSE31773) and from TB patients vs control (GSE19443) archived in Gene Expression Omnibus. Shared (differentially expressed both in TB and BA as compared to controls) and specific (differentially expressed in one disease only as compared to control) gene sets were revealed and subsequently analyzed for enrichment with LRpath and ConceptGen software. These sets were also used as seed genes for construction of gene networks using MetaCore software.

We found genes with opposite direction of the expression in some shared KEGG pathways (Ribosome biogenesis in eukaryotes; RNA transport; Apoptosis; DNA replication; Proteasome; Spliceosome) and we hypothesize that these genes are responsible for rare co-occurrence of BA and TB.

CONGENIC STRAINS FOR STUDYING ACCELERATED SENESCENCE IN OXYS RATS: RETINAL TRANSCRIPTOME AND CANDIDATE GENE ANALYSIS BY RNA-SEQ

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Key words: age-related macular degeneration, retinal transcriptome, RNA-Seq, snp-calling, senescence-accelerated OXYS rats, congenic strain

Motivation and Aim: Complex etiology of multifactorial age-related disorders, such as cataract and neurodegenerative diseases (e.g. age-related macular degeneration (AMD) and Alzheimer's) remains poorly understood due to the paucity of animal models, fully replicating human disease. Previously, two quantative trait loci (QTLs) for early cataract, AMD-like retinopathy, and behavioral signs of accelerated senescence in OXYS rats were demonstrated in a cross between OXYS and WAG rats. Two congenic strains, each introgressing the OXYS QTL on to the WAG genetic background, WAG/OXYS-1.1 and WAG/OXYS-1.2, were constructed and characterized. Animals of both strains displayed early cataract and retinopathy, but differed from OXYS rats on clinical and histological features. Thus the impact of the transferred loci on disease development was proved. The present study has focused on further disease-promoting pathway analysis in congenic rats by means of RNA-Seq.

Methods and Algorithms: Above 40 mln single-end reads of 50-bp length were obtained for each sample of OXYS, WAG, WAG/OXYS-1.1 and WAG/OXYS-1.2 male retina RNAs (in triplicate or pool) using Illumina non-stranded sequencing (Illumina GA IIx). Rn5/TopHat/Cufflinks (or DESeq R) pipeline was used to determine differential transcript expression; GeneOntology, DAVID, PANTHER, KEGG tools - for functional annotation; Bowtie/Samtools, Bcftools/My SQL/ENSEMBL pipeline - for SNP-calling.

Results: Comparative analysis of SNPs on 1st chromosome defined two (62.4 and 87.4 Mb) QTLs for accelerated-senescence phenotype in OXYS rats to congenic loci, each < 20 Mb; in the case of WAG/OXYS-1.1 rats – with a match to a QTL-flanking region, beyond expectation. Retinal transcriptome analysis in 20-days-old OXYS and congenic rats revealed significant differences in apoptosis, Ca2+-homeostasis, antigen processing and presentation, inflammation mediated by chemokine and cytokine signaling pathways, associated with cataract and retinopathy progression in both rats and humans. DE genes between two congenic strains were enriched in genes generally associated with *Wnt-*, *Integrin-*, and *Tgf-β*-signaling pathways, widely known to be involved in neurodegeneration. Genes, containing non-synonymous SNPs with a potentially relevance to complex traits under study, were revealed in the congenic loci. Additional consideration of gene position and expression suggested several more candidate genes, including *Prx, Fcgbp, Lim2, Ldha*, *Hbb-b1, Gp2, Asrgl1, Anxa1, Sypl2, Synpo2, Pitx2, Emp1, Mgp*. Taken together, the results provide the basis for studies of relationship between candidate genes within the QTL regions and the retinal DE genes as potential causal mechanisms that underlie age-related disorders. Significantly, our study represents the first analysis of rat retinal transcriptome with 40 mln sequencing read depth by RNA-Seq technology to our knowledge. *This work has been supported by Russian Foundation for Basic Research (project 14-04-00376).*

IMPLICATIONS OF HOSTILE ENVIRONMENT AND SOCIAL INSTABILITY IN ADOLESCENT MICE

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Key words: animal models, anxiety, depression, neurodevelopment, adolescents

Individuals exposed to social stress in childhood have been shown to be more predisposed to developing psychoemotional disorders in adulthood. The study aims to elicit the influence of hostile social environment and social instability in childhood on the behaviors in adult life. One-month-old adolescent male mice were placed for 2 weeks in a common cage divided by perforated transparent partitions with an adult male. The partitions were removed daily for 5 minutes to allow the adult males to demonstrate aggression toward the adolescent males. The former attacked and chased the young males which, in turn, demonstrated flight and defense behavior. Adolescents of other group were placed daily in an unfamiliar cage with a strange adult partner living on its own territory (social instability). The animal behaviors were studied in the partition, social interactions, elevated plus-maze, open field and Porsolt swim tests to evaluate the level of communicativeness with a strange partner in home and unfamiliar cages, anxiety and depressiveness, respectively. After exposure to hostile environment, some of the adolescent mice were placed for three weeks in comfortable conditions, into common cages with friendly partners of the same age to estimate extended effects of social stress. The male mice of respective age living in littermate groups were used as control. Additionally adult mice exposed to social hostile environment in childhood were engaged in agonistic interactions with non-aggressive strange male mice. BrdU positive cells in the hippocampal subgranular zone of dentate gyrus were analyzed in adolescent and adult mice. We found that 2 weeks of living in hostile environment decrease of communicativeness in the home cage and diminished social interactions on the novel territory. Stressed adolescents demonstrated a high level of anxiety and helplessness. Furthermore, the number of dividing (BrdU- positive) cells in the subgranular zone of the dentate gyrus was significantly lower in stressed adolescents. After 3 weeks of rest, most behavioral characteristics in different tests, as well as the number of BrdU-positive cells in the hippocampus, did not differ from those of the respective control mice. However, the level of anxiety remained high in adult males exposed to hostile environment in childhood. Furthermore, these males were more aggressive in the agonistic interactions. 2 weeks of social instability also resulted in high level of anxiety depressiveness and decrease of explorative activity. Thus, chronic early life stress has long term effects and may cause anxiety and depression in adulthood.

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AGING OF RAT RETINA: TRANSCRIPTOME STUDY

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Key words: aging, retina, transcriptome

Motivation and Aim: The aging process is the major risk factor for age-related diseases, including age-related macular degeneration (AMD). AMD is complex and polygenic, resulting from cooperation of a variety of genes. Mechanisms that trigger the transition from normal age-related changes to the disease and the changes that are common to normal aging of the retina and its diseased state are not well understood. It was shown that the senescence-accelerated OXYS rats are a suitable model for the study of pathogenesis AMD [1]. In order to identify the impact of aging process on in response to normal aging and progression of AMD-like retinopathy, we compared gene expression profiles of retina from 3- and 18-month-old OXYS and control Wistar rats.

Methods: Analysis of RNA-Seq data by DESeq and Cufflinks methods, pathway analysis, real-time PCR, immunohystochemistry

Results: We found method-specific changes in transcript abundance. However most differently expressed (DE) genes were overlapped between methods. We identified over 100 age-regulated genes in Wistar and OXYS retinas. The majority of them are related to the immune system and extracellular matrix turnover. Aging had significant effects on the expression of inflammatory genes but their composition was different in the retina of OXYS and Wistar rats. Only 24 age-regulated genes were common for the two strains, suggestive of different rates and mechanisms of aging. Over 600 genes showed significant differences in expression between the two strains. Main disease-associated pathways were immune response, inflammation, apoptosis, Ca^{2+} homeostasis, and oxidative stress. Interestingly, the majority of DE genes were downregulated in OXYS as shown by both methods. At both ages in OXYS retinas, genes that were underexpressed compared to Wistar rats included many regulators of immunity, such as leukocyte markers, chemokines, cytokines, complement components, interferon-inducible proteins, and major histocompatibility complex genes.

Conclusion: We observed transcriptional alterations in a variety of genes in the retina due to the genetic background and aging process. The development of retinopathy in OXYS rats is associated with an imbalance in immune and inflammatory responses. Data support the view that any disturbances in immune defenses can accompany retinal disease, not only upregulation, but also downregulation, which can be explained within the framework of immunosenescence theory.

*Acknowledgments***:** This work has been supported by Russian Foundation for Basic Research (project 14-04-00376 A).

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RELATIONSHIPS BETWEEN HUMAN GENE SET AND SET OF GENE DISORDERS

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Key words: gene density, gene disorders, gene diseases, human genome

Motivation and Aim: Now the human genome is a ground of research concerning genetic disorders and diseases not treated as genetic earlier. The aim of our research is basic relationship between gene set and set of gene disorders. Chromosome distribution of gene disorders allows us to compare gene density with density of gene disorders in the human genome.

Methods and Algorithms: Data of genes located on human chromosomes and size of chromosomes were obtained from the reference genome (release GRCh38) [1]. Information about known gene disorders is accumulated by National Library of Medicine [2]. Gene density (GD) is a ratio of a number of genes located on a chromosome per its size in million base pairs (Mb). Disorder density (DD) is a ratio of a number of known disorders associated with genes located on a chromosome per its size in million base pairs (Mb).

Results: The Pearson's correlation coefficient is used to evaluate the strength of connection between GD and DD, its value is 0.93. The ratio of all autosomal gene disorders per all genes on autosomes is 3.47%. The ratio of DD per GD for human autosomes is in the range of 2.41% - 4.41%. For X chromosome this ratio is 6.87%. For Y chromosome this ratio is 1.09%. For mitochondrial DNA - 24.32%.

Conclusion: Revealed close correlation between GD and DD for human autosomes points to random character of gene disorders' distribution. Gene disorders appear to be uniformly dissociated in all genes and form very tiny foam layer in the gene set. The majority of detected genes don't have described gene disorders. It seems to be a right to fail of a genomic information carrier. These rights vary depending on a type of carrier (autosomes, sex chromosomes or mitochondrial DNA). The level of rights can be evaluated by the percent ratio of DD per GD. The rights are equal for human autosomes. If it is considered as the point of reference then Y chromosome is cut in rights. This ratio for Y chromosome is three times lower than for autosomes. For X chromosome and especially for mitochondrial DNA the rights are expanded. The ratio for X is almost twice higher than for autosomes, the ratio for MT – over ten times higher. In our opinion it is related to different biological role and evolution history of genomic information carrier.

We observed general budget ratio of gene disorders in the human genome. This parameter is likely to be aligned and strictly controlled. The personalized difference in values of this parameter may contribute to diagnostics, therapy and prevention of genetic-based disorders of patients.

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COMPUTER ANALYSIS OF HUMAN GENE EXPRESSION DATA IN BRAIN USING MICROARRAYS

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Key words: gene expression, microarray, gene network, human genome

Motivation and Aim: Fast expansion of neurobiology frontiers is taken place last years, following explosive growth of experimental data on structure, function and evolution of nervous system at different hierarchical levels of its organization. Using high-throughput sequencing technologies and gene expression data analysis allows statistically analyze gene expression for thousands of genes taking into account special location of the cell in the brain. Affymetrix microchips GeneChip U133 series are widely used including Allen Brain Atlas data and clinical data from BioGPS (biogps.org/).

Methods and Algorithms: Computer program in C++ was developed to work with existing databases, process Affymetrix GeneChip microarray data. We used quality control estimates for probe sets of Affymetrix microarrays described earlier [1]. We have analyzed specific genome features of genes differentially expressed in brain cells, such as exon length, number of alternate transcripts, and median level of gene expression across the tissues. Some genes with overexpression in brain structures are connected to neurological diseases. Numbers of exons and active transcripts were estimated for genes differentially expressed in different organs. Statistical difference by such parameters for genes actively expressed in brain and other organs is shown. We discuss examples of such differentially expressed genes related to neurological deseases.

Results: We prepared set of genes differentially expressed in brain tissues (cerebellum, hippocampus, cortex, etc.) from BioGPS database. Context characteristics of these genes were studied [2] including correlation matrix for genes with higher expression in brain

Conclusion: Among samples microchip Affymetrix U133, that are represented in the BioGPS database, were identified some with high expression, collected samples of genes for which the expression is higher in brain structures, created a tool that allows to create a chart for correlations of expression of gene pairs. Structural features of genes with high expression (number of exons, the length of the transcript, link with alternative splicing) were detected. Created tool allows integrate gene expression and gene annotation data. It is planning to integrate this tool into a package for statistical data processing expression of genes that is developed in ICG SB RAS.

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HIPPOCAMPAL NEUROCHEMICAL PROFILE IN NEONATAL RATS: EFFECTS OF ANESTHESIA

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Key words: brain development, hippocampus, anesthesia, magnetic resonance spectroscopy, unanesthetized

Motivation and Aim: In vivo study of cerebral metabolism in neonatal animals by high-resolution magnetic resonance spectroscopy (MRS) is an important tool for deciphering the developmental origins of adult diseases. Up to date, all *in vivo* spectrum acquisition procedures have been performed in neonatal rodents under anesthesia. However, it is still unknown if commonly used anesthetics could affect metabolite levels in the brain of neonatal rats. Moreover, the unanesthetized MRS preparation that uses neonatal rodent pups is still lacking.

The goals of this study were to develop a restraint protocol to perform MRS experiments in immobilized unanesthetized rat pups and to investigate if the hippocampal metabolic profiles in neonatal pups determined by ^{1}H MRS are affected by a commonly used anesthetics – isoflurane, urethane and pentobarbital.

Methods: The immobilization procedure required for spectrum acquisition in unanesthetized pups was developed in accordance with the European Directive 2010/63/EU. All MRS experiments were performed on a horizontal high-field 11.7T magnet (Bruker, BioSpec 117/16 USR, Germany) interfaced with a digital spectrometer operating at a resonant frequency of 500 MHz (TE=3 ms, TM=20 ms, TR=4 s, 230 scans, VOI = 7.5-μL).

Results: The developed protocol for immobilization of unanesthetized neonatal pup shares the same gradation of severity as the protocol for non-invasive magnetic resonance imaging of animals with appropriate sedation or anesthesia. This procedure made possible the detection of metabolites in the hippocampi of unanesthetized neonatal rats. The peaks for nine target metabolites – creatine, phosphocreatine, choline compaunds, N-acetylaspartate, myo-inositole, taurine, glutamine, glutamate and lactate were clearly observed, and the peaks of three other compounds – GABA, alanine and aspartate were also detected. The treatment with noninhaled anesthetics urethane and pentobarbital resulted in the significant increase of lactate level in the hippocampi of anesthetized pups in comparison with unanesthetized rats. At the same time, the short-term isoflurane treatment did not affect the levels of key metabolites in the hippocampi of anesthetized pups.

Conclusion: Only isoflurane anesthesia is suitable for MRS studies in neonatal rodents when the interaction between anesthetic and target drugs is not expected. However, when the interaction between anesthetic and target drugs is suspected, the immobilization of unanesthetized neonatal rats should be used for MRS studies.

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ESTIMATION OF IMPACT OF ALPHA-CRYSTALLIN'S ALTERATIONS ON THE DEVELOPMENT OF AMD-LIKE RETINOPATHY IN OXYS RATS

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Key words: age-related macular degeneration, α-crystallin, OXYS rats

Age-related macular degeneration (AMD) - the most common form of severe blindness and vision loss among those over the age of 60, - is a multifactorial disease involving a complex interplay of genetic, environmental, metabolic, and functional factors. Evidence for the association of AMD development with changes in the expression and the functional activity of small heat shock proteins, namely α-crystallins, is strong. However, the function of α-crystallins in healthy and affected retina remains poorly understood. Here we examine the impact of expression of α-crystallins on the development of AMD using senescence-accelerated OXYS rats as a model. OXYS rats develop clinical signs of AMD-like retinopathy, that occurs by the age of about 3 months against the background of the reduction in retinal pigment epithelium (RPE) cell number and alterations in choroidal microcirculation. Significant pathological changes of RPE as well as clinical symptoms of advanced stages of retinopathy are observed in OXYS rats older than 12 months. Eventually primary cellular degenerative changes develop in the RPE cells, leading to choriocapillaris atrophy and resulting in a complete loss of photoreceptor cells in the OXYS retina by the age of 24 months. Recently to identify alterations in response to normal aging and progression of AMD-like retinopathy, we compared gene expression profiles of OXYS and control Wistar rat's retina at the age of 20 day, 3 and 18 months, by means of RNA-Seq [1]. Among differentially expressed genes, both *Cryab* and *Cryaa* were significantly downregulated in OXYS rat's retina when compared with Wistar rats, even at 20 days, when any clinical signs of retinopathy were not observed. The confirmation was made by RT-PCR analysis. The level of αB-crystallin protein in OXYS rat's retina, determined by Western blot, was more than 50% lower than in Wistar rat's retina within each age group. The level of αA-crystallin protein in the retina showed no difference between two strains at all the ages examined. We confirmed this by immunohistochemical analysis. The comparably low level of αB-crystallin, found in OXYS rat's retina (particularly in RPE cells), could lead to their susceptibility to oxidant-induced cell death, and contribute to further worsening with the age. Thus, our results pointed to a decline of the reserve capacity of neurons in OXYS retina, as evidenced by the fact that the degenerating area in 24-mo-old OXYS rats retina was shown to extend in the inner and outer nuclear layer; while photoreceptors were disorganized and fragmented. In conclusion, the inheritable impairment of Cryab mRNA expression combinated with reduced level of αB-crystallin protein in OXYS retina can contribute to the development of AMD-like retinopathy in OXYS rats, especially in conjunction with the inability to fully respond to stress. This study was supported by the Russian Foundation for Basic Research (Grant # 14-04-00376A).

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GENOME-WIDE PROFILING OF DNA COPY NUMBER AND METHYLATION IN ATHEROSCLEROSIS

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Key words: DNA methylation, copy number variation, copy number neutral loss of heterozygosity, coronary atherosclerosis, vascular tissues, microarrays

Motivation and Aim: Different vascular beds vary in their susceptibilities for development of atherosclerosis. The coronary arteries had a higher prevalence of atherosclerotic plaques in comparison with the other arteries. The internal mammary arteries are resistant to the development of atherosclerosis. We hypothesize that genetic and epigenetic heterogeneity of arteries can contribute to regional vascular bed differences in susceptibility to atherosclerosis. However, the copy number changes and DNA methylation alterations of affected and healthy vascular tissues had not been investigated in detail.

Methods and Algorithms: The Agilent SurePrint G3 Human CGH+SNP 2Ч400K and Illumina HumanMethylation27 BeadChip microarrays were used for DNA testing from right coronary arteries in the area of advanced atherosclerotic plaques (CAP) and atherosclerotic-resistant internal mammary arteries (IMA) of six patients with coronary artery disease. The DNA methylation of four CpG-sites located within *MIR10B* gene sequence and upstream about 1 Kb of the *HOXD4* gene was further quantified by bisulfite pyrosequencing in paired vascular tissue samples.

Results: We detected the multiple copy number variations and copy number neutral loss of heterozygosity in all vascular tissues. Polyploidy was observed in CAP of one patient. Right coronary arteries in the area of atherosclerotic plaques presented a higher average copy number variations length and number of genes located in their vicinity in comparison with IMA. The DNA methylation profiles of individual samples within CAP group had a wider range of variability than in the IMA. The genes hypomethylated in atherosclerotic-resistant and athero-prone arteries were predominately involved in inflammatory and immune responses, embryonic skeletal system development. Four CpG-sites located within *MIR10B* gene sequence and upstream about 1 Kb of the *HOXD4* gene were also confirmed as hypomethylated in the data set of CAP in comparison with IMA.

Conclusion: The DNA copy number and methylation changes in atherosclerotic-resistant and athero-prone arteries indicate the importance of these mechanisms in disease. Further studies need to be carried out to investigate the genes and biological pathways that are involved in this pathological process and may serve as targets for prevention and treating atherosclerosis.

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MOLECULAR MECHANISMS OF METABOLISM, EXCRETION AND DRUG TOLERANCE IN HUMAN LIVER FLUKE *OPISTHORCHIS FELINEUS*

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Key words: helminth, xenobiotic biotransformation, CYP450, P-glycoprotein

Motivation and Aim: Opisthorchiasis is one of the most common and difficult to treat parasitic disease in Russia. *O. felineus* invasion can result in severe complications, such as cholangitis, cholecystitis, in some cases associated with the development of cholangiocarcinoma. Pathogenic mechanisms of helminths action on the surrounding host tissue may include an active export of excretory metabolites, including lipids and oxysterols. These metabolites can be generated by xenobiotic biotransformation and excretion system, that remains unknown in flatworms, despite its both fundamental and applied significance in terms of finding therapeutic targets for opisthorchiasis treatment. In addition, xenobiotic biotransformation and efflux proteins may be involved in drug resistance formation also. Our aims were (1) to identify xenobiotic biotransformation coding genes in parasitic flatworms, (2) to identify cell efflux coding genes of ATP-binding transmembrane channels in parasitic flatworms and (3) to test CYP activity in liver fluke *Opisthorchis felineus*.

Methods: CDD search, rpsblast search, MS-MASCOT, HPLC, Microscopy, Droplet digital PCR.

Results: The only one cytochrome P450 has been identified in various parasitic flatworms including Trematoda (Opisthorchiidae, Schistosomatidae, Fasciolidae), Cestoda (Taeniidae). Monooxygenase CYP activity *in vivo* was similar to the mammalian CYP2E1 and CYP2B and was almost fully inhibited by ketoconazole. Phase II conjugation consists of gluthathione S-transferases, methyltransferases ans sulfotransferases. No UGT enzymes were found in parasitic flatworms. We found 20 ATP-dependent cell efflux genes including 4 homologs for human PgP1 (multi drug resistance genes) in *O. felineus*. One of four PgP genes (P4) have no close homologs in *C. sinensis*. P4 was differentially expressed through the parasite life cycle, being the most expressed PgP gene in adult worms, and was upregulated by drugs.

Conclusion: We found functionally active xenobiotic biotransformation and ATP-dependent efflux system in *O. felineus* with conservative organization and species-specificity. Our results demonstrate that the system plays an important role in parasite metabolism of exogenous and endogenous compounds. This points to high probability of synthesis and efflux of different metabolites including drugs and the existence of potential drug resistance mechanism.

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AHR-DEPENDENT GENES *CYP1B1* AND *CYP2J3* ARE INVOLVE IN CARDIOVASCULAR PATHOLOGY OF SENESCENCE-ACCELERATED OXYS RATS

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Key words: cardiovascular pathology, OXYS rats, cytochrome P450

Motivation and Aim: AhR-dependent genes coding 1st phase biotransformation enzymes are involved in arachidonic acid metabolism: cytochromes CYP1A1, CYP1B1 and CYP2J2 (CYP2J3 for rats) can form or disrupt anti-inflammatory/vasodilatory, or on the contrary, vasoconstrictor/cardiotoxic compounds [1]. Recent data demonstrated a real involvement of AhR-dependent genes especially *CYP1B1* in cardiovascular pathology [2] and association between cardiac hypertrophy and CYP expression level [3]. We compared mRNA level of *CYP1B1*, *CYP2J3* and *CYP1A1* in heart departments of senescence-accelerated OXYS rats as model of cardiovascular pathology, OXYSb rats with normal arterial pressure and Wistar rats without pathology. According to ECG 3-month-old OXYS rats characterized by severe disorders of the heart functional state

Methods and Algorithms: Left and right ventricles (LV and RV), left and right atrium (LA and RA) and aorta from 3-months-old male OXYS, OXYSb and Wistar rats were used. Total RNA was isolated and converted to complementary DNA. Evaluation of *CYP1B1*, *CYP2J3* and *CYP1A1* gene expression was carried out using TaqMan real-time PCR. The data were analyzed by standard calibration curve method. Statistical analyses were performed using the *STATISTICA* software using one-way ANOVA and Newman-Keuls post-hoc test.

Results: A global trend is that OXYS rats have more low mRNA level of *CYP1B1* and *CYP2J3* in all heart departments except LA compared Wistar и OXYSb rats. Significant differences were as follows. In RA *CYP1B1* and *CYP2J3* mRNA level was decreased in OXYS compared to Wistar and *CYP1B1* mRNA level was decreased in OXYS compared to OXYSb. In RV *CYP2J3* mRNA level was decreased in OXYS compared to Wistar. In LV *CYP1B1* and *CYP2J3* mRNA level was decreased in OXYS compared to Wistar and OXYSb. In aorta *CYP1B1* and *CYP2J3* mRNA level was decreased in OXYS and OXYSb compared to Wistar. In LA no differences was found. *CYP1A1* mRNA level was only higher in OXYSb compared to Wistar in left ventricle.

Conclusion: Transcriptional activity of *CYP1B1* and *CYP2J3* is different in «healthy» rats and rats with cardiovascular pathology. CYPs can be regarded as potential targets for new approaches to the treatment of cardiovascular diseases.

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THE SNP-MED SYSTEM FOR PERSONAL MEDICINE: ANALYZE THE EFFECT SNP TO THE FUNCTION OF GENES ASSOCIATED WITH DISEASES

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Key words: bioinformatics, single nucleotide polymorphism, disease, personalized medicine, computer systems

Motivation and Aim: Basis of personalized medicine is information about personal genomes and the modern technology in risk assessment of diseases associated with genomic polymorphisms. Genome-wide association studies have discovered many genetic loci associated with disease traits, but the functional molecular basis of these associations is often unresolved. But most currently available tools for associating genome variations to diseases focus on coding regions, disregarding relevant information present in the promoter regions of genes, such as variations that alter the binding affinity of transcription factor binding sites, which have been shown to play an important role in the regulatory machinery of the cell. Because with this, the developing the computer system that could automatically carry out assessment of the risks of socially significant diseases based on personal genome and systematization and analysis the huge amount of information including the known association of polymorphisms with diseases and predicted associations based on bioinformatics is challenging.

Methods and Algorithms: A program for analyzing and identifying the most dangerous genomic variation is developed on the basis of modification of complex algorithm ANNOVAR, integrating a large number of well-proven algorithms and data sources in the functional annotation of variations. Functional assessment of the role of polymorphisms in the gene function was performed using the original methods of evaluating the impact of single nucleotide polymorphisms (SNPs) in the regulatory regions of genes on their function and original methods of evaluating the impact of SNPs on the structure and function of proteins.

Results: The modular computer-based information system SNP-MED for estimation of single nucleotide polymorphisms influence on the function of genes associated with risk of the socially significant diseases based on personal genome and information including the known association of polymorphisms with diseases and predicted associations based on bioinformatics is developed.

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ANNOTATIONS *IN SILICO* OF 388 SNPS OF THE CORE-PROMOTERS OF 68 HUMAN FEEDING BEHAVIOR GENES IN TERMS OF THEIR POTENTIAL ASSOCIATIONS WITH NERVOUS DISORDERS

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Key words: SNP, TATA-box, TBP, feeding behavior genes, nervous disorder, annotation

Motivation and Aim: Investigations of single nucleotide polymorphisms (SNPs) in genes associated with nervous disorders of broad range including predispositions to alcoholism, drug addiction, narcolepsy, depression, and so on is of high importance because according to World Health Organization (WHO) data by the year 2020 that disorders will become second after cardiovascular diseases in number of deaths and disabilities worldwide.

Methods and Algorithms: We used our earlier developed computer-based model of TBP binding to DNA [1] that was verified at both equilibrium [2] and non-equilibrium [3] experimental conditions by the significant linear correlation ($r=0.82$, $p<10^{-3}$) between the predicted and measured equilibrium dissociation constant, K_D, of TBP/DNA-complexes. That is the only one reason why we applied this model to annotate the all 388 SNPs in-between -20 bp and -70 bp relative to each of the all transcription start site noted by hg19 (this is the region where all known TATA boxes reside) of 68 genes of human feeding behavior. The 1000 Genomes Project stored these SNPs within dbSNP as "unannotated".

Results: We found ninety SNPs each of which may significantly decrease (increase) K_D-values of the TBP/promoter-complex and, hence, may cause an excess (deficiency) of proteins encoded by the gene containing this SNP. We excluded 298 SNPs remaining neutral in the sense described above. As an illustrative example, SNP -28g → A of the non-canonical TATA-box, cctttgTGT(g → A)CCTctgctc, of the gene NPB in human, rs111897318, may decrease K_D-value, 18 nM → 6 nM, and, thus, may cause an excess of neuropeptide B encoded by this gene. Using a knockout mice, Hirashima and co-workers [4] identified that neuropeptide B induces slow wave sleep. Based on this experimental result [4], we heuristically associated rs111897318 with a potential sleepwalking that is a well-known nervous disorder often met during the slow wave sleep enhanced in-depth.

Conclusion: In this manner, we first annotated *in silico* ninety SNPs of 68 genes of human feeding behavior in the terms of their potential associations with nervous disorders.

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WHOLE EXOME SEQUENCING IN ALTAIAN FAMILIES (THE ALTAI REPUBLIC, SOUTHERN SIBERIA) WITH CONGENITAL HEARING LOSS

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Motivation and Aim: Hereditary hearing loss (HL) is characterized by extremely high genetic heterogeneity. About 140 genetic loci associated with non-syndromic HL (NSHL) have been mapped, with over 70 genes identified to date (http://hereditaryhearingloss.org). Mutations in *GJB2* (Cx26) gene account for a significant portion of autosomal recessive NSHL (ARNSHL) worldwide. Establishing a genetic diagnosis of HL is of great importance for a clinical evaluation of deaf people and recurrence risks for their families. However, identification of HL-responsible genes is a great challenge due to extreme genetic heterogeneity of disorder. Recently, the next generation DNA sequencing technologies, including whole exome sequencing (WES) have been successful in identifying candidate mutational variants causing deafness. In this study we explored the utility of WES for identifying candidate causal variants in patients with undiagnosed congenital deafness from the Altai Republic.

Methods: Genomic DNA samples of seven Cx26-negative individuals with congenital ARNSHL from four unrelated Altaian families have been sequenced on Illumina HiSeq 2000 using Agilent SureSelect exome enrichment kit followed by the standard BWA alignment, GATK post processing and deleterious variants annotation with Annovar and PolyPhen2. All variants not shared by affected persons from one family were filtered out. Shared "probably damaging" (PolyPhen2) variants have been further analyzed for homozygocity or compound heterozygocity in the context of NSHL candidate gene list (http://hereditaryhearingloss.org). If no candidate mutations among the NSHL genes were found, search has been extended beyond the NSHL panel. Variants found have further been validated by Sanger sequencing on remaining family members. Mutational screening for identified deleterious variants was performed in other Cx26-negative deaf patients and in Altaian control sample.

Results: We applied WES for identifying candidate causal variants in patients with undiagnosed ARNSHL and revealed the plausible causes of their deafness: homozygosity for a novel mutation c.1111G>C in *OTOF* gene, for mutation c.5254G>A in *RAI1* gene previously unknown in association with NSHL, and for already known deleterious mutation c.2168A>G (*SCL26A4*) cosegregating with HL in studied Altaian families. Based on these data we evaluated contribution of the identified mutations to deafness in other Cx26-negative deaf patients and estimated its prevalence in the Altai Republic population.

Conclusion: Whole exome sequencing is a suitable method to discover common and rare causative genes for a highly heterogeneous monogenic disease like hearing loss.

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GENETIC DISSECTION OF INHERITED HYPERTENSIVE STATE IN ISIAH RATS USING KIDNEY GENE-EXPRESSION AND GENOME MAPPING

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Key words: QTL and microarray analyses, kidney weight, candidate genes, stress-induced arterial hypertension, ISIAH rats

Motivation and Aim: Hypertension is the most common disease in industrialized societies. The basic mechanism of essential hypertension is the inability of the kidneys to excrete an adequate volume of sodium and water at a normal arterial pressure. The ISIAH rat strain is one of the advantageous models to study the mechanisms of the stress-induced hypertension and its complications. ISIAH rats were characterized by increased kidney mass and some alterations in kidney histology. The aim of the present study was the search for genetic determinants for kidney hypertrophy in ISIAH rats.

Methods and Algorithms: The genetic dissection of the traits (absolute and relative kidney weight) was performed using genome mapping (quantitative trait loci (QTL) analysis) and gene-expression studies (microarray analysis). The QTL approach was performed using F_2 hybrids (ISIAH x WAG) of 6-month old male rats. The genome was scanned with 148 polymorphic markers. Linkage analysis was done using MAPMAKER/EXP 3.0 and MAPMAKER/QTL 1.1 programs. Microarray analysis was performed in kidney of 6-month old rats in JSC Genoanalytika (Moscow, Russia) using Illumina RatRef-12 Expression BeadChip microarray platform containing probes for 22,228 rat genes. The functional analysis of differentially expressed genes was performed using DAVID Bioinformatic Resources (http://david.abcc.ncifcrf.gov/). The Gene Ontology (GO) option was utilized to determine the most significant biological processes possibly related to kidney function and blood pressure regulation. The KEGG Pathway Database was used to identify pathways that were most significant to the data set.

Results: Six suggestive loci were detected for absolute kidney weight. One significant and 3 suggestive loci were found for relative kidney weight. The presence of the ISIAH alleles in some loci caused a significant increase in the kidney weight traits and several loci were characterized by negative effect of ISIAH alleles on the traits appearance. Comparative analysis of gene expression profiling in kidney of hypertensive ISIAH and normotensive WAG rats revealed 126 differentially expressed genes in renal cortex and 65 genes in renal medulla. Both in renal cortex and renal medulla the differentially expressed genes associated with GO terms 'response to stress' and 'transport' were revealed. The group 'response to stress' contained genes for response to oxidative stress, hypoxia, inflammatory response and regulation of response to stress. The genes associated with GO term 'transport' related to ion transport, lipid transport, and organic alcohol transport. KEGG analysis showed that the interstrain differences in gene expression both in renal cortex and renal medulla were related to cell adhesion molecules and tyrosine metabolic pathways. Among genes found to be differentially expressed in kidney of ISIAH and WAG rats, 12 genes were reported in Rat Genome Database (http://rgd.mcw.edu/) as genes related to hypertension. Several differentially expressed genes mapped in the QTL for absolute and relative kidney weight are considered as candidate genes for renal hypertrophy in ISIAH rats.

Conclusion: The current study demonstrated the usefulness of combined genome mapping and gene-expression approaches for detection of candidate genes for disorders with polygenic inheritance.

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MELATONIN IN PREVENTION OF ALZHEIMER'S DISEASE-LIKE PATHOLOGY IN OXYS RATS

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Key words: accelerated aging, Alzheimer's disease, melatonin, senescence-accelerated OXYS rats

Motivation and Aim: Melatonin (M) is one of the most powerful antioxidants acting at various levels, and the level of M reduces during aging and in Alzheimer's disease (AD) patients. Its indirect antioxidant effects and anti-amyloid-β (Aβ) effects are based on the support of appropriate circadian phasing and anti-excitotoxic actions. Thus, it is not surprising that M is protective in numerous experimental systems and has been proposed as a treatment for AD. However, the question of to whom and at what age to start treatment with M ceases open. It is difficult to give correct estimate effects of any anti-aging medicines on humans in connection with individual peculiarity of their age-related dysfunction accumulation and quality of life. The studies with AD models are necessary to confirm the role of M at the pathology of AD. To investigate a link between M secretion and AD pathology and whether M treatment could have beneficial effects on cognitive impairment and Aβ burden in a rat model AD, we treated OXYS rats with M as the disease pathology progressed and found that M efficiently prevented daily rhythms' disturbances and cognitive impairment and Aβ burden in the brain.

Methods and Algorithms: 1, 1.5, 3, 12 and 16 month-old OXYS and Wistar rats (n=15-30) were used. Animals got M ("Melaxen" medicine) in dosage 0.04 mg/kg of body weight with food from 1.5 to 4 (experiment 1) and from 12 to 18 (experiment 2) months of age. Animals were subjected to behavioral testing in Elevated Plus-Maze test, Open Field test, Eight-Arm Radial Maze (EARM) and Morris water maze (MWM). Light microscopy was used for analysis of hippocampal neurons. ELISA was used to quantify levels of melatonin in serum and Aβ in the cortex and hippocampus. ANOVA and nonparametric tests were performed on StatSoft Statistica 6.0.

Results: The levels of M secretion decline with age in OXYS and Wistar rats with more rapidly reduction in OXYS rats. Daily rhythms' disturbances of M secretion in OXYS rats develop to 3 and increase to 16 month of age. Treatment with M from 1.5 to 4 months of age, in the critical period for development of AD-like pathology in OXYS rats, prevented disturbances of daily rhythms of M secretion in these rats. Also M prevented degenerative alterations of neurons in all regions of hippocampus. Likewise M prevented increasing of anxiety, slowed down reduce of locomotor and exploratory activities, learning and memory deficits in OXYS rats and it didn't affect the similar points in young Wistar rats. Treatment with M from 12 to 18 months didn't affect the endogenous M's serum levels but prevented degenerative alterations in neurons of hippocampus as well as accumulation of Aβ in the brain of OXYS rats. In addition, M slowed down increased anxiety, learning and memory deficits in OXYS rats in the MWM as well as the learning ability in EARM in adult Wistar rats.

Conclusion: M is a potentially useful agent in the prevention and treatment of AD.

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VIRAL EXPRESSION ASSOCIATED WITH GASTROINTESTINAL ADENOCARCINOMAS IN TCGA HIGH-THROUGHPUT SEQUENCING DATA

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Keywords: cancer, papilloma virus, herpes virus

Background: Up to 20% of cancers worldwide are thought to be associated with microbial pathogens, including bacteria and viruses. The widely used methods of viral infection detection are usually limited to a few a priori suspected viruses in one cancer type. To our knowledge, there have not been many broad screening approaches to address this problem more comprehensively.

Methods: In this study, we performed a comprehensive screening for viruses in nine common cancers using a multistep computational approach. Tumor transcriptome and genome sequencing data were available from The Cancer Genome Atlas (TCGA). Nine hundred fifty eight primary tumors in nine common cancers with poor prognosis were screened against a non-redundant database of virus sequences. DNA sequences from normal matched tissue specimens were used as controls to test whether each virus is associated with tumors.

Results: We identified human papilloma virus type 18 (HPV-18) and four human herpes viruses (HHV) types 4, 5, 6B, and 8, also known as EBV, CMV, roseola virus, and KSHV, in colon, rectal, and stomach adenocarcinomas. In total, 59% of screened gastrointestinal adenocarcinomas (GIA) were positive for at least one virus: 26% for EBV, 21% for CMV, 7% for HHV-6B, and 20% for HPV-18. Over 20% of tumors were co-infected with multiple viruses. Two viruses (EBV and CMV) were statistically significantly associated with colorectal cancers when compared to the matched healthy tissues from the same individuals ($p = 0.02$ and 0.03, respectively). HPV-18 was not detected in DNA, and thus, no association testing was possible. Nevertheless, HPV-18 expression patterns suggest viral integration in the host genome, consistent with the potentially oncogenic nature of HPV-18 in colorectal adenocarcinomas. The estimated counts of viral copies were below one per cell for all identified viruses and approached the detection limit.

Conclusions: Our comprehensive screening for viruses in multiple cancer types using next-generation sequencing data clearly demonstrates the presence of viral sequences in GIA. EBV, CMV, and HPV-18 are potentially causal for GIA, although their oncogenic role is yet to be established.

MUTATION ANALYSIS OF *MYCOBACTERIUM TUBERCULOSIS* GENOME AND ASSOCIATION WITH DRUG RESISTANCE

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Key words: genome-wide association studies, multi drug-resistant tuberculosis, genotype, single nucleotide polymorphisms

Motivation and Aim: Tuberculosis (TB) is one of the major public health threats in Belarus and worldwide. In recent years it has been complicated due to the appearance and development of multi drug-resistant tuberculosis (MDR TB) or extensively drug-resistant tuberculosis (XDR TB) which requires long-term treatment. The ability of TB agent to resist treatment is strongly connected with variations and mutations in specific parts of the bacteria genome. Information about resistance mutations and their interrelationships may become very valuable for preventing therapy failure. Here, we introduce a framework for analyzing TB genome data generated in our own research. Our final efforts are directed to creation of a knowledgebase that it is updated with new information available due to research projects in this field.

Methods and Algorithms: We perform a genome-wide association study (GWAS) that seeks to identify single nucleotide polymorphisms (SNPs) in M. tuberculosis genome mapped against reference H37Rv strain and check them for association with emergence of drug-resistance. Practically, we compare DNA sequences isolated from drug-resistant (cases) and drug-susceptible (control) organisms. Data analysis procedure comprises several steps organized into a pipeline. The initial steps are aimed to analyze new pathogen genomes (reveal population structure, site covariations, identify signals of natural positive selection under specific treatment regimen) [1,2]. Next steps uncover associations of genome variations with results of phenotype resistance tests [3] and comprise discovered associations into probabilistic network of dependencies.

Results: Since TB genome sequencing is still in process, we have tested elements of this approach on sample M.Tuberculosis data collected from public databases and used synthesized data as a source of assigned quantity traits. However, results of the example data demonstrated that the choice of the methods for each step may significantly affect the outcome and its interpretation requires interdisciplinary efforts.

Availability: Elements of this approach are used in current to establish the Belarus tuberculosis portal (http://tuberculosis.by) and conduct comprehensive study of obtained MDR and XDR TB strains. In the nearest future we plan to significantly expand the functionality of the portal to share our bioinformatics data on this research.

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GENOME-WIDE ENVIRONMENTAL SENSITIVITY ANALYSIS OF HUMAN METABOLOMICS DATA

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Key words: genome-wide association study, metabolomics, gene interactions

Motivation and Aim: In most published Genome-wide association studies (GWAS) interactions between both genetic factors themselves and environmental and genetic factors were ignored. Complex genetic models, which include many interacting loci and environmental factors, can help to identify new genes and improve our understanding of the genetic architecture of complex traits.

Methods and Algorithms: Promising approach to detect interactions is based on screening for the heterogeneity of the trait variance. A significant difference in the trait variance between genotypes indicates that this locus is potentially involved in interactions with unknown factors. This approach has been implemented in the Squared residuals Value Linear Model (SVLM) method. We applied SVLM to analyze data of the population based KORA study (2,901 individuals, 420,000 SNPs). 151 concentrations of metabolites measured in human blood serum were analyzed.

Results: We found 6 loci, which are potentially involved in interactions in the control of the human metabolome. 5 out of 6 loci were already published in previous GWAS. On the next step we founded factors, which are interacting with the identified loci. Finally we found 5 gene-gene interactions in the genetic control of glycine and different carnitines levels.

Conclusion: We found gene interactions, which were previously unknown. Our results need to be verified using independent sample. These results are the basis for a future discovery of interaction between genetic and environmental factors.

Availability: In our study we used “GenABEL” and “VariABEL” packages, which are available from {{http://www.genabel.org}}

CAN OPIOID AND SUBSTANCE P SYSTEM INTERACTION ANTOGONIZE MORPHINE-INDUCED RESPIRATORY DEPRESSION?

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Key words: respiration, rhythm generation, newborn, hypoxia, morphine, substance P

Motivation and Aim: Opioids are widely used to treat pain in preterm and newborn infants, and respiratory depression is the most common acute side effect of morphine during intensive care. We have demonstrated recently [1] that morphine considerably modified hypoxic response in neonatal mice with knock-out of substance P (KO Tac -/-) which is deeply involved in central respiratory rhythm and pattern generation. KO mice exposed to morphine both *in vivo* and *in vitro* developed exaggerated hypoxic/anoxic response. Therefore, our results suggest certain interaction of opioid and substance P mediator systems [2], throughout neuronal networks responsible for the respiration which may result in resistible to morphine hypoxic/anoxic response.

Methods and Algorithms: To optimize the experimental analysis/search we applied the Associative Network Discovery approach [3]. This approach allows to operate with different databases and construct associative networks describing semantic relationships between different biological objects, processes, and diseases.

Results: We constructed several interactive networks and identified associative connections between respiratory side effects of opioids and polymorphism in activity of substance P mediator system which is potentially involved in protection of respiratory activity. Using the created networks and a web-based interface to interpret the between-object connections we identified main nodes of interest which, besides metabolites of substance P and endomorphines, include also several paths within nitric oxide and serotonergic systems.

Conclusion: Our results suggest that protective influence of interaction between SP and morphine on respiration may operate through activation of main SP metabolite, SP(1-7) binding site via the products of morphine degradation, and this proposal has to be further confirmed with experiments.

Availability: http://pbiosoft.com/

Acknowledgements: Supported by "HKH Kronprinsessan Lovisas foreningen för barnasjukvård".

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ACTIVATION OF HIPPOCAMPAL CELL PROLIFERATION AND DECREASE OF C-FOS EXPRESSION IN THE AMYGDALA UNDER POSITIVE FIGHTING EXPERIENCE IN MALE MICE

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Key words: repeated aggression, cell proliferation, BrdU, c-fos, dentate gyrus, amygdala

The *aim* was to examine hippocampal neurogenesis and neuronal activity in the amygdala and hippocampus of male mice with prolonged positive fighting experience in daily agonistic interactions.

Methods: The male mice were groups of animals that had won 20 daily encounters (winners) before and after a no-fight period for 14 days and animals that were not exposed to agonistic interactions (controls). Brains of the winners and controls were analyzed for cell proliferation (BrdU-positive cells) in the hippocampus and neuronal activity (c-fos-positive cells) in the amygdala and hippocampus.

Results: We found that the winners demonstrate an enhanced aggression after the no-fight period as compared to their level of aggression before fighting deprivation. In the winners the number of BrdU-positive cells was increased in the subgranular zone of the hippocampal dentate gyrus, and reverted back to the control level after the no-fight period. Neuronal activity, estimated by the number of c-fos-positive cells, was suppressed in basolateral amygdala in the winners and was not recovered to the control level after a no-fight period. We did not find differences in the number of c-fos-positive cells in the hippocampus of the winners and controls.

Conclusions: Long positive fighting experience affects hippocampal cell division and neuronal activity in the basolateral amygdala. These findings may be relevant for understanding the mechanisms of enhanced aggression in fighting deprived winners.

Acknowledgements: Supported by RFBR, grant 14-04-31299 мол_а; "Molecular and Cellular Biology" program of Russian Academy of Sciences, grant 6.25; grant 11.G34.31.0071 from Russian Ministry of Education and Science.

THE SENESCENCE-ACCELERATED OXYS RATS AS A MODEL OF ALZHEIMER DISEASE

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Key words: Alzheimer disease, brain aging, senescence-accelerated OXYS rats

Motivation and Aim: More than 35 million people worldwide have Alzheimer disease (AD), the most common type of age-related dementia, with dramatically increasing of the incidence as a consequence of the ageing population. Research efforts on aging and age-related deficits rapidly expanded and greatly advanced our understanding of the mechanisms that result in neuronal dysfunction and death and the risk factors of AD. Nevertheless, many questions remain to be answered about how changes in the sporadic form of AD, which accounts for over 95% of all cases, lead to neurodegeneration and how to prevent these changes. To date, there has been only limited success in an animal model of sporadic AD. Our studies of senescence-accelerated OXYS rats demonstrated that this strain constitutes an interesting rodent model of sporadic form of AD.

Methods and Algorithms: 1, 3, 12, 18, and 24 month old OXYS and Wistar rats (n=6-15) were used. Animals were subjected to behavioral testing in Elevated Plus-Maze test, Open Field test, and Morris water maze. MRI were used for the study of neurodegenerative changes. Light and electronic microscopy was used for analysis of neurons of the hippocampus. Standard immunofluorescence techniques, peroxidas immunocytochemistry, Western-blot analysis and ELISA were used to quantify levels or localization of some key proteins, involved in neurodegeneration. ANOVA and nonparametric tests were performed on StatSoft Statistica 6.0.

Results: OXYS rats are characterized by the progressive cognitive and behavioral dysfunction, loss of synapses, neuronal cell death, hyperphosphorylation of tau, increased load of amyloid precursor protein (AβPP) and amyloid-β (Aβ) in the brain, especially the peptides 42 amino acids, amyloid plaques, and mitochondrial dysfunction. OXYS rats overproduce AβPP protein by 12 months of age and the protein levels remain high subsequently at 24 months of age. Total Aβ content increases by 333% between the ages 3 and 24 months. It is noteworthy that in 3-month-old OXYS rats, when the behavioral alterations become manifested and first signs of neurodegeneration become detectable by MRI, light and electronic microscopy, there are no differences between OXYS and Wistar rats in the $A\beta_{1-42}$ and AβPP levels. The excessive accumulation of Aβ in the brain of OXYS rats by 12 months of age happens after the appearance of mitochondrial aberrations, which take place at 3 months of age and increasing with age. Ultrastructural changes include a decrease in mitochondrial specific area and appearance of mitochondria with destroyed cristae and lysed matrix. The hyperphosphorylation of tau in OXYS rats appears before accumulation of Aβ. The significant increase in the level of the tau protein and its phosphorylation in the cortex and hippocampus were found already in 3-month-old OXYS rats and increasing with age; the phospho-tau T181 level increases by 102% between 3 and 24 months of age.

Conclusion: We believe that further experiments with the OXYS rat strain will yield new insights into the complex mechanisms underlying AD and may lead to new therapeutic strategies to combat this disease. *This work was supported by grant from the Russian Foundation for Basic Research (projects # 12-04-00091).*

ALTERATIONS OF RAT RETINAL PIGMENT EPITHELIUM WITH AGE AND AMD-LIKE RETINOPATHY

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Key words: retinal pigment epithelium, aging

Motivation and Aim: Age-related macular degeneration (AMD), the leading cause of blindness in the elderly, is a degenerative disease that severely impacts the retinal pigment epithelium (RPE). RPE is essential for retinal homeostasis by transporting of nutrients into and removal of waste products from photoreceptor cells. The mechanisms of age-related cell loss in retina, especially of the RPE cells during AMD, are not fully understood. To decipher the age-related changes in RPE we compared RPE morphology during aging of senescent-accelerated OXYS rats with clinical signs of AMD-like retinopathy and disease-free Wistar rats.

Methods: RPE structures were examined at 20 days, 4 and 18 mo. Analysis of RPE morphology were conducted by confocal microscopy using flat-mounts phalloidin staining; assess the apoptosis in at different stages of OXYS rat's retinopathy was conducted by Tunnel immunohistochemistry; the distribution of microglia/macrophages were assessed using IBA-1 staining.

Results: Morphometric analyses demonstrated age-related, topographical and interstrain differences in RPE cell size, shape, and granule content. At 20 days we observed relatively homogeneous RPE cells distribution with a regular hexagonal shape which was replaced by more heterogeneous pattern already in 4 mo. Majority of the RPE cells were binucleated at all ages. The RPE cell density was decreased in OXYS rats compared to age-matched Wistar rats: 1369±143 vs 1724±28 cells/mm^2 at 3 months, 1238±90 vs 1379±112 cells/mm2 at 18 months. The aged RPE in 18 mo was characterized by structural changes, which were more pronounced in OXYS rats. RPE abnormalities included the increase in cell size, the increase in variability in cell size, the increase in proportion of multinucleated cells, disruptions of the cell form, changes in the number of cell neighbors, and irregular immunolabeling suggesting alterations of cell junctions. According to RNA-seq data several components of signaling pathways known to inhibit apoptosis (*Birc3, Cdc2, Bcl2l10*, *Aven* and others) demonstrated downregulated expression. Moreover, *Hmgb1*, which is considered a marker of late apoptosis, was found greatly upregulated in the OXYS retina [1].

Conclusions: Our data support the view that RPE is a mosaic of variable cells rather than a homogeneous monolayer [2]. The inherent cell heterogeneity of RPE cells probably contribute to the polymorphic clinical picture of retinal diseases including AMD.

Acknowledgments: Microscopy was performed in the Microscopy Centre of ICG SB RAS. This study was supported by RFBR (project 14-04-00376 A).

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COMPARATIVE NGS ANALYSIS OF miRNA EXPRESSION IN RAT BRAIN AND BLOOD PLASMA AFTER TRANSIENT FOCAL ISCHEMIA

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Key words: NGS, miRNA expression, transient focal ischemia, plasma, brain

Motivation and aim: Despite the existence of different therapeutic approaches in the treatment of hypoxic/ischemic brain injury, favorable results have not been achieved yet. It may be attributed to the lack of complete understanding of the underlying pathological processes as well as the late diagnostics of the disease when massive death of neurons has already occurred and effective therapeutic intervention is problematic. Screening analysis of the expression profile of blood plasma miRNAs appeared in circulation as a sequence of cell death or secretion/excretion from injury area is proposed to be non-invasive and highly sensitive approach to reveal specific markers of ischemic brain injury at early stages of stroke development.

Methods and algorithms: We analyzed miRNA expression profiles in brain and blood plasma samples of rats subjected to transient focal ischemia following 24 hours after stroke onset using Illumina Next Generation Sequencing technology. miRNA expression fold changes were determined in perinfarct zone in reference to contralateral cortical region as well as in plasma samples before and after ischemia onset. Variance analysis of miRNA expression level was performed on the number of sequence reads obtained after alignment of FASTQ-files to miRBase and NCBI using Novoalign tool (Novocraft Technologies) resulted in 11 million and 1.5 million 19-24-mer RNA reads on average per brain and plasma samples, respectively.

Results: Linear regression analysis of the data revealed 109 miRNAs in plasma and 23 miRNAs in brain tissue with more than two-fold expression level changes following 24 h after focal ischemia onset. Among differentially expressed miRNAs, miR-423-3p and miR-21-3p were found to be up-regulated in plasma and brain during ischemia. Besides that, the expression of 5 miRNAs (miR-145-3p, miR-375-3p, miR-19b-3p, miR-19a-3p and miR-188-5p) in plasma and brain samples was found to be changed in opposite directions during ischemia development. This fact may be explained, in part, by different regulatory mechanisms of individual miRNA intracellular production and/or secretion/excretion into the circulation. According to miRWalk and PANTHER databases, the 187 validated gene-targets of differentially expressed miRNAs are involved in such biological processes as apoptosis, cell adhesion, transcription regulation, immune response, inflammation, synaptic vesicle exocytosis, neurotransmitter secretion, synaptic transmission, intracellular protein transport, nitric oxide biosynthesis, cellular glucose homeostasis and angiogenesis – the processes known to be involved in ischemic brain injury. The functional role of each miRNA found to be dysregulated in the present study is the subject of our further research.

Conclusion: Our data indicate that miRNA analysis in systemic circulation may have implications for early diagnostics of brain damage after ischemia.

IDENTIFICATION OF NEW GENETIC MARKERS FOR PREECLAMPSIA USING AN INTEGRATED APPROACH

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Key words: transcriptional profiling, placental tissue, gene, tagSNPs, preeclampsia

Motivation and Aim: Preeclampsia (PE) is a common pregnancy-specific disorder with unknown etiology diagnosed in 5-17% of pregnancies. It is the leading cause of maternal and perinatal morbidity and mortality. Candidate genes associated with PE have not been fully described. To investigate how the expression of maternal genes contributes to the mechanisms underlying the progression of the disease, we investigate global placental gene expression using microarray technology. We studied the role of variability in some of these genes in the genetic susceptibility to PE also.

Methods and Algorithms: Genome-wide transcriptional profiling was performed on decidua basalis tissue from preeclamptic (n=10) and normal (n=11) pregnancies. We analyzed 51 tagging single nucleotide polymorphisms (tagSNPs) in 12 genes (*ANKRD37, BCL6, BHLHE40, CCSAP, CORO2A, DGKG, GPT2, PLIN2, RDH13, SIGLEC6, SYDE1* and *ZNF175*) in 514 patients with preeclampsia and 627 women with uncomplicated pregnancies from Russian, Buryat and Yakut populations using MassArray iPLEX (Sequenom).

Results: Among the 47000 transcripts that were screened, 63 were found to be differentially expressed between normal and preeclamptic tissues (Fold Change >1.5, FDR<0.1). Among these candidates, 50 were up-regulated and 13 were down-regulated. The up-regulated genes included LEP, BHLHB2, SIGLEC6, RDH13, BCL6, SYDE1, which are well-known differentially expressed genes (DEG) for PE, as well as CORO2A, CEBPA, HK2 which was recently proved to be linked with the etiology of this disease. Gene ontology analysis further revealed several biological processes that could be associated with the development of PE, including response to stress, immune system process, regulation of cell communication, intracellular signaling cascade etc. We have detected significant associations for PE with tagSNPs in *PLIN2, BHLHE40, DGKG, RDH13, SYDE1* genes in Russian and Buryat population. In Yakut population, only three genes *(BHLHE40, CORO2A, GPT2*) are associated with increased risk of PE. tagSNPs in *ANKRD37* and *ZNF175* genes were associated with PE in Buryat population only. Interestingly, we found an association with preeclampsia for twenty out of the fifty-one studied polymorphism.

Conclusion: This finding may provide insight into the pathophysiology of the disorder and lead to new therapeutic possibilities for this disease. This results demonstrate the high informative value of the integrative approach in studies of the genetic components of PE and show that allelic variations of the differentially expressed genes in placental tissue are associated with PE in different ethnic groups.

Acknowledgements: This work was supported by the Russian Foundation for Basic Research (grant №14-04-01467).

CONSERVATIVE mTOR SIGNALING PATHWAY AS A TARGET FOR PROPHYLAXIS OF ACCELERATED BRAIN AGING

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Key words: neurodegeneration, OXYS rats, aging, rapamycin, mTOR, Alzheimer's disease

Motivation and Aim: Aging is associated with increased risk of cognitive decline and neurodegenerative diseases. Mounting evidences suggest that widely used immunosuppressor rapamycin (R) (selective inhibitor of mTORC1 kinase complex) is able to extend lifespan in a wide group of organisms including mouse. Several reports have indicated that R can impact multiple age-related diseases. Particularly, there was shown ability of rapamycin to treat some manifestations of Alzheimer's disease (AD) and other neurodegenerative diseases, but the background of this process is still under investigation. OXYS rat's strain is the unique model of accelerated aging, which has signs of early-onset (from 3 months old) neurodegeneration with relevance to abnormalities of AD. So, the aim of this study is to investigate potential ability of R to delay the neurodegenerative manifestations of OXYS rats.

Methods and Algorithms: 1.5 month old Wistar and OXYS rats (n=45 each group) were randomly assigned on 6 equal groups (n=15 each group): control diet or control diet supplemented with R, 0.1 or 0.5 mg/kg body weight per day for each strain. After 2 month of treatment, animals were subjected to behavioral testing in following order: assessment of the degree of anxiety in Elevated Plus-Maze test (EPM) followed by observation of locomotor and exploratory activity in an Open Field 48 hours after the completion of the 1st one. High-resolution T2-weighted MRI images were used to investigate the area of brain regions on coronal brain sections and to reveal demyelization. Western-blot analysis and ELISA were used to quantify levels of some key proteins, involved in neurodegeneration and mTOR signaling pathway. ANOVA and nonparametric tests were performed on StatSoft Statistica 6.0.

Results: MRI revealed hydrocephalus in control group of OXYS rats – the area of lateral ventricles was about 2 times larger than that of Wistar rats. Also there were no foci of demyelization in Wistar rats, but all of the OXYS rats had such pathology. R treatment eliminated difference in the area of ventricles between strains and decreased percentage of animals with demyelization in half. In both behavioral tests R improved exploratory activity and locomotor activity in OF test of OXYS rats. The level of anxiety of OXYS rats was significantly decreased by R in OF test and in EPM test at tendency. The quantification of proteins, involved in the progression of AD, revealed no impact of R on level of Aβ but the drug progressively reduce phosphorylation of Tau peptide. There wasn't found a decrease in the phosphorylation level of S6 protein which is a target of mTOR kinase activity.

Conclusion: Rapamycin has an ability to reduce important pathological manifestations of accelerated brain aging of OXYS rats.

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POLYMORPHISM RS1625895 GENE *TP53* AND EFFECTIVENESS OF TREATMENT OF DLBCL

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Key words: genetic polymorphism, *TP53*, rituximab, prognosis, survival, Non-Hodgkin's lymphomas

Motivation and Aim: Deficiency of gene *TP*53 function is one of the adverse prognostic factors in Non-Hodgkin's lymphomas. In Diffuse large B-cell lymphoma (DLBCL) incidence of *TP53* mutations and 17p deletion in the onset of the disease is rare [1]. Earlier studies have shown that the polymorphism rs1625895 gene *TP53* is associated with altered white blood cell apoptosis in cancer [2]. The purpose of the present study was to study association of rs1625895 with effectiveness of R-CHOP DLBCL treatment.

Methods and Algorithms: We studied 106 unrelated patients with DLBCL treated by R-CHOP. Genotyping of rs1625895 was carried out with use of PCR-RFLP. Overall survival (OS) and relapse-free survival (RFS) probabilities were estimated with the use of the Kaplan–Meier method and were compared by means of the log-rank test. Multivariate analyses were conducted with the use of the Cox model with forward selection to identify independent prognostic variables influencing the OS and RFS.

Results: For rs1625895 genotype distribution in DLBCL patients was as follows: genotype G/G - 75,5%, G/A - 22.6 % and A/A - 1.9 %. The response rate in subgroups of patients with rare allele and homozygous genotype G/G was 73.1% and 50.0%, respectively (p=0.0396). Odds ratio response to R-CHOP therapy in patients with genotype G/G, was 0.37 [95 % CI: 0,15; 0,99, P <0,05]. In patients with DLBCL with genotype G/G rs1625895 5 - year OS was 42.5% vs 65.4% in patients with genotypes G/A and A/A (p =0.014). In the subgroup of patients with homozygous genotype G/G rs1625895 relapse-free survival was 36.3 %. It was significantly lower (p=0.030) than in the subgroup of patients with genotypes G/A and A/A - 57.7%. In multivariate analysis by Cox regression method was shown that rs1625895, along with the index IPI, can serve as a predictor of the likelihood of achieving OS and DFS.

Conclusions: The present study showed that genotype G/G of rs1625895 gene *TP53* is associated with a high probability of failure R-CHOP therapy of DLBCL patients. Future studies to confirm these data are required.

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ATTRACTOR BASED CLASSIFIERS FOR PREDICTION OF POST-TREATMENT SURVIVAL IN CANCER AND DETECTION OF NON-MALIGNANT DISEASES

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Key words: functional genomics, cancer attractor, predictive model, supervised classification

Motivation and Aim: Recently, we proposed that the holistic measures defined as distances between the entire gene expression profile of a tumor and the center of the space occupied by normal samples could be useful as diagnostic and prognostic classifiers in the management of cancer. The use of distance measures allows one to depart from the classical two-bin prediction model (e.g. "bad prognosis/good prognosis") in favor of a continuous prognosis model, where each sample is located in the neighborhood of other samples analyzed post-hoc and associated with known survival. This paradigm aligns itself with the theory of cancer attractors, which considers that differentiating cell travels in a multi-dimensional state space defined by expression profile of this cell. In this model, a trajectory of a cell is defined by the underlying dynamics of its gene regulatory network which drives their migration to corresponding tissue or cancer attractors. In this study, we demonstrate the utility of the attractor-based predictive model to estimate the stage of a tumor and as a correlate to the post-treatment survival.

Methods and Algorithms: Microarray data was collected from Rembrandt, a Repository for Molecular Brain Neoplasia Data. Gene expression and survival data were analyzed for astrocytoma, glioblastoma multiforme (GBM), and oligodendroglioma samples. Additionally, Non-coding RNA profiles of 20 healthy controls and 47 psoriasis biopsies (GSE31037) were retrieved from GEO. Using whole transcriptome expression signatures, we constructed a high-dimensional Euclidean space, in which individual tissue-specific attractors were defined. To measure how far away each individual tissue sample drifted from this attractor, Pearson's or Mahalonobis distances were used. Similarly, attractors were defined for either primary or metastatic tumors or other pathophysiological states. Therefore, each issue sample was associated with multiple measure of distance in the space that could be parsed by 2- or 3-dimensional clustering and used to construct predictive models.

Results: In all tumor datasets we studied, normal samples were found to group within a compact area close to normal attractor, while tumor samples were further removed, forming distinct attractors of their own. In the datasets with both primary and metastatic tumor samples, we observed distinct clustering pattern of metastatic cells that were further away from normal attractor than their less malignant counterparts, suggesting the existence of multiple attractors and endorsing the application of the distance analysis as a measure of malignancy. Moreover, in all tumor types analyzed, the distance to normal attractor showed significant positive correlation with relative degree of malignancy as measured by histological type of the tumor and negative correlation to the duration of survival, thus allowing predictive classification of tumors. Additionally, an analysis of psoriasis dataset showed the utility of attractor theory and distance analysis for the study of non-cancerous human diseases.

Conclusion: The distance analysis of molecular portraits is robust and versatile in its application as it is equally attributable to gene expression profiles collected by microarrays and by RNA-seq. The distance-based continuous predictive models depart from the classical two-bin prediction model (e.g. "bad prognosis/good prognosis") by placing each sample in the neighborhood of other samples analyzed post-hoc and associated with known survival.

Availability: on collaborative request

Acknowledgements: Dr. Ganiraju Manyam (MD Anderson Cancer Center, TX, USA). Some calculations were run on ARGO, a research computing cluster provided by the Office of Research Computing at George Mason University, VA.

ASSOCIATION OF THE DOPAMINE RECEPTOR D4 (DRD4) GENE POLYMORPHISM WITH CARDIOVASCULAR DISEASE RISK FACTORS

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Keywords: blood pressure, LDL cholesterol, dopamine D4 receptor gene, genetic polymorphism

Motivation and Aim: Dopamine receptor genes are candidates for cardiovascular disease susceptibility. Dopamine-4 receptor (DRD4) gene has a 16 amino acid (48 base pairs) repeat polymorphism located in exon 3 where a G-protein binding area is encoded. The long allele (defined as at least one 7 to 10 repeat) has been associated with higher systolic and diastolic blood pressure (BP) in US white population [1]. Having a 2- or 5-allele DRD4 polymorphism was related to high high-density lipoprotein (HDL) cholesterol levels in men, but to low HDL cholesterol levels in women of Finnish population [2]. Our aim was to analyze the association of the DRD4 gene polymorphism with cardiovascular disease risk factors (BP, the levels of HDL cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides) in Russian population.

Methods and Algorithms: We genotyped 257 female and 425 male subjects of Slavic ethnicity at the DRD4 repeat polymorphism site. The subjects were divided either in 7+ (those having from 7-8 repeated allele) and 7- (those having from 2-6 repeated allele) groups or in 2+5+ (those having 2 or 5 repeated allele) and 2-5- (those having 3, 4, 6, 7, and 8 repeated allele) groups. The relationship of DRD4 polymorphism and traits was analyzed using general linear model procedures of Statistica 8.0.

Results: We found association between the 7+ alleles and increased systolic BP (P= .029) and diastolic BP (P= .009). There was no association between DRD4 polymorphism and pulse BP. Having a 2- or 5-allele DRD4 polymorphism was associated with low HDL cholesterol levels in women (P= .040) and was not associated with HDL cholesterol levels in men. There was significant interaction between 2+5+genotype and sex and LDL cholesterol levels (P= .017) but the difference between 2+5+ and 2-5- groups was insignificant for comparison of men or women alone. DRD4 gene variants showed no association with triglyceride levels.

Conclusion: We replicated previous finding of association of DRD4 polymorphism and BP. We also replicated finding of association of DRD4 polymorphism and HDL cholesterol levels in women. Lack of association of DRD4 polymorphism and HDL cholesterol levels in Russian men in comparison with Finnish men may be connected with a history of regular alcohol use.

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Компания Хеликон – крупнейший поставщик в России самого современного оборудования и реактивов для широкого спектра научных исследований, молекулярной диагностики, криминалистики. Клиентами Компании Хеликон являются исследователи в области молекулярной и клеточной биологии, протеомике, молекулярной диагностике, медико-генетической экспертизе и идентификации личности, а также ветеринарии и селекции в сельском хозяйстве.

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