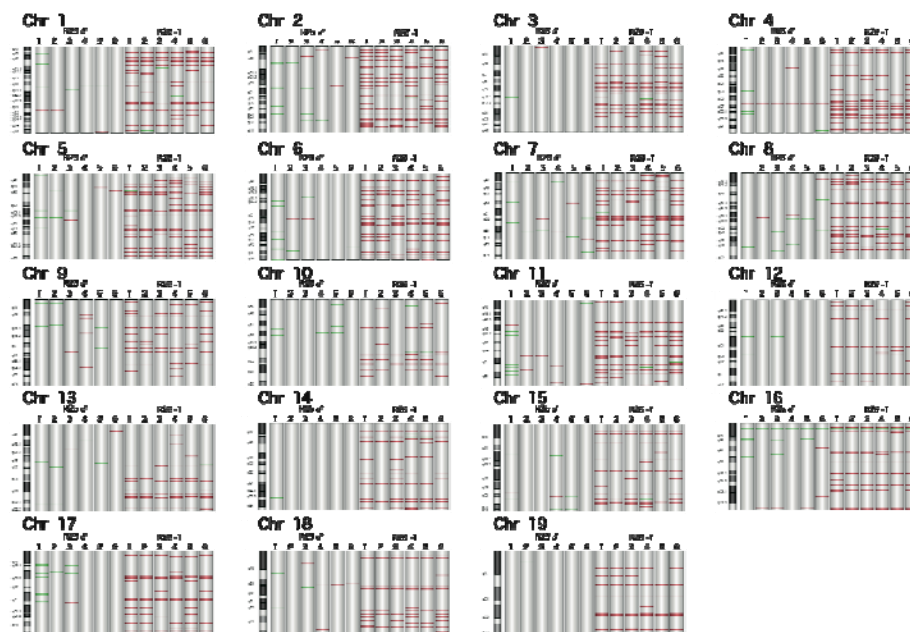


# Transgenerational Effects in Mice Exposed to Continuous Low-Dose-Rate Gamma-Rays – Genome-Wide Detection of Germ Cell Mutation –

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## Abstract

Transgenerational effects of continuous low-dose-rate  $\gamma$ -ray irradiation of male mice have not been well studied. First of all to establish the method to detect newly generated and inherited mutations by offspring from an irradiated male mice, we tried to screen germ cell mutations using an oligo microarray CGH method. Acutely  $\gamma$ -ray irradiated (8 Gy at 0.9 Gy/min), 8-week-old, specific-pathogen-free (SPF) C57BL/6J male mice were mated to non-irradiated C57BL/6J female mice to get their offspring. The genome-wide molecular comparisons of tail DNAs from irradiated male mouse, its mate and their offspring were performed to identify mutations that may have been newly generated and inherited by offspring from the irradiated male mice. Here we show that the oligo microarray CGH analysis could screen mutation candidates of an average of 196 loci per one progeny from irradiated male, in contrast to an average of 0.4 loci per one progeny from non-irradiated male.

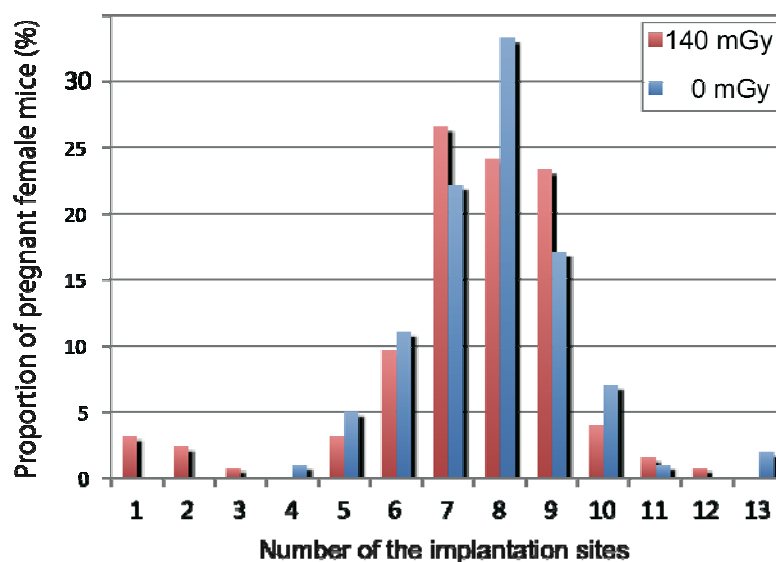


**Fig. 1** An example of results obtained by oligoarray CGH in one progeny. DNAs from irradiated male mouse (R25, shown in left side lanes of each chromosome) and its one child (R25-1, shown in right side lanes of each chromosome) were repeatedly analyzed (6 times, as shown in lane number 1-6). To screen the mutation, we performed "differential aberration" protocol using these twelve array results. Red lines: genomic loss. Green lines: genomic gain.

**Table 1** The number of mutation candidate loci in the fetuses from acutely irradiated (8Gy) or non-irradiated male mice

| Irradiated male mouse No. | Progeny No. | No. of detected loci | Non-irradiated male mouse No. | Progeny No. | No. of detected loci |
|---------------------------|-------------|----------------------|-------------------------------|-------------|----------------------|
| R25                       | 1           | 261 (5)              | C1                            | 1           | 0                    |
|                           | 2*          | 323 (4)              |                               | 2           | 0                    |
|                           | 3*          | 757 (16)             |                               | 3           | 0                    |
| R28                       | 1*          | 70 (5)               |                               | 4           | 0                    |
|                           | 2*          | 32 (1)               |                               | 5           | 1 (0)                |
| R32                       | 1           | 210 (1)              |                               | 6           | 0                    |
|                           | 1*          | 3 (0)                |                               | 7           | 1 (0)                |
| R36                       | 2*          | 27 (10)              | C3                            | 1*          | 1 (0)                |
|                           | 3*          | 62 (25)              |                               | 2*          | 0                    |
|                           | 1           | 171 (1)              |                               | 3*          | 1 (0)                |
| R41                       | 2           | 222 (19)             |                               | 4*          | 0                    |
|                           | 1*          | 128 (19)             |                               | 5*          | 1 (0)                |
| R42                       | 2*          | -                    |                               | 6*          | 1 (0)                |
|                           | 3*          | -                    |                               | 7*          | -                    |
| R45                       | 1           | 281 (1)              |                               |             |                      |
| R49                       | 1           | 32 (10)              |                               |             |                      |

Summary of mutation candidate loci detected by oligoarray CGH. The fetuses were sampled at 16-19 embryonic days old fetus from acutely irradiated (R) or non-irradiated (C) male mice. All analyses used 224 K arrays except those marked with an asterisk (\*) that used 1M arrays. Numbers shown in parentheses indicate large deletions containing more than two flanking array probes.



**Fig. 2** Distribution of the number of the implantation sites detected from the uterus of the pregnant female mice exposed to low-dose-rate (20 mGy/22h/day) gamma-ray for 7 days (total dose of 140 mGy). There were more females having implantation sites from 1 to 4 in irradiated group than in non-irradiated group ( $P < 0.05$ ).