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Absence of mutations in the human interferon alpha-2b gene in workers chronically exposed to ionising radiation

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Individuals chronically exposed to low-level ionising radiation (IR) run the risk of harmful and long-term adverse health effects, including gene mutations and cancer development. The search for reliable biomarkers of IR exposure in human population is still of great interest, as they may have a great implementation potential for the surveillance of occupationally exposed individuals. In this context, and considering previous literature, this study aimed to identify mutations in the human interferon alpha-2b (hIFNα-2b) as a potential biomarker of occupational chronic low-dose IR exposure linking low-IR exposure to the effects on haematopoiesis and reduced immunity. The analysis was performed in the genomic DNA of 51 uranium miners and 38 controls from Kazakhstan, and in 21 medical radiology workers and 21 controls from Italy. hIFNα-2b gene mutations were analysed with the real-time polymerase chain reaction (PCR) or Sanger sequencing. However, none of the investigated workers had the hIFNα-2b mutation. This finding highlights the need for further research to identify biomarkers for early detection of health effects associated with chronic low-dose IR exposure.

KEY WORDS: hIFNα-2b mutations; genotoxicity; radiology workers; uranium miners

In addition to cellular DNA damage and damage response, exposure to ionising radiation (IR) triggers non-targeted effects mainly related to the immune system (1–4). Two recent studies reported an association between mutations in the human interferon alpha-2b (hIFNα-2b) gene and chronic exposure to low-level of IR in medical personnel (5) and residents from Pakistan (6). The IFNα-2b gene encodes a protein belonging to the class of cytokines with immunomodulatory, antiviral, anti-proliferative, and anti-tumour activities (7–10). Mutations, typically the frameshift ones or single nucleotide changes in the human IFNα-2b gene could therefore compromise the functioning of the immune system. In addition, these mutations have been detected in brain tumour patients exposed to different environmental stressors, including high levels of naturally occurring IR (10), and other cancer patients (11, 12). We considered these findings interesting because of the health consequences associated with chronic exposure to low doses of IR. As mutations in the human IFNα-2b gene might compromise immunity, we wondered if they could also serve as biomarkers for health monitoring of occupational and environmental chronic exposure to low IR doses. The aim of this study was therefore to verify the hypothesis in a group of uranium miners from Kazakhstan and in a group of Italian radiology workers.

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MATERIALS AND METHODS

Study population

Volunteer coal miners, all men (n=51) were recruited from two Kazakh regions, Aksu and Zavodskoy (Northern Kazakhstan). In Aksu the mean effective dose is 4 mSv/year, while in Zavodskoy it is 4.95 mSv/year (13). Figure 1 shows a map of the area with the sources of IR exposure. Matched controls (n=38) were anonymous blood donors recruited at the Almaty Blood Donation Centre. Formal written consent was signed by all participants before inclusion in the study.

Figure 1 IR sources of exposure at locations of the Kazakh study arm

The Italian study arm consisted of 42 hospital workers, 21 of whom were occupationally exposed to IR and 21 were healthy, unexposed controls. These participants represent a subset of our previous study in radiological workers (14), whose DNA samples had been stored for future sub-studies. They had signed informed consent for the use of their samples in sub-studies such as this, according to Helsinki Declaration and its later amendments.

Both the Kazakh and Italian participants were taken 10 mL of blood in heparinised tubes during regular medical examination. Blood samples were stored at -80 °C until DNA isolation and subsequent IFNα-2b mutation analysis.

DNA isolation and IFNα-2b mutation analysis

Genomic DNA was isolated from frozen peripheral blood lymphocytes using a standardised phenol-chloroform extraction method. The DNA was purified with 70 % ethanol, air dried overnight at room temperature, and re-suspended in approximately 100 µL of deionised nuclease-free water. Before we analysed the samples for mutations, we selected ten IFNα-2b mutations appearing at the frequency higher than 5 %, as reported by Shahid et al. (5, 6), which, according to these authors, were associated with chronic low-dose IR exposure and its potential to induce point mutations in single genes and modulate the expression of a variety of genes (15–17).

We then analysed our samples for these ten mutations using real-time polymerase chain reaction (PCR) with a customised Applied Biosystems™ Taqman Assay® (Thermo Fisher Scientific, Waltham, MA, USA) as recommended by manufacturer or using Sanger sequencing with an ABI Prism™ 310 Genetic Analyzer (Thermo Fisher Scientific) as described by Shashid et al. (5) (Table 1). Blanks were included in each reaction for quality control.

RESULTS AND DISCUSSION

Surprisingly, none of the analysed samples had any of the ten mutations of this gene. Reasons for our negative findings may be several. One of them is a possible bias in the selection of mutations. Instead of sequencing the entire coding region of the hIFNα-2b gene, we selected ten most frequent mutations identified by Shahid et al. (5, 6). Another

Table 1 Characteristics of the IFNα-2b gene mutation

Mutation (base position)§	Frequency*	Methods
C insertion (8–9)	5.4 %	Real-time PCR
A to T (187)	8 %	Real-time PCR
T to A (219)	8 %	Real-time PCR
A to G (256)	8 %	Real-time PCR
C insertion (330–331)	8 %	Real-time PCR
A deletion (435)	3.3 %	Sanger sequencing
G to A (436)	4.3 %	Sanger sequencing
A to G (437)	3.3 %	Sanger sequencing
A deletion (439)	4.3 %	Sanger sequencing
A deletion (477)	3.3 %	Real-time PCR

* Frequency reported by Shahid et al (5, 6); § gene accession number NM_00605

reason could be the sample size, which is particularly small for the Italian arm. Yet another could be differences in genetic make-up, environmental background, lifestyle, and exposure between our study participants and the populations described by Shahid et al. (5, 6).

With regard to the Kazakh study arm, we did not have information about the effective annual dose received by each occupationally exposed worker participating in the study but only the mean doses for Aksu and Zavodskoy (4 and 4.95 mSv/year, respectively). While not knowing the exact magnitude of occupational exposure in this particular group may seriously limit the interpretation of our findings, the reported annual mean doses did not differ significantly from those reported by Shahid et al. (6).

As for the Italian arm, occupational exposure was routinely monitored by personal devices (film badges). The dose equivalent of IR to the whole body obtained from the personal dosimetry records ranged from 0.90 to 116.74 mSv (mean ± standard deviation = 40.61±37.70 mSv) over the entire working life (which ranged from four to 34 years). Considering the wide range of the years of employment, the idea was also to investigate whether IFNα-2b mutations could identify a subgroup of occupationally exposed individuals more prone to IR-induced DNA damages as an important step towards a timely establishment of effective health surveillance programmes. However, none of the radiological workers showed mutation in the IFNα-2b gene. In addition, a routine occupational health examination showed no clinical or haematological abnormalities in any of the study participants (14). This differs from the report by Shahid et al. (5), in which complete blood count (CBC) revealed that radiation-exposed workers had more abnormal CBC findings than controls. Therefore, the lack of mutation in the Italian study group could also be due to a better health status of these workers. We also cannot exclude the involvement of a different ethnic susceptibility (i.e. different genetic make-up). DNA repair mechanisms may have an important role in eliminating the genetic stress caused by exposure to genotoxic agents, including IR. In this context, the presence of polymorphisms in DNA repair genes, which characterises individual genetic make-up, may contribute to different levels of mutational load and/or genetic damage as highlighted in several studies (18–24). With regard to genetic damage, our earlier studies (14, 19, 20), however limited by the small sample size, found significantly higher micronucleus (MN) frequency in radiological workers chronically exposed to low level of IR than in controls. These and other studies (19, 20, 22, 24) clearly confirm the association between occupational exposure to low-dose IR exposure and genotoxicity. MN formation is considered a reliable biomarker of exposure to clastogenic and aneugenic agents, including IR (26–28). In addition to MN, other cytogenetic tests, including the comet assay (29–32) and chromosome aberration assays (33–36), have confirmed the association between genotoxicity and low-dose radiation exposure.

CONCLUSIONS

Despite their promising potential, none of the available cytogenetic tests has become part of routine biodosimetry surveillance of occupationally and/or environmentally exposed individuals. These tests are traditionally manual and labour-intensive, even with the recently proposed automation protocol for MN and chromosomal aberration analysis (37, 38). Despite our enthusiasm about the promising potential of the IFNα-2b gene as a reliable biomarker of IR-associated immunological risk (5, 6), we did not observe any IFNα-2b gene mutation or changes in blood cell counts.

Unfortunately, we do not have any cytogenetic data (i.e. chromosome aberrations, MN, or the comet assay findings) or data about health consequences associated with low-dose radiation for the Kazakh study arm. In conclusion, our failure to find mutation in the IFNα-2b gene in either arm calls for further research that would identify reliable biomarkers for early detection of health effects associated with low-dose IR.

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Izostanak mutacija humanoga interferon alfa-2b gena u radnika kronično izloženih ionizirajućem zračenju

Kronična izloženost niskim razinama ionizirajućega zračenja povezana je s rizikom od dugoročnih štetnih posljedica za zdravlje, što obuhvaća i mutacije gena te nastanak raka. U tijeku je potraga za pouzdanim biopokazateljima izloženosti ionizirajućem zračenju u ljudi, budući da njihova primjena može značajno unaprijediti praćenje profesionalno izloženih osoba. U tom smislu, a s obzirom na ranija saznanja, cilj je ovoga istraživanja bio utvrditi mutacije gena za proizvodnju humanoga interferona alfa-2b (hIFNα-2b gena) kao mogućega biopokazatelja profesionalne kronične izloženosti niskim dozama ionizirajućega zračenja, koje je usto povezano s djelovanjem na hematopoezu i pad imuniteta. Analiziran je genomski DNA 51 rudara u rudnicima uranija te 38 kontrolnih ispitanika iz Kazahstana, odnosno genomski DNA 21 zdravstvenoga radnika na radiologiji i 21 kontrolnoga ispitanika iz Italije. Mutacije hIFNα-2b gena utvrđivane su metodom lančane reakcije polimerazom u stvarnom vremenu (engl. *real-time PCR*) odnosno sekvenciranjem prema Sangeru, ali se pokazalo da niti jedan radnik nije imao niti jednu od deset traženih mutacija toga gena. Stoga ne preostaje drugo nego i dalje tražiti pouzdane biopokazatelje za rano otkrivanje štetnih zdravstvenih učinaka povezanih s kroničnom izloženosti niskim dozama ionizirajućega zračenja.

KLJUČNE RIJEČI: hIFNα-2b; genotoksičnost; radiologija; rudari, uranij; zdravstveni radnici