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Homologous recombination between HERVs causes duplications in the AZFa region of men accidentally exposed to cesium-137 in Goiânia

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ABSTRACT. In September 1987, in Goiânia, Brazil, one of the most serious radiological accidents occurred at a radiation therapy unit involving a source of cesium-137. The current study examined the occurrence of possible germline mutations at the AZF region of the exposed men and in their male offspring. Genomic DNA samples of 16 individuals were analyzed for microdeletions. All exposed individuals amplified sequence tagged sites; however, sY84 and sY86 showed a duplication in 75% (12/16) of the exposed group. Exposed families designated as B and E showed a duplication of sY84 and sY86, both in the fathers and their sons. Fathers of families A, C, D, and F did not show a duplication in the AZF region, but their sons did. The children in A and D had duplications of sY84 and sY86, while children in families C and F had a duplication exclusively of sY84. Family G showed a duplication of sY84 in all three generations from grandfather to grandson. Two human endogenous retroviral sequences (HERV) exist in the AZFa region, and non-allelic recombination between these sequences could cause chromosomal rearrangements, such as deletions or duplications, and a mutational mechanism intrinsic to non-allelic recombination could be

increased by individual exposure to ionizing radiations from cesium-137. Consequently, the hotspots inside HERV mediated recombination in AZFa, and the duplication diversity was compatible with male fertility, since to date, none of the exposed individuals have demonstrated fertility disorders.

Key words: Y chromosome; Rearrangement; Ionizing radiation; Male fertility

INTRODUCTION

In September 1987 there was a radioactive accident in Goiânia, involving a source cesium-137 at a radiation therapy unit. On such occasion, 150 individuals were exposed from low to high doses of ionizing gamma radiation (0.2 up to 7 Gy) (da Cruz et al., 1996). Cellular exposure to ionizing radiation induces a genotoxicity stress in cells that could result in damage of their genetic material. A 3-year follow-up study of the population accidentally exposed to high doses of ionizing radiation found 40% of missense mutations involving A:T pairs (da Cruz and Glickman, 1997; da Cruz et al., 1997). Recently, our group surveyed the genetic variation of 12 microsatellite loci in 10 families of exposed individuals and their offspring and the mutation rate was found to be higher in the exposed families compared to the control group (da Cruz et al., 2008).

Initially, a 4-Gy dose of ionizing radiation produces approximately 120-160 double-strand DNA breaks (DSB), 1000-2000 single-strand DNA breaks and a similar number of base damaging events in the cell (Wyman and Kanaar, 2006). Some of these occur very soon after radiation exposure and, in certain instances, may result in longer-term alterations, affecting the maintenance of chromosome stability. Furthermore, strand break repair possibilities are influenced by the manner in which the break is created. These two ends can, in principle, be rejoined by non-homologous DNA end-joining or be repaired by homologous recombination with their intact sister chromatid as the repair template (Gasior et al., 2006).

During the annual medical follow-up of people accidentally exposed to cesium-137, we also inquired about the occurrence of germline mutations in the AZF region in male offspring of exposed men. The AZF region is located at Yq11, and it was first described in cytogenetic studies carried out by Tiepolo and Zuffardi (1976). Recent studies indicated that microdeletions in Yq are related to male infertility (Arruda et al., 2007; Choi et al., 2008; Rodovalho et al., 2008).

The human Y chromosome has been recently shown to contain dispersed repeats, including Alu and HERV (human endogenous retrovirus) sequences, at a significantly higher frequency than in the autosomes (Blanco et al., 2000; Sun et al., 2000; de Parseval and Heidmann, 2005). The AZFa region contains two sequences of HERV that measure ~10 kb and flank 780 kb of the region. The 5' HERV sequence comprises an L1 fragment of ~1.5 kb (Kamp et al., 2000; Bosch and Jobling, 2003) and also contributes to the duplication of complete chromosome segments and transposable elements found in the human genome, consequently, causing genomic reorganization.

Intrachromosomal recombination events can also occur between elements that are located apart on the chromosome, leading to large deletions or duplications. Both mitotic and meiotic recombination events can be stimulated by agents that produce DSBs. DSBs are the major contributor to the cytotoxicity of ionizing radiation because ionization tracts yield high frequencies of multiply damaged sites (Agarwal et al., 2006). Increasing numbers of genetic disorders have been shown to result from the recombinogenic effect of flanking repeat sequences (Kirsch et al., 2005).

Herein, we report the results of a case-control study carried out in 16 individuals in which the father was exposed to cesium-137 ionizing radiation. Our aim was to test whether ionizing radiation could cause paternal genetic mutations that are transmitted to male offspring.

MATERIAL AND METHODS

Probands

In August 2005, 8 men accidentally exposed to the ionizing radiation of cesium-137 and their offspring were studied (Table 1). The parental generation included was directly exposed to ionizing radiation. The control group comprised 55 healthy men, without previous history of ionizing radiation. All participants contributed voluntarily with 5 mL peripheral blood collected in Vacutainer tubes containing sodium heparin.

Table 1. Data of the 7 families showing the ages, the absorbed doses of the fathers and/or grandfather, the groups (classified according to generation - F1 or F2) and the presence of a duplication in the AZFa region.

Family	Individual	Age (years)	Radiation (Rad)	Group	sY84	sY86
A	19 Father	48	30	I	-	-
	955 Son	4	---	II	=	=
B	11 Father	32	22.2	I	=	=
	952 Son	3	---	II	=	=
	954 Son	10	---	II	=	=
C	9 Father	49	101	I	-	-
	943 Son	13	---	II	=	-
D	28 Father	61	>20.025	I	-	-
	970 Son	6	---	II	=	=
E	26 Father	70	---	II	=	=
	1008 Son	16	---	II	=	=
F	15 Father	66	>22.2	I	-	-
	950 Son	16	---	II	=	-
G	30 Granfather	64	150.3	I	=	-
	6 Father	39	>20.04	I	=	-
	965 Grandson	12	---	II	=	-

- One band; = Two bands.

Genetics analysis

Genomic DNA was isolated from peripheral blood lymphocyte samples using the GFX™ Genomic DNA Purification Kit (GE Healthcare, USA) according to manufacturer instructions and stored at -20°C until analysis. DNA samples were separated on a 1% agarose gel impregnated with ethidium bromide to check for DNA quality. PCR followed the protocol proposed by Simoni et al. (2004) for analyzing the AZF region. Primer sequence tagged sites (STS) were used for the three sub-regions: AZFa (sY84, sY86), AZFb (sY127, sY134) and AZFc (sY254, sY255). SRY (sex determining region of the Y chromosome) gene and ZFX/Y gene for the presence of human genomic DNA were used as internal control. These STS primers are recommended by the European Academy of Andrology and are useful in detecting 90% of mutations in AZF loci (Arruda et al., 2007). PCR products were separated by electrophoresis on a 1.5% agarose gel impregnated with 5 µg/mL ethidium bromide using a constant and uniform electric field (8 V/cm) for 2 h. The

gels were analyzed in a Video-Documentation System (Amersham Pharmacia Biotech, USA) using the Image Master 1D software (Total Lab). Band sizes were determined by comparing with specific ladders (Promega Corporation, USA) added in every run in each gel.

RESULTS

The average age of the control group was 33 years old, and all individuals showed amplifications to all STS of the AZF region analyzed, including SRY and ZFX/Y genes.

The exposed individuals had an average age of 53 years, and 10 years for the offspring. All STS analyzed were amplified in the tested samples, including SRY and ZFX/Y genes. However, it was observed that the amplifications of the STS sY84 and sY86, located in the AZFa region, displayed a duplication in 12 (75%) of the individuals analyzed (Figure 1).

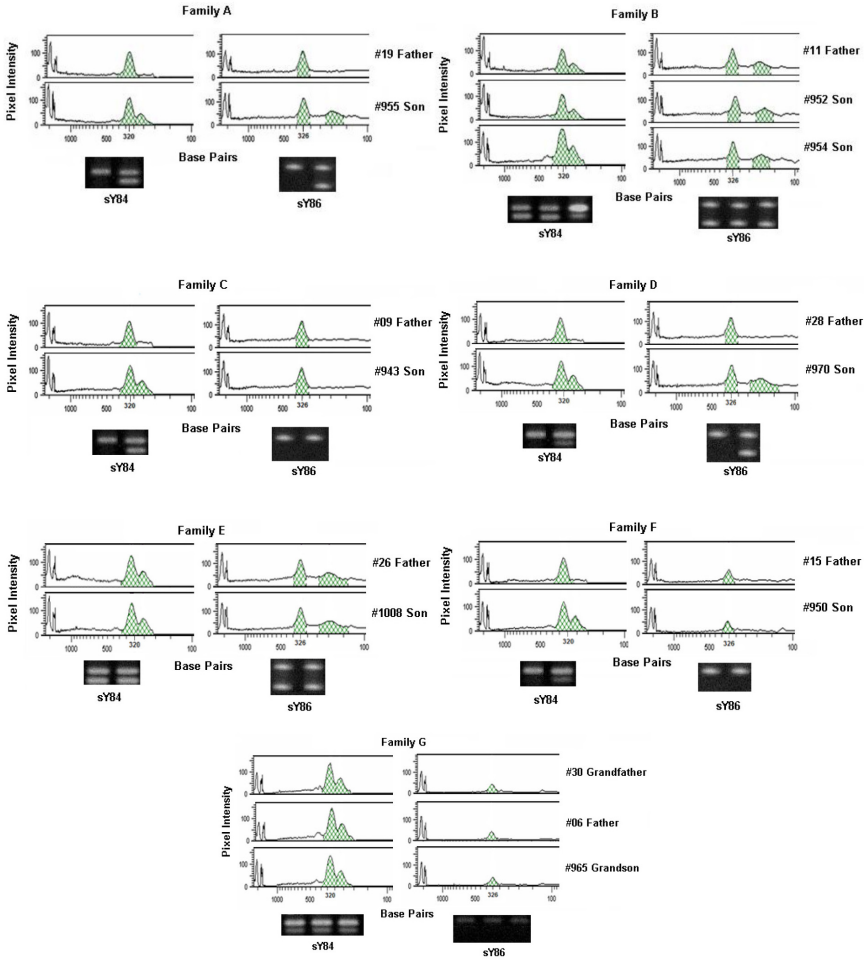

Figure 1. Electropherograms and gels of the seven families showing all duplications found in this study. The arrows indicate the duplications.

Of these individuals, families B and E (father #11 and children #952 and #954; father #26 and child #1008) showed a duplication, both in the fathers and children. In families A, C, D and F, the fathers did not show a duplication in the AZFa region, but their children did (father #19 and child #955; father #9 and child #943; father #28 and child #970; father #15 and child #950). Of these four families, children A and D showed a duplication of sY84 and sY86. There was only a duplication of sY84 in families C and F. Family G (grandfather #30, father #6 and grandson #965) showed a duplication of sY84 STS from the grandfather to grandson. Of the 16 individuals analyzed, 5 showed duplication only of sY84 STS (31.25%) and none of sY86, while 7 showed the two duplicated STS (43.75%).

DISCUSSION

Several studies have detected and observed the potential genetic effects of radiation at the DNA level in children of exposed individuals (Furitsu et al., 2005; da Cruz et al., 2008). Duplications play a significant role in eukaryotic genome evolution, but it is unclear how these events are generated (Fredman et al., 2004). Although the pathogenic effects of AZFa were not totally elucidated in our study, we demonstrated that some children had the same AZFa duplication as their parent.

Diseases involving chromosome rearrangements are referred to as genomic disorders, and they are mostly mediated by low-copy repeats that can induce non-allelic homologous recombination and account for 5-10% of the human genome. Specific repeat sequences are the substrates for a homologous recombination event (Rozen et al., 2003; Bosch et al., 2004; Stankiewicz et al., 2004). This suggests that there may be a correlation between regions of gene conversion or recombination and susceptibility to rearrangements (Eichler, 2001).

Experimental tests suggest that gene conversion is a prominent homology-repair mechanism of double stranded breaks in mammalian cells (Agarwal et al., 2006). Interallelic gene conversion seems to be four to 15 times more frequent than crossovers (Bosch and Jobling, 2003). Another possibility is that occasional double crossovers transfer indels between the duplicated segments. While the precise mechanism remains unclear, our results indicate that indel transfer between segmental duplications is possible (Kirsch et al., 2005; Sankaranarayanan, 2006).

Crossover hotspots drive allelic exchanges at meiosis. It is not clear if they can also promote unequal crossover (ectopic exchange, non-allelic homologous recombination) between related DNA sequences or if they could drive genomic rearrangements, such as deletions and duplications, triggered by recombination-initiating DSB (Pavlicek et al., 2005). Such genome instability is of utmost importance given the frequency of segmental duplications in the human genome (Gasior et al., 2006).

Several studies indicate that exposures of 2 to 4 Gy of ionizing gamma radiation could increase DNA damage associated with radiation exposition (Eichler, 2001; Farkash et al., 2006). Genomic rearrangements could happen during meiosis and the duplications in AZFa also support the idea that men who carry such alterations are capable of producing children with this duplication (Hurles et al., 2004). Chromosomes with duplications are probably a target for natural selection (Bosch et al., 2004). The complete deletion of the AZFa region could cause male infertility with the lack of germinative cells (Krausz et al., 2006; Choi et al., 2008). Thus, since the exposed men did not show fertility problems until now, we suggest that there is an

association between ionizing radiation and the mechanisms of duplication and chromosomal rearrangements identified in this study (Bosch and Jobling, 2003; da Cruz et al., 2008).

The genome is not uniformly stable and contains fragile sites implicated in chromosome breaks and DSB formation (Wyman and Kanaar, 2006). The human Y chromosome is known to have undergone a number of intrachromosomal duplication events and more detailed analysis will be required to reveal the dynamics of such events. These structural properties should elucidate the exact nature of this association and provide insights into the general mechanisms underlying chromosomal rearrangements.

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