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The effect of low-dose exposure on germline microsatellite mutation rates in humans accidentally exposed to caesium-137 in Goiânia

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A serious radiological accident occurred in 1987 in Goiânia, Brazil, which lead to extensive human and environmental contamination as a result of ionising radiation (IR) from caesium-137. Among the exposed were those in direct contact with caesium-137, their relatives, neighbours, liquidators and health personnel involved in the handling of the radioactive material and the clean-up of the radioactive sites. The exposed group consisted of 10 two-generation families, totalling 34 people. For each exposed family, at least one of the progenitors was directly exposed to very low doses of γ-IR. The control group consisted of 215 non-irradiated families, composed of a father, mother and child, all of them from Goiânia, Brazil. Genomic DNA was purified using 100 μl of whole blood. The amplification reactions were prepared according to PowerPlex® 16, following the manufacturer's instructions. Genetic profiles were obtained from a single polymerase chain reaction amplification. The exposed group had only one germline mutation of a paternal origin in the 'locus' D8S1179 and the observed mutation presented a gain of only one repeat unit. In the control group, 11 mutations were observed and the mutational events were distributed in five loci D16S539, D3S1358, FGA, Penta E and D21S11. The mutation rates for the exposed and control groups were 0.006 and 0.002, respectively. There was no statistically significant difference ($P = 0.09$) between the mutation rate of the exposed and control groups. In conclusion, the quantification of mutational events in short tandem repeats can provide a useful system for detecting induced mutations in a relatively small population.

Introduction

A serious radiological accident occurred in 1987 in Goiânia, Brazil, which led to extensive human and environmental contamination and exposure to ionising radiation (IR) of caesium-137. The accident began when two individuals entered the premises of the Institute of Radiotherapy of Goiás and removed from an abandoned teletherapy unit which contained 19.26 g of ^{137}CsCl (50.9-TBq) as the radioactive source. These individuals interested in selling the lead shield as scrap metal, dismantled the apparatus into pieces, releasing the capsule containing the radioactive salt (1,2). As a consequence of handling the radioactive material and the subsequent clean-up of the radioactive sites, several people were exposed to gamma radiation, both from the public and from the aid personnel who assisted with the care and management of the accident in Goiânia. Approximately 250 people received significant exposure to ionising gamma radiation from caesium-137, resulting in four immediate casualties. Individual absorbed doses ranged from 0 to 7 Gy with over a hundred people being identified as having signs of acute radiation syndrome. For a comprehensive study regarding the accident, please refer to previously published material (3).

Human cells have sophisticated DNA repair systems and the mechanisms underlying the loss of gene function in a cell are complex and multifactorial (4). Both genetic predisposition and exposure to physical, chemical and biological agents are the main reasons that result in uncontrolled cell cycle, underlying neoplastic development. Exposure to IR is one of the most important environmental risk factors for cellular stress in higher eukaryotes. Consequently, DNA damage in mammalian cells exposed to IR promotes or delays cell death. Due to the stochastic effects of IR exposure on living systems, induced changes in cellular physiology are important carcinogenic steps in humans. Tissues from the thyroid, lung, breast and bone marrow are especially susceptible to the development of radiation-induced malignancies (5–9). Therefore, understanding the biological effects of IR on somatic cells is very important to properly estimate the genetic risks for the potentially exposed populations (10–14).

The induction of germline mutation in mice exposed to IR remains an important source of experimental data to assess and derive genetic risk for humans. Risk estimations obtained from experimental models are particularly difficult because the extrapolations have not been completely validated; consequently, it remains poorly understood. Several studies have used the mutation ratio in hypervariable tandem repeat DNA 'loci' as a novel approach for monitoring radiation-induced germline mutation in mammalian cells (11–24).

The population exposed to IR of caesium-137 during the radioactive accident in Goiânia (Brazil) and their offspring offered an opportunity to understand the biological effects of radiation exposure on human germ cells. Thus, the main objective underlying the work described here was to estimate the induced germline mutation rates on 15 short tandem repeats (STR) loci of a human population exposed to low absorbed doses of γ-IR in Brazil.

Material and methods

Studied groups

The exposed group consisted of 10 two-generation families (Table I), totalling 34 people. For each exposed family, at least one of the progenitors was directly

Table I. Time elapsed between progenitor's radiation exposure and childbirth in the exposed group from the Brazilian population accidentally exposed to IR in Goiânia in 1987

Family	Exposed progenitor	Data of birth	Time elapsed (years)
Family 1	Mother	September 24, 1988	1
Family 2	Mother	October 10, 1999	12
Family 3	Father	Child 1—March 26, 1989	Child 1—2
		Child 2—June 15, 1991	Child 2—4
Family 4	Father and mother	June 12, 2000	13
Family 5	Father	August 20, 1988	1
Family 6	Father	December 12, 2006	19
Family 7	Father	July 13, 2005	18
Family 8	Mother	February 26, 1993	6
Family 9	Mother	December 29, 1990	3
Family 10	Mother	F1—September 20, 1989	F1—2
		F2—Januaru 17, 1992	F2—5
		F3—June 10, 1993	F3—6

exposed to very low doses of γ-IR from caesium-137. Individual absorbed doses of the exposed population were estimated as ≤0.2 Gy. As reported in (3), the exposed individuals incurred initial acute whole-body external exposures, followed by chronic whole-body exposure at low doses rates from internally deposited ^{137}Cs. The lack of precise information on individual exposure history further obscured the dose estimations for the Goiânia population. However, most of the individuals were exposed over a protracted period at varying dose rates with exposure ranging from acute to chronic. In the exposed group, the average parental age at the time of conception was 22 ± 6 years (Table I), and all subjects were non-smokers. The control group consisted of 215 non-irradiated families, composed of a father, mother and child. The control group was from Goiânia (Brazil), thus presenting the same environmental background of the exposed group. At the time of conception, the parental age in the control group was 24 ± 2.7 years. Exposed and control groups were also matched by age, smoking and drinking habits. For both exposed and control groups, biological paternity of the offspring was confirmed using DNA testing at an established paternity index of 99.99%. All subjects voluntarily donated 5–10 ml of peripheral blood. Samples were collected in disposable vacuum blood collection tubes containing sodium heparin. This study received ethical approval by the Comissão Nacional de Ética em Pesquisa com Seres Humanos (CONEP//MS).

Molecular analyses

Genomic DNA was purified from 100 µl of whole blood using a commercial kit for DNA extraction and isolation (Easy-DNA™ Kit, Invitrogen, Carlsbad, CA, USA). Isolated DNA samples were stored at −20°C. All families had their genotype profiles established using 10 ng of human genomic DNA. The amplification reactions were prepared according to PowerPlex® 16 (Promega Corporation, Madison, WI, USA), following the manufacturer's instructions. Genetic profiles were obtained with a single polymerase chain reaction (PCR) amplification, which increased time efficiency and sample conservation, as well as a reduction on sample handling and consequently potential laboratory errors associated with it. Fluorescent PCR products for the 15 microsatellite polymorphic markers were separated using a MEGABACE 1000 (Amersham Biosciences, Pittsburgh, PA, USA). Amplicons were analysed using the software Fragment Profiler® v.1.2 (AmershamBiosciences). All electropherograms were manually reviewed to ensure the quality of the runs prior to analysing family profiles. The mutations were scored without knowing the individual exposure status. Mutation data were obtained from three independent experiments.

Mutation analyses

The microsatellite mutation in a child was defined by the presence of a PCR product whose size was different from both paternal and maternal alleles. Thus, the mutation included a longer or a shorter repeat unit in the core of each PCR amplified microsatellite. The parental origin of mutations was determined according to (25–27), based on a stepwise mutation model (28). The mutation rates for both exposed and control groups are described in Tables II–IV.

Statistical analyses

To assess whether or not exposure to caesium-137 IR presented any effect on the rate of germline mutation, a binomial test of two proportions was performed using BioEstat 5.0 (29).

Results

In the exposed group, the arithmetic means of the ages of the fathers and mothers at the time of the conception were 22 (SD ± 6) years (Table I). The accidentally exposed group contained five and six exposed fathers and mothers, respectively. For the exposed group, only one germline mutation of a paternal origin in the locus D8S1179 was found (Tables II and III). The observed mutation presented a gain of only one repeat unit (Figure 1). In the control group, 11 mutations were observed and the mutational events were distributed in five loci D16S539, D3S1358, FGA, Penta E and D21S11 (Tables II and IV). The control group had six mutations of maternal origin and five of paternal origin (Table II). A total of 330 alleles were investigated for the exposed group, resulting in a mutation rate of 0.003. For the control group, 12 900 alleles were analysed and a mutation rate of 0.0009 was found. Thus, the cumulative mutation rate observed for the exposed group was about three times higher than the mutation rate observed for the control group, despite a substantial period of time having passed between exposure and conception. According to the paternal and maternal mutation rates, the full profiles for 15 STR loci were obtained for members of 10 families, including 20 parents and 14 children. The set of data represents 165 and 6450 meioses for the exposed and control groups, respectively. The observed cumulative mutation rate for the 15 STR loci was 0.006. In the control group, the observed cumulative mutation rate was 0.002 (Table II). There was no statistically significant difference ($P = 0.09$) between the mutation rate of the exposed and control groups according to the binomial test for either one of the two approaches used to estimate mutation rates in both groups (Table III). The statistical power of this study was 20%.

Discussion

In the current study, the exposed and control groups were composed of 34 and 645 family members, respectively. The exposed group showed only one mutation at the locus D8S1179 in a child born after the accident. This mutation was of paternal origin inherited from a male accidentally exposed to caesium-137. According to Table I, especially on Families 2, 4, 6 and 7, a substantial period of time had elapsed between the time of exposure and the time of conception. However, we found that the child who carried the mutation was born 19 years after the accident. This child was born to a family which had another male child born before the accident, who had no observed mutation in his genetic profile. Although only one mutation was found in the exposed group, the mutation rate was nearly three times higher than the mutation rate observed in the control group. The chi-square test with Yate's correction showed no statistically significant difference between the two distinct mutation rates. However, with respect to human populations, there is no safe threshold dose for IR exposure. Thus, even low doses are potentially harmful to the genome and may contribute as an effective biological dose, therefore imposing some genetic risk, generally proportional to the absorbed dose.

On the other hand, finding one germline mutation in association with an exposed person, causing a 3-fold increase in mutation rate for that particular group, could be considered as evidence of one inherited mutation related to the paternal exposure to low doses of gamma radiation. This assumption is in conformity with the linear-no-threshold model to predict health effects in humans exposed to radiation (24). Moreover,

Table II. Germline mutations observed in the exposed and unexposed progenitors from Goiânia (Brazil)

	Exposed group		Control group	*P*value*
	Exposed alleles	Unexposed alleles	Unexposed alleles	
Number of alleles examined	165	145	6450	
Mutations of paternal origin	1	—	5	
Mutations of maternal origin	—	—	6	
Mutation rate	1/165 = 0.006	—	11/6450 = 0.002	$P = 0.09$

*Binomial test of two proportions.

Table III. Distribution of mutations in the 15 STR loci study to investigate the effect of human exposure to IR in the population accidentally exposed to caesium-137 in Goiânia (Brazil)

Locus	Paternal origin			Maternal origin			Total		
	Number of meiosis	Number of mutations	Mutation rates	Number of meiosis	Number of mutations	Mutation rate	Number of meiosis	Number of mutations	Mutation rate
D16S539	5	0	0	6	0	0	11	0	0
D3S1358	5	0	0	6	0	0	11	0	0
FGA	5	0	0	6	0	0	11	0	0
PENTA E	5	0	0	6	0	0	11	0	0
D21S11	5	0	0	6	0	0	11	0	0
D18S51	5	0	0	6	0	0	11	0	0
TH01	5	0	0	6	0	0	11	0	0
TPOX	5	0	0	6	0	0	11	0	0
D8S1179	5	1	0.2	6	0	0	11	1	0.09
vWA	5	0	0	6	0	0	11	0	0
PENTA D	5	0	0	6	0	0	11	0	0
CSF1PO	5	0	0	6	0	0	11	0	0
D7S820	5	0	0	6	0	0	11	0	0
D13S317	5	0	0	6	0	0	11	0	0
D5S818	5	0	0	6	0	0	11	0	0
Total	75	1	0.013	90	0	0	165	1	0.006

transgenerational studies carried out using mouse models showed that STR mutation rate is a sensitive endpoint capable of detecting alterations in a very small population (30). As discussed by Slebos *et al*. (18), small sample sizes could result in limited statistical power for germline mutation rates. Therefore, despite the evidence in this study, the reduced number of mutational events observed in the exposed group may be a result of its limited population size.

It has been well documented that the mutation rate increases linearly with the dose of absorbed radiation. Thus, a higher mutation rate reflects proportionately more damage to cell DNA and, consequently, a larger individual absorbed dose. Furthermore, exposure of biological systems to discrete IR doses seems to stimulate the repair system, which behaves more efficiently (3). Thus, in the current study, parental exposure to doses <0.2 Gy could explain the low mutation rate observed in the F1 generation of exposed parents. However, the molecular endpoints used in the current study were neutral genomic markers and their results cannot be used to infer damage to the genetic health for the filial generation. In general, the effects of human exposure to IR are still very controversial (31).

The results found in the current study were similar to the results of other studies that reported no increase in the frequency of germline mutations in the offspring of irradiated fathers (14,18,21,32). On the other hand, a statistically significant increase in mutation rate was found in the exposed families from the Semipalatinsk district, where nuclear tests were carried out in Kazakhstan (17) and in the germline of exposed fathers in the Ukraine region (12). Additionally, an increased minisatellite germline mutation rate was observed in the Techa River population (23). Our laboratory previously reported increased germline mutation rate in the offspring of the population accidentally exposed to gamma radiation of caesium-137. In that study, 10 mutational events in STR markers were found in the F1 generation of the population who was directly exposed to radiation doses >0.2 Gy. Although the exposed group in the current report comes from the same Brazilian population, it differs from the group previously investigated as they were acutely exposed to very low doses of caesium-137 IR during the Goiânia radiological accident.

Other studies have also evaluated the adverse effects on human health in populations directly exposed to IR (20). Some of these studies used experimental strategies of exposure *in vivo* and *in vitro* usually on animal models, mostly mice (17). Some results have shown that rates of induced mutations on germ cells remained high for a considerable period of time after the initial exposure, a common feature associated with genomic instability, reflecting an increase in cancer risk (30). Our group is currently investigating the spatial distribution of cancer clusters in Goiânia in order to estimate the potential evidence of elevated cancer incidence among the studied cohorts as a genetic consequence of their radiation exposure over two decades ago.

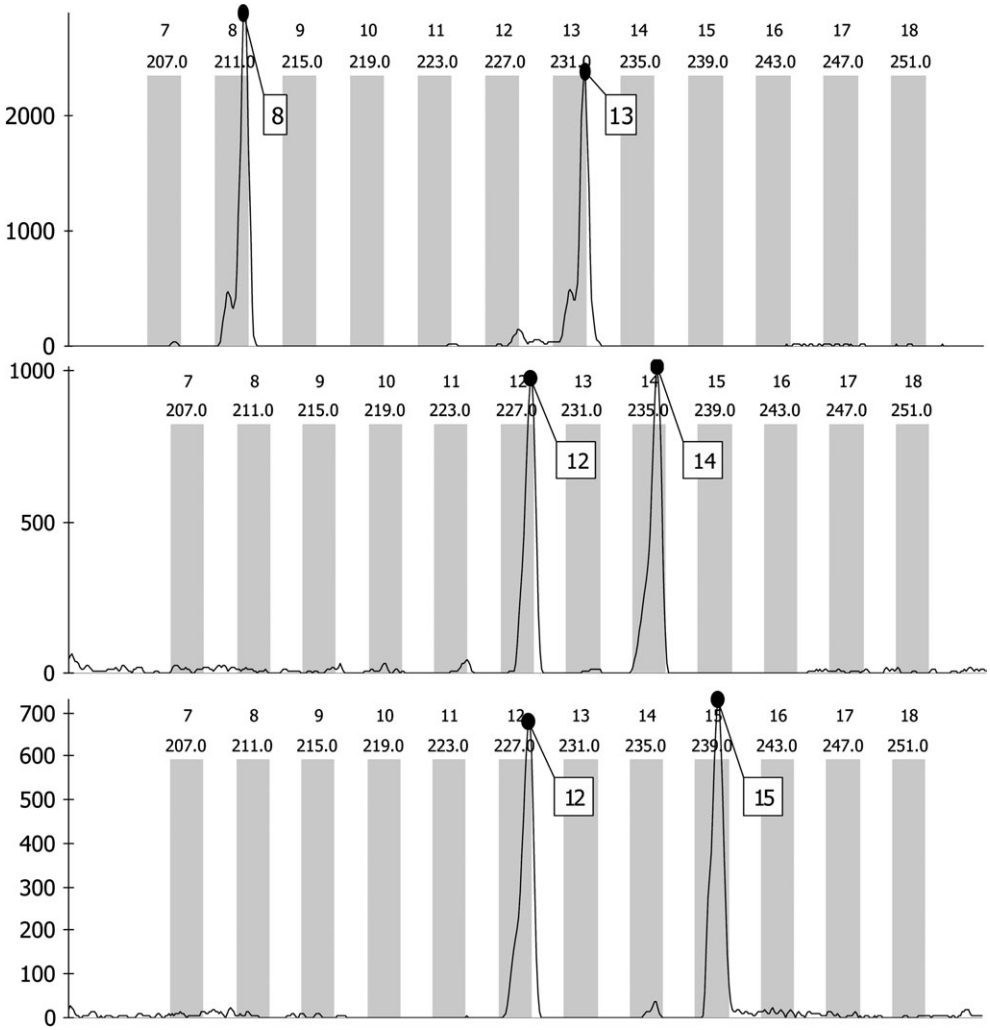

Fig. 1. Electropherogram showing the mutation at D8S1179 locus in Family 6. It observed the allelic profiles of the father (8, 13), son (12, 14) and mother (12, 15), indicating a change of the obligatory paternal allele, which gained one tetranucleotide repeat unit.

Table IV. Distribution of mutations in the 15 STR loci study to investigate the effect of human exposure to IR in the control population from Goiânia (Brazil)

Locus	Paternal origin			Maternal origin			Total		
	Number ofmeiosis	Number of mutations	Mutation rates	Number of meiosis	Number of mutations	Mutation rates	Number of meiosis	Number of mutations	Mutation rates
D16S539	215	0	0	215	1	0.005	430	1	0.002
D3S1358	215	0	0	215	1	0.005	430	1	0.002
FGA	215	3	0.014	215	0	0	430	3	0.007
PENTA E	215	0	0	215	2	0.009	430	2	0.005
D21S11	215	2	0.009	215	2	0.009	430	4	0.009
D18S51	215	0	0	215	0	0	430	0	0
TH01	215	0	0	215	0	0	430	0	0
TPOX	215	0	0	215	0	0	430	0	0
D8S1179	215	0	0	215	0	0	430	0	0
Vwa	215	0	0	215	0	0	430	0	0
PENTA D	215	0	0	215	0	0	430	0	0
CSF1PO	215	0	0	215	0	0	430	0	0
D7S820	215	0	0	215	0	0	430	0	0
D13S317	215	0	0	215	0	0	430	0	0
D5S818	215	0	0	215	0	0	430	0	0
Total	3225	5	0.0016	3225	6	0.002	6450	11	0.002

One of the advantages of the STR, as exposure biomarkers, lies in the fact that they are highly polymorphic and present higher mutation rates than coding sequences. Thus, the performance features of particular STRs make them efficient in tracking genetic effects induced in the genome by mutagens. Human exposure to IR could increase the frequency of somatic mutations in experimental studies. Moreover, the effects of IR in the induction of germline mutations in humans are not well defined. Thus, the results of this study corroborates the evidence reported elsewhere that germline mutation rates of STR can be used satisfactorily in a retrospective study of populations exposed to IR, with a reliable discriminatory power for parental exposure (10,13,14,16,21,23).

In conclusion, the quantification of mutational events in STR can provide a useful system of detecting induced mutations in a relatively small population size, thereby increasing to our knowledge of the biological effects of exposure of human populations to IR. Despite their usefulness, the results of several studies using the STR system to estimate the rate of germline mutations remain controversial, mostly because of the limited population size of available exposed families.

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