
DNA and radiobiological material bank of persons exposed to ionising radiation

M.B. Freidin*, R.M. Takhauov, A.B. Karpov,
E.O. Vasilyeva, I.A. Goncharova,
P.V. Ivanina, E.V. Skobelskaya,
E.V. Nemykina, I.A. Ten, G.B. Nekrasov and
A.S. Izosimov

The Seversk Biophysical Research Centre,
Federal Department of the Medical Biological and Emergency Problems,
Russian Ministry of Public Health and Social Development, Seversk
E-mail: fmb@sbrc.ru E-mail: tm@sbrc.ru
E-mail: kab@sbrc.ru E-mail: veo@sbrc.ru
E-mail: irina.goncharova@medgenetics.ru
E-mail: ivaninap@mail.ru
*Corresponding author

Abstract: The structure of the DNA and radiobiological material bank of the Seversk Biophysical Research Center is described. Workers of the Siberian Group of Chemical Enterprises (SGCE) and their relatives are the subjects of the storage. Cytogenetic analysis, including G-banding, is being performed for the participants. Currently, the bank contains 700 DNA and blood samples of workers of SGCE, 123 DNA and blood samples of cancer cases, about 500 DNA samples of children from Seversk city, and 100 blood samples of SGCE workers who survived acute myocardial infarction. DNA and blood accumulation is still in progress, but by this time, it is ready for large scale scientific projects devoted to the study of the genetic basis of individual radiosensitivity and consequences of low dose irradiation effect on the human genome.

Keywords: DNA bank; Siberian group of chemical enterprises workers; low dose irradiation; genetic basis of individual radiosensitivity.

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Biographical notes: M.B. Freidin was a student of the Biology and Soil Faculty of Tomsk State University during 1992–1998. In 1998–2001, he was a post-graduate student at Medical Genetics Research Institute, Tomsk, Russia, gaining there a PhD in Genetics. Since 2001 to 2003, he was a research assistant at Medical Genetics Research Institute and a senior research assistant at the Seversk Biophysical Research Centre, Seversk. Since 2003 to present time, he is a senior research assistant at Medical Genetics Research Institute and the head of Genomic Medicine Laboratory of the Seversk

Biophysical Research Centre. His research accents are genetics of complex diseases and traits and genetics of radiosensitivity.

R.M. Takhauov graduated from Tomsk State Medical Institute in 1987. After graduation, he was sent to work in the Cancer Research Institute of the Tomsk Scientific Centre of the Siberian Branch of RAMS, where he worked from the Junior Research Assistant to the head doctor of clinics. He was distinguished by the Institute administration and the Siberian Branch of RAMS for great achievements and active participation in public life. In 2000, he was invited to head the Seversk Biophysical Research Centre. He is a Doctor of Medical Science.

A.B. Karpov graduated from Treating Faculty of Tomsk State Medical Institute in 1982. From 1982 to 2000 he worked first as a junior and later a senior research assistant in the Cancer Research Institute of the Tomsk Scientific Centre of the Siberian Branch of RAMS. Since 2000 he has been a Deputy Director for Research of the Seversk Biophysical Research Center, Doctor of Medical Science. His papers are published in a great number of advanced journals. His research activity is oriented towards radiation medicine, pathological physiology, oncology.

E.O. Vasilyeva is the head of Division of Screening and Prophylaxis of Main Disorders at SBRC. She is a candidate of Biological Sciences. She graduated from Tomsk State University in 1987 with specialisation in cytology and genetics. After graduation till 2004 she worked as a Laboratory Doctor in Cytogenetics at the Medical Genetics Research Institute (Tomsk). In 2004 she was invited to be the head of Medical Genetics Consultation in Seversk, which was reformed to the Division of Screening and Prophylaxis of Main Disorders.

I.A. Goncharova is a research assistant at SBRC. She is a candidate of Biological Science. She graduated from Tomsk State University in 1990 with specialisation cytology and genetics. Since that she has been working as a researcher at Cytogenetics Laboratory at the Medical Genetics Research Institute (Tomsk) and later as a researcher at Population Genetics Laboratory. In 2001 she was invited to the SBRC as a junior researcher in Genomic Medicine Laboratory. Since 2003 she is a researcher at same laboratory.

P.V. Ivanina is a junior research assistant at SBRC. She graduated from Tomsk State University in 2002 with specialisation cytology and genetics. After graduation she has been working at Genomic Medicine Laboratory at SBRC. Starting since 2004, she is a junior research assistant.

E.V. Skobelskaya is a Laboratory Doctor at SBRC. She graduated from Siberian Medical University in 1996 as a Biophysical Doctor. In 1997–2001, she worked in the commercial institute 'Poisk' (Tomsk) as a Physical Doctor. In 2002, she was the head of marketing division of Siberian pharmaceutical company (Tomsk). In 2003, she was invited to SBRC as Laboratory Medical Attendant in Genomic Medicine Laboratory and since 2004, she is a Laboratory Doctor in the Division of Screening and Prophylaxis of Main Disorders at SBRC.

E.V. Nemykina was a student of Treating Faculty of Siberian State Medical University from 1996 to 2002. During 2002–2003, she completed her pediatrics internship in Siberian State Medical University. In 2003, she received a certificate of Siberian State Medical University proving continuing education in medical genetics. From 2003 to 2004, she worked as a Medical

Geneticist in the Seversk Biophysical Research Centre. Since 2004 to present day, she has been a Medical Geneticist in Seversk Central Medical Department No. 81.

I.A. Ten was graduated from Siberian Medical University in 2002 as a Biophysical Doctor. Since that time she is a junior researcher at the Genomic Medicine Laboratory and a postgraduate student at Medical Genetics Chair of Siberian Medical University. She is process of preparation of PhD thesis in genetics devoted to biotransformation enzymes genes polymorphisms in common diseases.

G.B. Nekrasov graduated from Treating Faculty of Tomsk State Medical Institute in 1973. From 1973 to 1975, he completed his medical residency in Tomsk State Medical Institute. Since 1975 to present day, he is a head of pathologoanatomic department of Seversk Central Medical Department No. 81. Since 2002, he has worked as a Medical Statistician in the Seversk Biophysical Research Centre. He has several publications.

A.S. Izosimov was graduated from Treating Faculty of Tomsk State Medical Institute in 1981. During 1981–1983 he worked as a Medical Pathologist in the pathological morphology laboratory of Tomsk Cardiologic Centre. Since 1983 to present day, he is a Medical Pathologist in Seversk Central Medical Department No. 81. Since 2004, he has worked as a Medical Statistician in the Seversk Biophysical Research Centre.

1 Introduction

The necessity of collecting and preserving genetic material appeared to be growing with development of molecular genetic technologies. The method of preserving DNA in a bank which represents a system of storage of nucleic acids samples, seems to be the most optimal one (ASHG, 1988).

Interest in human DNA banks is shown not only by scientists but also by politicians, the authorities and ordinary citizens. Recently to conduct research in various world laboratories, DNA banks were created and are being created, the donors of which are different contingents of patients, social and ethnic groups and healthy persons. The aim of these investigations seems to be the search for genes of predisposition to widespread diseases, genes responsible for narcotic dependence development, alcoholism, ‘genes of longevity’, estimates of the frequency of definite genetic variants in various populations.

In addition to being an instrument to conduct research, a DNA bank can represent a commercial institution as well. For example, in Ekaterinburg at the Ural Medical Academy the unique institution, i.e., the bank for human DNA storage is being opened where anyone can give his/her own DNA for certain payment.

In the USA, the question of DNA bank creation is being discussed, the aim of which is complete information on suspects in terrorism.

The formation of a bank for storage of genetic information on ethnic minorities of the North as well as the population conducting underground nuclear explosions is being planned in the Yakutiya Republic, to evaluate the effect of unfavourable climatic and ecological conditions on human health and genetic fund preservation (Maksimov, 2003).

In the South-Ural Institute of Biophysics a DNA bank has been created, of people exposed to radiation to estimate the risk of distant radiation consequences. At present this DNA bank contains the genetic material of 1,000 former and present workers of the nuclear enterprise 'Mayak'. Some of these workers received chronic radiation at maximum permissible doses during the first years of functioning. The work on enlisting the families of PA 'Mayak' to create the bank of genetic material has been realised. The electronic database of the DNA bank, including individual medico-demographic, occupational and dose characteristics as well as qualitative and quantitative DNA indices is created (Rusinova et al., 2001, 2002).

In spite of the aims concerning the DNA bank creation, there should be special norms of collection, material processing and information storage, as these processes are accompanied by certain ethical and technical problems.

2 Ethical problems of DNA bank creation

Over the last decade the questions of biomedical ethics have been the centre of attention and discussion on the fora of such International Organizations as the United Nations Organization, UNESCO, Council of Europe, and World Health Organization which adopt legal documents directed to juridical regulation of medico biological investigations and public health practice. The most important questions of bioethics seem to be an informed consent, property ownership of biological material and confidentiality (Benda, 1997).

In 2002 in our country, according to the International and Russian legislative acts, an Expert Council on medicine HEB of the Russian Ministry of Education adopted a resolution, on the basis of which all research work with man as an object of investigation should be accompanied by the written informed consent of persons participating in research (Expert Council on Medicine HEB Ministry of Education of Russia, 2002). The informed consent should be obtained on the basis of complete information, namely:

- what information should be given by a person or what information will be obtained as a result of studying the donor's biomaterial
- for what purpose this information will be used
- what information will be recorded
- how and during what period of time this information will be kept
- whether this information can be given to other people for other purposes (Benda, 1997).

There are standard documents concerning the availability of informed consent, but the problem of property rights to biological material has not been solved, yet. Some problems about possessing DNA or biological material as well as the necessary activities of bank holders in case of demands to withdraw DNA from the bank by the donor or his relatives remain unsolved at present.

Confidentiality seems to be an essential condition of conducting medico biological research. So, different ways of encoding information on DNA and a donor and its restricted access are developed to preserve confidentiality. According to recommendations of the American Committee on Ethics, information encoding should be

done by disinterested and specially selected people. And even in that case, the researchers cannot answer for a donor's absolute confidentiality (PPPC, 2003).

3 Technical problems of DNA bank creation

The creation of a DNA bank seems to be a labour-intensive and responsible procedure. DNA, forming the basis of the bank and liable to storage for long duration, should correspond to definite characteristics, namely:

- on spectrophotometric analysis the optical absorption ratio at the wavelength 260/280 nm should be in the range of 1.8–2.0 that testifies to a high DNA sample cleaning of proteins
- on electrophoregrams obtained in DNA samples distillation in 0,8% agarose gel the samples should be in the form of one narrow band at 1–1.5 cm distance from the start that is indicative of a high molecular structure of the sample and the absence of degraded DNA.

At present different methods to obtain DNA samples are used in medicine. And each procedure has its own advantages and shortcomings.

Standard method are: using proteinase K and phenol for protein hydrolysis and precipitation (Maniatis et al., 1984). The given method allows us to obtain DNA samples of the greatest quality which meets the above requirements. However, the given method appears to be the most expensive and the least ecologic one.

Method using Na perchlorate and chloroform for protein hydrolysis and precipitation. This method is not so expensive but there is no possibility to obtain the greatest quality of the DNA sample, due to residual salts.

Express method of DNA isolation. It allows us to isolate a great amount of DNA samples for a short period of time. However, this method is not suitable for a long DNA storage in the bank, so DNA samples are used in examinations within the next two to three years.

The methods of DNA storage in the bank can be varied as well. The most optimal ways are the following: storage at +5°C in TE-buffer; storage at –20°C in ethanol; storage at –70°C in deionising water.

In addition to DNA and biological material samples for DNA bank functioning of full value, it is necessary to create the electronic database containing demographic, occupational (occupational register, the length of service), dosimetric (doses of external radiation or Plutonium-239 content in an organism) and medical data. The numbers or sample codes should be inserted in the electronic database to make a search for database of members of one family (sample code, family code). Information in DNA bank can be duplicated on electronic and paper media.

Among the significant technical issues of DNA bank creation one should mention the material and technical basis formation. The main problem seems to be high costs of equipment and reagents.

Taking into account the above problems the Genomic Medicine Laboratory of the Seversk Biophysical Research Centre (SBRC) is engaged in creating the DNA bank of the Siberian Group of Chemical Enterprises (SGCE) workers to be the biggest one among the atomic industry enterprises in the world exposed to long term occupational

radiation – external, internal (by incorporated ^{239}Pu) and combined. The purpose of DNA bank creation is to study the effect of ionising radiation on hereditary structures of the exposed, their descendants and to determine genetic grounds of individual human radiosensitivity as well as genetic aspects of main disease development under conditions of long term effects of radiation in the range of low doses (Goncharova et al., 2003, Takhauov et al., 2004).

To create optimal long term conditions for DNA storage and use, the Seversk Biophysical Research Centre has developed the following approach: for each subject of DNA and biological material bank, there are three units of storage including DNA samples isolated by means of standard method using proteinase K (the main sample), DNA sample isolated by means of express method (the sample under work) and blood sample (the reserve sample). Besides, within the frameworks of the main research direction of the SBRC Genomic Medicine Laboratory, i.e., hereditary grounds of individual radiosensitivity, for each subject, a cytogenetic analysis including estimation of frequency and spectrum of chromosome aberrations has been performed.

At present, the SBRC DNA and biological material bank contains about 700 DNA and blood samples of the main SGCE production personnel, including 318 DNA samples of reactor production (RP) workers, 119 – radiochemical production (RCP) and 261 – plutonium production (PP). Besides, a collection has been made, of 123 DNA and blood samples of SGCE workers suffering from cancer, about 500 DNA samples of children – SGCE workers' offspring and Seversk residents, and 100 DNA samples of SGCE workers who suffered from an acute myocardial infarction.

The samples obtained from SGCE males (78%) make up the main part of the bank. The average length of service at SGCE amounts to 27.7 years at the moment of research. The age boundaries of DNA bank donors lie between 37–68 years of age, with the average value being 53.1 years. The radiation dose of bank donors is between 0.1 – 1631.071 mSv, that totals 205.4 mSv on average. Distribution of SGCE workers – the subjects of storage – by incorporated plutonium content and the length of service at the enterprise are presented in Tables 1–3.

Table 1 Distribution of SGCE personnel: the subjects of DNA bank storage – by doses of external γ -radiation (mSv)

<i>Production</i>	<i>0–99</i>	<i>100–199</i>	<i>200–299</i>	<i>300–399</i>	<i>400–499</i>	<i>500–699</i>	<i>Higher than 700</i>
Reactor	91	78	34	37	24	28	26
Radiochemical	75	22	12	5	4	1	0
Plutonium	168	38	25	13	5	3	9

Table 2 Distribution of SGCE personnel: the subjects of DNA bank storage – by incorporated plutonium content (nCi)

<i>Production</i>	<i>0.4–4.9</i>	<i>0.5–2.9</i>	<i>3.0–9.9</i>	<i>10.0–19.9</i>	<i>Higher than 20</i>
Radiochemical	49	40	13	5	12
Plutonium	79	69	44	31	38

Table 3 Distribution of SGCE personnel: the subjects of DNA bank storage – by the length of service at the enterprise (years)

<i>Production</i>	<i>Lower than 10</i>	<i>10–19</i>	<i>20–29</i>	<i>30–39</i>	<i>40 and higher</i>
Reactor	78	65	97	36	42
Radiochemical	59	16	26	12	6
Plutonium	50	68	64	53	26

The structure of oncologic pathology in 100 of 123 SGCE workers suffering from cancer with DNA stored in SBRC is presented in Table 4.

Table 4 The structure of oncologic diseases in SGCE personnel: the subjects of DNA bank storage

<i>Disease</i>	<i>Males</i>	<i>Females</i>	<i>In all</i>
Carcinoma of the stomach	11	5	16
Carcinoma of the prostate	14	–	14
Breast cancer	–	12	12
Carcinoma of the intestine	9	2	11
Lung cancer	8	1	9
Renal carcinoma	5	4	9
Thyroid gland cancer	–	3	3
Carcinoma of the skin	2	2	4
Other	16	6	22
In all	63	34	100

Information on 23 other patients is being specified. In the above group, 48 persons were exposed to external radiation (36 males and 12 females) in the range of 3.6–1555.71 mSv. 22 individuals had doses of internal radiation, 17 males and 5 females were among them. Pu content was between 0.2–45 nCi. Combined radiation (both external and internal) was recorded in 19 persons: 15 males and four females. The duration of a disease in the groups of patients was between two to six years (3.25 years on average). The age at the beginning of the disease was between 32 and 60 years.

For all SGCE workers whose DNA and blood are stored in DNA and biological material banks, including patients with oncologic and cardiovascular pathology, cytogenetic preparations have been prepared and cytogenetic analyses have been performed, including karyotyping and estimation of chromosome aberrations frequency among these persons. Data on cytogenetic anomalies are supposed to be used for further prognosis purposes to reveal groups at risk, primarily, in oncologic pathology, as well as in research performed in the Genomic Medicine Laboratory of the Seversk Biophysical Research Centre dedicated to the search for hereditary determinants of individual radiosensitivity.

At present, an electronic database for DNA bank accompaniment is being formed which has the following fields: the serial number corresponding to the family code with the designation of all its members as 1f: father, 1d¹: the first child, 1d²: the second child, 1m: mother; the number of basic DNA which is given on blood sampling, the number of

DNA under work which is given on DNA isolation; surname, name, patronymic; the date of birth; the length of service; dose, accumulated radiation dose in the course of work; sex; age; plant; workshop. In further work on creating the DNA bank, the electronic database will be broadened, including demographic data about donors (nationality, birthplace) and information concerning DNA samples (the ratio of optical absorption, DNA concentration). Application of demographic data is necessary for conducting molecular genetic investigations due to different gene frequencies among representatives of various races, nationalities and populations. Non-observance of unification of the compared groups by demographic parameters can lead to receiving false associations of genotypes under study with a disease or erroneous increase of mutation frequencies of definite genome parts (Risch, 2000).

The electronic database is filled by entering the subsequent samples, the sample is given a serial number, the family code, the number of basic DNA; other fields of database are completed as well. Integration of the electronic database with the regional medico-dosimetric register of the Seversk Biophysical Research Centre is in being planned.

Along with the DNA and biological material bank, the Seversk Biophysical Research Centre is creating the radiobiological bank of tissues, the components of which are the samples of autopsy materials of persons (former SGCE workers) exposed to ionising radiation during their lifetime as well as the samples of the malignant neoplasm of SGCE workers and Seversk residents. At present, the radiobiological bank of tissues includes autopsy material of 63 former SGCE workers and 362 samples of malignant neoplasm.

Thus, DNA and biological material bank as well as the radiobiological bank of tissues of the Seversk Biophysical Research Centre seem to be a unique boundless material storage for research aimed at revealing the molecular grounds of individual radiosensitivity, study of the development of the genetic aspects of the main diseases (primarily, oncologic ones), in the conditions of long term radiation effect in the range of low doses as well as other investigations on radiation and medical genetics. The available methods allow us to preserve DNA samples for long periods of time (for decades) as well as genomic amplifications that determine the immensity of the DNA bank being the source of scientific information. As for the workers of harmful production, in particular, the personnel of nuclear chemical industry, it should be noted that creation of a DNA and biological material bank as well as the radiobiological bank of tissues seems to be a foreground task. This is connected with a necessity to store DNA and biological material of persons involved in nuclear chemical production for many years (20–40 years) the amount of which is decreasing. Biological material of these individuals seems to be a significant source of scientific information about the medico biological effect of low and high doses of external and internal radiation and other technogenic factors as well. Moreover, DNA and biological material accumulation of relatives (children and spouses) of nuclear chemical production workers is of great importance for base formation to reveal the long term effects of technogenic factors.

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