Predicting Individual Radiation Sensitivity: Current and Evolving Technologies

Multiple Genetic Variants Associated With Risk of Adverse Reactions Following Radiotherapy in Cancer Patients: A large-scale Candidate Genes Approach

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Multiple Genetic Variants Associated With Risk of Adverse Reactions Following Radiotherapy in Cancer Patients: A large-scale Candidate Genes Approach

1. Strategy

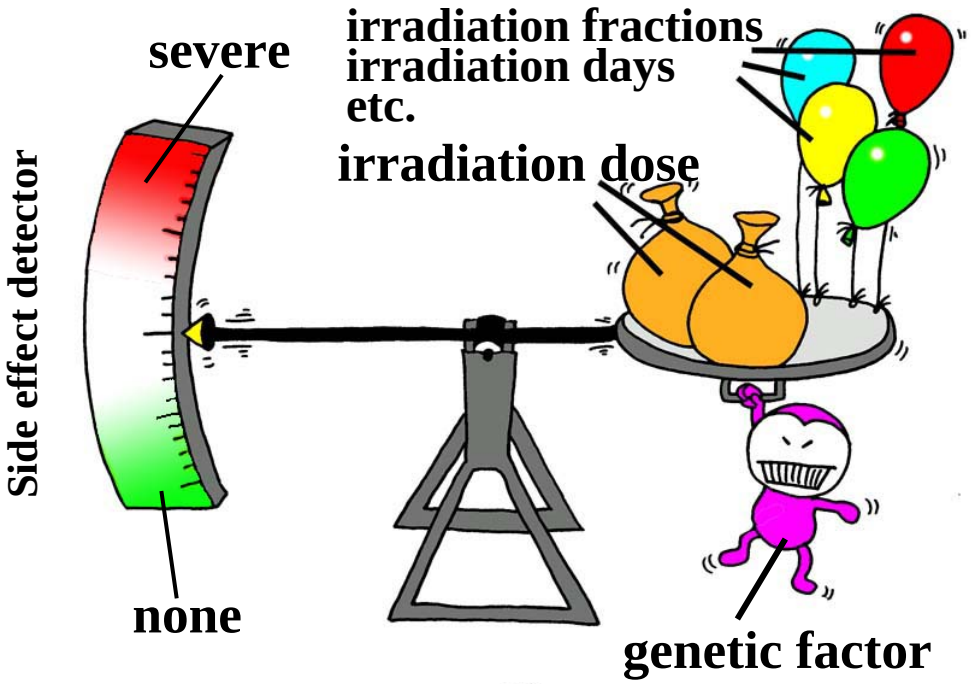
   Clinical samples

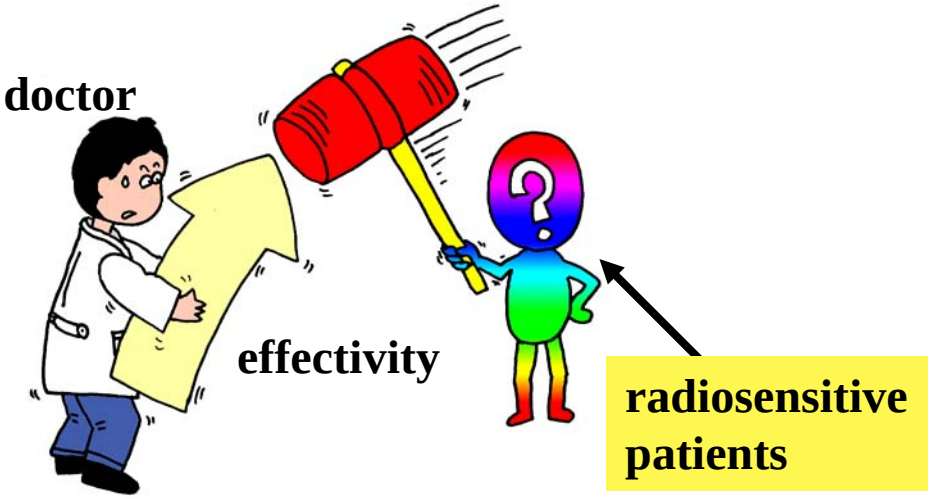
   Candidate genes for SNP typing

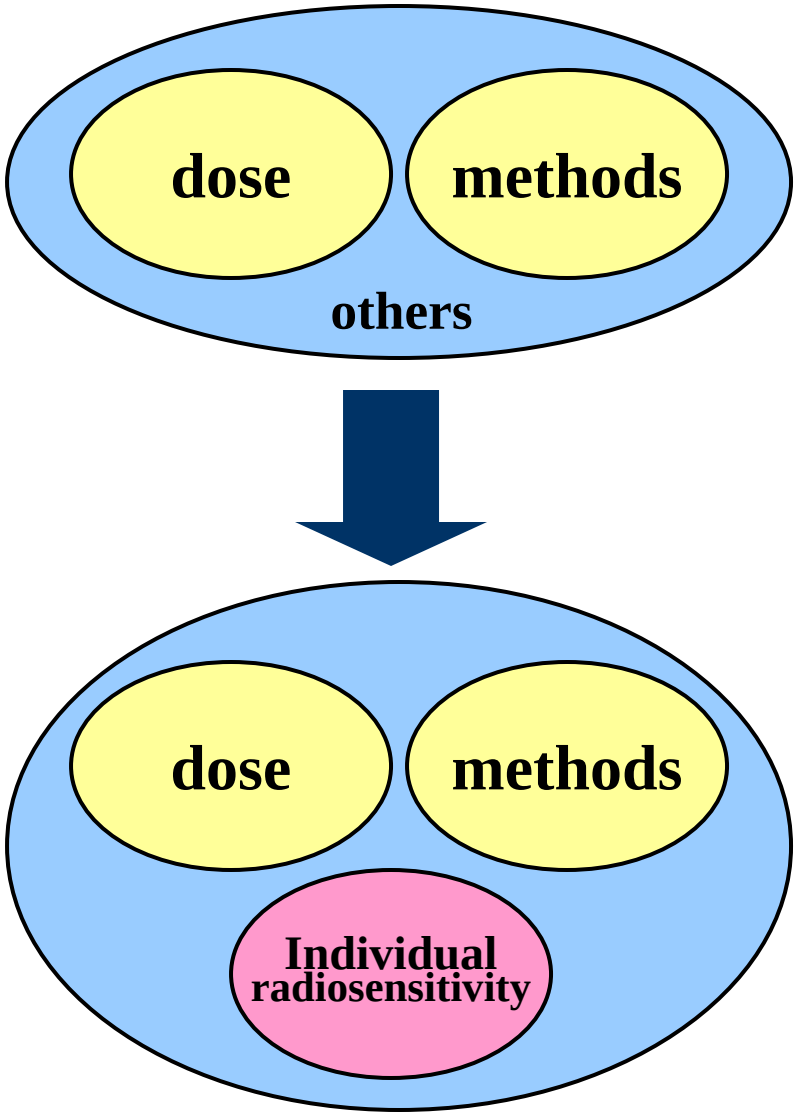
2. Results of haplotype analysis of genes associated with risk of adverse skin reaction in breast cancer patients

3. Summary

Genetic factors cause adverse reactions







How can we find SNPs associated with a risk of adverse reactions?

To perform a genetic analysis:

1. Patients: Which cancer and adverse reactions should be analyzed first as a model case?

2. Genes: Which genes should be analyzed?
   How? A genome scan method or candidate genes approach?

3. Statistics: Which genetic analytical methods would provide the most useful information?

Patients: which cancer and adverse reactions to analyze genetic variance in radiosensitivity?

1.

Breast Cancer

- Breast-conserving RT: 50 Gy/25 Fr
- Stage I, II

The procedure for the breast-conserving radiotherapy is established and the resultant skin reactions are easy to score.

2.

Prostate Cancer

- Carbon-ion: 66 or 60 GyE/20 Fr/5 w
- Stage I, II

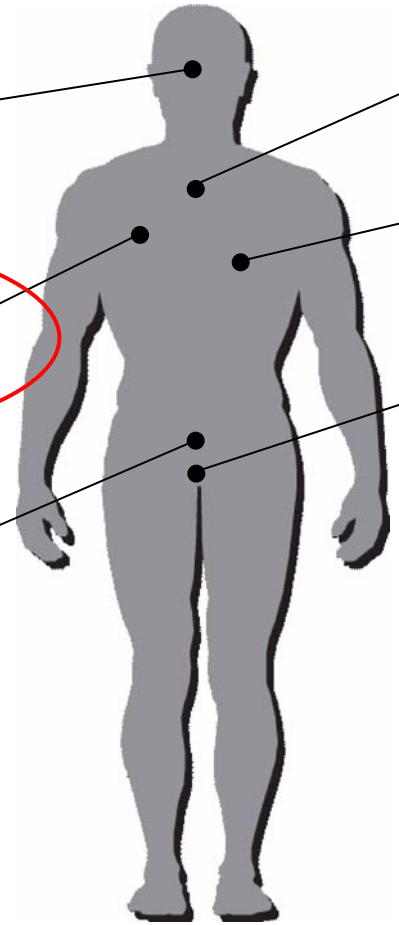
We had access to patients involved in two phase I/II clinical studies performed in our institute's hospital.

One advantage of analyzing a group of patients from a single hospital was that factors such as variations in therapeutic protocols and possible scoring differences between examiners from multiple institutions, could not affect the results.

3, 4, 5 ---- will be described tomorrow afternoon by MI.

Clinical blood samples

Cancer patients 2327
(Oct. 2001〜Dec. 2007)



Head and Neck	269
Breast	748
Cervical	309
Esophagus	71
Lung	241
Prostate	624
Others	65

Healthy donors 227

RadGenomics NIRS

A genome scan method or candidate genes approach?

- <u>Genome scan</u>

  Powerful
  few published papers at 2001
  too expensive
  labor-intensive

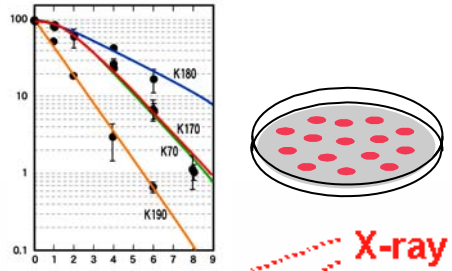
- <u>Candidates genes approach</u>

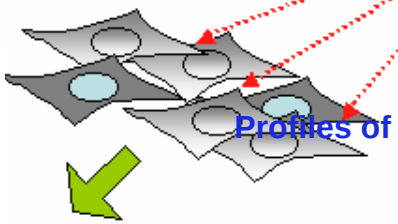
  Comprehensive gene expression analysis was becoming popular.
  Microarray methods to identify genes involved in radiosensitivity
  SNPs in the public databases

  This approach may not detect all SNPs that play a role in radiosensitivity.

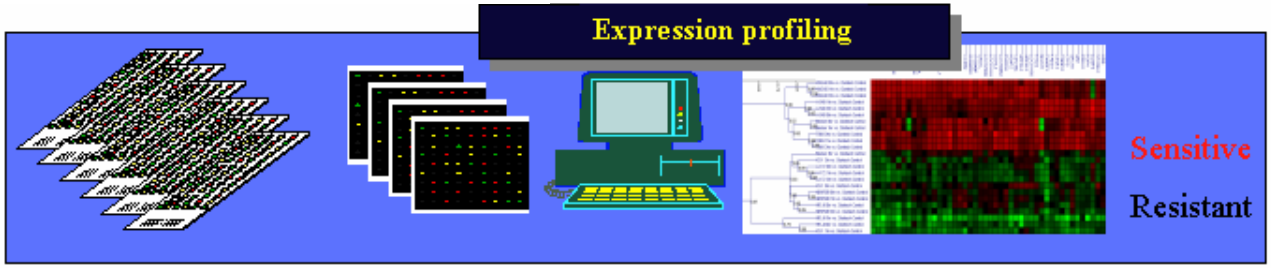
Selection of candidate genes for SNP typing

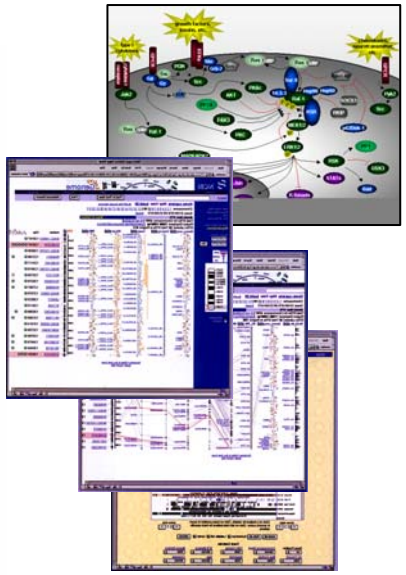
Human cell lines with highly variable in vitro radiosensitivity



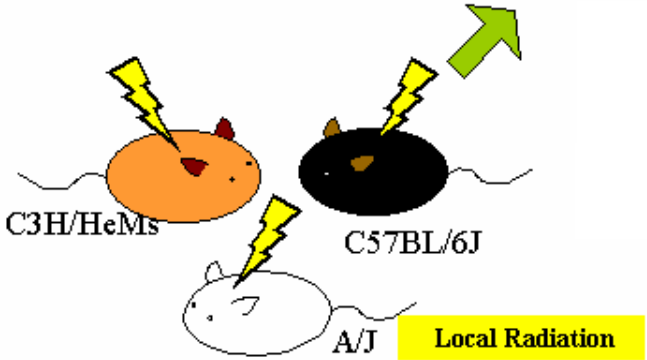


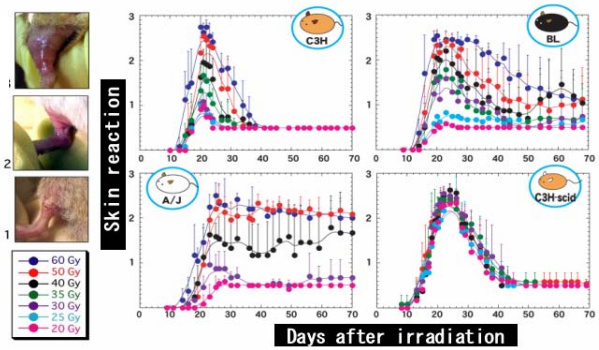
Comparison of gene expression among the cell lines could reveal factors which contribute to the variation in the radiosensitivity of the cells





Selecting genes identified in the literature as playing a role in radiation sensitivity.





Mouse strains with differing radiosensitivity

Analysis of gene expression profiles of cell lines with different radiosensitivity

Genes which can classify the cell lines into two groups based on their D_{10} values

Oligo-array (25K genes)

↓

Cluster analysis

↓

Cluster analysis

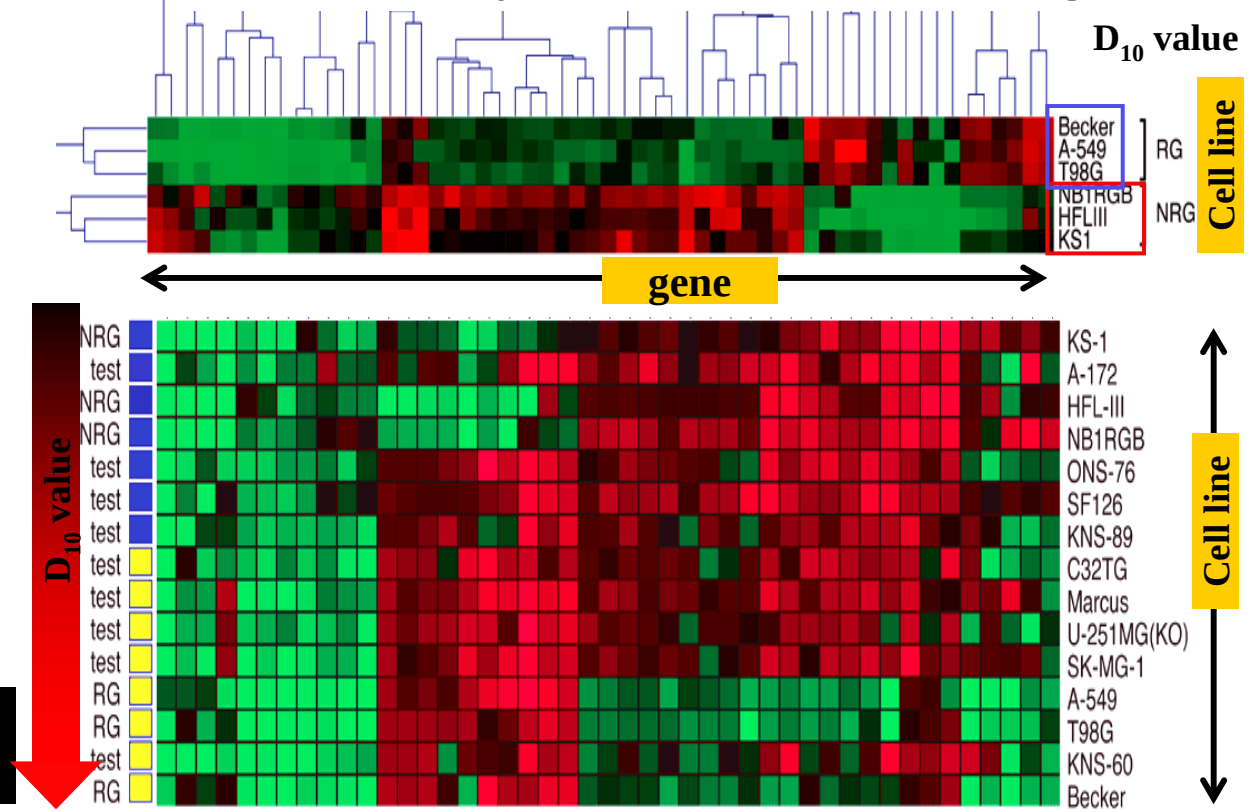
↓

Cluster analysis

↓

Classifier analysis
44 genes were selected

2D cluster analysis of the cell lines with 59 genes



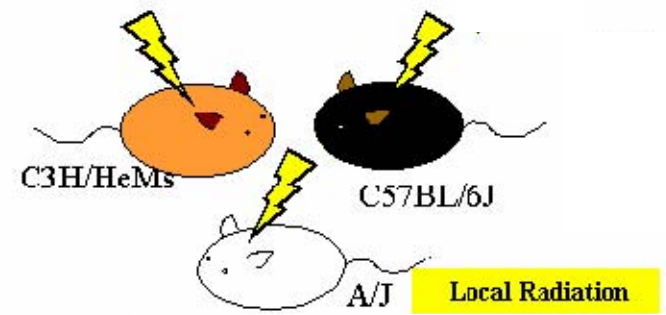
Gene expression : low Gene expression : high

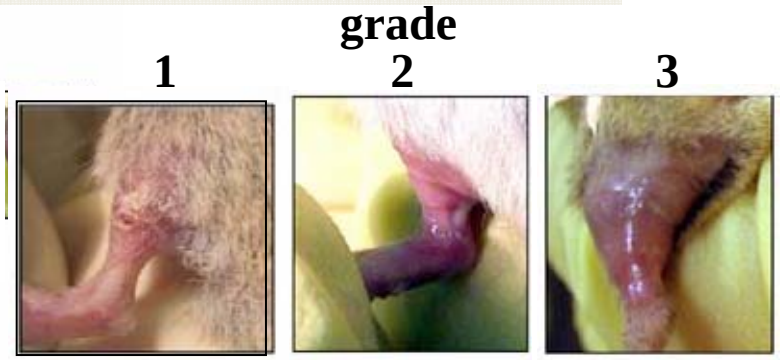
If a change in gene expression or gene function was the result of genetic variation, then the SNPs we were looking for were likely to be found within these genes.

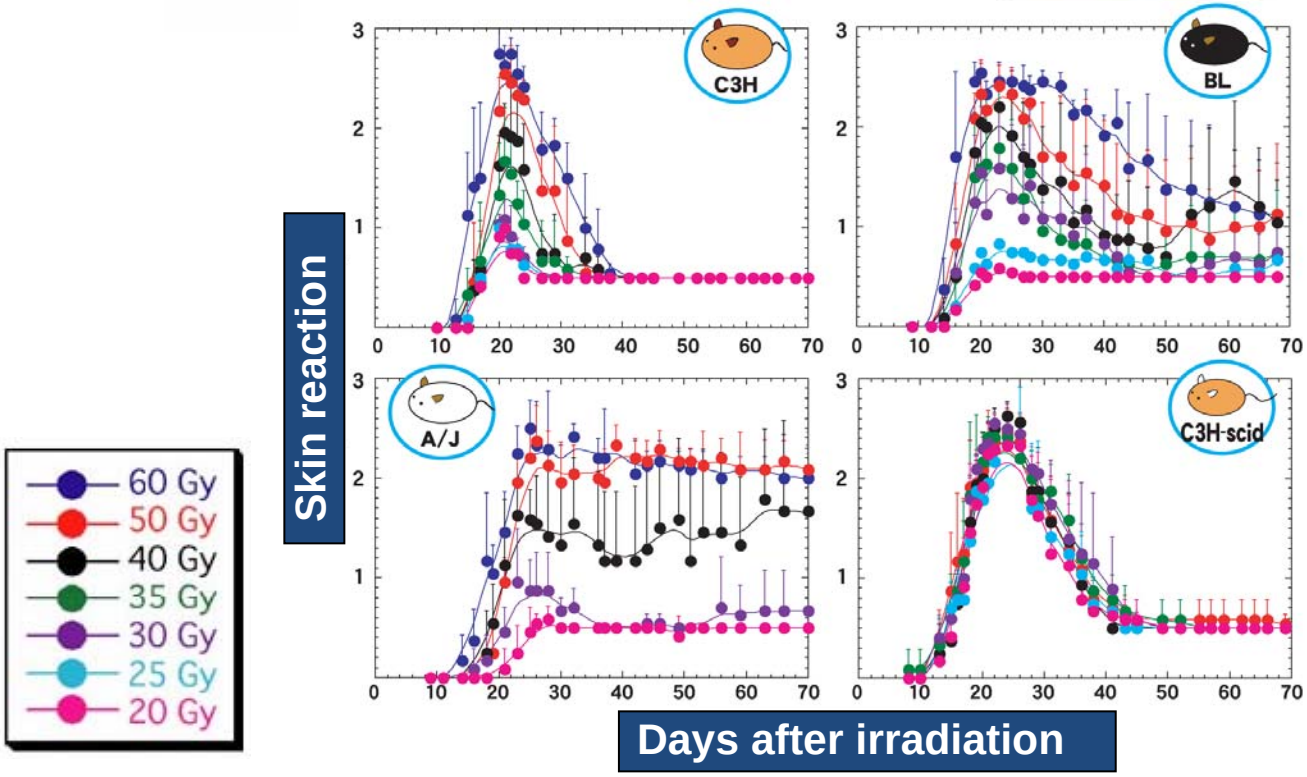
Ishikawa et al. IJROBP 65: 234-245, 2006

RadGenomics NIRS

Strain-dependent radiosensitivity in mice







Iwakawa M. et al. JRR 2003, 2004
Oota T. et al. JRR 2004
Noda et al. Cancer Detect Prev 2005

<u>Skin reactions in three strains following a single localized radiation dose.</u>

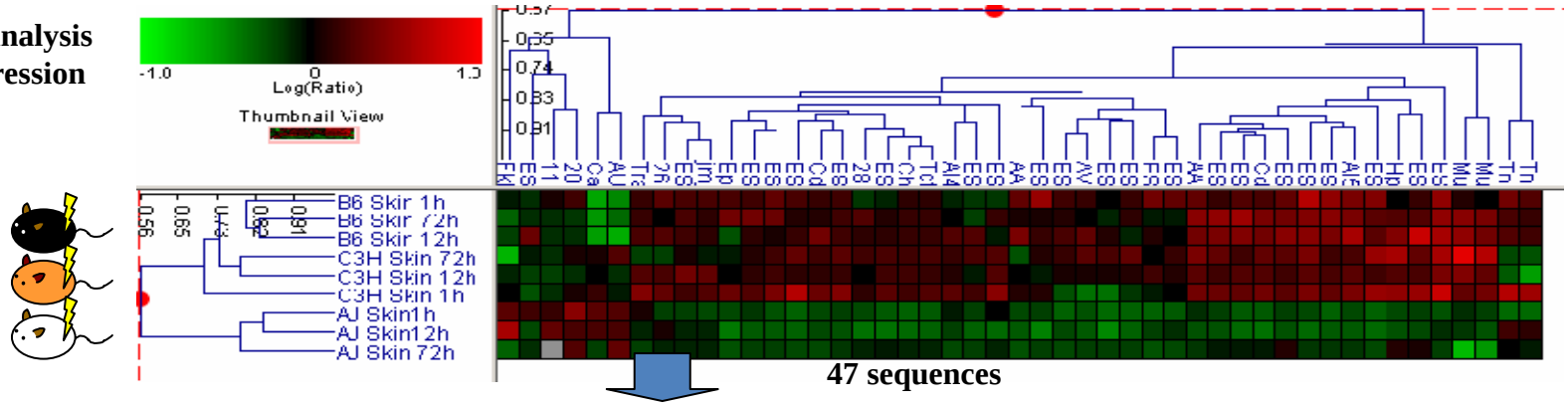
Different degrees of skin reactions between strains of mice were observed during the period between irradiation and healing.

All strains showed dose-dependent skin damage, but regardless of the dose, C3H showed the skin recovered to a state of mild damage within 40 days after irradiation.

Recovery times in A/J was longer than in C57BL mice.

Expression profiles of skin among 3 strains after irradiation

2D cluster analysis of gene expression

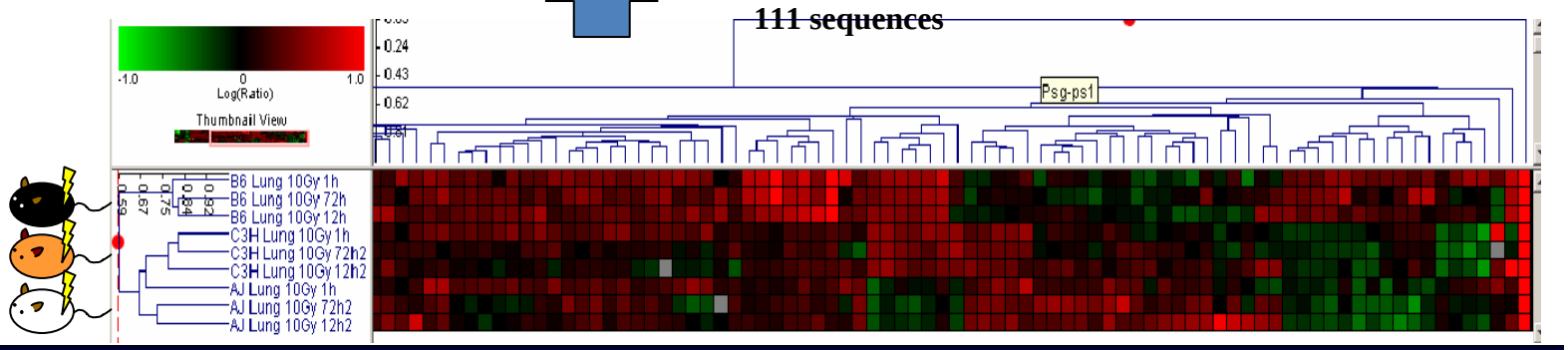


47 sequences

Select genes

"Bioset Compare"

111 sequences



Expression profiles of lung among 3 strains after irradiation

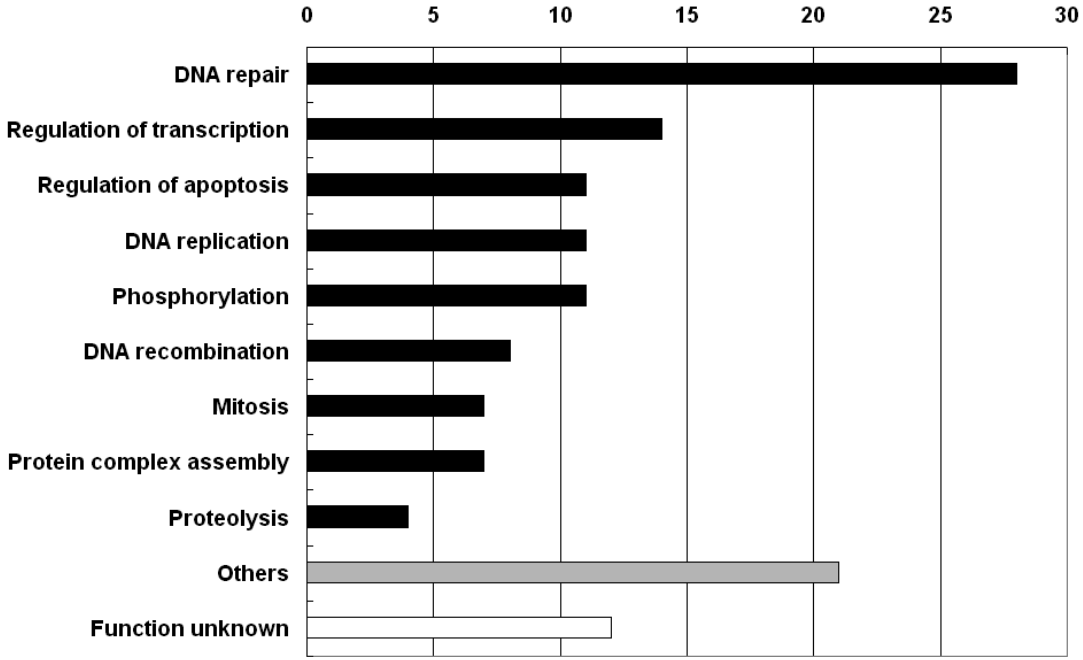
The gene expression data revealed clusters formed by strains but not time or dose parameters.

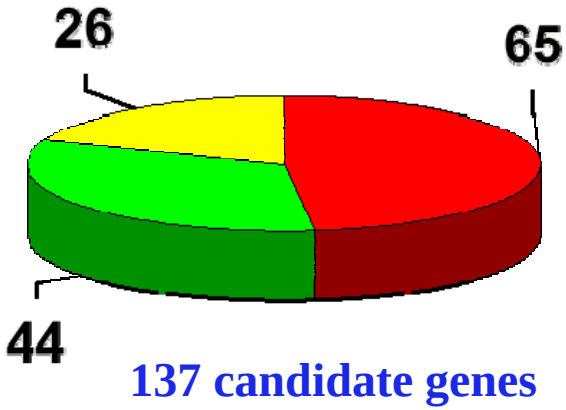
<u>A similar analysis of RNA extracted form the lung of irradiated mice</u>
The expression profiles were strain-dependent but not time or dose dependent.

The genes selected form the 2D cluster analysis could contribute to strain difference in radiosensitivity.

Iwakawa et al. 2004

Ontological classification of the candidate genes for typing

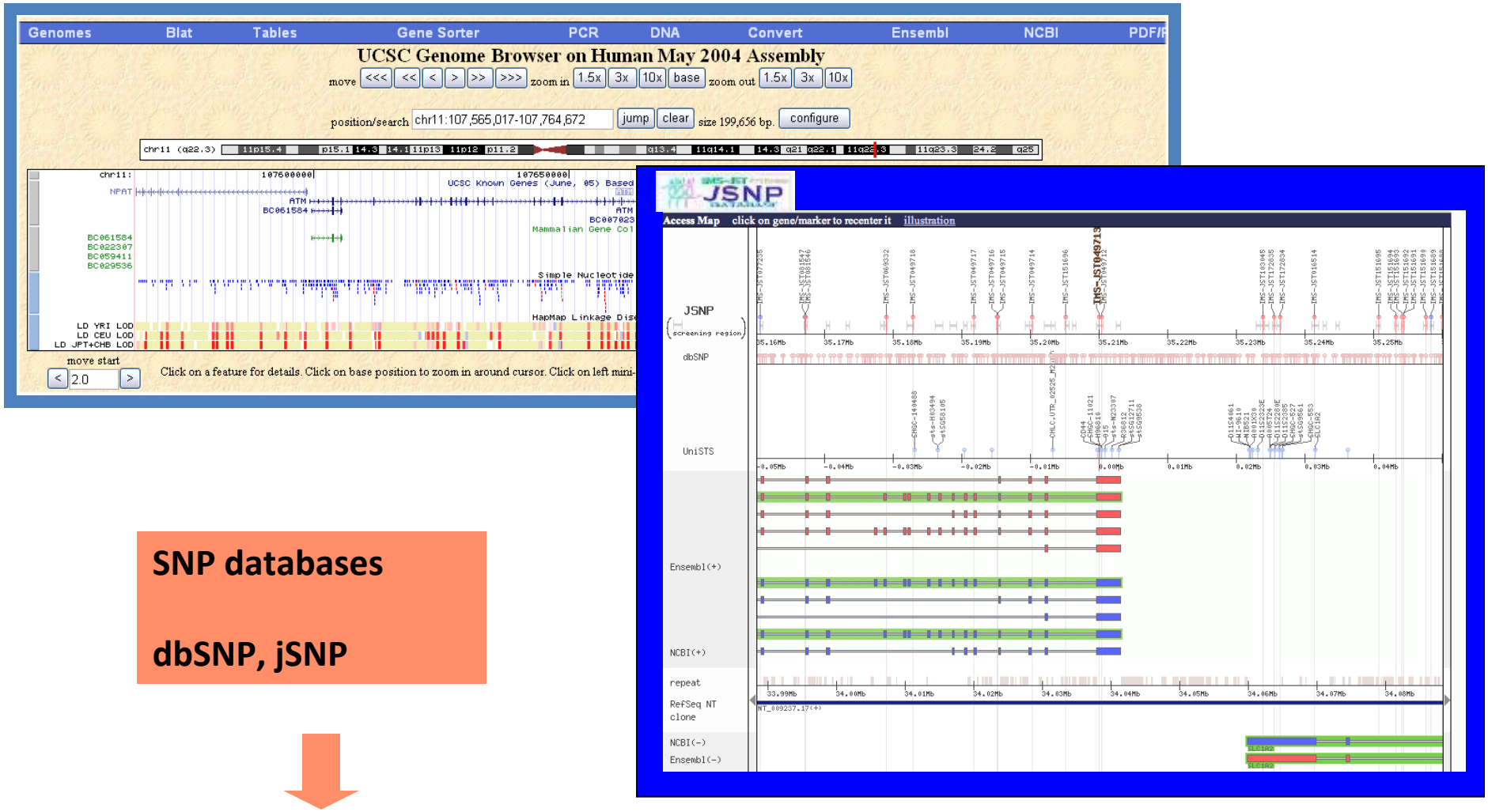




- human cell lines analysis
- mouse inter-strain variance
- document retrieval

A total of 137 candidate genes were assigned to biological categories by the Gene Ontology database at hierarchy level 7.

SNP selection



SNP databases

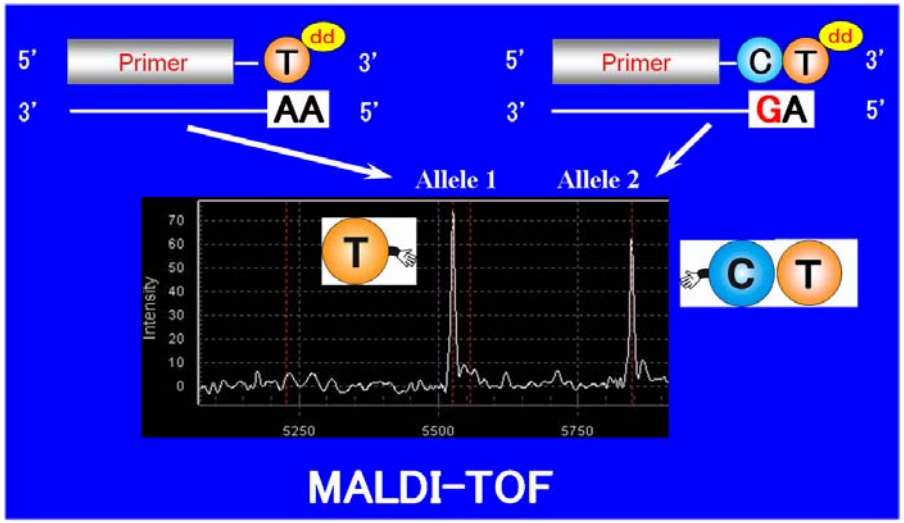
dbSNP, jSNP

1264 SNPs for 182 Genes (2008)

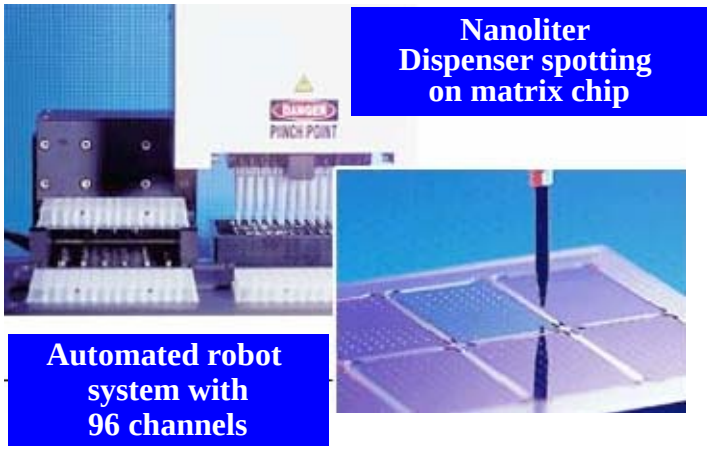
The mean value of the selected SNP sites per gene was 7 in this study.

SNP typing system

Allele-specific termination of primer extension generates the weight differences between two alleles.



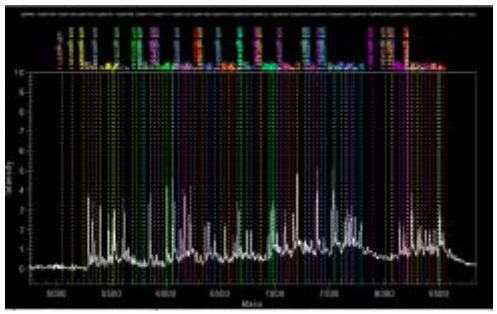
MassARRAY system



Nanoliter Dispenser spotting on matrix chip

Automated robot system with 96 channels

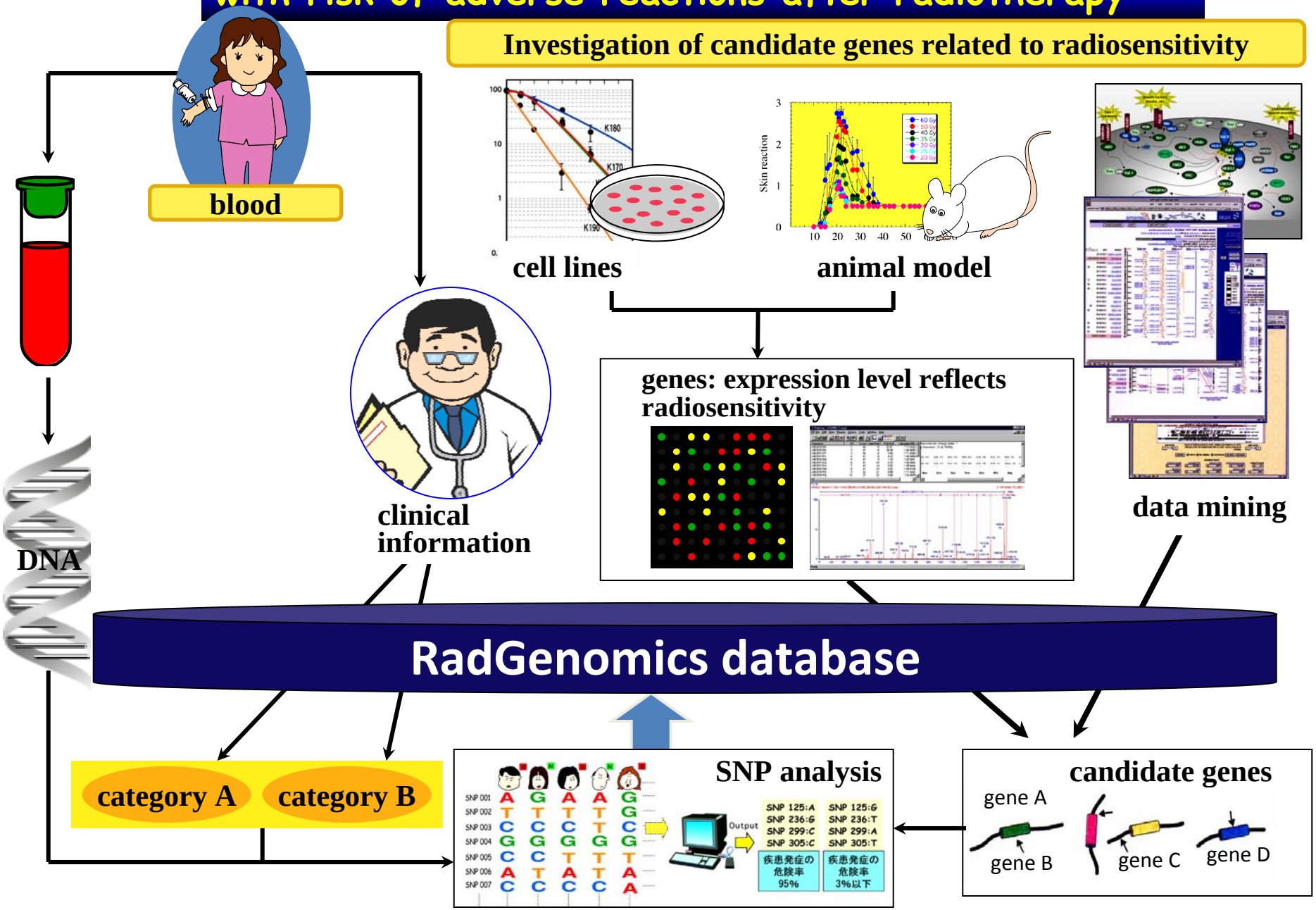
29 multiplex



MALDI-TOF analyzer

The weight differences can be accurately measured by mass spectrometry.
30 reaction-products can be detected in a single run.

Strategy for identification of variants associated with risk of adverse reactions after radiotherapy



Multiple Genetic Variants Associated With Risk of Adverse Reactions Following Radiotherapy in Cancer Patients: A large-scale Candidate Gene Approach

1. Strategy

   Clinical samples

   Candidate genes for SNP typing

2. Results of haplotype analysis of genes associated with risk of adverse skin reaction in breast cancer patients

3. Summary

Clinical samples - 1

•Initially, we assessed 284 breast cancer patients who underwent radiotherapy with breast-conserving surgery from 4 collaborating institutions in Japan.

<u>Patient & treatment Information</u>

Patients who underwent mastectomy were excluded, and the collaborating institutions were all equipped with appropriate treatment modalities.

We graded radiation dermatitis according to the NCI-CTC scoring system.
A total of 45 clinical factors were examined for adverse effects.

Clinical samples - 2

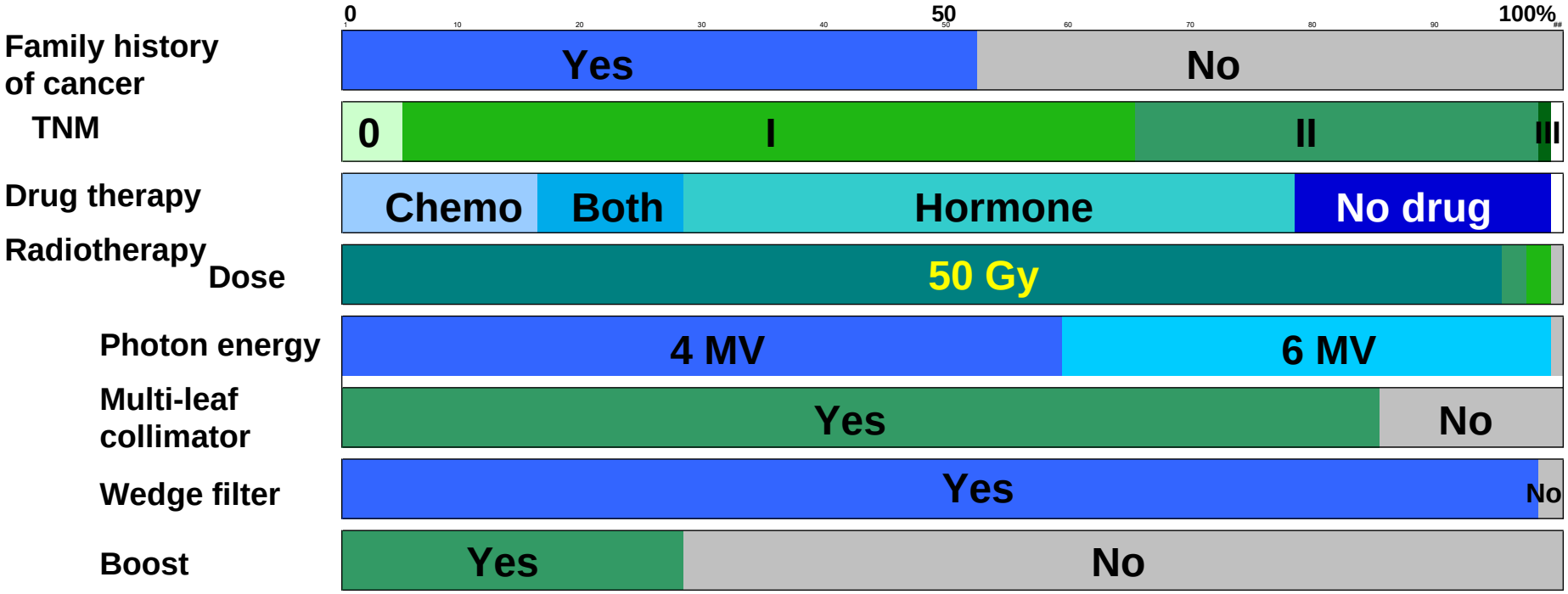
Select eligible institutions

The results showed that the institution, operative procedure, and magnitude of photon energy were associated with adverse skin reactions.

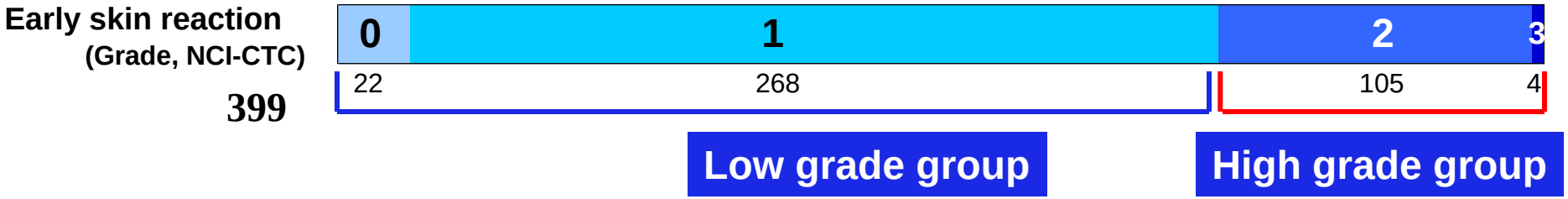
Finally, out of the 284 patients, 154 from two institutions were deemed eligible for genetic investigation, and an additional 245 new patients were subsequently included.

Iwakawa M. *et al*. *Breast Cancer* 2006;13:300-307.

Clinical features of the 399 eligible patients



No association between these factors and early skin reaction



For genetic analysis, the patients were divided into two groups according to their skin reactions: the low-grade (LG) group: having a skin reaction grade equal to or less than 1, n = 290, and the high-grade (HG) group: patients with a skin reaction grade greater than or equal to 2, n = 109.

Clinical features of the two categorized groups of the patients

Characteristics	LG (n = 290)		HG (n = 109)		Difference *P*-value
Age at radiotherapy: Mean ± SD (Range)	54 ± 10	(26-88)	50 ± 11	(30-77)	0.032 (CA)
Family history of cancer	157	54.1%	52	47.7%	0.26 (FE)
TNM stage classification*					0.58 (FE)
0	13	4.5%	5	4.6%	
I	179	61.7%	61	56.0%	
II	94	32.4%	42	38.5%	
III	3	1.0%	0	0.0%	
unknown	1	0.3%	1	0.9%	
Drug therapy					0.32 (FE)
Chemotherapy	50	17.2%	12	11.0%	
Hormone therapy	136	46.9%	47	43.1%	
Both	35	12.1%	14	12.8%	
No drug	69	23.8%	33	30.3%	
Unknown	0	0.0%	3	2.8%	
Radiotherapy					
Photon energy level*					0.46 (FE)
4-MV	176	60.7%	60	55.0%	
6-MV	111	38.3%	47	43.1%	
4-MV and 10-MV	3	1.0%	2	1.8%	
Dose (Gy) : Mean (Range)	49.97	(46.0-60.0)	49.87	(46.0-50.0)	
Multi-leaf collimator	244	84.1%	96	88.1%	0.35 (FE)
Wedge filter	285	98.3%	106	97.2%	0.46 (FE)
Boost	86	29.7%	26	23.9%	0.26 (FE)

Outline of statistical analysis

489 SNPs were excluded from the analysis because of a lack of polymorphisns at that site or very low frequency (MAF < 5%).

999 SNPs (137 loci)

↓

510 SNPs (123 loci)

↓

Allele and genotype frequencies

↓ SNPAlyze ver 6.0

25 SNPs (19 loci)

↓

Pairwise linkage disequilibrium (LD) analysis

↓ Haploview, Genome Browser

19 loci

↓

Haplotype analysis

↓ haplo.stats

6 loci

↓

Estimation of risk haplotypes

haplo.stats

↓

5 loci (7 haplotypes)

999 SNPs for 135 Genes

Typed for 399 breast cancer patients

510 SNPs for 123 genes

Used for association study

Example

Association of SNPs between high grade (HG) and low grade (LG) groups

SNPs	Gene			Genotype		Recessive model	
				HG	LG		
rsSNP ID		Chr	Mm	MM/Mm/mm	MM/Mm/mm	*P*-value	Odds ratio (95% CI)
rs2294638	MAD2L2	1	GC	48/45/16	86/146/58	0.0087	1.87 (1.18-2.97)
rs8193	CD44	11	CT	27/64/18	118/133/39	0.0034	0.48 (0.28-0.76)

To avoid false negatives, we set the significance level at 0.05.

showed association with the grade of EASRs in either allele or genotype frequency

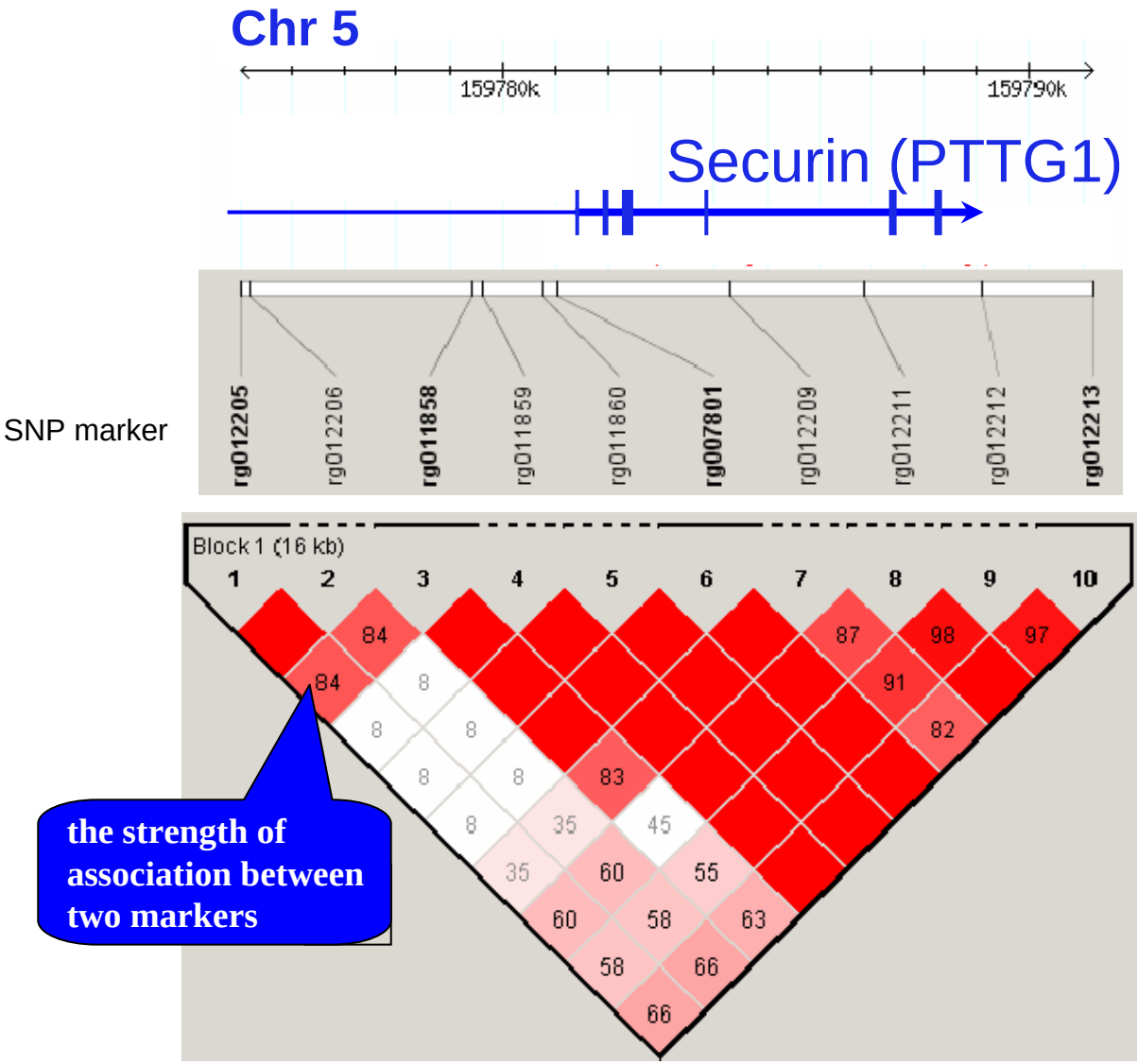
25 SNPs for 19 genes → **Haplotype analysis**

Haplotype analysis

- According to the principles of population genetics, common genetic variation is structured into haplotypes, and the statistical power of association tests using phased data is likely to increase.

- Genetic analysis of haplotype frequencies enables the detection of predisposing haplotypes, even without typing the true functional SNP, by using SNPs for which single-locus analysis shows no association.

Linkage disequilibrium (LD) of securin locus



the strength of association between two markers

Tag SNPs

rg012205 rg011858 rg007801 rg012213

01	03	06	10	
A	C	T	G	.254
G	C	T	G	.248
A	C	T	A	.157
G	T	T	G	.144
G	C	G	G	.089

Determine possible haplotypes consisting of the tag SNP selected from each LD block

Assess the association between haplotypes and the risk of adverse skin reaction.

Overall association between haplotypes and risk of adverse skin reactions

Gene	SNPs for haplotype	P-value*	FDR
RAD9A	rs2255990_rs2286620_rs917570	0.015	0.033
securin	rs2910190_rs3811999_rs1862391_rs2961351	0.016	0.033
LIG3	rs3744355_rs2074518_rs3744357	0.017	0.033
REV3L	rs190246_rs240962	0.023	0.033
CD44	rs187116_rs3794116_rs3794107_rs8193	0.026	0.033
MAD2L2	rs2294636_rs746218	0.04	0.041

FDR, false discovery rate. Haplotype differences with P <0.05 and FDR < 0.05 were detected.

<u>Tag SNPs</u>: haploview (http://www.broad.mit.edu/mpg/haploview/)
Barrett JC et al. *Bioinformatics* 2005;21:263-265.

<u>The overall differences in the haplotype distribution between the two groups</u>:
haplo.stats (http://cran.r-roject.org/src/contrib/Descriptions/haplo.stats.html)
Schaid DJ et al. *Am J Hum Genet* 2002;70:425-434.

Estimated frequency of haplotypes and association with risk of adverse skin reactions (1)

Gene	Haplotype*	Estimated frequency		Effect		
		LG (n = 580)	HG (n = 218)	Odds ratio (95% CI)	P-value	
CD44	GGTC	0.42	0.33	1.0 (Reference)		
	GGTT	0.22	0.30	2.17 (1.07-4.43)	0.01	*
	AGTC	0.12	0.14	1.79 (0.82-3.90)	0.18	
	AGTT	0.058	0.093	2.09 (0.93-4.67)	0.04	
	AGAC	0.054	0.034	1.07 (0.36-3.18)	0.68	
	AGAT	0.052	0.037	0.80 (0.27-2.40)	1	
	AATC	0.041	0.03	0.88 (0.26-3.01)	0.82	
	AATT	0.026	0.014	0.84 (0.12-5.83)	0.77	

*Haplotypes observed with > 1% frequency in pool.

CD44 locus as an example.
4-tag SNPs were selected.
Haplotypes consisting of these 4 SNPs and the haplotypes observed with more than 1% frequency in both low-grade and high-grade patients.
More than 8 haplotypes could be estimated in this locus.

Suga T. et al. IJROBP 2007

Estimated frequency of haplotypes and association with risk of adverse skin reactions (2)

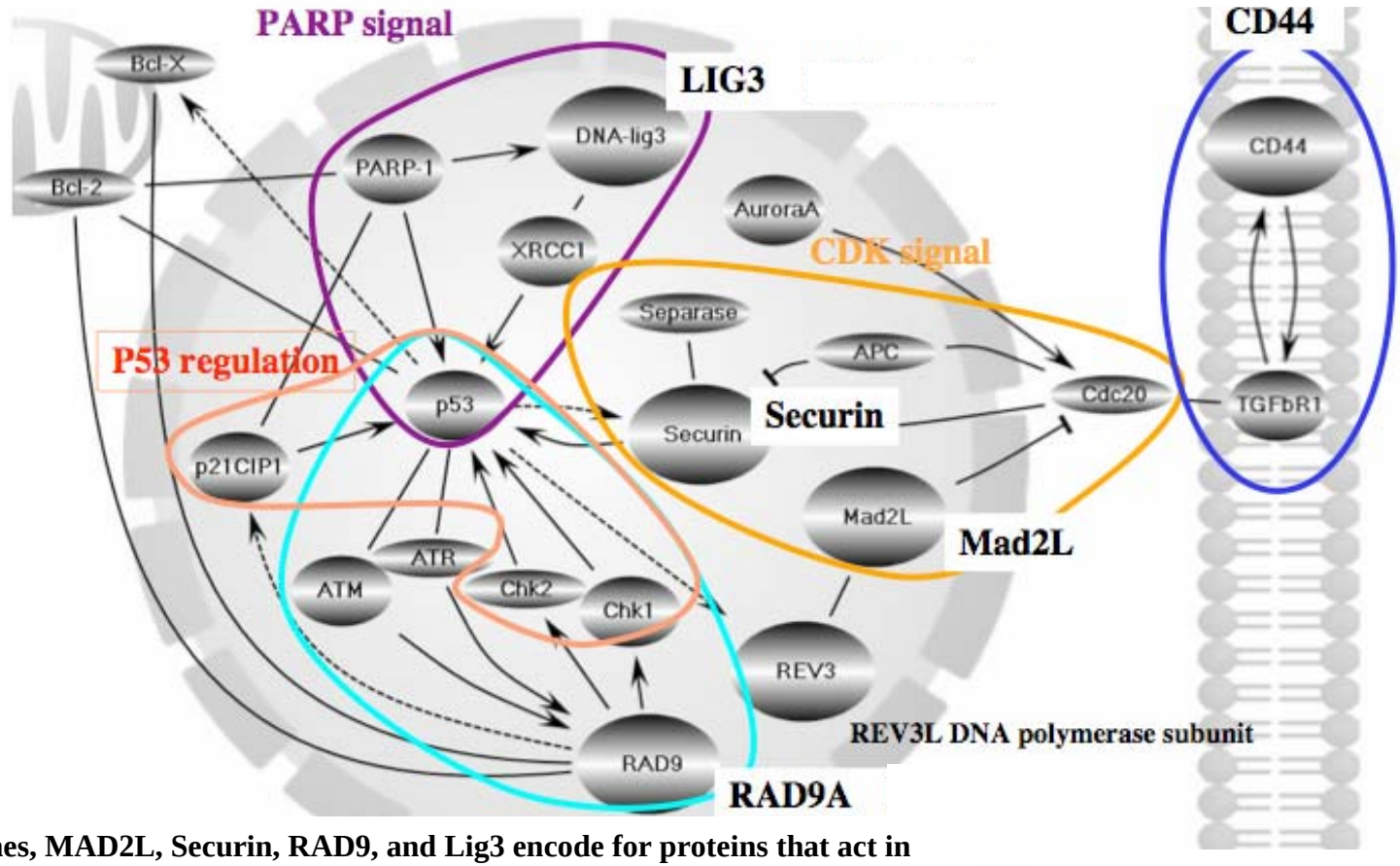
Gene	Haplotype*	Estimated frequency LG (n = 580)	Estimated frequency HG (n = 218)	Effect: Odds ratio (95% CI)	Effect: P-value
MAD2L2	GG	0.54	0.64	1.0 (Reference)	
	CG	0.34	0.24	0.55 (0.35-0.87)	0.0044 *
	CA	0.12	0.11	0.81 (0.46-1.42)	0.45
Securin (PTTG1)	GCTG	0.23	0.27	1.0 (Reference)	
	ACTG	0.22	0.19	0.94 (0.51-1.71)	0.29
	ACTT	0.19	0.23	1.29 (0.73-2.27)	0.82
	GTTG	0.15	0.079	0.48 (0.24-0.96)	0.0081 *
	GCGG	0.094	0.076	0.86 (0.41-1.82)	0.29
	ACGG	0.064	0.061	0.86 (0.39-1.90)	0.6
	GCTT	0.025	0.059	2.17 (0.78-6.03)	0.13
	GTTT	0.022	NA	NA	NA
RAD9A	CTC	0.71	0.82	1.0 (Reference)	
	TCC	0.13	0.073	0.48 (0.26-0.89)	0.017 *
	CCG	0.12	0.073	0.50 (0.27-0.92)	0.022 *
	CTG	0.036	0.023	0.51 (0.18-1.39)	0.28
LIG3	GCC	0.41	0.45	1.0 (Reference)	
	CCC	0.24	0.29	1.14 (0.68-1.90)	0.63
	GTC	0.21	0.15	0.76 (0.45-1.29)	0.1
	GCT	0.13	0.041	0.46 (0.22-0.93)	0.0004 *
	CCT	0.007	0.033	3.73 (0.66-21.05)	0.021

*Haplotypes observed with > 1% frequency in pool.

CD44: GGTT

Function of genes related to risk of EASR

A transmembrane adhesion receptor that is the major cell surface receptor for the nonsulfated glycosaminoglycan hyaluronan, and is reported to be involved in lymphocyte extravasation.



The genes, MAD2L, Securin, RAD9, and Lig3 encode for proteins that act in the nucleus. The MAD2L and securin proteins assist in chromosome maintenance, including sister chromatid separation and the mitotic spindle checkpoint. This suggests that functional variation in these genes could lead to a malfunction of cell cycle regulation and to genome instability.

We previously analyzed the interstrain variation in irradiated murine lung and found an increase in the number of CD44-positive cells in radioresistant mice.

Conclusion (Skin reactions in breast cancer patients)

- We analyzed the haplotypes of genes that were candidates for influencing the risk of adverse skin reactions following radiation therapy.
- 123 genes with 999 SNPs were subjected to the first screening.
- We found haplotypes associated with the risk of early adverse skin reaction in *CD44*, *MAD2L2*, *PTTG1*, *RAD9A* and *LIG3*.
- Combinations of these haplotypes in multiple loci may determine an individual's radiosensitivity.

Issues to be resolved (1)

Function of SNPs

The functions of the SNPs in the haplotypes we identified have yet to be characterised.

Identifying functional SNPs in the selected genes would facilitate the understanding of the mechanisms contributing to patient variation in radiation susceptibility.

Issues to be resolved (2)

The candidates

The use of selected genes imposes some limitations on our findings.

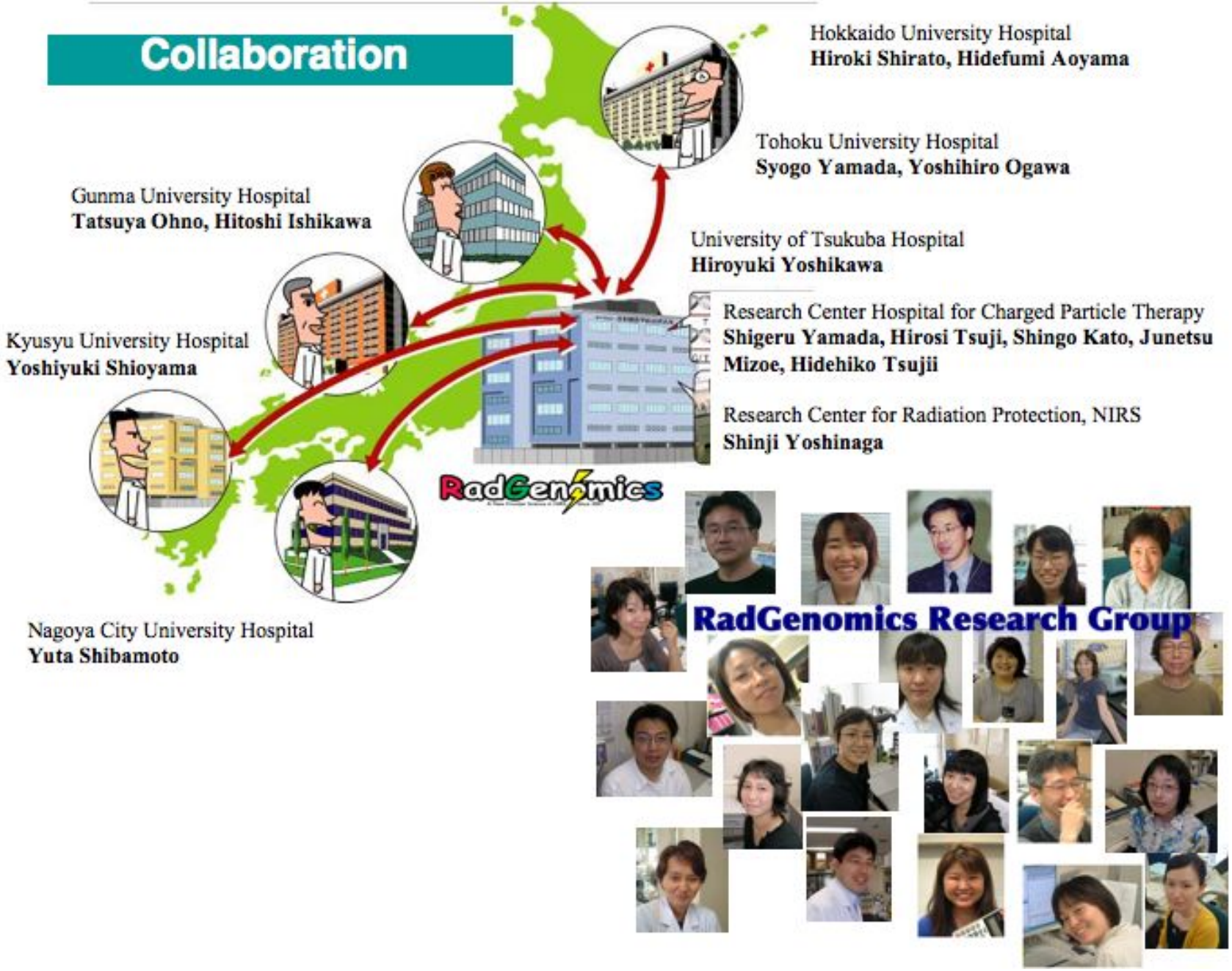
The candidate genes for genotyping were selected using a limited number of gene expression analyses, in addition to genes we selected from the literature.

As HapMap data is now available, it may be possible to effectively perform a genome-wide association study for radiosensitivity in cancer patients.

Number of patients

The need for a subsequent association study with a large number of patients and/or meta-analysis in multiple populations.

Collaboration



Conclusion (Skin reactions in breast cancer patients -2)

- We found haplotypes associated with the risk of early adverse skin reaction in *CD44*, *MAD2L2, PTTG1, RAD9A* and *LIG3.*

- Combinations of these haplotypes in multiple loci may determine an individual's radiosensitivity.