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# Search for polymorphic variants of candidate genes contributing to individual radiosensitivity

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## ABSTRACT

**Background.** Cytogenetic damage (CD) in lymphocytes induced by low doses (up to 0.1 Sv) of ionizing radiation (IR) is the main cytogenetic sign of individual radiosensitivity of the human body. In addition to DNA repair and cell death, which affect the formation of CD and its elimination, IR effects on the cell can be manifested through changes in proliferation of cells with unrepaired DNA damage. The system of cyclins and cyclin-dependent kinases (CDK), which provide coordination of mitotic events during passage of a cell through the cell cycle, plays a crucial role in regulation of cell proliferation.

**Aim** To evaluate the relationship of single-nucleotide polymorphisms (SNPs) of cell cycle genes with an increased frequency of CD in workers of a nuclear power plant affected by chronic occupational radiation exposure in the dose range of 100–500 mSv.

**Materials and methods.** The object of the study was blood of 55 conditionally healthy workers of Siberian Chemical Plant (SCP) who were affected by chronic occupational radiation exposure (gamma radiation) in the dose range of 100–500 mSv. A standard cytogenetic analysis of blood lymphocytes was performed for all examined individuals. Genomic DNA was isolated from the blood of the workers using the QIAamp DNA Blood Mini Kit (QIAGEN, Germany). DNA was genotyped using 257 SNPs of cyclin genes and neighboring intergenic regions using DNA microarrays from the high-density CytoScan HD Array (Affymetrix, USA).

**Results.** Taking into account the Bonferroni correction, only statistically significant associations of SNPs with the frequency of dicentric chromosomes were found; all other types of chromosomal aberrations did not show statistical significance. The rs803054 *CCNI2* was associated with an increased frequency of dicentric chromosomes arising under the influence of chronic occupational radiation exposure.

**Conclusion.** The discovered SNP (rs803054), whose recessive genotype is associated with an increased frequency of dicentric chromosomes in workers of SCP exposed to radiation at doses of 100–500 mSv over a long time, can be considered as a potential marker of individual radiosensitivity. To confirm the identified associations, further validation studies are needed on an expanded sample of people affected by chronic occupational radiation exposure.

**Keywords:** ionizing radiation, individual radiosensitivity, chromosomal aberrations, gene polymorphism, microarray analysis

**Conflict of interest.** The authors declare the absence of obvious or potential conflicts of interest related to the publication of this article.

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# Поиск полиморфных вариантов кандидатных генов индивидуальной радиочувствительности

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## РЕЗЮМЕ

**Актуальность.** Цитогенетические нарушения (ЦН) лимфоцитов, индуцированные «малыми» дозами (до 100 мЗв) ионизирующего излучения (ИИ), являются основными цитогенетическими признаками индивидуальной радиочувствительности организма человека. Помимо репарации ДНК и гибели клеток, которые влияют на формирование ЦН и их элиминацию, вклад в последствия воздействия ИИ на клетку может реализоваться за счет изменений пролиферации клеток с нерепарированными дефектами ДНК. Определяющую роль в регуляции пролиферации клеток играет система циклинов и циклин-зависимых киназ, которые обеспечивают координацию митотических событий при прохождении клеточного цикла.

**Цель.** Оценить связь однонуклеотидных полиморфизмов (ОНП) генов клеточного цикла с повышенной частотой ЦН, возникших у персонала объекта использования атомной энергии, под действием долговременного техногенного профессионального облучения ИИ в диапазоне доз 100–500 мЗв.

**Материалы и методы.** Объектом исследования служила кровь 55 условно здоровых работников Сибирского химического комбината (СХК), подвергавшихся в процессе профессиональной деятельности долговременному техногенному радиационному воздействию (γ-излучение) в дозах 100–500 мЗв. Для всех обследованных лиц проводили стандартный цитогенетический анализ лимфоцитов крови. Геномную ДНК из крови работников выделяли с помощью набора QIAamp DNA Blood mini Kit (Qiagen, Германия). Генотипировали ДНК по 257 ОНП генов циклинов и межгенных областей вблизи генов циклинов с помощью ДНК-чипов высокой плотности CytoScan HD Array (Affymetrix, США).

**Результаты.** Установлено, что с учетом поправки Бонферрони имеются только статистически значимые связи ОНП с высокой частотой дицентрических хромосом, все остальные типы изученных ЦН не показали достоверных отличий. С повышенной частотой дицентрических хромосом, возникающих под действием долговременного техногенного профессионального облучения ИИ, ассоциирован rs803054 *CCNI2*.

**Заключение.** Обнаруженный ОНП (rs803054), рецессивный генотип которого ассоциирован с повышенной частотой дицентрических хромосом у работников СХК, подвергавшихся в процессе профессиональной деятельности долговременному техногенному радиационному воздействию (γ-излучение) в дозах 100–500 мЗв, можно рассматривать в качестве потенциального маркера индивидуальной радиочувствительности. Для подтверждения выявленных ассоциаций необходимы дальнейшие валидационные исследования на расширенной выборке людей, подвергавшихся долговременному техногенному профессиональному облучению ИИ.

**Ключевые слова:** ионизирующее излучение, индивидуальная радиочувствительность, хромосомные аберрации, полиморфизм генов, микроматричный анализ

**Конфликт интересов.** Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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## INTRODUCTION

Cytogenetic signs of individual radiosensitivity (IRS) of the human body include frequency of induced cytogenetic damage (CD) and formation of radiogenic disease, among which tumor diseases are the most prevalent [1–3]. Lymphocytes are cells in the human body that are the most sensitive to the effect of ionizing radiation (IR). Blood lymphocytes with induced CD are eliminated from the body through various types of cell death, such as apoptosis, necrosis, necroptosis, autophagic cell death, mitotic catastrophe, and accelerated aging of irradiated cells [4–6]. However, when these mechanisms of death are impaired or their activity decreases due to normal genomic variation, CD-affected lymphocytes can accumulate, which is manifested through an increase in the frequency of CD even at low doses of IR.

Another mechanism in the frequency of CD is proliferation of lymphocytes, since it is well known that chromosomal aberrations are eliminated during proliferation [1, 2]. A system of cyclins plays a crucial role in the regulation of proliferation. They function as regulators of cyclin-dependent kinases (CDKs) and contribute to temporal coordination of each mitotic event. Therefore, the formation and elimination of CD are directly affected by the variability in the mechanisms of DNA repair and cell death and indirectly affected by the variability in the mechanisms of proliferation of cells with unrepaired CD.

A genome-wide study of the association of 162 single-nucleotide polymorphisms (SNPs) in cyclin genes (*CCNA1, CCNA2, CCNB1, CCNB2, CCNB3, CCND1, CCND2, CCND3, CCNE1, CCNE2, CCNF, CCNG1, CCNH, CCNI, CCNI2, CCNJ, CCNJL, CCNK, CCNY*) and 95 neighboring intergenic SNPs with high frequency of CD was carried out in workers of Siberian Chemical Plant (SCP, 55 people) who experienced occupational radiation exposure in the dose range of 100–500 mSv. In a preliminary study on the dose – effect relationship, a plateau was observed, i.e. the frequency of CD did not change with the increasing dose [7, 8], and it is this dose range that should be used for studying the association of SNPs with increased frequency of CD to assess IRS.

The aim of the study was to evaluate the relationship of SNPs in cyclin genes and their promoters with the increased frequency of CD in workers of a nuclear power plant who experienced chronic occupational radiation exposure in the dose range of 100–500 mSv.

## MATERIALS AND METHODS

The study used whole venous blood obtained from 38 conditionally healthy SCP workers who were not exposed to IR in their professional activities (the control group) and 55 conditionally healthy SCP workers who experienced chronic occupational radiation exposure (gamma radiation) in the dose range of 100–500 mSv (the experimental group). The characteristics of the studied groups are presented in Table 1.

Table 1

**Characteristics of the studied groups of SCP workers**

| Parameter | | Control group, $n = 38$ | Experimental group, $n = 55$ |
|---|---|---|---|
| Men / Women | | 38/0 | 55/0 |
| Age, years | *Me* | 52.00 | 59.00 |
| | *L–R* | 37.00–58.00 | 54.00–69.00 |
| Work experience, years | *Me* | 20.00 | 34.00 |
| | *L–R* | 12.00–34.00 | 29.00–42.00 |
| External radiation dose, mSv | *Me* | – | 203.35 |
| | *L–R* | – | 164.00–276.15 |

Information about donors of the biological material was collected and clarified using the database in the medical and dosimetric register of SCP staff and the Archives of Seversk Biophysical Research Center, containing medical information about all SCP workers [9]. In accordance with the Federal Law No. 323-FZ of 21.11.2011 "On the Basics of Public Health Protection in the Russian Federation", each donor signed an informed consent to participate in the study.

Blood was taken from donors from the ulnar vein. It was collected in the volume of 9 ml in Vacuette K3 EDTA tubes (Greiner Bio-one, Austria) using a Vacuette Visio Plus needle 38 × 0.8 mm, 21G × 11/2 (Greiner Bio-one, Austria) for subsequent DNA isolation and microarray. To prepare cytogenetic suspensions, blood was collected in the volume of 9 ml in Vacuette LH Lithium Heparin tubes (Greiner Bio-one, Austria) using a Vacuette Visio Plus needle 38 × 0.8 mm, 21G × 11/2 (Greiner Bio-one, Austria).

The individuals included in the study underwent a cytogenetic analysis of mononuclear leukocytes. For lymphocyte culture, whole venous blood was used. It was mixed with a nutrient medium (15% fetal bovine serum and 85% RPMI 1640 medium supplemented with glutamine, phytohemagglutinin, and penicillin) and incubated (at 37 °C) in culture tubes (Corning, USA) in the dry air shaker – incubator (Biosan, Latvia). The cytogenetic analysis was performed using the Leica DM2500 microscope (Leica, Germany). At least 300 metaphase plates were analyzed for each individual. The results were presented as the frequency of CD per 100 metaphase plates. The following types of CD were determined: aberrant cells, polyploid cells, multi-aberrant cells (more than 5 chromosomal aberrations), chromosomal and chromatid fragments, ring and dicentric chromosomes, chromatid exchanges (crossing over), and translocations.

DNA was isolated from mononuclear leukocytes using the QIAamp DNA Blood Mini Kit (Qiagen, Germany). The purity (A260 / A280 = 1.80–2.00, A260 / A230 = 1.90–2.15) and concentration (50–150 ng / ml) of DNA were determined on the NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). DNA integrity was determined using capillary electrophoresis – DNA fragments were larger than 48 kb.

The studied SNPs were genotyped using DNA microarrays from the high-density CytoScan™ HD Array (Affymetrix, USA). Sample preparation, hybridization, and scanning were carried out in accordance with the manufacturer's protocol. The microarray results were processed using Chromosome Analysis Suite 4.3 software (Affymetrix, USA). To clarify and identify SNPs and genes to which they belong, the Affymetrix genotype database, NCBI, OMIM, GWAS Catalog, and SNPedia databases were used.

The following SNPs of cyclin genes (*CCN*) and neighboring intergenic regions were studied ($n = 257$):

– *CCNA1 (cyclin A1)*: rs7997378, rs3814803, rs17188012;

– *CCNA2* (*cyclin A2*): rs3217753, rs3217772, rs3217771;

– *CCNB1* (*cyclin B1*): rs1128761;

– *CCNB2* (*cyclin B2*): rs28383518, rs28383514, rs75767699, rs169410487, rs16941042, rs16941046, rs28383551;

– *CCNB3* (*cyclin B3*): rs12848359, rs12009873, rs7063886, rs6614336, rs12007902, rs17003332;

– *CCND1* (*cyclin D1*): rs3212869, rs649392;

– *CCND2* (*cyclin D2*): rs3217805, rs4765775, rs3217916, rs3217812, rs3217848, rs3217830, rs11063072, rs3217882, rs3217898, rs3217933;

– *CCND3* (*cyclin D3*): rs9369318;

– *CCNE1 (cyclin E1)*: rs3218071, rs3218035, rs3218036, rs41520849, rs3218038, rs3218068, rs3218042, rs3218064, rs3218066, rs3218044;

– *CCNE2* (*cyclin E2*): rs2467670, rs16893774, rs2278891;

– *CCNF* (*cyclin F*): rs8052046;

– *CCNG1* (*cyclin G1*): rs299322, rs2069345, rs2069347;

– *CCNH* (*cyclin H*): rs6879293, rs3752862, rs74582239, rs16902635, rs16902632, rs75949864, rs6891010, rs10067098, rs115516306, rs114916935, rs3827607, rs16902623, rs16902625, rs11745338, rs16902631, rs77996308, rs1062035;

– *CCNI* (*cyclin I*): rs803054, rs803057, rs10006033, rs62302339;

– *CCNJ* (*cyclin J*): rs4921132, rs57334361, rs17057562, rs6875660, rs4921270, rs74734346, rs17057596, rs78444213, rs17111275, rs2303059, rs915506, rs6874570, rs6556488, rs11949221, rs17057577, rs6899125, rs754112, rs2421777, rs2421778, rs17057631, rs10052876, rs2421779, rs2421780, rs10038395, rs28595384, rs72814336, rs12657051, rs17057641, rs9313842, rs11596126;

– *CCNK* (*cyclin K*): rs10144895, rs3918051, rs3918094, rs2069492, rs2069493, rs3918139, rs3918048;

– *CCNY* (*cyclin Y*): rs12261552, rs113182825, rs115589270, rs11816866, rs111374708, rs2295417, rs115469285, rs2504352, rs2504350, rs2474533, rs4934749, rs35745247, rs17593103, rs75954134,

rs16936030, rs3003980, rs16936032, rs3013364 rs114206731, rs12241755, rs16936035, rs74979754, rs10508817, rs10827506, rs11010151, rs4934753, rs112496700, rs11591533, rs4934754, rs1345561, rs12248732, rs10508818, rs116338411, rs75609581, rs11010178, rs74866156, rs2086153, rs12242002, rs10827509, rs112818779, rs11595699, rs11010213, rs16936102, rs3003981, rs7067539, rs61449529, rs10827512, rs4934551, rs11010225, rs7910421, rs17500653, rs116009947, rs12249814, rs112091952;

– neighboring intergenic regions: rs6817626, rs2138940, rs9566153, rs13153588, rs6509615, rs9547604, rs4865924, rs73537845, rs79959089, rs79226566, rs17053967, rs12508668, rs4241604, rs413127, rs11097684, rs115693938, rs9315437, rs2323125, rs12902628, rs5961171, rs59776629, rs6887755, rs6826342, rs4557282, rs375299, rs4502705, rs6818356, rs7682171, rs13133761, rs4518274, rs6849124, rs6849534, rs6871154, rs323746, rs112520532, rs9603050, rs114501411, rs323758, rs323757, rs1441709, rs2919902, rs984026, rs34383364, rs17285919, rs9547632, rs73770251, rs35556022, rs1517886, rs35000040, rs6892636, rs11749408, rs11749439, rs3909481, rs7250135, rs4805497, rs10422957, rs17002403, rs35204615, rs16963260, rs4998568, rs11882235, rs56400371, rs28582702, rs11881322, rs255259, rs1811302, rs255263, rs77475690, rs11084309, rs410468, rs17053969, rs10514840, rs17053994, rs76165140, rs74045329, rs17054069, rs12866109, rs1517893, rs9315426, rs7489996, rs7692898, rs9594152, rs9603064, rs1474085, rs6822060, rs17054113, rs9603072, rs4943389, rs7317651, rs17191516, rs17054217, rs9547595, rs660005, rs16963219, rs594452.

The genotyping data for each studied SNP were analyzed according to four genetic models: dominant, recessive, additive, and over-dominant. According to the dominant model, the frequency of CD was compared in homozygous recessive and heterozygous individuals and in homozygous dominant individuals. According to the recessive model, the frequency of CD was compared in homozygous dominant and heterozygous individuals and in homozygous recessive individuals. The additive model was used to compare the frequency of CD in homozygous dominant, homozygous recessive, and heterozygous individuals. The over-dominant model was used to compare the frequency of CD in homozygous dominant and homozygous recessive individuals, on the one hand, and heterozygous carriers, on the other hand.

Statistical processing of the results was carried out using Statistica 8.0 software (StatSoft, USA). The results were presented as the median and the interquartile range *Me (L–R)*.

The genotype distribution was tested for deviations from the Hardy – Weinberg equilibrium using the Court lab HW calculator program in Excel. The Mann – Whitney test with the Bonferroni correction ($p < 0.05$) was used to determine the significance of differences in the frequency of CD.

## RESULTS

At the first stage, a cytogenetic study of the frequency of CD in the SCP workers of both groups was carried out to confirm the increased frequency of CD in the workers of the experimental group. The results are presented in Table 2.

Table 2

**Comparison of the frequency of CD in SCP workers of the control and experimental groups, per 100 cells *Me* (*L–R*)**

| Parameter | Control group, *n* = 38 | Experimental group, *n* = 55 | *p* |
|---|---|---|---|
| Number of aberrant cells | 1.0000 (0.3333–2.3333) | 2.5641 (1.4285–3.3333) | **0.0002** |
| Chromatid fragments | 0.3316 (0.0000–0.6666) | 0.6269 (0.0000–1.3071) | 0.1294 |
| Chromosomal fragments | 0.3268 (0.0000–0.6557) | 0.3225 (0.0000–0.9118) | 0.5140 |
| Ring chromosomes | 0.0000 (0.0000–0.0000) | 0.2724 (0.0000–0.3333) | **0.0050** |
| Dicentric chromosomes | 0.3322 (0.0000–0.9493) | 0.7712 (0.0000–1.2578) | **0.0280** |
| Multi-aberrant cells | 0.0000 (0.0000–0.0000) | 0.0000 (0.0000–0.0000) | 0.8819 |
| Chromatid exchanges | 0.0000 (0.0000–0.0000) | 0.0000 (0.0000–0.0000) | 0.2687 |
| Translocations | 0.0000 (0.0000–0.0000) | 0.0000 (0.0000–0.0000) | 0.8819 |
| Polyploid cells | 0.0000 (0.0000–0.0000) | 0.0000 (0.0000–0.0000) | 0.8820 |

Note: here and in Table 3, statistically significant differences are highlighted in bold.

As can be seen from Table 2, the frequency of most types of CD, which are not markers of radiation exposure, do not differ in both groups of SCP workers. It may be due to the fact that all the employees of SCP included in the study live and work in approximately identical conditions, i.e. the samples were thoroughly stratified, and the control and experimental groups do not differ significantly, except for markers of radiation exposure – ring and dicentric chromosomes.

It is well known that lifestyle (smoking, alcohol consumption, and other bad habits), environmental factors (to a lesser extent), and industrial exposure to harmful chemical and toxic substances can drastically increase the frequency of CD in humans. The absence of differences in the frequency of chromatid and chromosomal fragments, multi-aberrant cells, chromatid exchanges, translocations, and polyploid cells indicates the absence of the above differences in the workers of the studied groups. However, the frequency of radiation exposure markers (ring and dicentric chromosomes) and the frequency of aberrant cells (due to ring and dicentric chromosomes) are significantly higher in the workers who have been experiencing chronic occupational radiation exposure.

At the second stage, we assessed the association of the frequency of radiation-induced CD with the polymorphic variants of the studied genes in the experimental group. When analyzing the data, we excluded SNPs of any genotype with $n < 5$, since in conditions of a small sample, this increased the probability of type I errors. Further, we excluded SNPs that deviated from the Hardy – Weinberg equilibrium. Therefore, out of 257 SNPs, 58 SNPs were included in the final statistical analysis. Their association with high frequency of the identified CD was revealed.

Figure shows the significance levels for 58 selected SNPs (see above) by dicentric chromosomes.

The negative logarithm of the confidence level is depicted on the ordinate axis. The line above shows the confidence level with the Bonferroni correction.

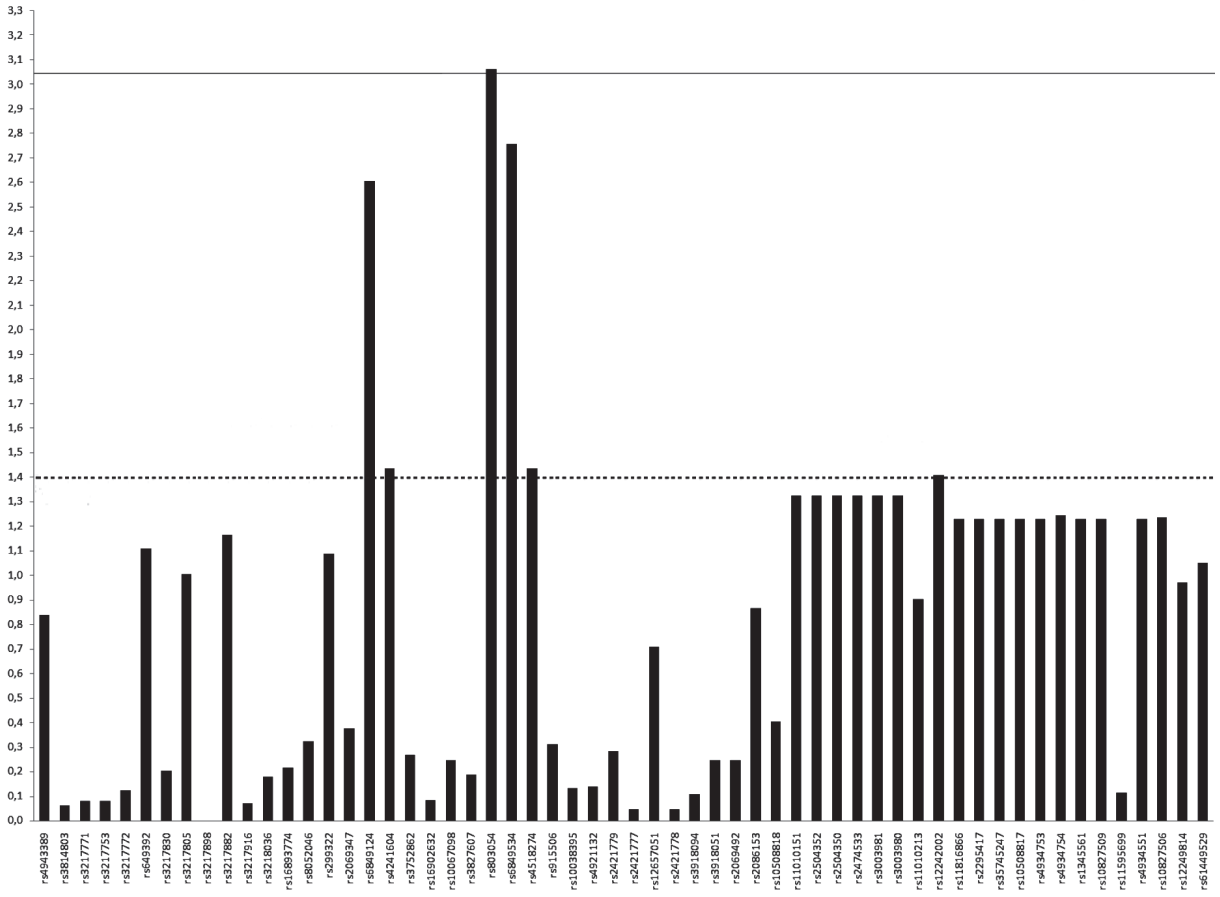


Figure. Significance levels for the recessive model based on the frequency of dicentric chromosomes. The ordinate axis is the $p$ value on the logarithmic scale – (log10); the dotted line marks the significance level of $p < 0.05$ calculated according to the Mann – Whitney test; the red line denotes the Bonferroni correction.

It equals to –log(0.05/57) = 3.0644. Therefore, Figure shows that, taking into account the Bonferroni correction, only 1 SNP is associated with high frequency of dicentric chromosomes in the SCP workers of the experimental group.

Of the 58 identified SNPs, the genotyping data analysis carried out for all four genetic models (dominant, recessive, additive, and over-dominant) showed an association with high frequency of dicentric chromosomes (markers of radiation exposure) for the recessive model for 1 SNP: *CCNI2* (*rs803054*) (Table 3).

Table 3

**The frequency of dicentric chromosomes depending on the genotypes of the studied genes in the experimental group of SCP workers, per 100 cells, *M (L–R)***

| Parameter | Frequency of dicentric chromosomes by the genotype | | | *p* | Bonfer-roni correction |
|---|---|---|---|---|---|
| *Dominant model* | | | | | |
| *CCNI2* rs803054 | *A/G + G/G*, *n* = 39 | *A/A*, *n* = 16 | | 0.0865 | 0.000874 |
| | 0.9740 (0.0000–1.6666) | 0.3928 (0.0000–1.0317) | | | |
| *Recessive model* | | | | | |
| *CCNI2* rs803054 | *A/A + A/G*, *n* = 42 | *G/G*, *n* = 13 | | **0.0008** | 0.000874 |
| | 0.5303 (0.0000–1.0899) | 1.4285 (1.0256–1.8750) | | | |
| *Over-dominant model* | | | | | |
| *CCNI2* rs803054 | *A/A + G/G*, *n* = 28 | *A/G*, *n* = 27 | | 0.1116 | 0.000874 |
| | 0.9890 (0.3928–1.5476) | 0.6060 (0.0000–1.0899) | | | |
| *Additive model* | | | | | |
| *CCNI2* rs803054 | *A/A*, *n* = 16 | *A/G*, *n* = 26 | *G/G*, *n* = 13 | 0.8645 | 0.000874 |
| | 0.3928 (0.0000–1.0317) | 0.6298 (0.0000–1.0899) | 1.4285 (1.0256–1.8750) | | |

## DISCUSSION

The polymorphic variant rs803054 is intronic, located at chr5:132750285 (GRCh38.p13), and belongs to the *CCNI2* gene. In 2008, S. Choudhry et al. suggested that 5q23.3 (the authors indicated that rs803054 is located at position chr5:132162193) is a potential region containing asthma genes in Puerto Ricans [10]. There is no other information about the contribution of this SNP to the regulation of the functional activity of *CCNI2*, including information in the SNPedia database.

In contrast to the results described by S. Choudhry et al., our work showed the association of rs803054 with radiation-induced CD. Thus, it was revealed that for rs803054 *CCNI2,* the frequency of dicentric chromosomes was 2 times higher in carriers of the recessive genotype than in carriers of the dominant genotype at relatively identical doses of IR. One of the reasons for the higher frequency of ring and dicentric chromosomes in workers of the experimental group was chronic occupational exposure to IR [11].

*CCNI2* is located at the long arm of chromosome 5 (5q31.1), it is considered a homolog of *CCNI*. *CCNI2* interacts with CDK5 and activates it. C. Liu et al. and J. Taneera et al. showed that the depletion of CCNI2 by siRNA inhibits passage of a cell through the cell cycle and cell proliferation [12, 13]. D.M.Lai et al. demonstrated a reduced level of CCNI2 expression, which, in turn, inhibited proliferation of colorectal cancer cells, stopped the cell cycle in the G2 phase, and stimulated apoptosis [14]. There are findings that a decrease in *CCNI2* expression slows down progression of gastric cancer by inhibiting proliferation of tumor cells, increasing susceptibility to apoptosis, and suppressing cell migration [15].

It is also known that CD is eliminated during proliferation, therefore, according to our results, carriers of the recessive rs803054 *CCNI2* allele may have a decrease in *CCNI2* expression, following which the proliferative potential of lymphocytes and the intensity of CD elimination (which includes dicentric chromosomes) decrease. The results obtained are in good agreement with the data obtained by [12, 13]. With chronic occupational exposure to IR, this leads to an increase in the frequency of dicentric chromosomes.

## CONCLUSION

For the first time, the rs803054 SNP was identified, which can be considered a potential marker of IRS. It was shown that the SCP workers who are homozygous for the recessive rs803054 allele had increased frequency of dicentric chromosomes in blood lymphocytes during chronic occupational exposure to IR in the dose range of 100–500 mSv. The identified

candidate marker of IRS can be used to develop a test system for detection of genetically determined IRS using a real-time PCR system.

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Isubakova D.S. – analysis and interpretation of the data, drafting of the article. Litviakov N.V. – conception and design. Tsymbal O.S., Usova T.V., Tsyplenkova M. Yu. – collection and processing of the material, carrying out of studies. Milto I.V. – critical revision of the manuscript for important intellectual content, editing of the article. Takhauov R.M. – critical revision of the manuscript for important intellectual content, editing of the article, final approval of the manuscript for publication.

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