

# Is Increased Low-Dose Somatic Radiosensitivity Associated with Increased Trans-Generational Germline Mutation Radiosensitivity?

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## ***Introduction***

This study addresses two issues central to understanding the risks to a general population exposed to low doses of ionizing radiation: The first is the issue of individual genetic susceptibility to low dose radiation, i.e. the possibility that a small fraction of the general population could be significantly more radiosensitive than average, as a result mutations in genes such as *ATM* or *BRCA1*. The second is the issue of the heritable effects of radiation, i.e., the effect of low-dose radiation exposure on the germline of the irradiated individual and, particularly, on the germline of the offspring of irradiated individuals.

While there is little doubt that mutations in genes such as *ATM* and *BRCA1* result in increased somatic radiosensitivity, nothing is yet known as to whether such genetic mutations could correspondingly result in increased germline radiosensitivity. Therefore we are investigating whether mice that are heterozygous for genes that confer somatic radiosensitivity also show an increased radiosensitivity for germline mutations, and whether an increased germline mutation rate is inherited by unirradiated offspring.

The long-term relevance of this work lies in the possibility of significant subpopulations with raised sensitivity for low-dose radiation-induced germline mutations. Such a scenario could be of broad societal relevance; for example, the radiation risk vs. benefit balance for mass screening mammography would be altered for these individuals - of particular relevance to younger women. More generally, radiation protection limits for somatic and genetic risks are currently premised on an essentially unimodal distribution of radiation sensitivity across the population; if a significantly-sized identifiable subpopulation were hypersensitive to radiation, a single radiation protection standard across the whole population would be of questionable relevance.

In this project we are studying the combined effects of low-dose exposure to ionizing radiation and DNA-repair deficiencies on mutation rate in the mouse germline. We use DNA-repair knock-out mice, which provide a useful *in vivo* model for the analysis of the genetic mechanisms of human susceptibility to environmental mutagens. Numerous studies have shown that mice carrying mutations in DNA repair and related genes show increased sensitivity to mutagens and carcinogens, often resulting in elevated cancer incidence. However, to date little is known about the effects of DNA-repair deficiency on spontaneous and induced mutation in the germline.

The results of our previous work have shown that expanded simple tandem repeat (ESTR) loci provide a useful and sensitive tool for monitoring germline mutation in mice (1,2). In this study ESTR mutation rates in the paternal germline are evaluated either by profiling the offspring of non-exposed and irradiated males or using single-molecule PCR (SM-PCR) technique. This approach involves diluting bulk sperm genomic DNA and amplifying multiple samples of DNA, each containing approximately one ESTR molecule, and allows the detection of indefinitely large number of *de novo* mutants in a single male (3).

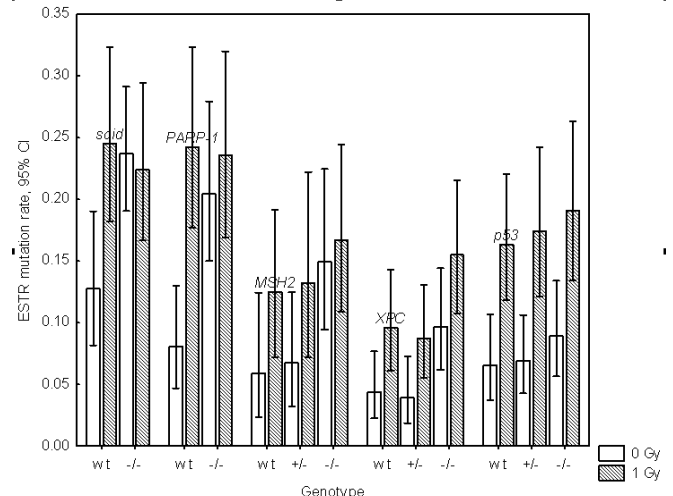
## ***Optimization of the SM-PCR technique***

Our recent work has shown that the initial protocol of the SM-PCR technique, developed by Yauk *et al.* (3), was prone to PCR artefacts and unreliable for the amplification of large and often medium-sized ESTR alleles. Using a combination of commercially available PCR kits and the DNA-stabilising agent Betaine, we have optimized the PCR conditions for a wide range of ESTR alleles (2-6 kb). Using this technique, we have analyzed the frequency of ESTR mutations in DNA samples prepared from sperm, bone marrow and spleen of the first-generation offspring of irradiated CBA/Ca and BALB/c male mice (4). For both strains, a statistically significant ~2-3 fold increase in the mean mutation frequency was found in all tissues of the offspring of irradiated males. Most importantly, the frequency of ESTR mutation was elevated in the germline (sperm) and somatic tissues of all the offspring of irradiated males. The results of this study therefore show that the modified SM-PCR approach provides robust estimates of individual mutation rates and can be used for the analysis of ESTR mutation rates in the germline of DNA-repair deficient mice.

### ***ESTR mutation rates in the germline of DNA-repair deficient mice***

To date, mutation rates have been analyzed in the germline of 6 DNA-repair deficient strains of mice, including severe combined immunodeficient (*scid*) mice, poly(ADP-ribose) polymerase (*PARP-1*), *mutS* homolog 2 (*MSH2*), xeroderma pigmentosum group C (*XPC*), DNA polymerase  $\kappa$  (*Polk*) and *p53* deficient male mice (5-7). ESTR mutation rates in the germline of these strains were evaluated using the pedigree approach. *scid* mice carrying a nonsense mutation in the catalytic subunit of DNA-protein kinase are deficient in the recognition / repair of DSBs by non-homologous end joining; *PARP-1* knockout mice are deficient in SSB recognition. Mismatch and nucleotide excision repair are compromised in *MSH2* and *XPC* knockout mice, respectively. The *Polk* gene encodes one of multiple DNA polymerases that are specialized for the replicative bypass of base damage in template strands of DNA (trans-lesion DNA synthesis). The efficiency of radiation-induced apoptosis is affected in *p53* deficient mice. Our results to date suggest that

1. *p53* status does not affect spontaneous or radiation-induced mutation rates in the paternal germline;
2. Spontaneous ESTR mutation rates in all five homozygous knockout strains significantly exceed those in the isogenic wild-type strains;
3. Acute exposure to ionizing radiation (1 Gy, x rays) does not affect ESTR mutation rate in the germline of homozygous *scid*, *PARP-1*<sup>-/-</sup> and *MSH2*<sup>-/-</sup> mice. The lack of mutation induction in these strains can be explained by the high cell killing effects of irradiation on the germline of deficient mice;
4. ESTR mutation rate in the germline of irradiated *XPC*<sup>-/-</sup> homozygotes significantly exceeds that in the wild-type / heterozygous males, indicating that the compromised nucleotide excision repair does not substantially affect the survival of irradiated germ cells and thus results in higher radiosensitivity;
5. Spontaneous and radiation-induced mutation rates in the germline of heterozygous *XPC*<sup>+/-</sup> and *MSH2*<sup>+/-</sup> mice do not significantly differ from those in the isogenic wild-type strains.



***ESTR mutation rates in the germline of DNA-repair deficient and wild-type male mice.***

In the next phase, we are irradiating male *Atm*<sup>+/-</sup> and wild-type control mice and, using SM-PCR, scoring ESTR mutations in the germline (sperm samples) of directly exposed and non-exposed males. We have verified that the *Atm* strain on a BALB/c background, currently maintained at Colorado State University, possesses amplifiable alleles at the *Ms6-hm* ESTR locus.

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