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**Study of the pathogenic mechanisms of metabolic syndrome at the  
background of genetically determined insulin resistance in childhood cancer  
survivors**

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**Study Title: Study of the pathogenic mechanisms of metabolic syndrome at the background of genetically determined insulin resistance in childhood cancer survivors****IND Holder:** Not Applicable**Brief Overview:**

The remarkable progress in developing curative therapy for childhood cancer over the last 4 to 5 decades has increased awareness of the serious cancer treatment-related late effects experienced by long-term survivors such as premature mortality, early deaths, second primary cancers, organ dysfunction (heart, lung, endocrine system), fertility impairment, cognitive deficits, and reduced quality of life. Endocrine disorders, which occur in 30% to 70% of childhood cancer survivors, are among the most frequent late effects of anticancer therapy. Survivors treated with radiation and alkylating agent chemotherapy for hematological malignancies and CNS tumors are at a particularly high risk for endocrine dysfunction.

Most anticancer drugs act directly or indirectly by modifying intracellular metabolism. Therefore, high frequency of acute and late cancer treatment-related organ toxicity can result in metabolic disorders. For example, steroid-induced hypercortisolism blocks glycolysis and results in insulin resistance of tissues. Insulin resistance is associated with earlier manifestation of diabetes mellitus, obesity etc. The clinical sequelae of metabolic syndrome developing in childhood cancer survivors may include insulin resistance, fasting hyperglycemia, endothelial failure, obesity, dyslipidemia, hypertension, chronic fatigue syndrome, motor and behavioral disorders.

Modern genetics make it possible to create a basis for a personalized approach to the prevention of early and late toxic effects caused by anticancer therapy and the rehabilitation of the childhood cancer survivors.

**Objectives:**

Specific Aim 1. Evaluate the frequency and clinical features of the metabolic syndrome in childhood cancer survivors.

Hypothesis 1A: Components of the metabolic syndrome are realized in children and adolescents at all stages of therapy of leukemia and lymphomas, can influence the development of complications and late toxic effects.

Hypothesis 1B: Initial health conditions (abnormal IBM, family history, comorbid diseases); drug's toxicity could influence to the appearance of early manifestation of metabolic syndrome.

Specific Aim 2: Evaluate the contribution of functional polymorphisms in candidate genes to metabolic syndrome outcomes among childhood cancer patients.

Hypothesis 2A: Genetic polymorphisms involved in the regulation of the insulin resistance and cancer medications during treatment contribute to the development of metabolic syndrome in childhood cancer survivors.

Specific Aim 3: Assess the extent to which genetic predictors, doses of drugs, risk factors improve the discriminatory performance of standard clinical prediction models for metabolic syndrome outcomes among childhood cancer survivors.

Hypothesis 3 A: Development of metabolic syndrome in cancer patients depends of genetic determinants and toxic effects of antitumor therapy.

Secondary Aim 1: Assess the definition of metabolic syndrome in cohort of patients of leukemia and lymphoma and survivors.

Hypothesis 1A: Episodes of triglyceridemic, insulin resistance (Hyperglycemia, HOMA $>2,7$ , Steroid Diabetes) during the treatment could be the base evidence marker of Metabolic Syndrome in patients treated by antitumor therapy.

Hypothesis 1 B: Endothelial dysfunction as a clinical component of metabolic syndrome is responsible for cardio-vascular abnormalities in cancer survivors.

**Exploratory aim 1:** Assess the association of biomarkers and genetic predictors among childhood cancer survivors with therapeutic exposures (chemotherapy and/or radiation therapy) and metabolic syndrome.

**Evaluation:**

Eligible persons who consent to participate in this trial will be asked to do the following:

- Vital sign measurement including resting heart rate, blood pressure, height, and weight.
- A total of 12 mL of blood will be collected in 3 test tubes. Biomarker analysis will be completed by Laboratory of Dmitry Rogachev National Medical Research Center.
- A Total of 4 mL of blood in 1 test tube will be used for genotyping for the presence of polymorphic variants in genes involved in biotransformation of xenobiotics, insulin resistance and carbohydrate metabolism in the biomolecular laboratory of Dmitry Rogachev National Medical Research Center.
- An echocardiogram and ultrasound will be performed to assess cardiac function.
- CAVI and ABI pulse wave velocity will be non-invasively measured by using the SphygmoCor VaSera VS-1500N. Arterial pressure waveforms will be recorded with a strain gauge pressure sensor placed lightly over the radial artery before and after “6-minutes physical activity”.

**Study Design:** Longitudinal

**Sample Size:** Aims of this study will be accomplished using a longitudinal assessment of 400 patients with ALL/NHL from Dmitry Rogachev National Medical Research Center and other Regional Clinics will be recruiting to the cohort at the start of the therapy MB-ALL/ BFM-NHL. Potentially 2100 survivors ALL/NHL will be treating in “Russkoe Pole” 2018-2021.

**Cases:** Participants for this study will be patients of “Russkoe Pole” cohort (RPC), a longitudinal study designed to evaluate health among children and adolescents survivors of childhood leukemia and lymphoma as they age. Participants in RPC undergo risk-based medical screening according to the Standards of Medical Insurance for rehabilitation of Cancer treated patients. Cases will be stratified by survival time (1-4, 5-9, and  $\geq$  10years).

To be eligible for RPC, cancer survivors must have been treated in Russian children oncology clinics by MB-ALL/ BFM-NHL and be 15 years of age or younger, and at least 6 months from the completion of the therapy. All the patients will have exhaustively full epicrisis of medical history with cumulative doses of medications. Because RPC is a retrospective cohort study with ongoing recruitment (additional survivors become eligible over time).

**Study Population:**

Eligibility criteria:

- Cancer survivors:
- Treatment with chemotherapy and/or radiation therapy for a primary ALL/NHL diagnosed prior to age 17 years.
- $\leq$  15 years of age at time of enrollment.
- No cytostatic drugs uptake during the study.

Exclusion criteria:

- Diagnosis of diabetes mellitus types 1 or 2 types before antitumor therapy
- Active oncological disease
- History of allogeneic hematopoietic cell transplant
- Absence of written consent for participation from the patient or legally authorized representative

<b>Randomization: N/A</b>
<b>Anticipated Primary Objective Completion Date: 10/09/2018</b>
<b>Anticipated Study Completion Date: 10/12/2021</b>
<b>Analysis of objectives:</b> Aims 1 and 2: Descriptive statistics will be used to summarize prior cancer therapy, collected from formal medical record abstraction. Epidemiological, clinical and laboratory variables, including genetic variants will be tested for their association with metabolic syndrome. McNemar's test for paired data will be used to compare the unmatched general characteristics of cases and controls. Allele frequencies will be estimated by two-side Fisher exact test, and the Chi-square test will be used to check for departures from Hardy-Weinberg equilibrium. Aim 3: The crude disease-exposure association will be determined by estimating the OR and its 95% confidence interval (CI). This will be done by univariate conditional logistic regression, to account for the matched design. The significance of the OR will be assessed by the Wald test. Exposures for which the P-value is less than 0.25 in univariate analysis will be entered into a multivariate conditional logistic regression model. Backward stepwise regression procedures will be used to develop the final multivariate model and possible interactions will be examined. The fit of the model will be assessed by the logistic regression diagnostics procedure. Haplotypes will be constructed where applicable, and pathway analysis will be used to construct multilocus genotypic groups, rather than a "one-gene-at-a-time" approach, where possible. Exploratory Aim: The multiple regression approach as described above (plan for primary Aims 1 and 2) will be implemented to assess the relationship between biomarker levels (HOMA index, triglyceridemic) and endothelial dysfunction (rigidness of arteries and positive biomarkers) with metabolic syndrome in cancer treated patients.
<b>Data management:</b> Data management for this study will be supervised by the Department of the study late effects of antitumor therapy, medical record data are contained in the data base of registry information of Dmitry Rogachev National Medical Research Center.
<b>Human Subjects:</b> The risk of harm anticipated in the proposed research is not greater than that encountered during daily activities. All assessments will be performed by the clinical practitioners and researches who has experience in their own scientific technology. Participants will not be required to complete any assessments they wish to omit. Dr. Zhukovskaya will be available to answer any questions participants have regarding their treatment or medical late effects. If a participant experiences undue discomfort during any of the testing, the test will be stopped immediately.

## Appendix 1 Genetic testing

The table summarizes specific polymorphisms that are candidate genes underlying genetic risk factors for metabolic syndrome after childhood cancer.

- i) A candidate gene approach, rather than genome-wide polymorphism detection, will be used,
- ii) Functional polymorphisms will be targeted, and
- iii) Genes will be identified by virtue of the fact that they have been associated with metabolic syndrome outcomes in adults, or with similar "de novo" diseases, or they are likely to affect the pharmacogenetics or pharmacodynamics of the most important drug-related risk factors for the adverse event.

Gene	SNP ID	Protein's function
<i>INSR</i>	rs2059806	The insulin receptor gene is a member of insulin signal transduction pathway which regulates the absorption and release of glucose, synthesis and storage of carbohydrates, lipids and protein. Mutations in the gene are associated with autosome-recessive hereditary syndromes, such as syndrome Donohue (leprechaunism) and Rabson-Mendenhall syndrome. It is possible that the heterozygote carriers of the mutations associated with these syndromes have hereditary predisposition to a metabolic syndrome.
	rs1051691	
	rs52800171	
<i>IRS1</i>	rs2943641	Binding of insulin to its receptor induces phosphorylation of the cytosolic substrates IRS1 and IRS2. The insulin receptor substrate 1 gene ( <i>IRS1</i> ) variants ( rs2972146, rs2943641, and rs2943634) are related to body fat percentage (BF%) and multiple metabolic traits.
<i>PPARG</i>	rs1801282	The protein PPAR-gamma is the regulator of adipocytes differentiation.
<i>KCNJ11</i>	rs5219	The protein KCNJ11 is a part of ATP-dependent K <sup>+</sup> channels of pancreatic beta cells, which play a key role in insulin secretion. As a result of 67A>G nucleotide change, the lysine in the position 23 is replaced with glutamine (Lys23Gln). The change in protein structure interferes with closing of channels and leads to decrease in insulin secretion from beta cells and to violation of blood sugar level control.
<i>TCF7L2</i>	rs7903146	Protein is a member of the TCF family (transcription factors with high mobility group domain). TCF7L2 can influence several biological pathways, including the Wnt signaling pathway. The single nucleotide polymorphism (SNP) within the TCF7L2 gene, rs7903146, is, to date, the most significant genetic marker associated with Type 2 diabetes mellitus (T2DM) risk. SNPs in this gene are especially known to be linked to higher risk to develop type 2 diabetes, gestational diabetes, and multiple other diseases.
<i>FTO</i>	rs9939609	Fat mass and obesity-associated protein also known as alpha-ketoglutarate-dependent dioxygenase FTO is an enzyme that in humans is encoded by the <i>FTO</i> gene. Certain variants of the FTO gene appear to be correlated with obesity in humans.
<i>APOB</i>	rs676210	The main apolipoprotein of chylomicrons and low-density lipoproteins. The polymorphism has protective effect, in the presence of a minor allele A dyslipidemia associated with methotrexate intake less often develops.
<i>APOE</i>	rs429358	The ApoE gene makes a protein which, when combined with fat, becomes a lipoprotein. The lipoprotein ApoE is a very low-density lipoprotein, responsible in part for removing cholesterol from the bloodstream. Variations in ApoE affect cholesterol metabolism.
	rs7412	
<i>ADIPOQ</i>	rs2241766	Adiponectin is a hormone secreted by adipocytes that

	rs266729	regulates energy homeostasis and glucose and lipid metabolism. It is an adipose tissue-specific plasma protein, the polymorphisms are associated with serum adiponectin concentrations.
<i>NR3C1</i>	rs56149945	The glucocorticoid receptor (GR, or GCR) also known as NR3C1 (nuclear receptor subfamily 3, group C, member 1) is the receptor to which cortisol and other glucocorticoids bind. The variant 1088A>G is associated with increase of sensitivity to glucocorticosteroids.
<i>CYP2D6</i>	rs3892097	Protein CYP2D6 responsible for 1 phase of biotransformation of a large number of exogenous and endogenous compounds. Gene polymorphisms may result in decrease in enzyme activity.
	rs35742686	
<i>CYP2C9</i>	rs1799853	It has been estimated that CYP2C9 is responsible for the metabolic clearance of up to 15-20% of all drugs undergoing phase I metabolism. Genetic variations cause changes in metabolic activity.
	rs1057910	
<i>CYP2C19</i>	rs4244285	The protein, a member of the cytochrome P450 mixed-function oxidase system, is involved in the metabolism of xenobiotics, including many proton pump inhibitors and antiepileptic. Genetic variations cause changes in metabolic activity.
<i>NAT2</i>	rs1801280	The gene encodes an enzyme N-acetyltransferase 2 that functions to both activate and deactivate arylamine and hydrazine drugs and carcinogens. Polymorphisms in this gene are responsible for the N-acetylation polymorphism in which human populations segregate into rapid, intermediate, and slow acetylator phenotypes. Allele T (341 T>C) is associated with increase insulin resistance.
	rs1799930	
	rs1799931	
<i>MTHFR</i>	rs1801133	The protein encoded by this gene catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a co-substrate for homocysteine remethylation to methionine. The polymorphism 677 C>T is known, substituting an alanine (A) for a valine (V), where the T(V) allele results in a thermolabile enzyme with reduced activity. The carriers of genotype T/T have higher plasma homocysteine level.

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