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РОССИЙСКОЕ КАРДИОЛОГИЧЕСКОЕ ОБЩЕСТВО

EPIDEMIOLOGY OF CARDIOVASCULAR DISEASES

Integration of biobanking technologies into protocols of large-scale prospective epidemiological studies

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Aim. To demonstrate successful experience in the development and implementation of biobanking methods for epidemiological research in the Russian Federation (RF), as well as the application of biobanking technology and the participation of a biobank created on the basis of the national medical research center in conducting a large-scale epidemiological study. This aims to improve the efficiency and quality of its implementation, as well as to ensure the possibility of conducting prospective studies.

Material and methods. The study included interviews with participants, instrumental measurements, biomaterial collection, laboratory diagnostics, and database creation. The protocols of the second and third stages of the study included biobanking regulations for collecting biological material from the regions of the RF and delivering it to the central biobank.

Results. The establishment of the biobank in 2014 for the storage of biospecimens collected within the Epidemiology of Cardiovascular Diseases and their Risk Factors in Regions of the Russian Federation (ESSE-RF) study enabled the planning and implementation of subsequent large-scale epidemiological projects. In accordance with the developed and validated biobanking algorithm, samples of whole blood, serum, and plasma from a representative sample of different Russian regions were collected in a centralized biobank. Information on biological samples, blood donors, and laboratory tests of all participants was combined into a single database. In total, the biobank currently stores samples and data from 79,516 participants from 41 regions of Russia.

Conclusion. The inclusion of biobanking technology in the study protocol made it possible to conduct a large-scale epidemiological study in compliance with high quality standards. The resulting biomaterial collection is stored and actively used in prospective studies to analyze the dynamics of the prevalence of risk factors and non-communicable diseases, as well as to study the frequency of gene variants involved in various pathological processes.

Keywords: biobank, biobanking, epidemiological study, prospective study, random sample, non-communicable diseases, risk factors.

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Introduction

A biobank is a collection of biological materials and related information that is securely stored in an organized system under appropriate conditions for current or future use in population and clinical research [1, 2].

Biobanks are currently becoming platforms for a wide range of research projects worldwide, including large-scale population-based studies of the prevalence of various diseases, drug resistance genes, etc. The use of biomaterials collected, processed and stored in accordance with uniform rules and standards guarantees the reliability and reproducibility of the results obtained[1] [3]. The role of a biobank as a structure regulating the collection of biomaterials in accordance with approved standard operating procedures (SOPs) in the organization of population-based studies is extremely important.

Since 2012, the National Medical Research Center for Therapy and Preventive Medicine (NMRC TPM) has been conducting a large-scale epidemiological study ESSE-RF to examine the prevalence of cardiovascular diseases (CVD), biological and behavioral risk factors for CVD, and their associations with various economic, climatic, and geographical characteristics. This study aims to profile each Russian region in terms of CVD prevalence and risk. Three stages of this study were conducted in 41 regions of Russian Federation (RF) to date. The study included three data collection phases: participant surveys using a specially developed questionnaire, instrumental measurements, and laboratory diagnostics. The biosamples for laboratory tests were collected in each region and delivered to the NMRC TPM biobank. The first stage implementation made it possible to formulate the need to develop a more modern, regulated approach to organizing procedures for collecting, processing, and transporting biosamples from various regions of the country, including geographically remote ones, to a centralized structure to create a unique collection of high-quality biomaterials for further responsible storage and laboratory research.

The creation of a biobank in accordance with the requirements of international standards, which is a center for the systematic collection and responsible storage of collections of high-quality biological samples and relevant clinical and other information, ensures the high-quality conduct of scientific work related to the use of biosamples, both within the framework of large-scale epidemiological projects and other research projects.

The aim of this work is to present the experience of developing and implementing biobanking methods for epidemiological studies, as well as the application of biobanking technology and the participation of a biobank created on the basis of a national medical research center in conducting a large-scale epidemiological study in order to improve the efficiency and quality of its implementation, as well as to ensure the possibility of conducting prospective studies.

Material and methods

The study included men and women aged 25-64 years during the first two stages of the ESSE-RF and 35-74 years at the subsequent stage, in a systematic stratified multi-stage random sample, which was formed on a territorial basis based on health care facilities using the Kish method [4–7]. In the structure of sample formation, the primary elective units were outpatient clinics and other health care facilities located on the territory of the study, the secondary ones were medical sites, and the tertiary ones were households or individual household members.

All stages of the population-based study ESSE-RF were conducted in accordance with the ethical principles of the Declaration of Helsinki and the Russian National Standard for Good Clinical Practice (GCP) (GOST R 52379-2005) and received approval from the Ethics Committee of NMRC TPM for conducting the study in the regions of the RF. Each participant examined during the ESSE-RF study and its subsequent stages signed an Informed Consent (IC) to participate in the study and to store their biosamples for research purposes only for an unlimited period.

All participants were examined, including questionnaires and instrumental measurements, as well as collecting and processing venous blood for laboratory tests.

The survey was conducted using a specially designed modular questionnaire based on validated methods for epidemiological studies. The questionnaire contained over 200 different questions on lifestyle, socioeconomic conditions, diseases, treatments, and various risk factors.

Instrumental measurements included parameters of height, weight, waist and hip circumference, blood pressure, heart rate and, in some regions, hand muscle strength assessment, electrocardiogram recording and analysis.

As part of the first stage of the ESSE-RF project, the biobank that meets international standards was created in 2014 to preserve and structure the collected biomaterial.

Preparations for the subsequent stages included expanding the biobank's infrastructure: new facilities and additional equipment were designed and commissioned in accordance with international biobanking best practices. Safe storage of collected biosamples under required conditions was ensured by modern equipment, including low-temperature freezers (-80 °C), an automated continuous monitoring system, uninterruptible

[1] Problems with scientific research: How science goes wrong. The Economist; 2013. Available from: https://www.economist.com/leaders/2013/10/21/how-science-goes-wrong.

power supply, and software that enables the systematic and secure storage of large volumes of biosamples-related data. Biobank staff developed a methodology for collecting, preparing, and transporting biosamples from the regions of the RF to the NMIC TPM biobank (the central biobank). Two collection and sample preparation sites and temporary storage facilities were established in each region.

The biological samples quality and related data stored in the NMIC TPM biobank was confirmed by the international certificate of conformity ISO 9001: 2015[2].

Results

Integration of biobanking into the research protocol

Based on of ESSE-RF (2012-2014) experience and literature data, an improved protocol for epidemiological research was developed, including a new section — planning and organizing biobanking of blood samples and its derivatives.

Equipping regional biomaterial collection points

A standard set of equipment and supplies was used to collect biosamples in all regions participating in the study. Each biosamples collection, processing, and temporary storage site included a medium-speed laboratory centrifuge with refrigeration, a freezer with a storage temperature of -25 °C, a computer with pre-installed software and internet access, a barcode scanner, variable-volume pipettes with disposable tips, vacuum blood collection systems, racks, and cryovials with 2D barcodes.

Software. Development of a single digital platform

To conduct a large-scale study, the "Instrumental Digital Platform for High-tech Biomedical Research" (the Unified digital platform) was developed and used, combining information about biosamples and donors into a database.

The platform is designed to automate the processes of conducting high-tech biomedical research, including:

- ensuring the organization and planning of biomedical research;
- access control based on a role-based management model.
- use for the development of information and registration cards;
- providing input of primary data using survey methods and information and registration cards;
- conducting preliminary control of research data;
- performing validation and quality control of data and biosamples;
- statistical processing of research data;
- use of tools for analyzing research data;
- ensuring integration (data exchange) with related medical information systems, laboratory systems, and biosample storage systems.

SOP development and personnel training

SOPs were developed by the employees of the biobank for the regulated collection of biosamples in the regions, transportation to the biobank in compliance with the cold chain, and receipt of biomaterial to the biobank in accordance with the research protocol based on international biobanking standards[3], ethical standards, and analysis of literature data [8]. These documents describe in detail the instructions for the blood collection procedure, sample preparation stages, entering information into the study database, preparing biosamples for transportation to the central biobank, as well as the conditions of transportation and rules for accepting biosamples at the biobank. Based on the developed SOPs, staff were trained in each region using video recordings and presentations.

This article presents a biobanking protocol developed, validated in three regions, and applied in the multicenter ESSE-RF studies. A general outline of the biobanking stages included in the study protocol is shown in Figure 1.

Main stages of biobanking

Blood collection procedure

The procedure of taking peripheral (venous) blood was carried out in accordance with the current recommendations and standards[4] in vacutainer tubes marked with special labels [9]. Each label contained encoded information about the participant's identification number (ID).

Tubes with a blood coagulation activator were used to produce serum; standard tubes with a dipotassium salt of ethylenediaminetetraacetic acid (EDTA) were used to collect whole blood and produce plasma with EDTA; tubes containing sodium citrate (3.2%) were used to produce plasma with sodium citrate.

Sample preparation of blood serum and plasma samples

The sample preparation procedure included centrifugation of blood and aliquoting of serum and plasma. Tubes containing sodium citrate for hemostatic studies were centrifuged no later than 30 minutes after blood collection, and tubes for obtaining serum and plasma

[2] ISO/IEC 9001:2015. Quality Management Systems Requirements; ISO Published: Geneva, Switzerland, 2015. Available from: https://www.iso.org/obp/ui/#iso:std:iso:9001:ed-5:v1:en.

[3] ISO 20387:2018. Biotechnology–Biobanking–General Requirements for Biobanking. Available from: https://www.iso.org/standard/67888.html.

[4] GOST R 59778-2021 Procedures for taking samples of venous and capillary blood for laboratory tests. Available from: https://protect.gost.ru/document.aspx?control=7&id=241615 (In Russ.)

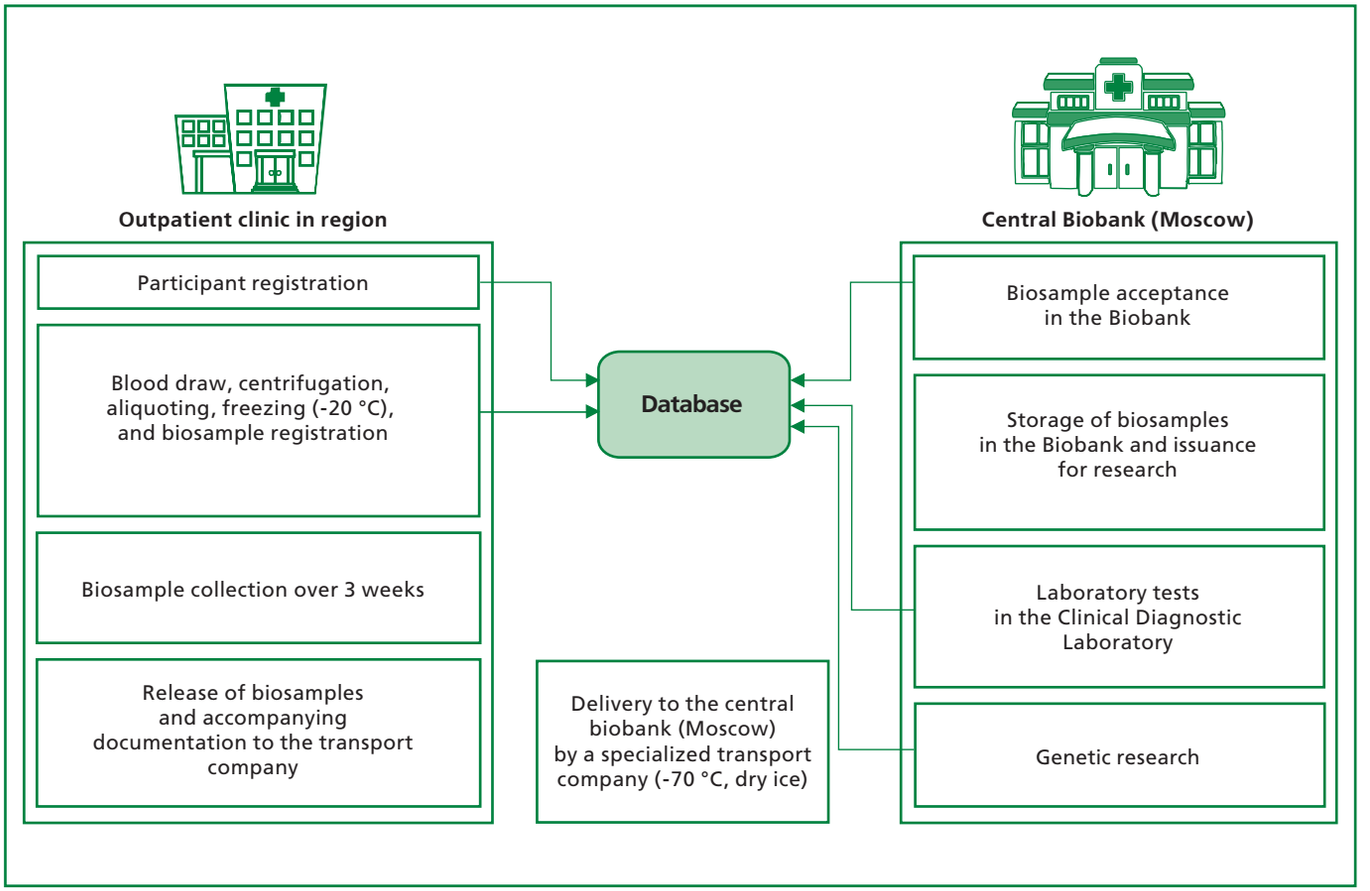

Figure 1. Biobanking stages included in the study protocol.

with EDTA were centrifuged no later than 1 hour. The standard centrifugation mode was used [10, 11].

Serum and plasma biological samples were aliquoted using an automatic pipette with disposable tips into cryovials labeled with factory-issued linear barcodes on the side and 2D barcodes on the bottom. Each participant received from 4 to 6 serum aliquots of 0.5-1.0 ml, at least two plasma aliquots with EDTA of 0.5-1.0 ml, and at least two plasma aliquots with sodium citrate of 0.5-1.0 ml. Each participant provided four to six aliquots of 0.5-1.0 ml serum, at least two aliquots of 0.5-1.0 ml EDTA plasma, and at least two aliquots of 0.5-1.0 ml sodium citrate plasma. During sample preparation, the start time of centrifugation and freezing, as well as the number and volume of aliquots obtained, were recorded in a special paper form "Information on biosamples".

For genetic studies, test tubes containing whole blood with EDTA and the cellular sediment obtained after centrifugation of such tubes and collection of plasma not below the level of the leukocyte layer were preserved.

Full information about each biosample was entered into the project database by scanning the barcodes on the labels of vacuum tubes and cryovials through a special input mask into the Unified digital platform, linking it to the ID of the corresponding study participant.

Freezing

After registration in the database, all samples were placed in a freezer at -25 °C in special racks. It is known that plasma and serum samples can be stored at this temperature for no more than 3 weeks, since it has been shown that storage under these conditions during this period does not significantly affect the quality of this biomaterial [12-14]. For long-term storage at a temperature of -72 °C, in accordance with the accepted standard, the obtained biomaterial was transported to the central biobank [13].

Preparing for transportation

To form a tranche at a regional biosamples collection point, a list of biosamples ready for shipment was generated in Excel spreadsheets using the Unified digital platform. In accordance with this list, a package of documents was prepared: IC (two for each participant), forms "Information on biosamples", a list of identification numbers and a certificate of transfer of biosamples with information on the number of tubes and racks.

Transportation of biomaterial to the central biobank

From regional collection points, samples were delivered by a transport company to the central biobank every 3 weeks in containers with dry ice at a temperature of -70 °C. Temperature recorders were used for continuously monitoring of the temperature inside the transport container at all stages of transportation. The biobank manager coordinated the date of transportation of the finished tranche with the transport company and coordinated the delivery of the biosamples. As part of the risk management procedure, the contract was concluded with two transport companies.

During transportation, biological samples were accompanied by a prepared package of documents.

Reception of biomaterial received from the regions into the central biobank

The central biobank received and registered incoming biospecimens and accompanying documents. Upon receipt of biospecimens, the central biobank assessed the integrity of the shipping container, the volume of dry ice, the presence and functionality of the temperature sensor, and the date and time of receipt on the shipping document. When placing biosamples in freezers, information about the sample number (ID), its type and location in storage was recorded in the information system of the central biobank [11].

Transfer of biomaterial to the clinical and diagnostic laboratory for analysis

To conduct the laboratory tests planned for the project, biomaterial obtained from the regions was transferred from the biobank to the clinical diagnostic laboratory of the at predetermined intervals. An example of laboratory tests performed during one of the three project phases is presented in Table 1.

Table 1. Types of laboratory tests.

№	Types of laboratory tests	Types of the analyzed biomaterial
1.	Total cholesterol, mmol/L	Blood serum
2.	High-density lipoprotein cholesterol, mmol/L	Blood serum
3.	Triglycerides, mmol/L	Blood serum
4.	Glucose, mmol/L	Blood plasma with EDTA
5.	Creatinine, mmol/L	Blood serum
6.	Uric acid, mg/Dl	Blood serum
7.	C-reactive protein of high sensitivity, mg/L	Blood serum
8.	Aspartate aminotransferase, U/L	Blood serum
9.	Alanine aminotransferase, U/L	Blood serum
10.	Gamma-glutamyltransferase, U/L	Blood serum
11.	Alkaline phosphatase, U/L	Blood serum
12.	Total bilirubin, mmol/L	Blood serum
13.	Fibrinogen, g/L	Blood plasma with sodium citrate

The results of the tests were automatically entered into the database on the Unified digital platform and were available both to researchers for statistical processing, and to doctors of the outpatient clinics in the participating regions for transmitting the results of laboratory tests in the form format to the examined participants. Due to the availability of several aliquots of each type of biomaterial for each participant, if additional, newly scheduled laboratory tests were needed, aliquots of the biomaterial with the required ID were issued from the biobank.

Quality assurance and quality control

ISO 9001:2015 quality management system (QMS) implemented at the central biobank. The accuracy of following the developed instructions was a fundamental factor affecting the quality of biological samples, the availability, completeness and reliability of the data associated with them. Timely identification of nonconformities and informing the employees of regional health care facilities made it possible to quickly develop corrective measures, monitor the implementation of each stage of the biobanking process, and reduce the likelihood of repeated errors [1].

The developed protocol was validated in three geographically remote regions. We analyzed the compliance of biosamples and associated data from the pilot regions with the requirements of the developed biobanking algorithm, the epidemiological study protocol, and the quality indicators developed within the biobank's QMS. We assessed the number of discrepancies related to biosamples labeling, failure to adhere to time intervals between sample collection and preparation, insufficient sample volume, the presence of hemolysis and chyle, and the completeness of database fields. The analysis results were within acceptable limits, with the proportion of biosamples with these discrepancies being no more than 1%, confirming the compliance of the developed biobanking algorithm with the study protocol's requirements for the quality of collected biosamples and data.

Creation of an extensive collection of biological samples from a representative sample of the population of the Russian regions

Using a validated, unified algorithm for collecting, preparing, transporting, and storing biosamples from various regions of Russia, a biobank was created to collect whole blood, serum, and plasma samples from a representative sample of the population. Information on the biosamples and donors was compiled into the single database for all participating regions on the Unified digital platform for high-tech biomedical research. This database currently

contains information from questionnaires, instrumental examination results, and laboratory tests for all participants in the three stages of the project. The biobank database contains the information on the biosamples linked to participant IDs and all other information collected on the Unified digital platform. In total, the biobank currently stores biosamples from 79,516 participants from all stages of the ESSE-RF study from 41 regions of Russia. The resulting collection of biomaterial provided the opportunity to conduct high-quality scientific research and became a resource for planning and conducting various new studies, including prospective ones.

Discussion

Conducting large-scale epidemiological studies, studying the prevalence of various diseases and risk factors for their development, markers of infectious and non-communicable diseases, drug resistance genes, etc., is an urgent area of medical sciences. Biobanking technology and population-based biobanks should be used to collect biomaterial for modern epidemiological projects. Only the use of biomaterial collected, processed, and stored in accordance with uniform rules and standards guarantees the reliability, reproducibility, and high quality of the results obtained in scientific research [1, 10].

The most well-known and striking example of the organization and implementation of large-scale collection and long-term storage of biosamples and associated data is the UK Biobank, which collected blood, urine, saliva, etc. biosamples from 500,000 Britons aged 40 to 69 years between 2006 and 2010. As a result of the survey of donors and the study of biosamples, a unique large-scale biomedical database of genetic and medical information was created, which is regularly updated with new data and is an inexhaustible resource for scientific research available to the world scientific community. This is confirmed by a large number of scientific publications using data from the UK biobank, which are presented in the PubMed database [15, 16].

No less interesting is the Qatar BioBank (QBB). QBB organizers are conducting a large prospective population-based cohort study that began in 2012. It is attended by more than 60,000 men and women over the age of 18. Follow-up visits with study participants are held every 5 years. This project collects and stores extensive data (clinical, metabolic, and phenotypic) and biosamples (blood, urine, saliva, DNA, RNA, and viable cells). The collected biomaterial and associated data are used in a wide range of studies to assess the relationship between various factors (diet, lifestyle, environment, genetics, etc.) and the development of diseases. Upon request from researchers, QBB provides biomaterials and associated anonymized information for various biomedical projects and developments [17, 18].

The material collected within the framework of the ESSE-RF studies, based on the study of a representative sample of the population from 41 regions of Russia, represents a valuable array of information, which served as the basis for a large number of scientific studies and publications. Based on the results of this large-scale project, the Russian healthcare system and the scientific medical community set priorities for preventive measures, develop a demographic and high-risk strategy [19]. The collection and storage of biomaterials in a biobank in accordance with strict standards within the framework of epidemiological studies allows, when new scientific questions arise, to use existing biosamples to solve new scientific problems [20]. Prospective observation of a cohort formed from participants in the ESSE-RF studies (2012-2018) allows us to study the contribution of risk factors and levels of certain biochemical markers to survival in Russia [21]. As part of this large-scale project, a large number of papers have been published, and research is ongoing and will continue in the future [22, 23]. The results of the one-step (cross-sectional) ESSE-RF study were used to develop indicators of the Russian National project "Demography", to model the risk of developing diseases at the population level, to assess the economic damage caused by risk factors, to predict the economic effect of population preventive measures, and to assess the possibility of adding new biomarkers to risk scales [24].

The biosamples and database collected under the project made it possible to conduct genetic studies related to the study of the prevalence of gene variants involved in various pathological processes [25-29]. For example, data obtained within the ESSE-RF studies were used to calculate the prevalence of heterozygous familial hypercholesterolemia and the carrier frequency of autosomal recessive diseases [26, 28, 29]. In the future, we will study pharmacogenetics in the epidemiological aspect on the basis of the collection. It is possible to conduct research by forming samples from the collection in accordance with the criteria, including within the framework of consortia. An important aspect of biosample storage in the biobank is the ability to expand the database with the results of new studies of the collected biomaterial, as well as the ability to conduct prospective studies, replenish the database as a result of tracking the health status of project participants and endpoints. Traditionally, the results of a survey of representative samples of Russian regions, including the results of questionnaires, instrumental and laboratory examinations, are of great interest to the scientific community and health care organizers. Experience shows that biosamples collected from a representative sample of the population of Russian regions are in demand by various specialists for conducting a wide range of studies.

Summing up the results of world practice and authors' experience, it can be argued that population biobanks are an almost inexhaustible source for biomedical research. A large volume of carefully annotated ac-

cumulated biosamples can be used to conduct research for decades. It should be noted that the more aliquots of serum and blood plasma that can be collected and stored from each donor/study participant at the biomaterial collection stage, the more promising the collection becomes in terms of its use for scientific and medical purposes.

Conclusion

Due to the application of the biobanking regulations for the collection, sample preparation, transportation and storage of blood and its derivatives, as well as the use of the Unified digital platform for combining databases of all stages of the study, a unique collection of biomaterial from a representative sample of the Russian population (41 regions) was created. The biobank is a necessary central link in the organization of large-scale epidemiological studies related to the collection of biomaterial.

The integrated approach to organizing biobanking within the framework of the ESSE-RF studies included the development of methodological recommendations (instructions for implementing each stage and training materials for personnel) and requirements for the technical means necessary for collecting, preparing samples, transporting biomaterials to the biobank, and creating a database.

Strict compliance with the rules of biobanking and the use of specialized software guarantees high quality of biomaterials and structured information support for the collection. The use of biomaterial collected during the three stages of the study and an extensive updated database allowed us to conduct a wide range of studies, including prospective ones. Long-term storage of biomaterials and obtaining new data on research participants will allow us to continue to conduct fundamental and applied biomedical research, study pharmacogenetics in the epidemiological aspect using the created collection of biosamples.

Relationships and Activities. State assignment "Circulating MicroRNAs in Plasma as Diagnostic and Prognostic Biomarkers of CAD".

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