Hereditary effects of radiation in men and in mice
Why don't we see the effects in humans?

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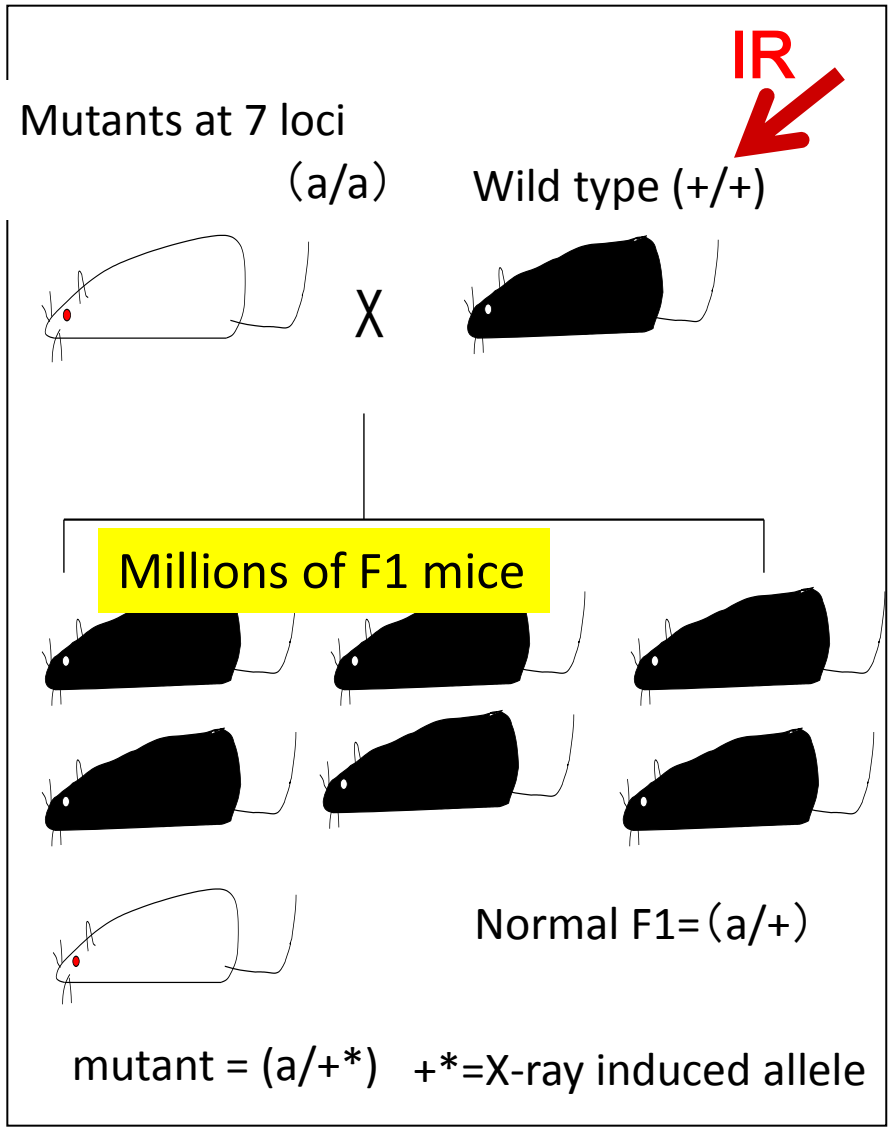
Observed facts

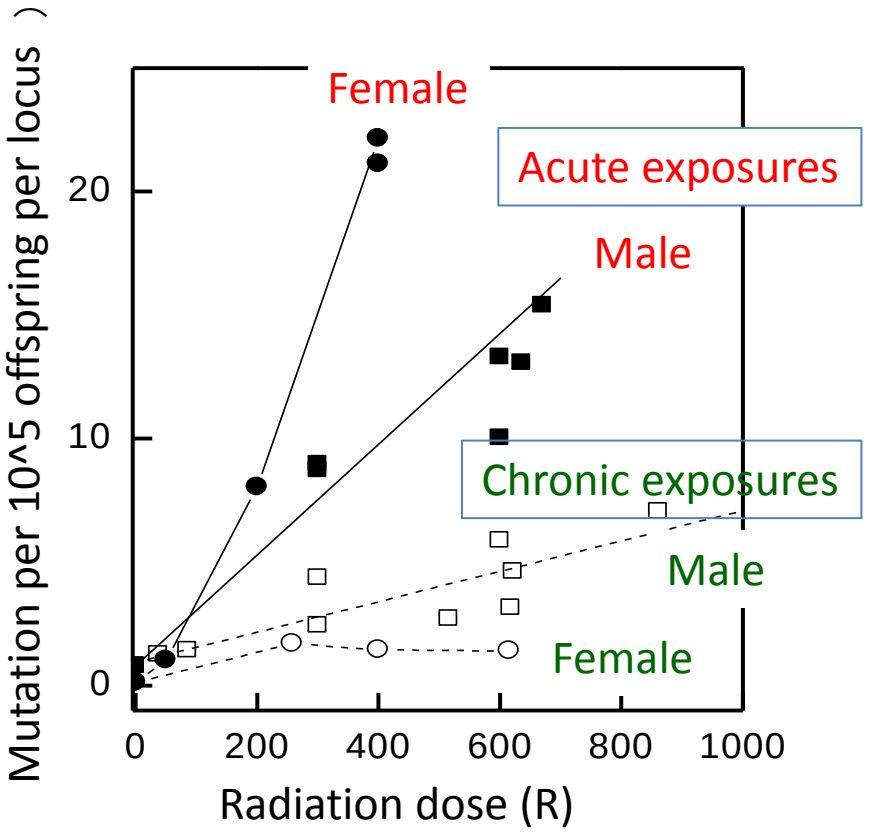
- Fruit flies (sex-linked recessive lethals)
- Mice (specific locus tests: SLT)
- Dominant lethal mutations
- Chromosome aberrations
- Malformations

Thus far, however, there is no clear evidence in humans

→Why?

Schema of SLT in mice





Genetic studies in the offspring of A-bomb survivors

Birth defect	77,000 (1948-1954)
Sex ratio	140,000 (1948-1966)
Chromosome	16,000 (1967-1985)
Biochemical tests	23,000 (1975-1984)
Mortality	80,000 (1946-present)
DNA study	1,000 families (on going)
F1 clinical study	10,000 (2002-on going)

In no study was found possible effect of parental exposures

It is therefore indicated that

Humans do not seem extremely sensitive to radiation than mice

(Even in mice, pre-meiotic stage is not sensitive for induction of malformation)

Information other then A-bomb survivors: Childhood Cancer Survivors

Cancer survivors who underwent radiotherapy received a large doses of IR to their gonads.
But there is no indication of genetic effects in the offspring.

e.g., Green et al. (2009 paper in J Clin Oncol 27, 2374-2381）
Mean gonadal dose = 1.26Gy (ovary) or 0.46Gy (testis)

	Offspring of	
Disease	Cancer patients (6,129)	Control (siblings) (3,101)
Chromosome aberration	7 (0.1%)	6 (0.2%)
Mendelian disorder	14 (0.2%)	8 (0.3%)
Birth defect	136 (2.2%)	97 (3.1%)
Total	157 (2.6%)	111 (3.6%)

Two alternative possibilities

1) Many mutations occur in germ cells but not recovered in the offspring (negative selection)
2) Genome does not mutate as expected from specific locus data (the 7 genes were exceptional)

Hypothesis 1

Negative selection theory

- Any evidence?
- Coat color gene could be exceptional? (no harm?)

→ *s* and *d* turned out to be lethal genes when inactivated

- 2DE assay of DNA fragments: GC-rich vs. AT-rich sequences

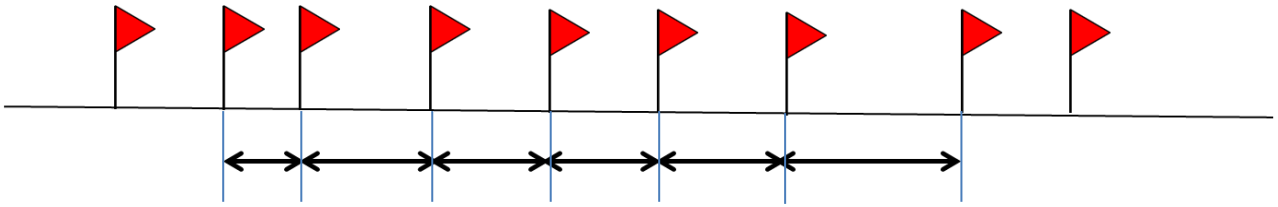
→No indication of difference

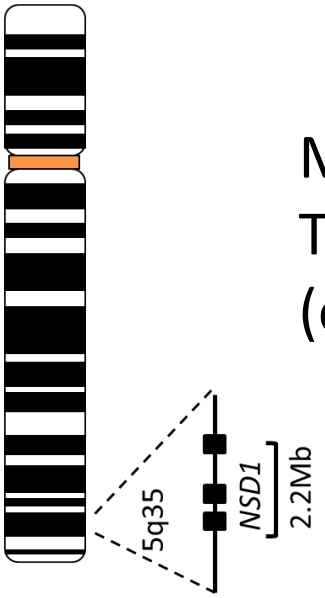
→No indication of negative selection

- But 2,000 essential genes* may be haplo-insufficient , and may lead to loss of deletion mutants

*Blomen VA et al. Science 350, 1092 (2015)
*Wang T et al. Science 350, 1096 (2015)

e.g., imagine 2,000 essential genes in the genome





Mean inter-gene distance = ~1500kb (1.5Mb)
This is sub-microscopic
(ordinary Giemsa one band ~ 10Mb)

Any deletion may include essential gene(s)
But essential genes = haplo-insufficient genes?

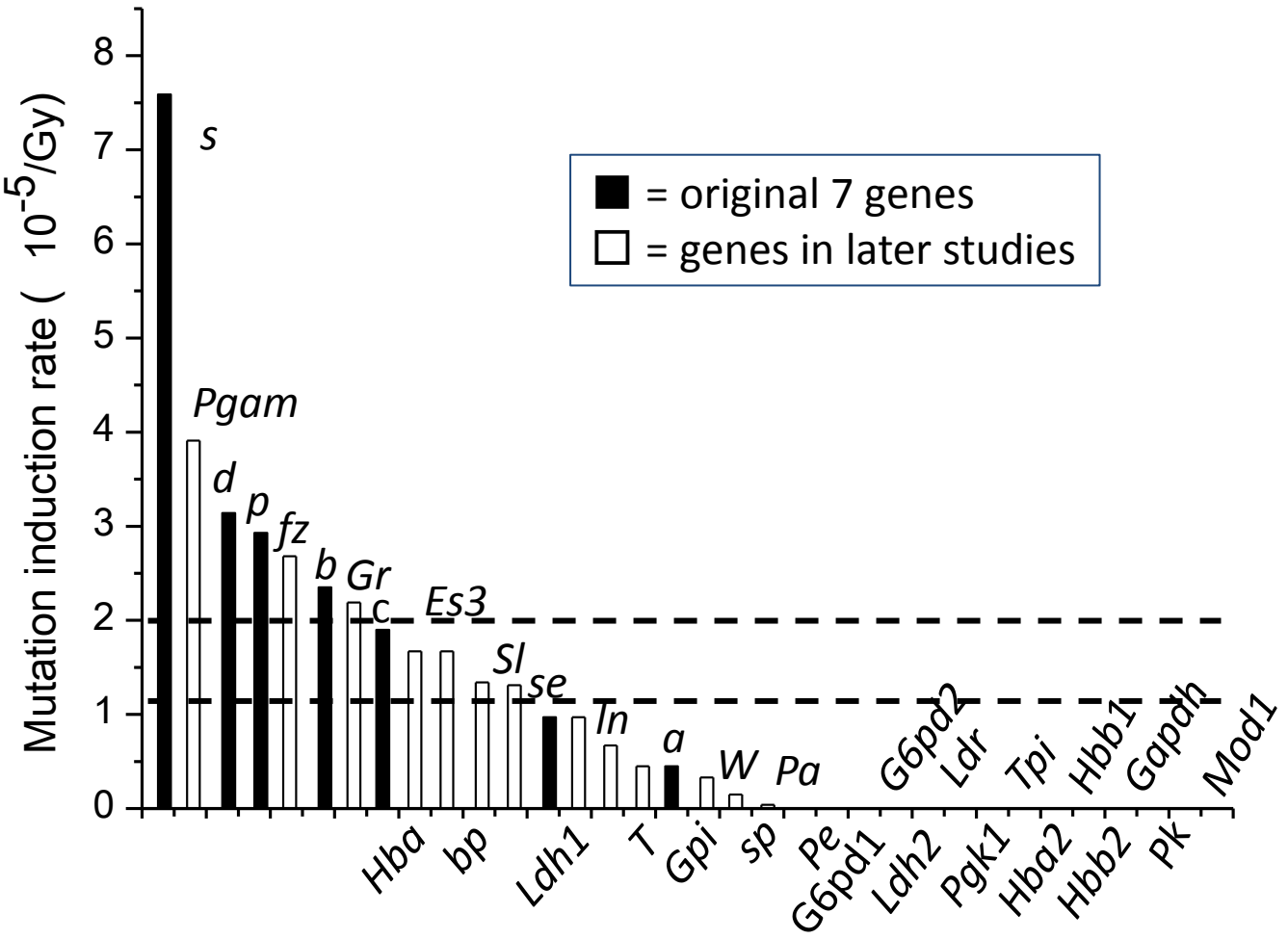
Hypothesis 2

"Genome does not mutate as expected from SLT data"

Any evidence?

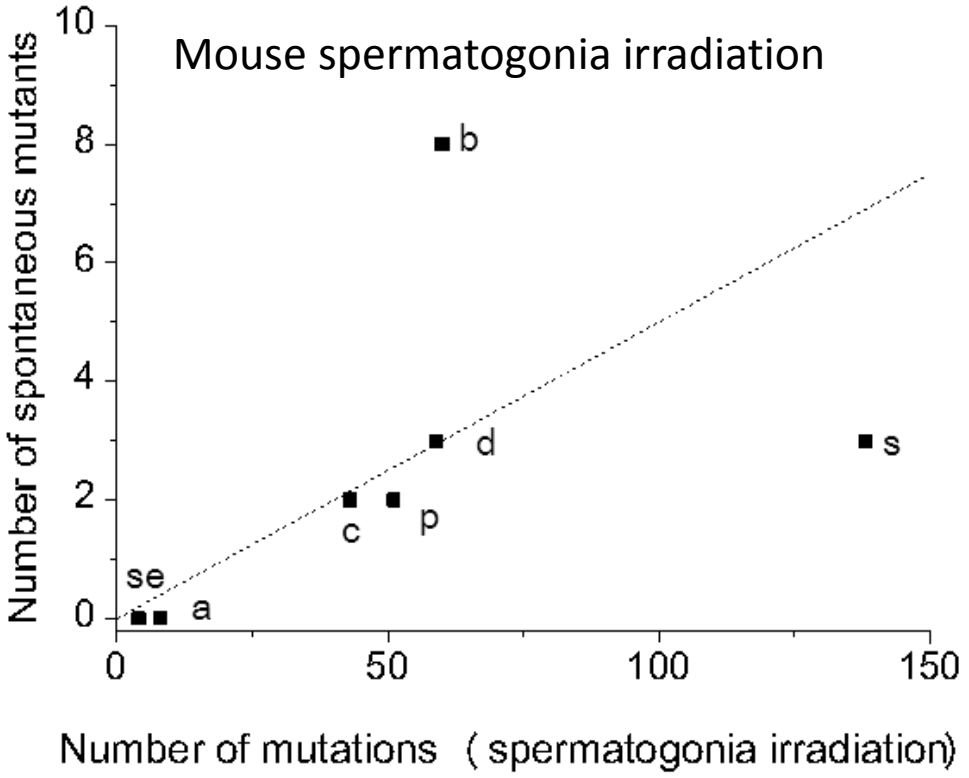
- SLT data shows large heterogeneity among genes

Mutation induction rate varies among genes



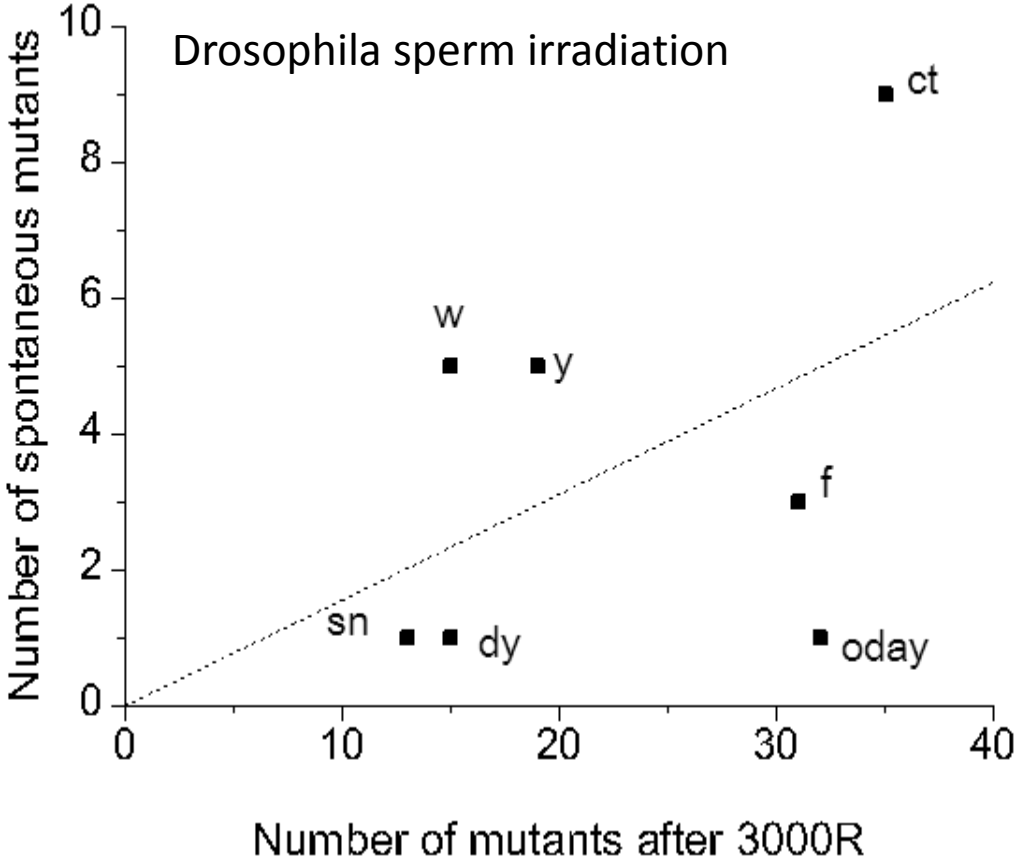
Spontaneous vs. induced mutations

Parallelism between spontaneous and induced mutation rates



Searle AG. Adv Radiat Biol 4, 131 (1974)

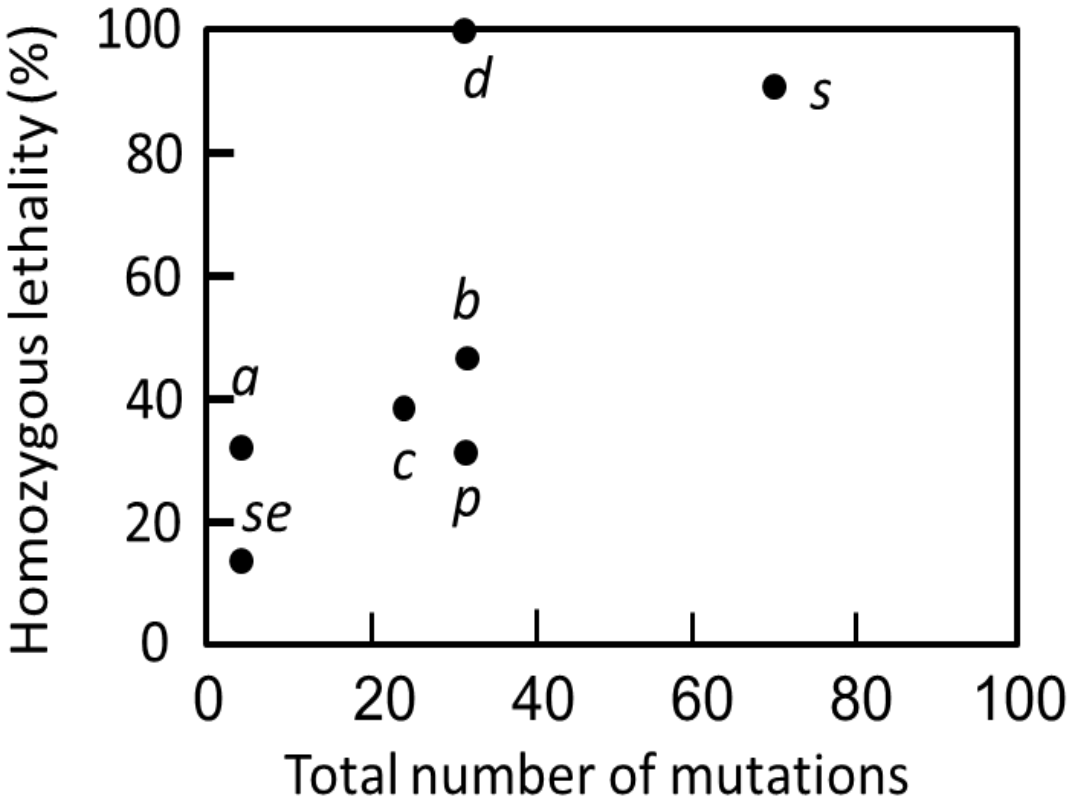
Similar trend in fruit flies



Shukla PT et al. Mutat Res 61, 229 (1979)

Another indication of heterogeneity among genes

Parallelism between spontaneous mutation rate and deletion size



Searle AG. Adv Radiat Biol 4, 131 (1974)

Other than gene mutation studies

Shortened Life span?

→No evidence

Kohn H et al (1965) (total 3,000 offspring)

Cosgrove GE et al (1993) (total 500 offspring)

Increased cancer?

→ NO

Catanach (UK; BALB/cJ, C3H/HeH strains)

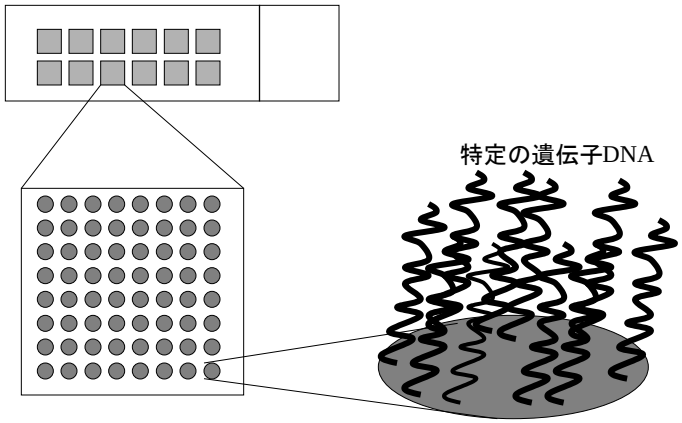
Cosgrove (USA; C3Hf strain)

Korn (USA; CAF1 strain)

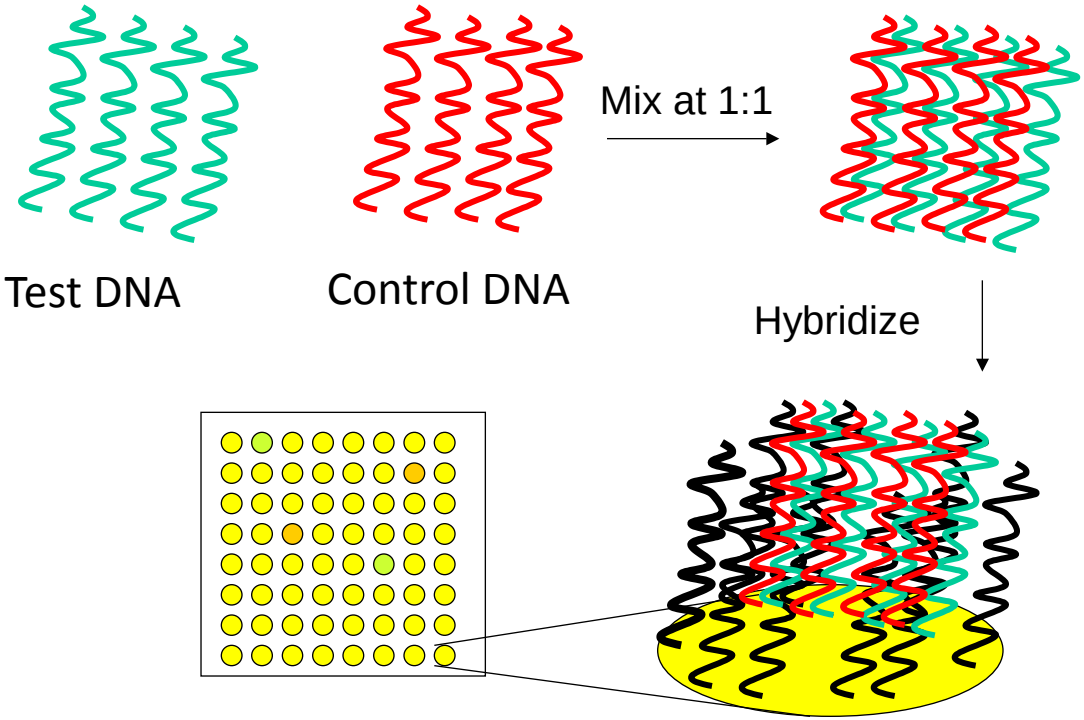
→ YES (but no earlier onset)

Nomura (Japan; ICR, LT, N5 strains)

Mutation detection with array-CGH

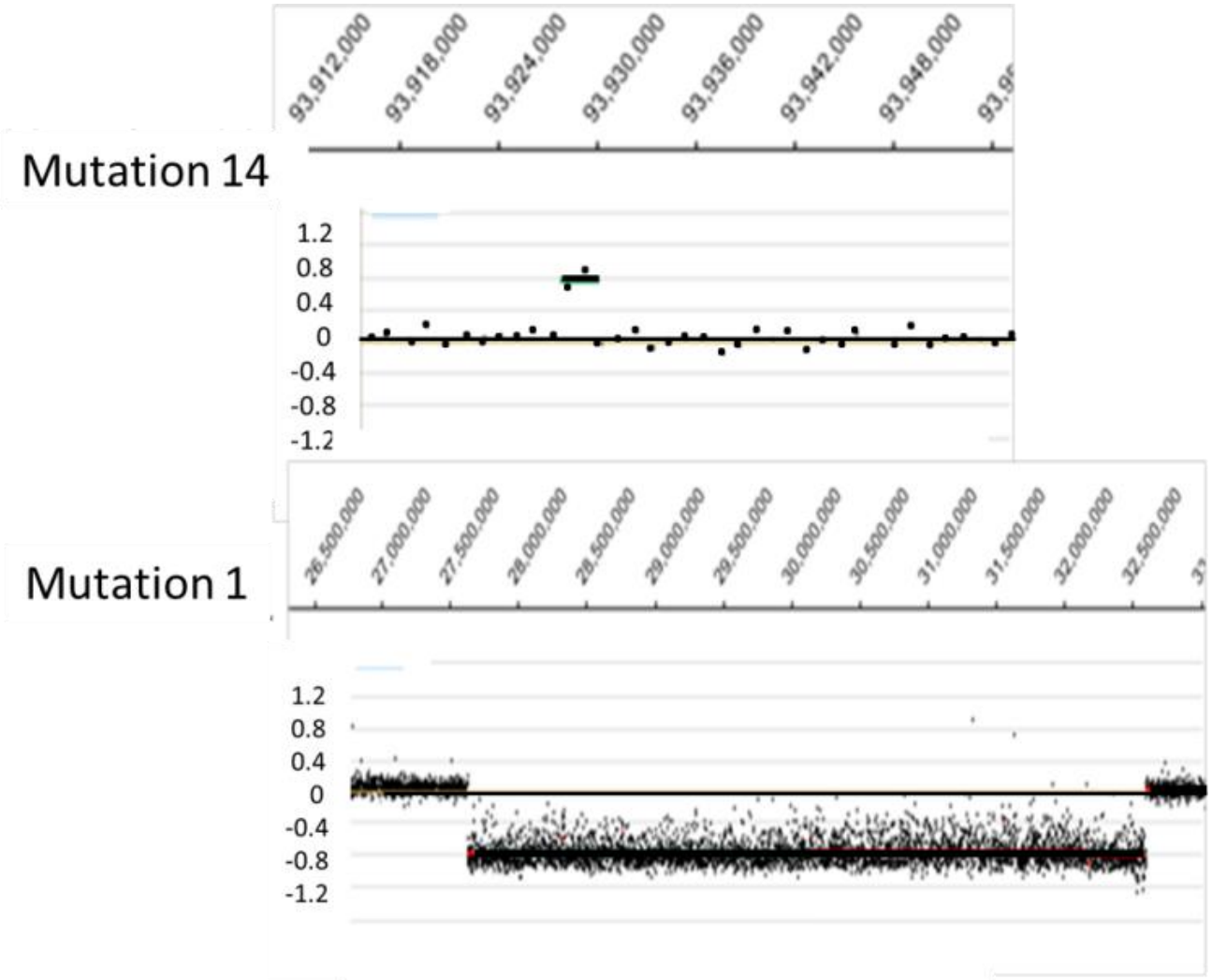


Each spot contains synthesized DNA (2M-array)



If test DNA had an deletion, the corresponding spot color turns to reddish

Examples of deletions detected with array-CGH



Asakawa et al. (submitted)

Summary of past studies

Mutation induction rate decreased with the increase of the number of genes tested

- Specific locus tests (7 genes)

  $1\sim2 \times 10^{-5}$/gene/Gy

- 2DE assay of DNA (~1,000 gene frg)

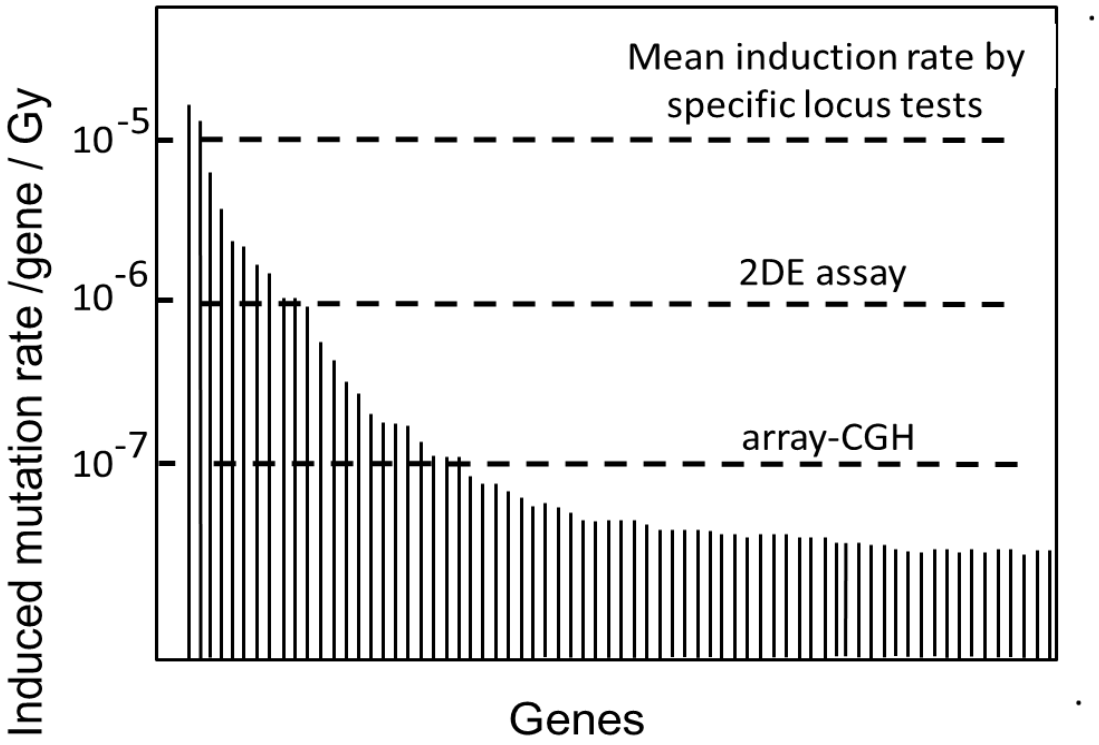
  2×10^{-6}/frg/Gy

- Microarray CGH (>1,000,000 sites)

  3×10^{-7}/10 kb frg/Gy

- The microarray data are in line with the estimate of genome-wide recessive lethal mutations

Schematic view



Different sensitivity among genes?
Or negative selective force?

Consequences of different responses among genes

Although the same number of genes are tested…

5 genes × 200,000 mice

1,000 genes × 1,000 mice

1,000,000 genes × 1 mouse → the meanings differ

How to express radiation risks?

- Mutation rate “per gene” does not work
- Number of genes lost per genome?
- Deletion size is larger after IR
- Deletion size may vary depending on the gene
- Deletion size may also vary by dose/dose rate

Conclusion

- There would be no species which does not undergo mutations by IR, thus humans are not exception
- But genetic risk is much more difficult to evaluate than previously thought

1. Mutations may NOT be induced in all genes?
   (ex. low oxygen conc., resting state etc.)
2. Mutations are induced but not recovered in live F1?
   (essential genes work to eliminate deletion-bearing cells)
3. Our genome is not quality controlled, which may cause noises in epidemiology

Thank you