

Characteristics of induced mutations in offspring derived from irradiated mouse spermatogonia and mature oocytes

Satoh, Yasunari, et al. Scientific Reports 2020

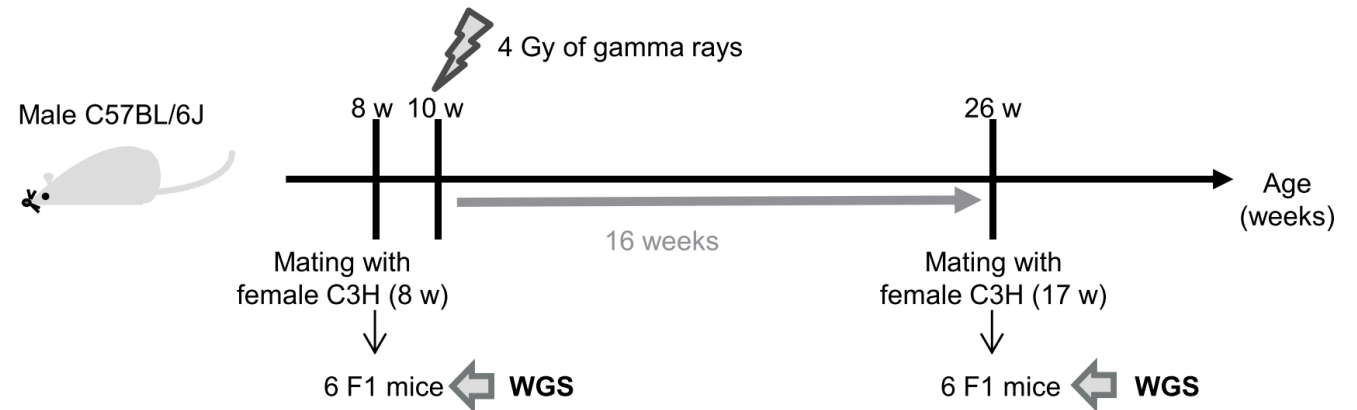
Recap – WGS after exposure to Ionizing Radiation

- Adewoje
 - Mice irradiated with 3Gy x-rays
 - 6 mice offspring 8 weeks after exposure
 - Found increase in multisite de novo mutations, indels and cnvs
- Pilot Study
 - Soldiers exposed to unknown quantities of IR
 - Increase in MSDNs

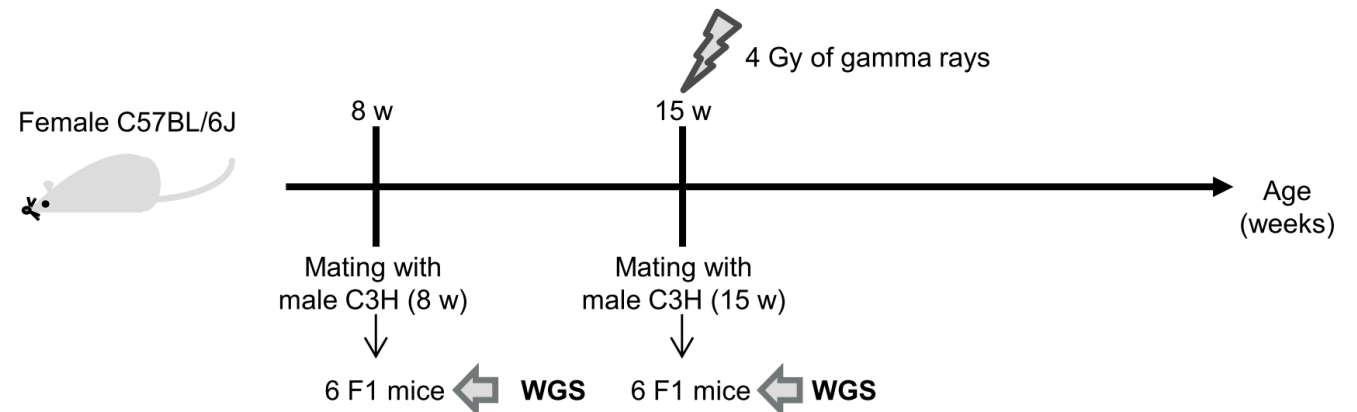
Study Setup

- Exposure: 4Gy gamma-rays
- Twofold Setup:
 - a) Irradiated male mice
 - Mating sixteen weeks after exposure
 - b) Irradiated female mice
 - Mating immediately after exposure
- Non-irradiated parents as control cohort
- 6 offspring and controls per experiment

a)



b)



Whole Genome Sequencing

- All mice were Whole genome sequenced
- Variants detected by HaplotypeCaller
- Called in Region (54% of total mouse autosomal genome):
 - $50\% < x < 300\%$ coverage compared to peak mapped to autosomal data
 - $80\% <$ ratio of high quality reads
 - Overlap of these regions in all samples
- Classes of mutation:
 - SNV (total 339)
 - Indel (total 79)
 - Multisite Mutation (Length \leq 100bp)
- CNVs not included

Filtering Criteria

- SNV:
 - AAF < 0.1 in parents
 - AAF > 0.25 in case
 - Review by manual inspection
- Indel:
 - Excluded:
 - Mutations in mononucleotide repeat sequences > 7 in length
 - Dinucleotide > 4
 - Trinucleotide (or more) > 2
- Validation by Sanger
 - 53/53 SNV, 40/40 Indels

Age Effects

- Estimate parental age effects for mice
- Base reference: de novo SNV count at 8 weeks parental age

	No. of increase of mutations (/ week)	Percentage of increase in the number of mutations per week to the number of spontaneous mutations in F1 of 8 week-old parents
Paternal effect	0.46 (= $[8.8-5.6]/7$) [-0.063, 0.99] ¹⁾	4.5% (= $0.46 / 10.3$)
Maternal effect	0.24 (= $[6.9-4.7]/9$) [-0.18, 0.70]	2.3% (= $0.24 / 10.3$)

1) 95% CI was calculated by 1,000 times of simulations based on Poisson distribution and binominal distribution.

SNV Mutation Rates

- Context:
 - No increase in Pilot Study, Adewoje
- After correcting for age effects:
 - No significant increase

Group	No. of mutations ^a	Mutation rate (95% CI)	<i>P</i> ^b	No. of mutations adjusted for parental age ^c	<i>P</i> ^d
<i>SNVs</i>					
Spermatogonia exposure					
Before IR	70	4.5×10^{-9} ($3.5\text{--}5.7 \times 10^{-9}$)		70	
After IR	128	8.3×10^{-9} ($6.9\text{--}9.8 \times 10^{-9}$)	0.0001	65.1	0.91
Mature oocyte exposure					
Before IR	54	3.5×10^{-9} ($2.6\text{--}4.6 \times 10^{-9}$)		54	
After IR	84	5.4×10^{-9} ($4.3\text{--}6.7 \times 10^{-9}$)	0.012	54.6	0.96

Non-SNV Mutations

- Indels and Multisite Mutations are increased after exposure

Group	No. of mutations ^a	Mutation rate (95% CI)	P ^b	No. of mutations adjusted for parental age ^c	P ^d
<i>Indels</i>					
Spermatogonia exposure					
Before IR	5	3.2×10^{-10} ($1.0-7.5 \times 10^{-10}$)		5	
After IR	42	2.7×10^{-9} ($2.0-3.7 \times 10^{-9}$)	<0.00001	35.9	<0.00001
Mature oocyte exposure					
Before IR	7	4.5×10^{-10} ($1.8-9.3 \times 10^{-10}$)		7	
After IR	25	1.6×10^{-9} ($1.0-2.4 \times 10^{-9}$)	0.0022	22.1	0.0095
<i>Multisite mutations</i>					
Spermatogonia exposure					
Before IR	2	1.3×10^{-10} ($0.16-4.6 \times 10^{-10}$)		2	
After IR	10	6.5×10^{-10} ($0.31-1.2 \times 10^{-9}$)	0.031	10	0.031
Mature oocyte exposure					
Before IR	0	0 ($0-2.4 \times 10^{-10}$)		0	
After IR	10	6.5×10^{-10} ($0.31-1.2 \times 10^{-9}$)	0.0031	10	0.0031

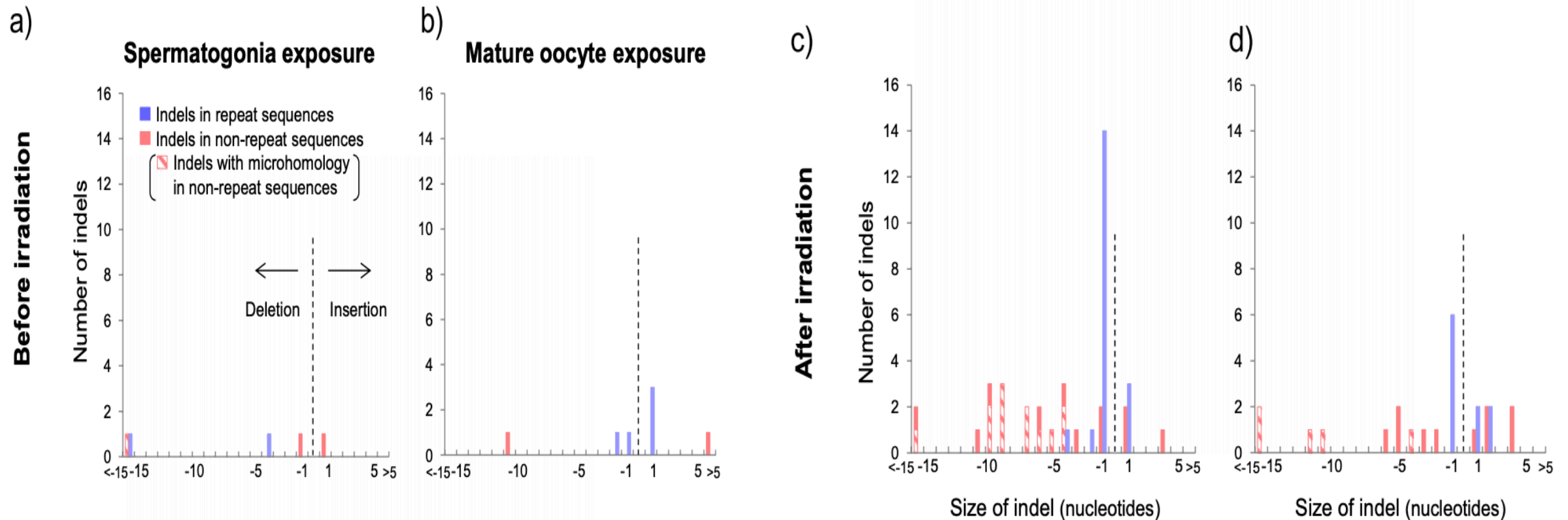
Characteristics of IR induced mutations

- Significantly increased number of Indels
- Two main types of deletions:
 - Single-nucleotide in mononucleotide repeat sequences
 - Deletions (1-35bp) in non-repeat sequences
- Both types appear in higher frequency in offspring of irradiated father

Experiment	Timing of mating	Length of mononucleotide repeats ^a	No. of indels ^b	
			Deletion (-1 base)	Insertion (+1 base)
Spermatogonia exposure	Before irradiation	Non-repeat	1	1
		2	0	0
		3	0	0
		4-7	0	0
	After irradiation	Non-repeat	2	2
		2	8	0
		3	3	1
		4-7	3	2
Mature oocyte exposure	Before irradiation	Non-repeat	0	0
		2	0	1
		3	1	0
		4-7	0	2
	After irradiation	Non-repeat	0	1
		2	4	2
		3	1	0
		4-7	1	0

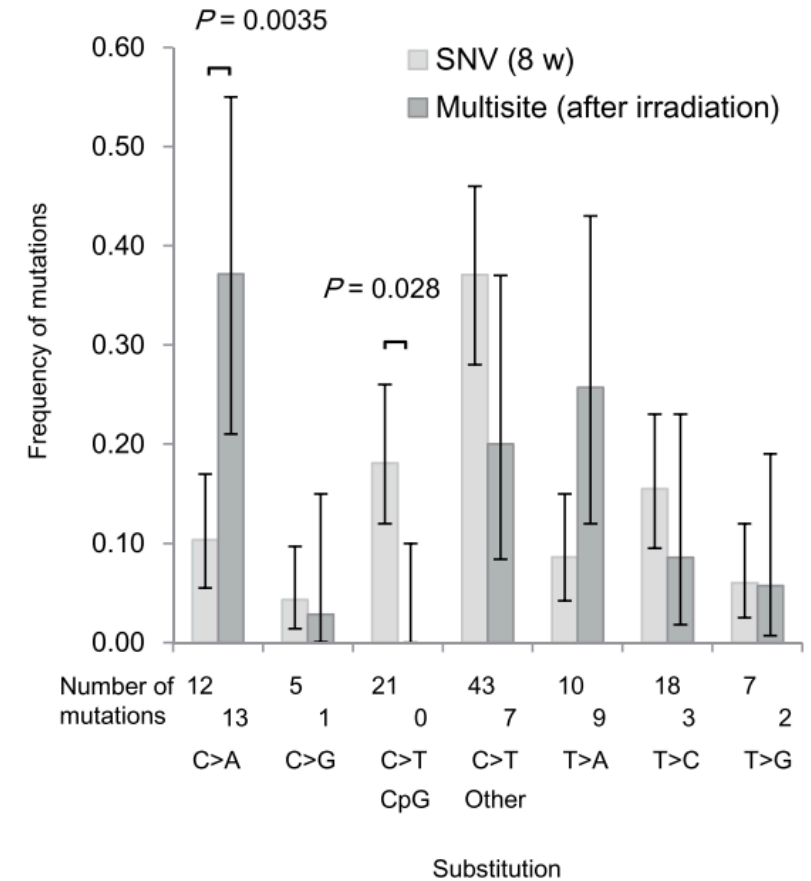
Characteristics of IR induced mutations

- Both Indel classes significantly increased compared to unexposed



Characteristics of IR induced mutations

- Multisite mutations (<100bp)
- “Most” within 15bp
- Total 20 mutations, 8 tandem
- C>A Substitutions increased
 - No multisites characteristic to error-prone polymerase (GC>AA, GA>TT)
 - No C>T at CpG sites



Conclusions

- DNA repair mechanisms has difficulty:
 - (micro-)homologous indels
 - Indels in repeat sequences
- DNA repair of double strand breaks, strand breaks at repeat sites
- Multisite mutations result from clustered lesions
 - Potential signature of IR induced mutations
- No phenotype observed

Thank you for your
attention!