

A Multidisciplinary Challenge to Assess the Next-generation Risks of Low-dose-rate Long-term Gamma-ray Exposure by Whole-genome Sequencing in the Mouse Model

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This study was supported by Research Project on the Health Effects of Radiation organized by Ministry of the Environment, Japan (Y.G.), KAKENHI 17H00789 (Y.G.), and 21K19842 (Y.M.).

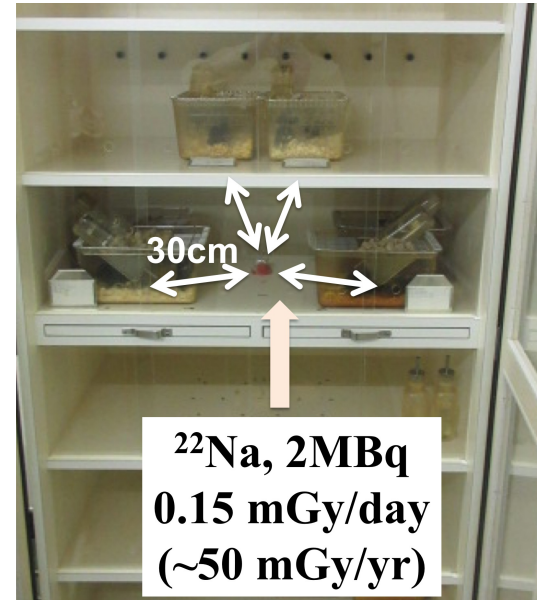
Low-dose-rate multigenerational exposure systems

Institute of Environmental Sciences (IES)



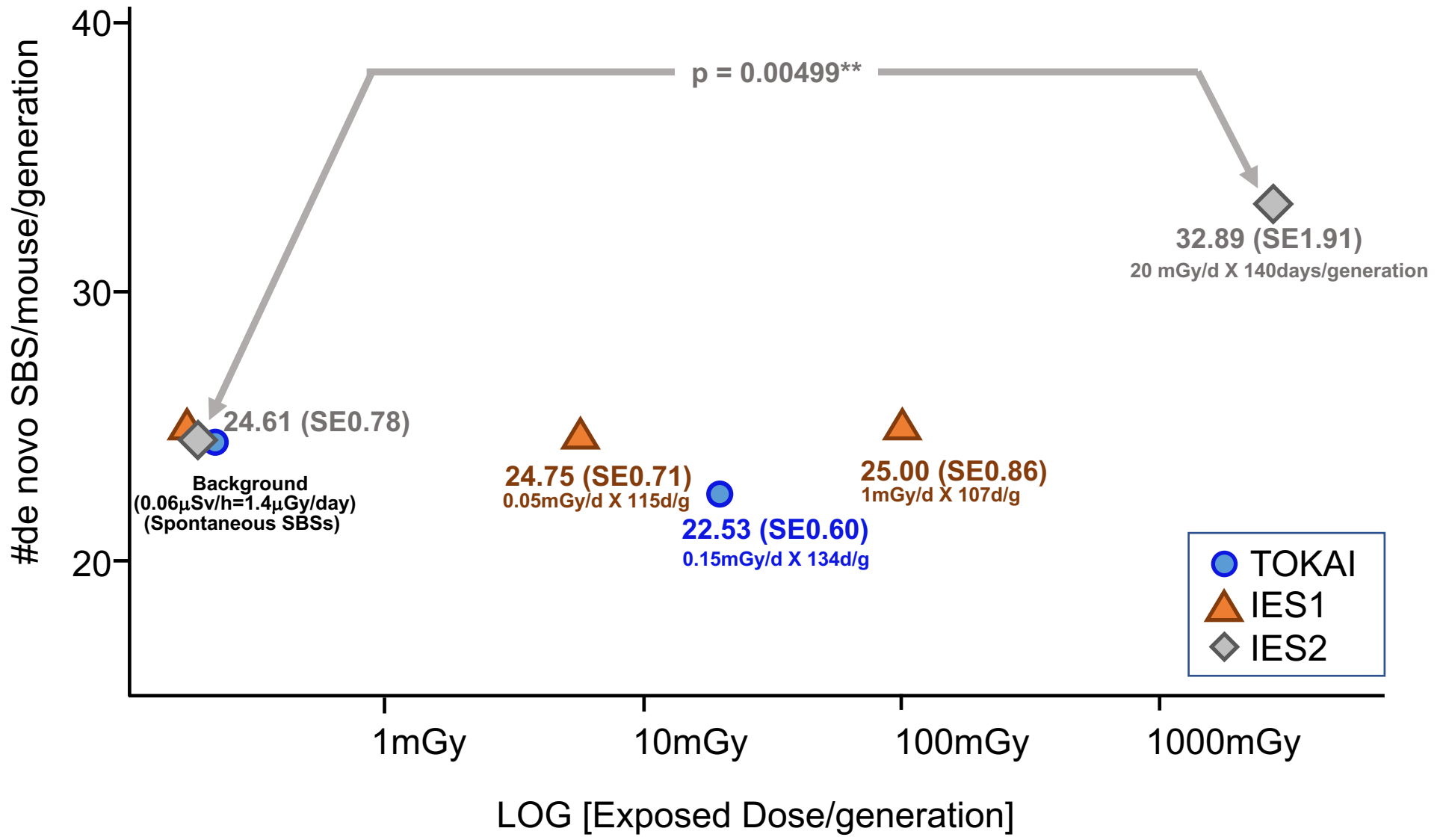
Three irradiation SPF mouse facilities with ^{137}Cs γ -ray: at the dose rate of:
0.05mGy/day
1 mGy/day and
20mGy/day
and an SPF mouse facility for negative controls.

Tokai University (TOKAI)



^{22}Na , 2MBq
0.15 mGy/day
(~50 mGy/yr)

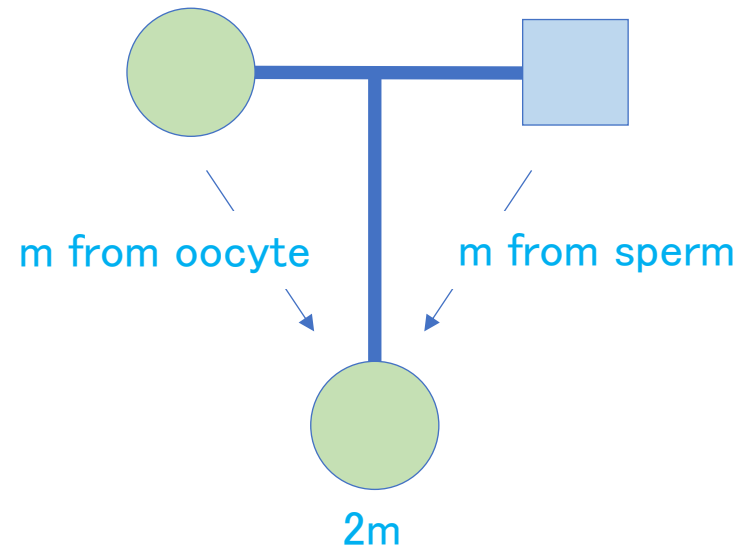
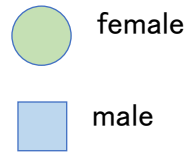
One mouse rack with ^{22}Na γ -ray:
at the dose rate of:
0.15mGy/day
and another rack for negative control.



Principle of TRIO analysis

by Whole-Genome Sequencing (WGS)
to detect de novo mutations. (DNMs)

m : average number of DNMs/gamete/generation



Expanded TRIO Analysis by WGS to detect DNMs

No. of **spontaneous** DNMs

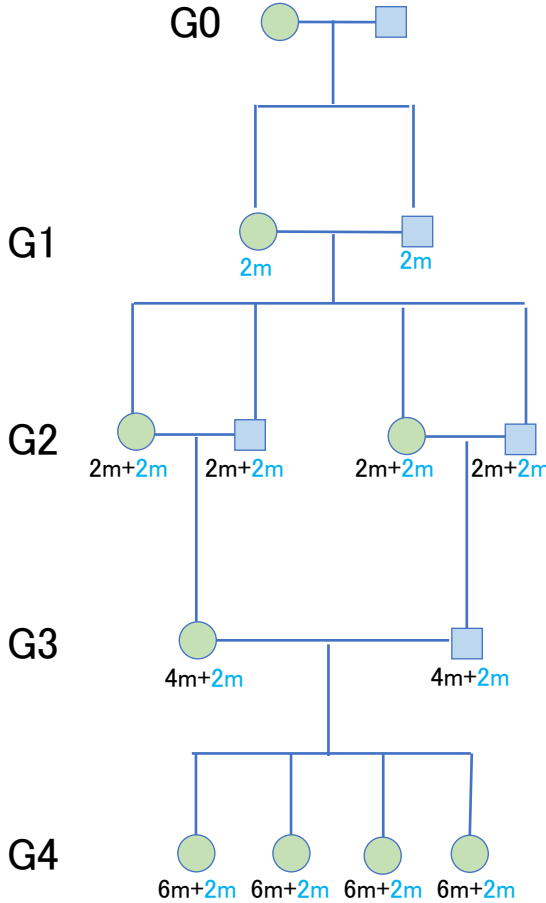
2m in G1

4m in G2

6m in G3

8m in G4

● female □ male

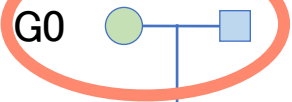


m : ave. no. of spontaneous DNMs/gamete/generation

m : ave. no. of inherited DNMs/gamete/generation

Expanded TRIO Analysis by WGS to detect DNMs

● female ■ male



m : ave. no. of spontaneous DNMs/gamete/generation
 r : ave. no. of induced DNMs/gamete/generation
 m, r : ave. no. of inherited DNMs/gamete/generation

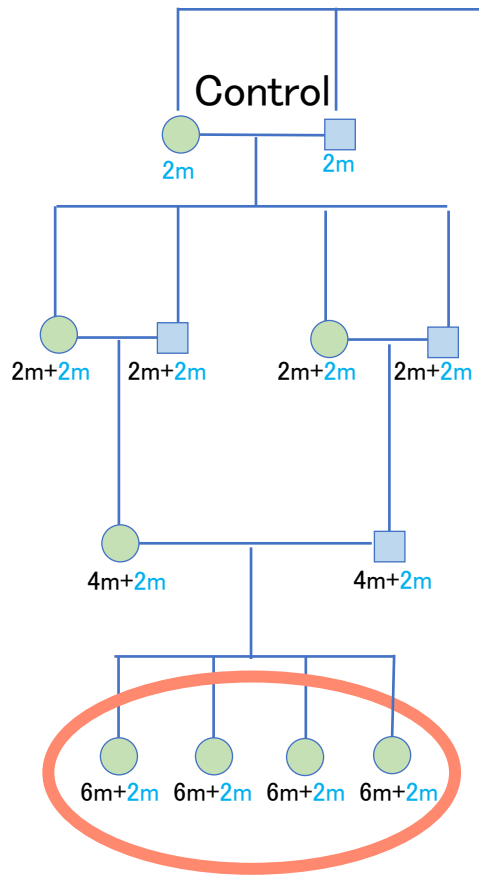
Accumulated
No. of **spontaneous** DNMs
 $2m$ in G1

$2m$ in G1

$4m$ in G2

$6m$ in G3

$8m$ in G4

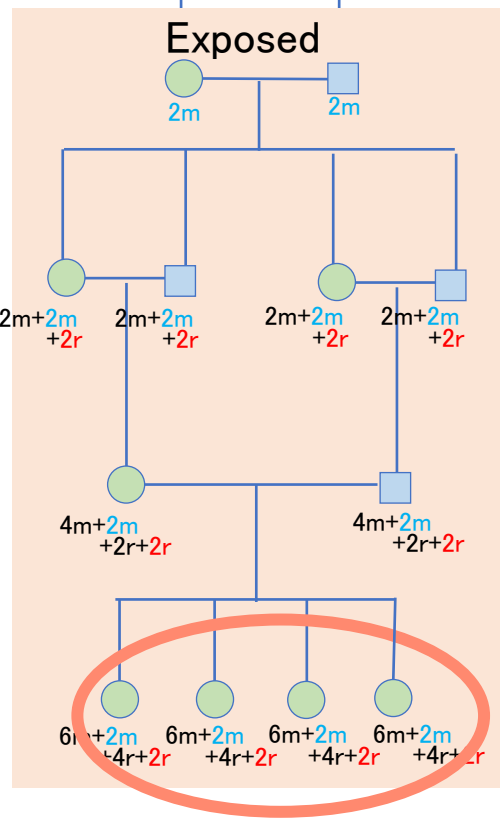


G1

G2

G3

G4



Accumulated
No. of **spontaneous**
and **induced** DNMs
 $2m$ in G1

$2m$ in G1

$4m + 2r$ in G2

$6m + 4r$ in G3

$8m + 6r$ in G4

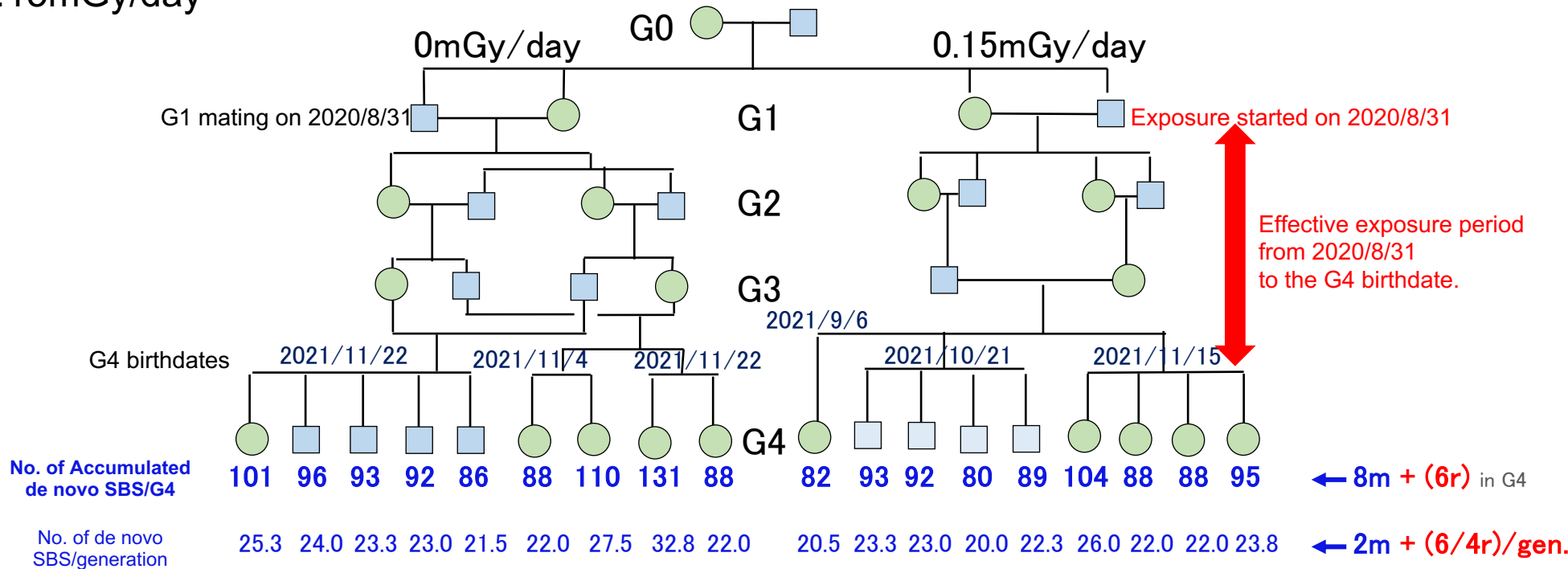
In this presentation, we focus on detected Single Base Substitutions (SBSs) on 19 pairs of mouse autosomes.

○ These mice were subjected to WGS.

TOKAI

^{22}Na γ -ray

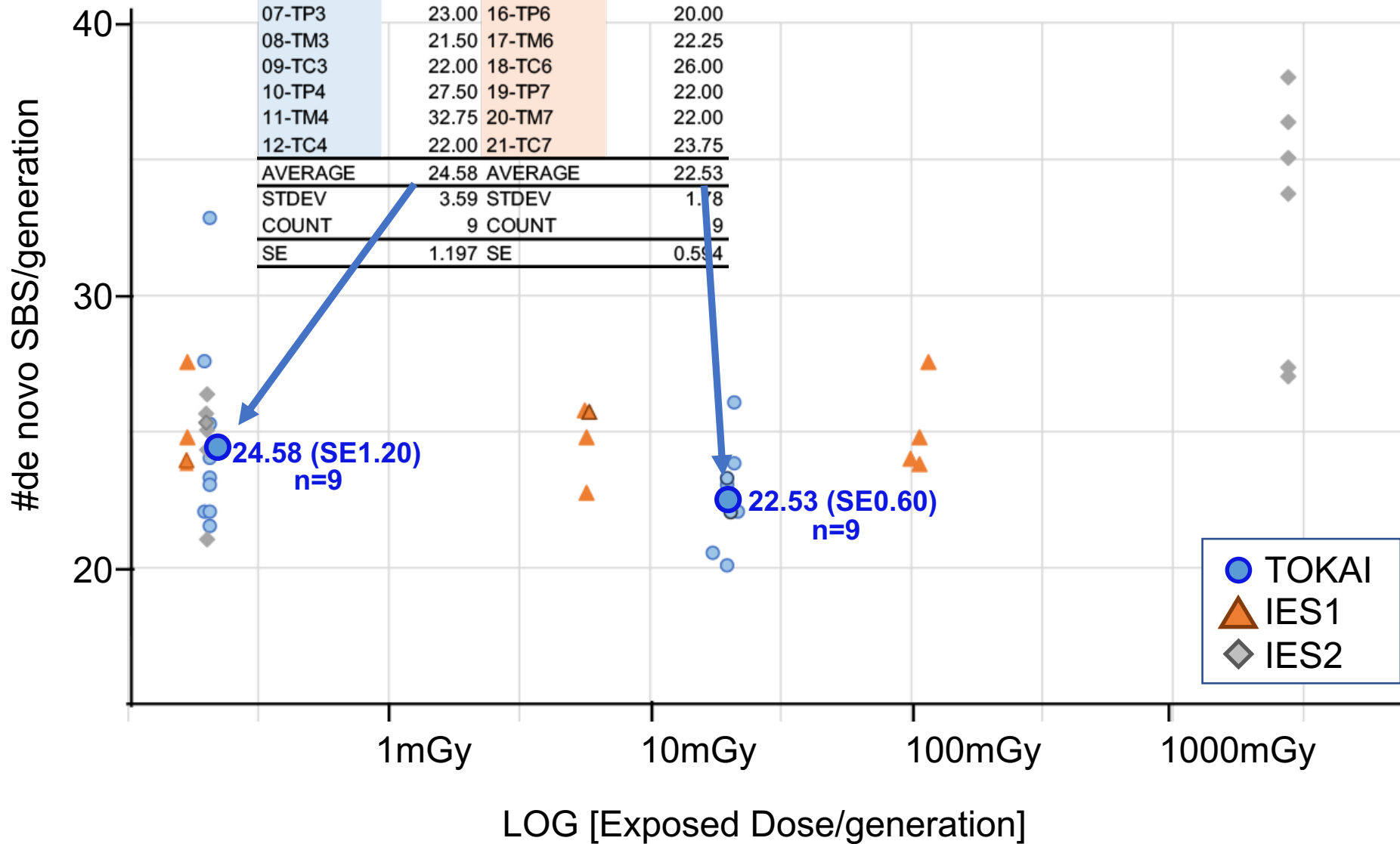
0.15mGy/day



In this presentation, we focus on detected Single Base Substitutions (SBSs) on 19 pairs of mouse autosomes.

TOKAI

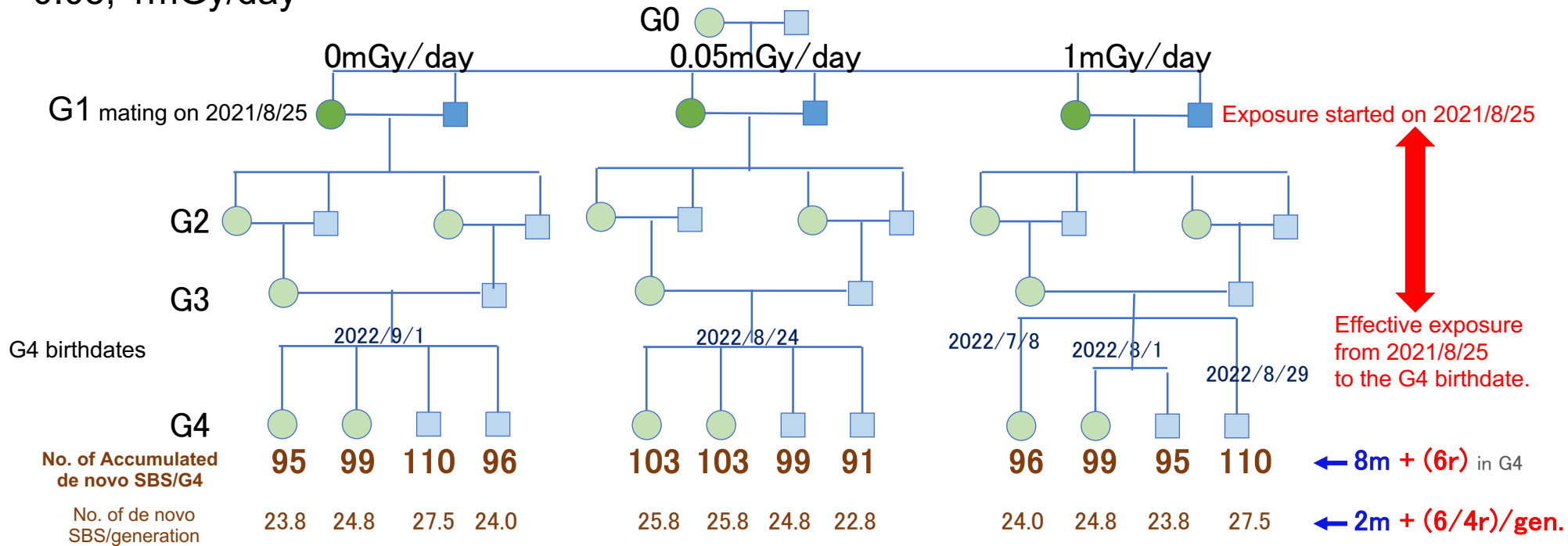
0mGy/d マウス検出新SNV	0.15mGy/d マ'検出新SNV
04-TP2	25.25
05-TM2	24.00
06-TC2	23.25
07-TP3	23.00
08-TM3	21.50
09-TC3	22.00
10-TP4	27.50
11-TM4	32.75
12-TC4	22.00
<hr/>	
AVERAGE	24.58
STDEV	3.59
COUNT	9
SE	1.197
<hr/>	
AVERAGE	22.53
STDEV	1.78
COUNT	9
SE	0.594

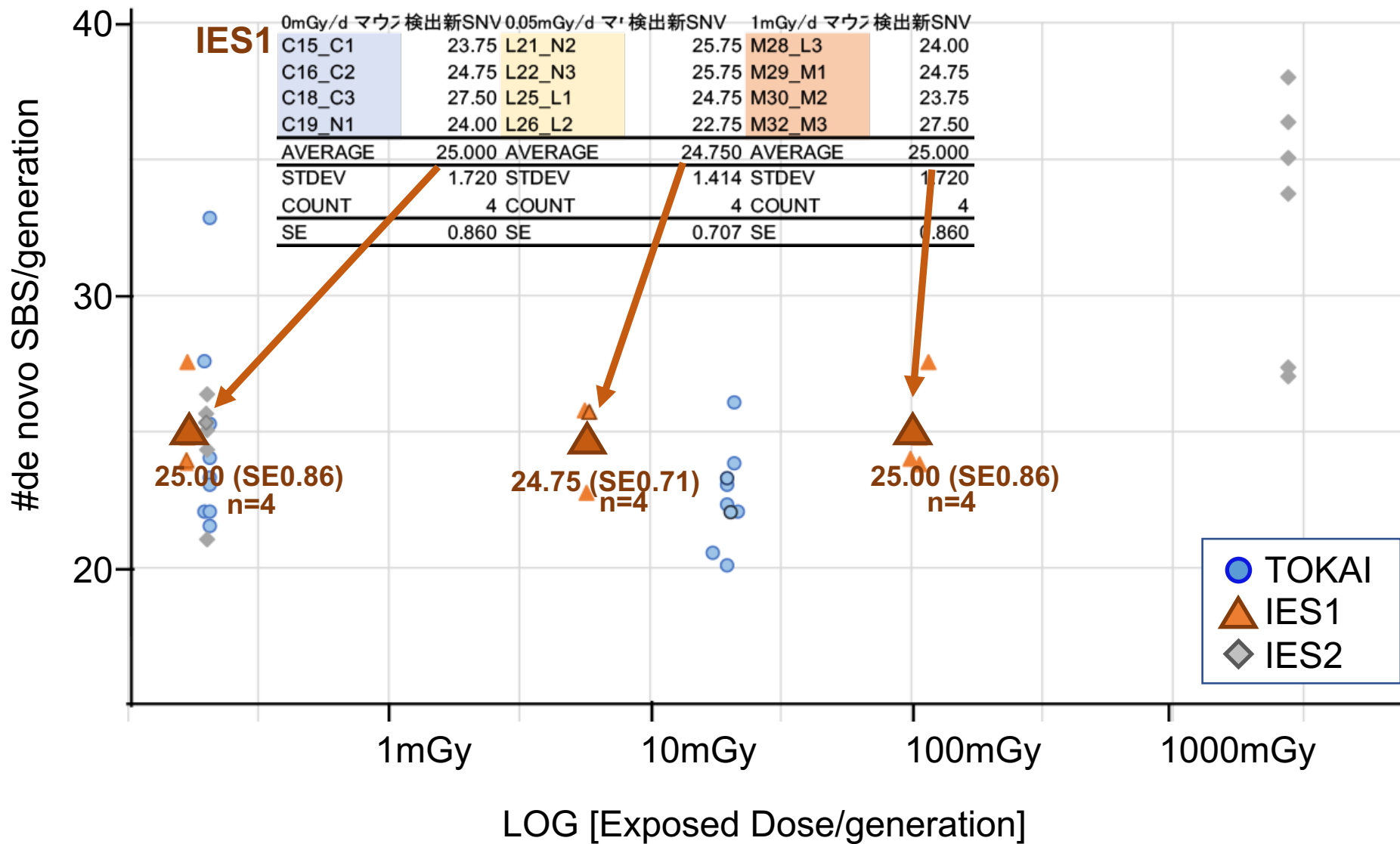


IES1

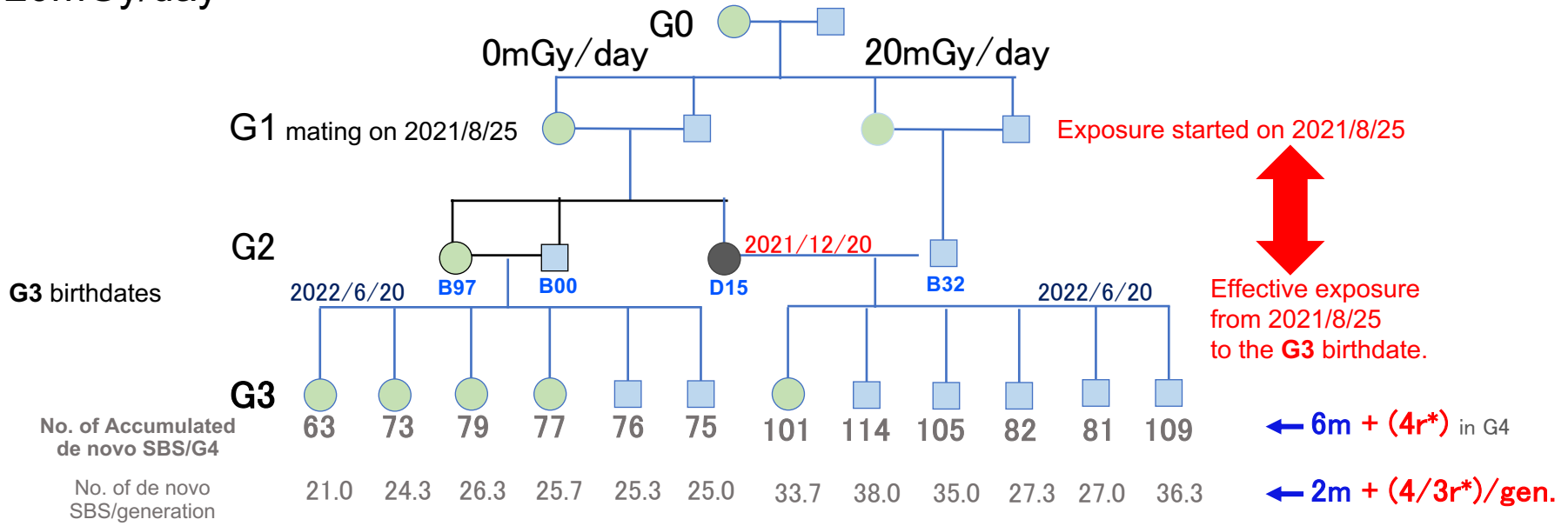
^{137}Cs γ -ray

0.05, 1mGy/day



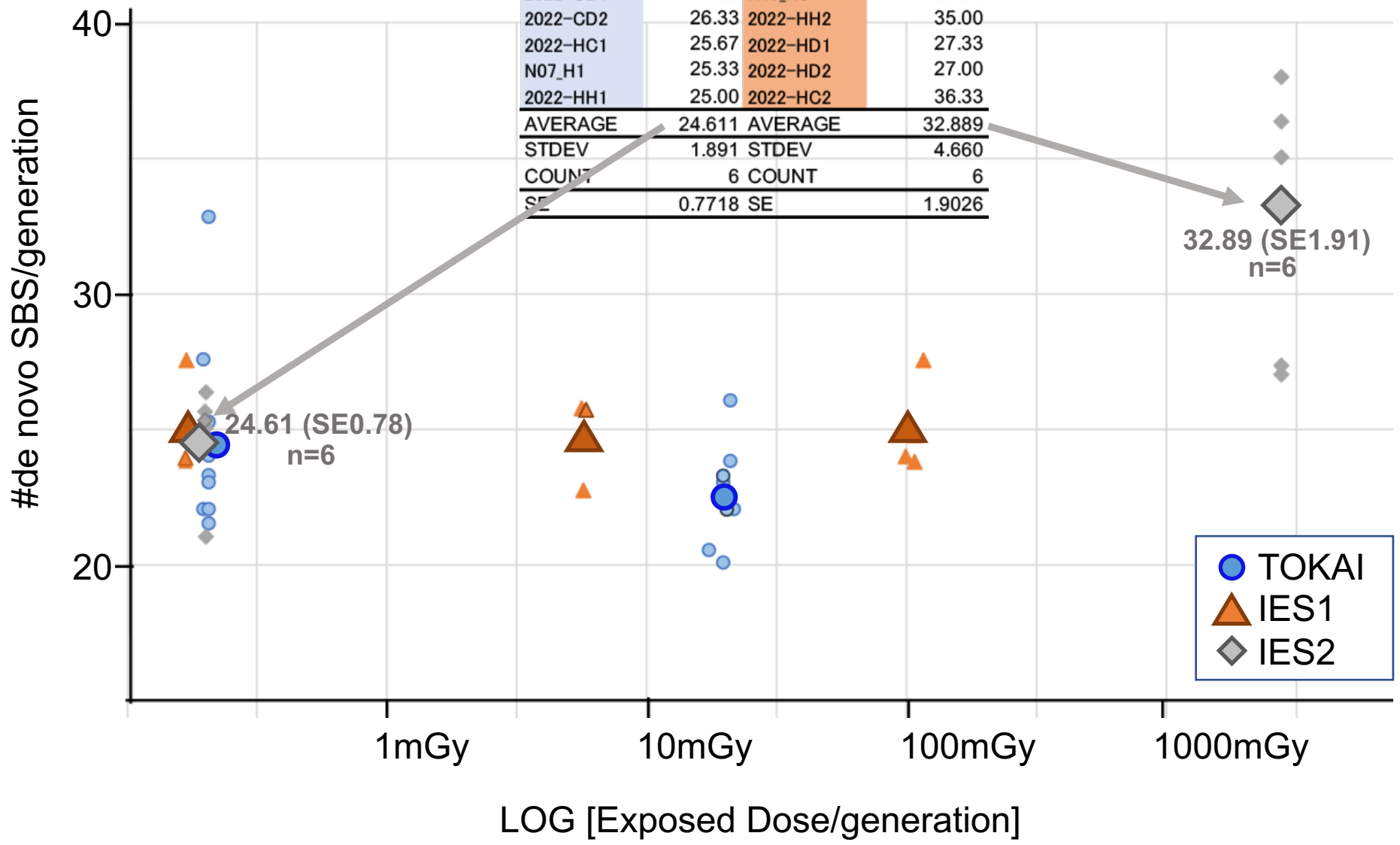


IES2
 ^{137}Cs γ -ray
 20mGy/day

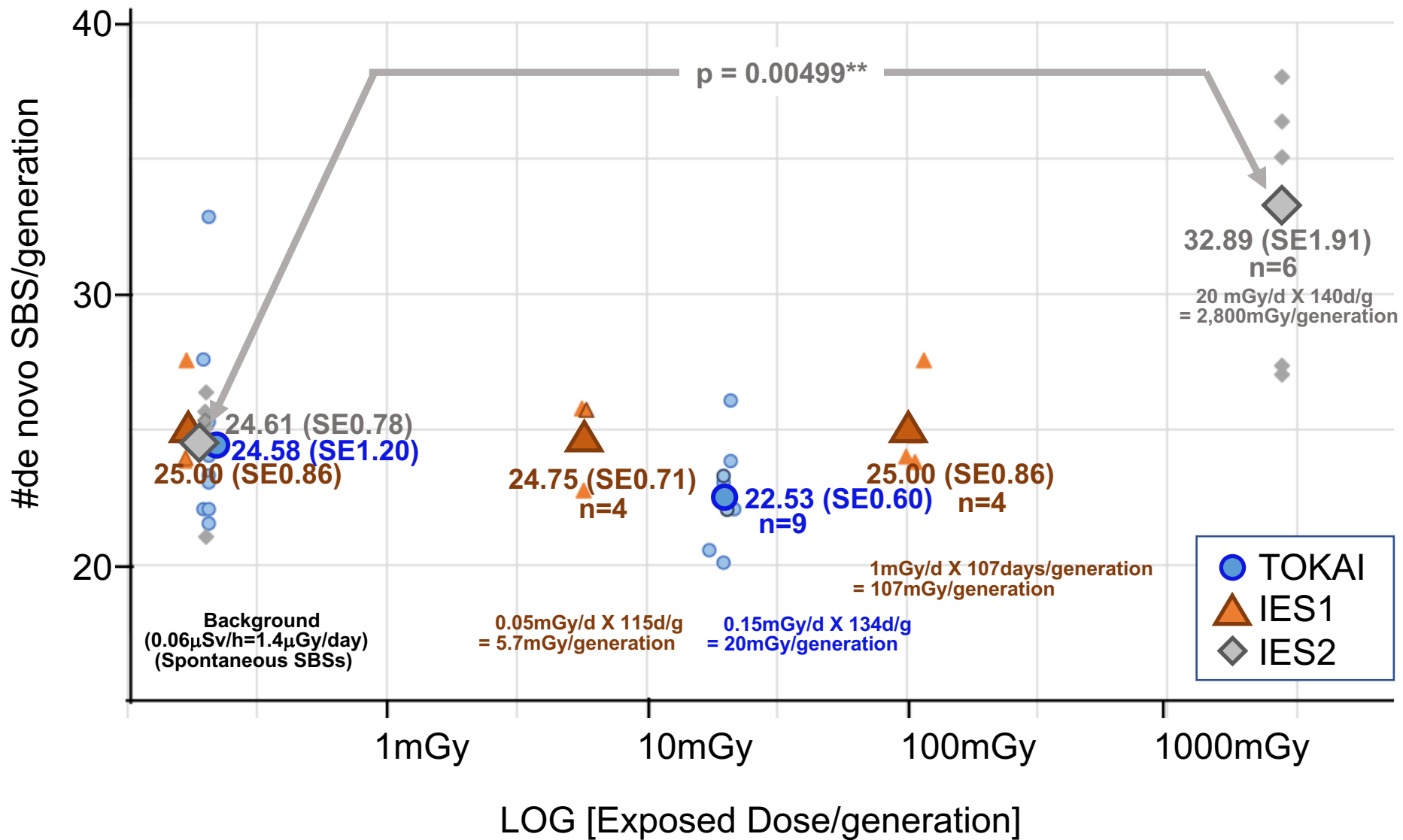


IES2

0mGy/d マウス 検出新SNV		20mGy/d マウス 検出新SNV	
N03_P3	21.00	H09_H2	33.67
2022-CD1	24.33	H10_H3	38.00
2022-CD2	26.33	2022-HH2	35.00
2022-HC1	25.67	2022-HD1	27.33
N07_H1	25.33	2022-HD2	27.00
2022-HH1	25.00	2022-HC2	36.33
AVERAGE		AVERAGE	32.889
STDEV		STDEV	4.660
COUNT		COUNT	6
SE		SE	1.9026



● TOKAI
 ▲ IES1
 ◆ IES2



Estimation of Mutation Rate

Experiment	Detected SBS/gen	EWC* (bp)	Mutation rate**
TOKAI (control)	24.58 (SD3.59)	1,973,810,746	6.23E-09/bp/generation
TOKAI (0.15mGy/d)	22.53 (SD1.79)		5.71E-09/bp/generation
IES1 (control)	25.00 (SD1.72)	2,016,473,366	6.20E-09/bp/generation
IES1 (0.05mGy/d)	24.75 (SD1.42)		6.14E-09/bp/generation
IES1 (1mGy/d)	25.00 (SD1.72)		6.20E-09/bp/generation
IES2 (control)	24.61 (SD1.90)	2,023,900,065	6.08E-09/bp/generation
IES2 (20mGy/d)	32.89 (SD4.66)		8.13E-09/bp/generation

*EWC: Effective whole-genome coverage (Uchimura et al., Genome Res 2015; Satoh et al. Sci Rep 2020); Uchimura, this session.

**Mutation Rate = (Detected SBS/gen) / (2 X EWC)

SUMMARY

- **~100 de novo SBSs were detected per G4 mouse with by the expanded TRIO analysis with WGS/bioinformatics pipeline.**
- **A total of 4085 (or 3927 on autosomal) de novo SBSs were detected from twelve G3 mice and thirty G4 mice.**
- **Quick (~1 yr for mating and 0.5 yr for WGS/bioinformatics),**
- **minimum number of mice,**
- **cost effective (42 mice X 1KUSD for WGS), and**
- **highly reproducible results (three control datasets!).**
- **SBSs were significantly induced by 2.8 Gy (20mGy/day X 140 days/generation). --- Further careful calculation is necessary to estimate precise mutation rate.**
- **All the WGS datasets will be freely open to public vis SRA/DRA.**
- **Whole bodies of irradiated and non-irradiated mice were kept at -80°C. Is this frozen mouse archive worth sharing by making it open to public?**

Some AGENDAs/TAKEHOME MESSAGEs

- **Validation of LNT at very low dose/dose rate?**
Further studies between 1mGy/d X 100 days/generation
and 20mGy/d X 140 days/generation.
- **Further assessments are feasible?**
e.g., mutational signature profiling, small indels, large structural variations,
females vs males, age effect, diet and other environmental factors, etc.
by using the same datasets and/or archived tissues and DNA samples.
- **Are mouse data applicable to human?**
or other species/different genetic backgrounds?
- **Need of new mathematical modeling?**
e.g., WAM model by Bando et al. Poster 2098@ICRP2023;
Toki et al. Poster 2099@ICRP2023; and
Tsunoyama et al. OS2-2@JRRS 2023.
- **Infrastructures for sharing tissues/DNA samples?**
A new budget(s) needed for archiving and shipping/handling.
Or, crowdfunding to enhance mutual communication with societies.
- **Collaborative efforts for various agendas ?**
by gathering more multidisciplinary researchers
and/or by organizing an international consortium ?