Transgenerational Effects in Mice Exposed to Continuous Low-Dose-Rate Gamma-Rays
– Genome-Wide Approach for Germ Cell Mutation –

Keiji OGURA, Satoshi TANAKA, Ignacia BRAGA-TANAKA III, Kazuaki ICHINOHE,
Yoichi OGHISO
Department of Radiobiology

Abstract

To elucidate whether germ cell mutations induced by chronic γ -ray exposure at low-dose-rates can be inherited by offspring, 8-week-old male specific-pathogen-free (SPF) C57BL/6J mice were exposed to γ-rays at a high-dose-rate of 889 mGy/min for 9 minutes, equivalent to a total dose of 8000 mGy at a low-dose-rate of 20 mGy/22 hr/day for about 400 days. Genome-wide molecular comparisons were made to identify mutations that may have been newly generated and inherited by offspring from a parent. These newly identified mutations were then classified based on their nucleotide sequences for comparison between irradiated and non-irradiated control groups. Here we show preliminary examination results on reliability of the array CGH method.

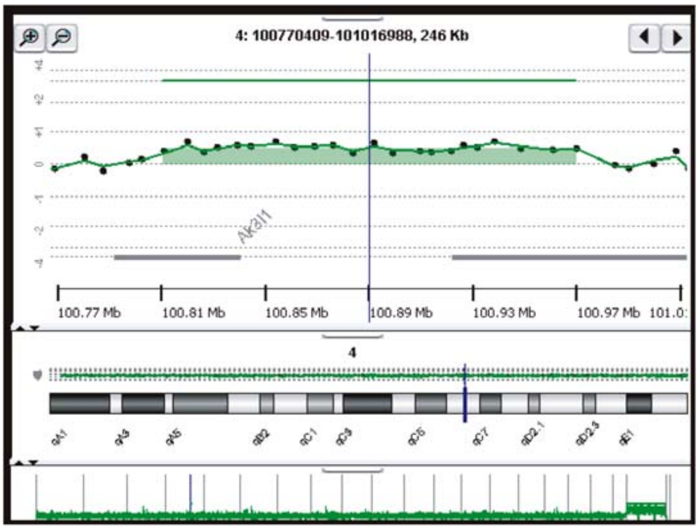


Fig. 1 Log$_2$ transformed ratios are plotted by CGH Analytics 3.4 (Agilent Tech.). Vertical lines represent the whole genome (lower figure), the fourth chromosome (middle figure), and part of the fourth chromosome (upper figure). Gains and losses are represented in the array CGH profiles on the opposite side across zero. The green belt in the upper figure suggests a deletion region in C1 female.

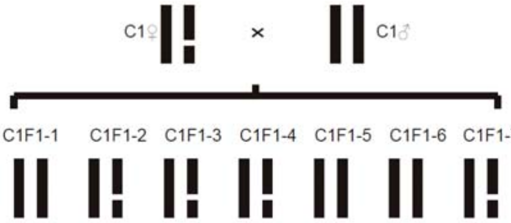
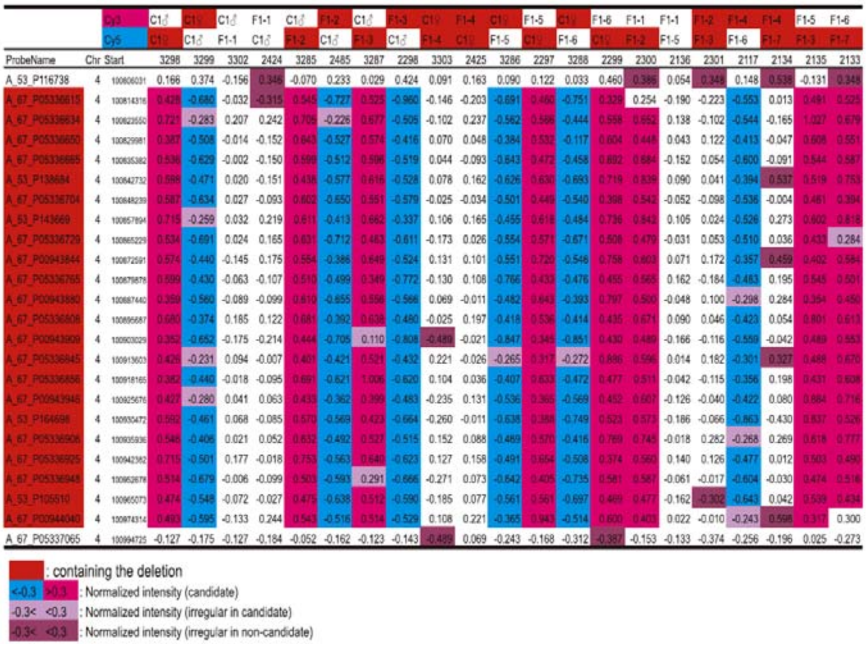


Fig. 2 Summary of deletions between positions 100,814,316 to 100,974,314 in the 4th chromosome. This was found in one non-irradiated control mouse 1 (C1)♀ and inherited by her offspring C1F1-2, C1F1-3, C1F1-4 and C1F1-7.

Table 1 Reliability of the array CGH in non-irradiated C1 parents and their progenies



ProbeName	Chr	Start																				
		Cy3	C1♂	C1♀	C1♂	F1-1	C1♂	F1-2	C1♂	F1-3	C1♀	F1-4	C1♀	F1-5	C1♀	F1-6	F1-1	F1-1	F1-2	F1-4	F1-4	F1-5	F1-6
		Cy5	C1♀	C1♂	F1-1	C1♂	F1-2	C1♂	F1-3	C1♂	F1-4	C1♀	F1-5	C1♀	F1-6	C1♀	F1-2	F1-5	F1-3	F1-6	F1-7	F1-3	F1-7
ProbeName	Chr	Start	3298	3299	3302	2424	3285	2485	3287	2298	3303	2425	3286	2297	3288	2299	2300	2136	2301	2117	2134	2135	2133
A_53_P116738	4	100806031	0.166	0.374	-0.156	0.346	-0.070	0.233	0.029	0.424	0.091	0.163	0.090	0.122	0.033	0.460	0.386	0.054	0.348	0.148	0.538	-0.131	0.348
A_67_P05336615	4	100814316	0.428	-0.680	-0.032	-0.315	0.545	-0.727	0.525	-0.960	-0.146	-0.203	-0.691	0.460	-0.751	0.329	0.254	-0.190	-0.223	-0.553	0.013	0.491	0.525
A_67_P05336634	4	100823550	0.721	-0.283	0.207	0.242	0.706	-0.226	0.677	-0.505	-0.102	0.237	-0.562	0.566	-0.444	0.558	0.652	0.138	-0.102	-0.544	-0.165	1.027	0.679
A_67_P05336650	4	100829981	0.387	-0.508	-0.014	-0.152	0.643	-0.527	0.574	-0.416	0.070	0.048	-0.384	0.532	-0.117	0.604	0.448	0.043	0.122	-0.413	-0.047	0.608	0.551
A_67_P05336665	4	100835382	0.536	-0.629	-0.002	-0.150	0.599	-0.512	0.506	-0.519	0.044	-0.093	-0.643	0.472	-0.458	0.692	0.684	-0.152	0.054	-0.600	-0.091	0.544	0.587
A_53_P138684	4	100842732	0.588	-0.471	0.020	-0.151	0.438	-0.577	0.616	-0.528	0.078	0.162	-0.626	0.630	-0.693	0.719	0.839	0.090	0.041	-0.394	0.537	0.519	0.753
A_67_P05336704	4	100848239	0.587	-0.634	0.027	-0.093	0.602	-0.650	0.551	-0.579	-0.025	-0.034	-0.501	0.449	-0.540	0.398	0.542	-0.052	-0.098	-0.536	-0.004	0.481	0.394
A_53_P143669	4	100857894	0.715	-0.259	0.032	0.219	0.611	-0.413	0.662	-0.337	0.106	0.165	-0.455	0.618	-0.484	0.736	0.843	0.105	0.024	-0.526	0.273	0.602	0.818
A_67_P05336729	4	100865229	0.534	-0.691	0.024	0.165	0.631	-0.712	0.483	-0.611	-0.173	0.026	-0.554	0.571	-0.671	0.508	0.479	-0.031	0.053	-0.510	0.036	0.433	0.284
A_67_P00943844	4	100872591	0.574	-0.440	-0.145	0.175	0.554	-0.386	0.649	-0.524	0.131	0.101	-0.551	0.720	-0.546	0.758	0.603	0.071	0.172	-0.357	0.459	0.402	0.584
A_67_P05336765	4	100879878	0.599	-0.430	-0.063	-0.107	0.510	-0.499	0.349	-0.772	-0.130	0.108	-0.766	0.433	-0.476	0.455	0.565	0.162	-0.184	-0.483	0.195	0.545	0.501
A_67_P00943880	4	100887440	0.359	-0.560	-0.089	-0.099	0.610	-0.655	0.556	-0.566	0.069	-0.011	-0.482	0.643	-0.393	0.797	0.500	-0.048	0.100	-0.298	0.284	0.354	0.450
A_67_P05336808	4	100895687	0.680	-0.374	0.185	0.122	0.681	-0.392	0.638	-0.480	-0.025	0.197	-0.418	0.536	-0.414	0.435	0.671	0.090	0.046	-0.423	0.054	0.801	0.613
A_67_P00943909	4	100903029	0.352	-0.652	-0.175	-0.214	0.444	-0.705	0.110	-0.808	-0.489	-0.021	-0.847	0.345	-0.851	0.430	0.489	-0.166	-0.116	-0.559	-0.042	0.489	0.553
A_67_P05336845	4	100913603	0.426	-0.231	0.094	-0.007	0.401	-0.421	0.521	-0.432	0.221	-0.026	-0.265	0.317	-0.272	0.886	0.596	0.014	0.182	-0.301	0.327	0.488	0.670
A_67_P05336856	4	100918165	0.382	-0.440	-0.018	-0.095	0.691	-0.621	1.006	-0.620	0.104	0.036	-0.407	0.633	-0.472	0.477	0.511	-0.042	-0.115	-0.356	0.198	0.431	0.608
A_67_P00943946	4	100925676	0.427	-0.280	0.041	0.063	0.433	-0.362	0.399	-0.483	-0.235	0.131	-0.536	0.365	-0.569	0.452	0.607	-0.126	-0.040	-0.422	0.080	0.884	0.716
A_53_P164698	4	100930472	0.592	-0.461	0.068	-0.085	0.570	-0.569	0.423	-0.664	-0.260	-0.011	-0.638	0.388	-0.749	0.523	0.573	-0.186	-0.066	-0.863	-0.430	0.837	0.526
A_67_P05336906	4	100935936	0.546	-0.406	0.021	0.052	0.632	-0.492	0.527	-0.515	0.152	0.088	-0.489	0.570	-0.416	0.769	0.745	-0.018	0.282	-0.268	0.269	0.618	0.777
A_67_P05336925	4	100942362	0.715	-0.501	0.177	-0.018	0.753	-0.563	0.640	-0.623	0.127	0.158	-0.491	0.664	-0.508	0.374	0.560	0.140	0.126	-0.477	0.012	0.503	0.490
A_67_P05336948	4	100952678	0.514	-0.679	-0.006	-0.099	0.503	-0.593	0.291	-0.666	-0.271	0.073	-0.642	0.405	-0.735	0.581	0.587	-0.061	-0.017	-0.604	-0.030	0.474	0.516
A_53_P105510	4	100965073	0.474	-0.548	-0.072	-0.027	0.475	-0.638	0.512	-0.590	-0.185	0.077	-0.561	0.561	-0.697	0.469	0.477	-0.162	-0.302	-0.643	0.042	0.539	0.434
A_67_P00944040	4	100974314	0.493	-0.595	-0.133	0.244	0.543	-0.516	0.514	-0.529	0.108	0.221	-0.365	0.943	-0.514	0.600	0.403	0.022	-0.010	-0.243	0.598	0.317	0.300
A_67_P05337065	4	100994725	-0.127	-0.175	-0.127	-0.184	-0.052	-0.162	-0.123	-0.143	-0.489	0.069	-0.243	-0.168	-0.312	-0.387	-0.153	-0.133	-0.374	-0.256	-0.196	0.025	-0.273

🟥 : containing the deletion
<-0.3 >0.3 : Normalized intensity (candidate)
-0.3< <0.3 : Normalized intensity (irregular in candidate)
-0.3< <0.3 : Normalized intensity (irregular in non-candidate)

Array CGH analysis was performed between non-irradiated C1 ♀, ♂ and their progenies. The red boxed probe IDs represent the deletions shown in Fig. 2. A combination of Cy3 -labeled normal mouse and Cy5-labeled deletion mouse usually showed normalized intensities in each probe of more than 0.3, whereas the reverse combination showeded normalized intensities in each probe of less than -0.3. Irregular results accounted for 4.2 % (13/ 300). Combinations between normal mice or deletion mice usually show normalized intensities.