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Analysis of individual differences in radiosensitivity using genome editing

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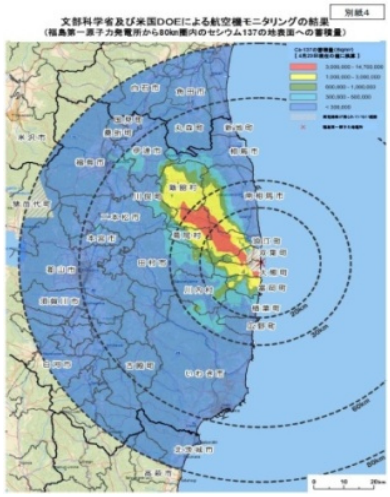
HIROSHIMA UNIVERSITY RIRBM

Social anxiety about the effects of radiation on the human body has increased

The Fukushima Daiichi nuclear power plant disaster on March 11, 2011

The Great East Japan Earthquake

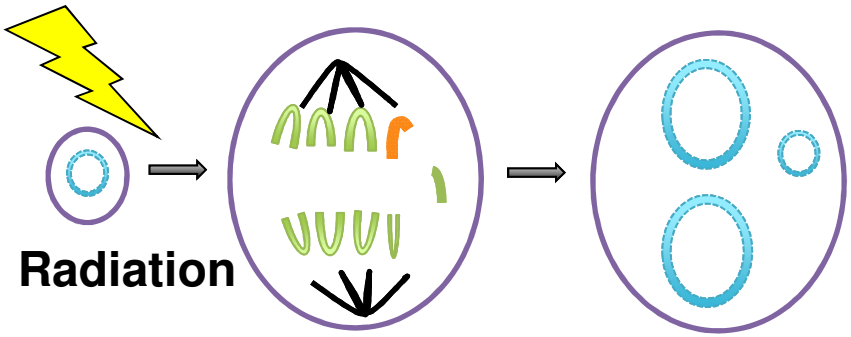


Current standards for radiological protection of the public have been uniformly established. However, individual differences in radiosensitivity are suggested to exist in human populations, which could be caused by nucleotide variants of DNA repair genes.

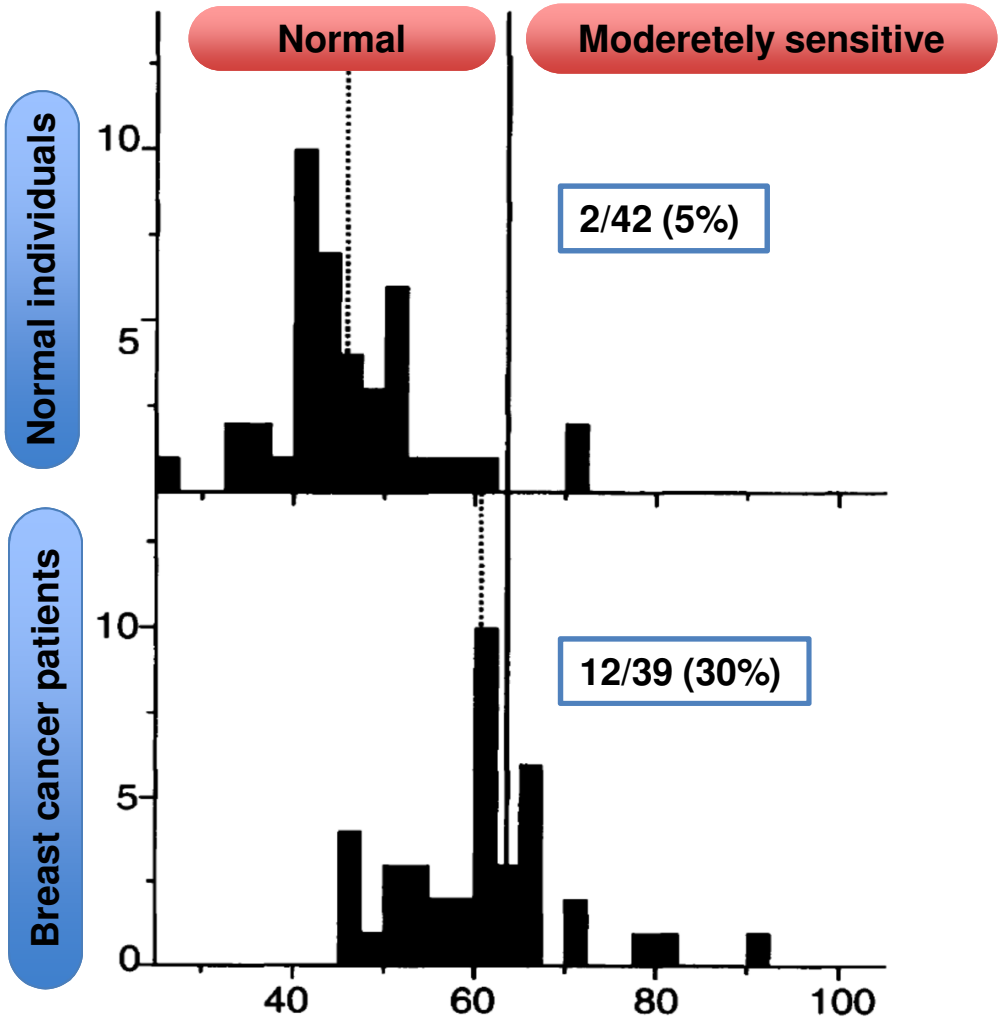
Genome editing is a useful tool to investigate individual cellular radiosensitivity

Individual differences in radiosensitivity are suggested to exist in human populations

Cytokinesis-block micronucleus (CBMN) assay



Micronuclei are formed from unrepaired DNA fragment



Scott et al. *Br J Cancer* 1998

Protocol of CBMN assay for peripheral blood lymphocytes

Peripheral blood was obtained from 6 healthy volunteers.

1st day — **3rd day** — **4th day**

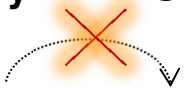
Irradiation

0 Gy, 1 Gy, 2 Gy

Blood

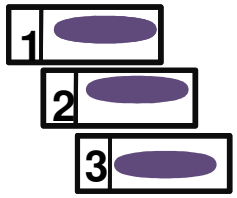
Phytohemagglutinin (PHA) stimulation

Cytochalasin B for blocking cytokinesis

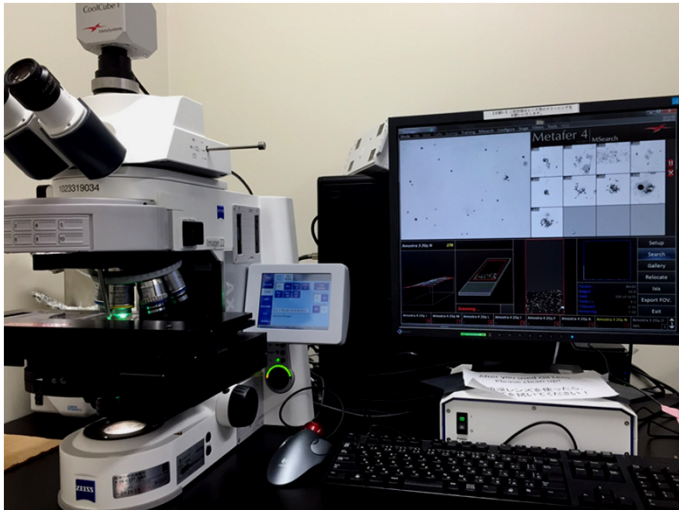


G-actin **F-actin**

Preparation of samples and counting



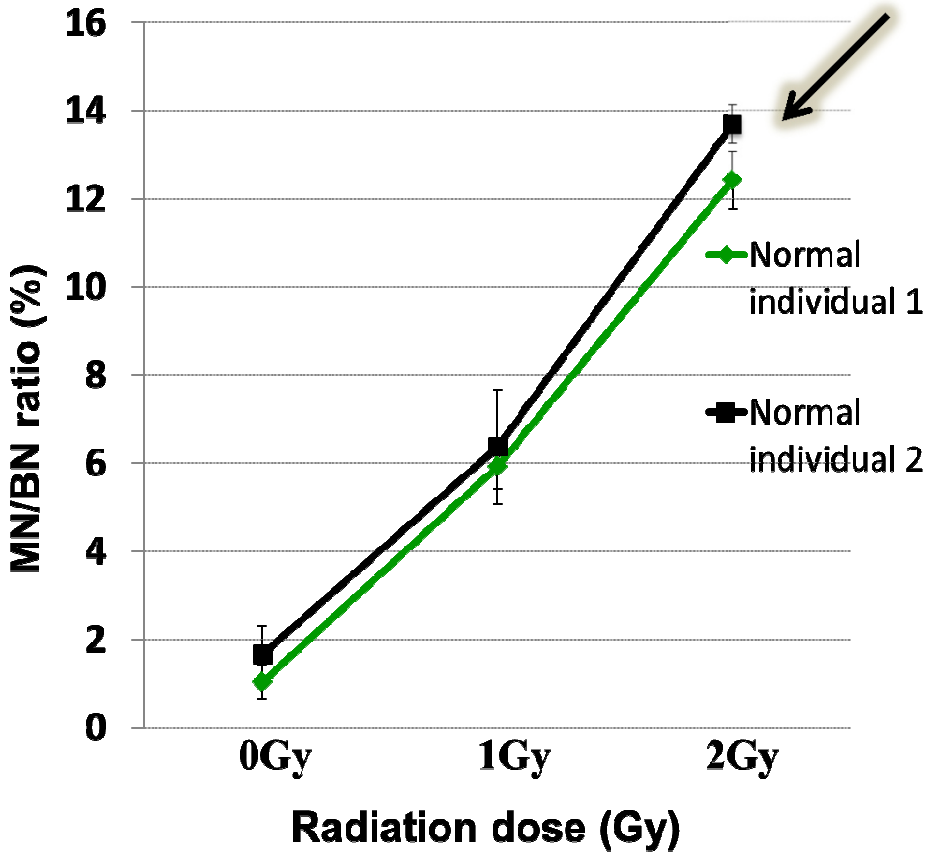
Metafer system, Zeiss



CBMN assay detects individual differences in radiosensitivity among normal individuals

Two individuals showed difference in radiation sensitivity

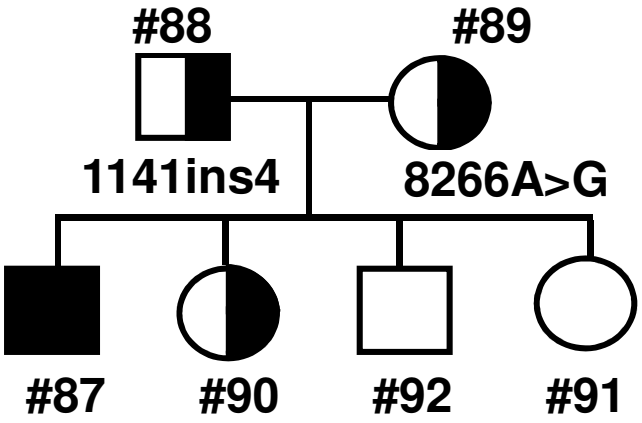
Parameter	MN/BN		
	0 Gy	1 Gy	2 Gy
Average (Normal 1)	1.03	5.9	12.4
Average (Normal 2)	1.7	6.4	13.7
S.D. (Normal 1)	0.4	0.5	0.6
S.D. (Normal 2)	0.6	1.3	0.4
p-value	0.08	0.1	0.03

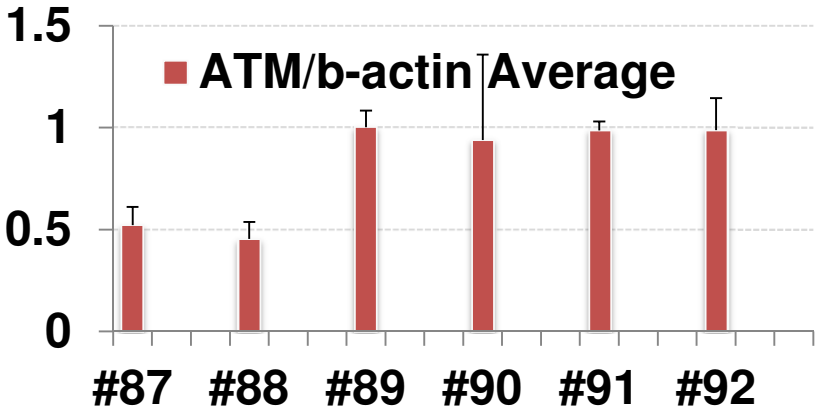


Volunteer 1 was a 53-year-old man and volunteer 2 was a 46-year-old woman

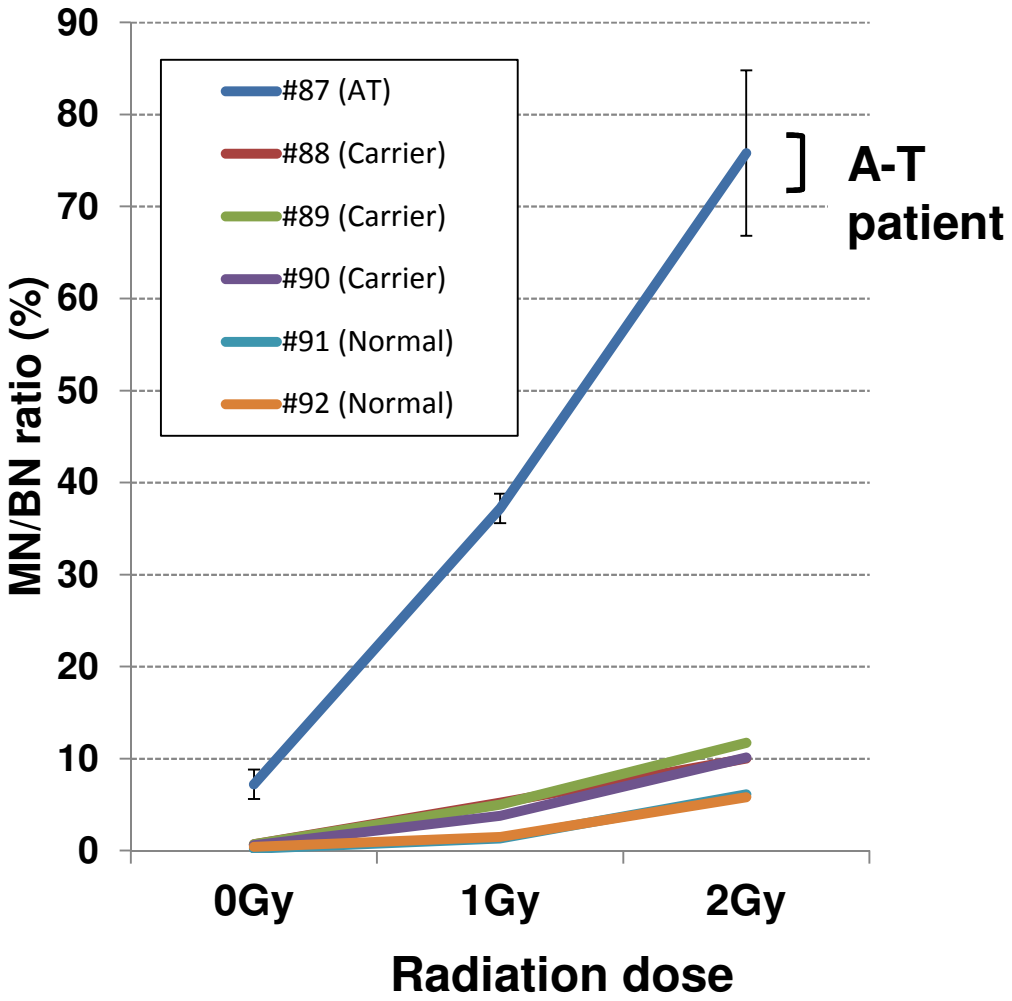
CBMN assay of Ataxia-telangiectasia (A-T) family members

Skin fibroblasts were obtained from A-T family members, and were analyzed by CBMN assay.

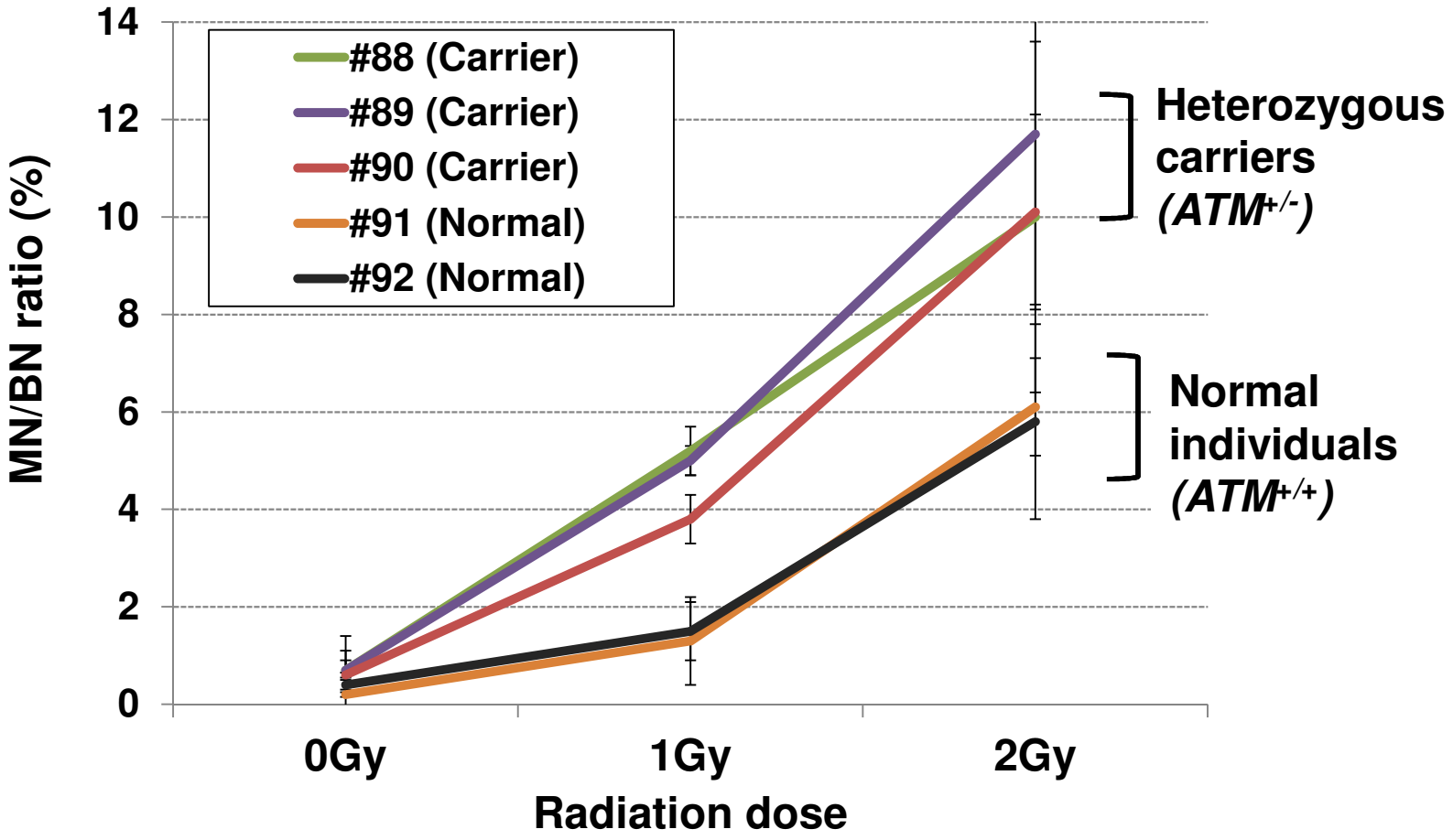




qRT-PCR analysis of *ATM* mRNA

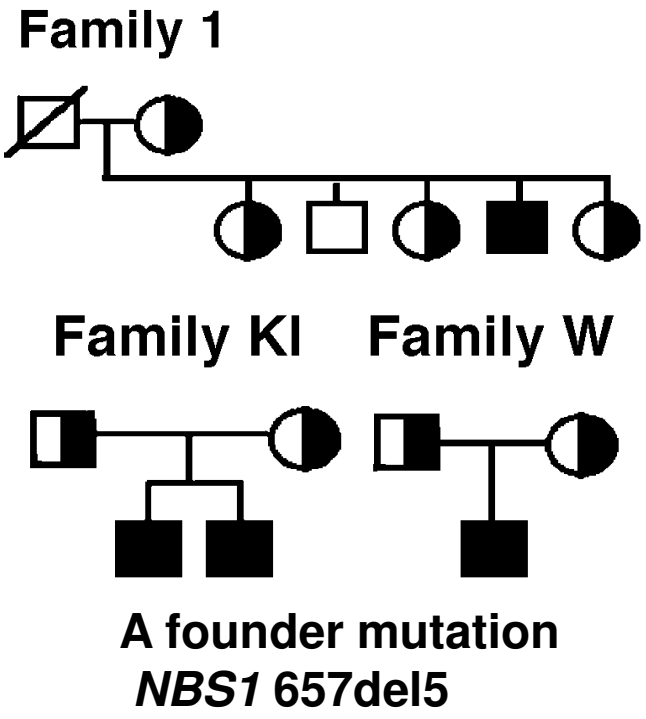


A-T heterozygous carries showed increased frequency of MN formation as compared to normal individuals

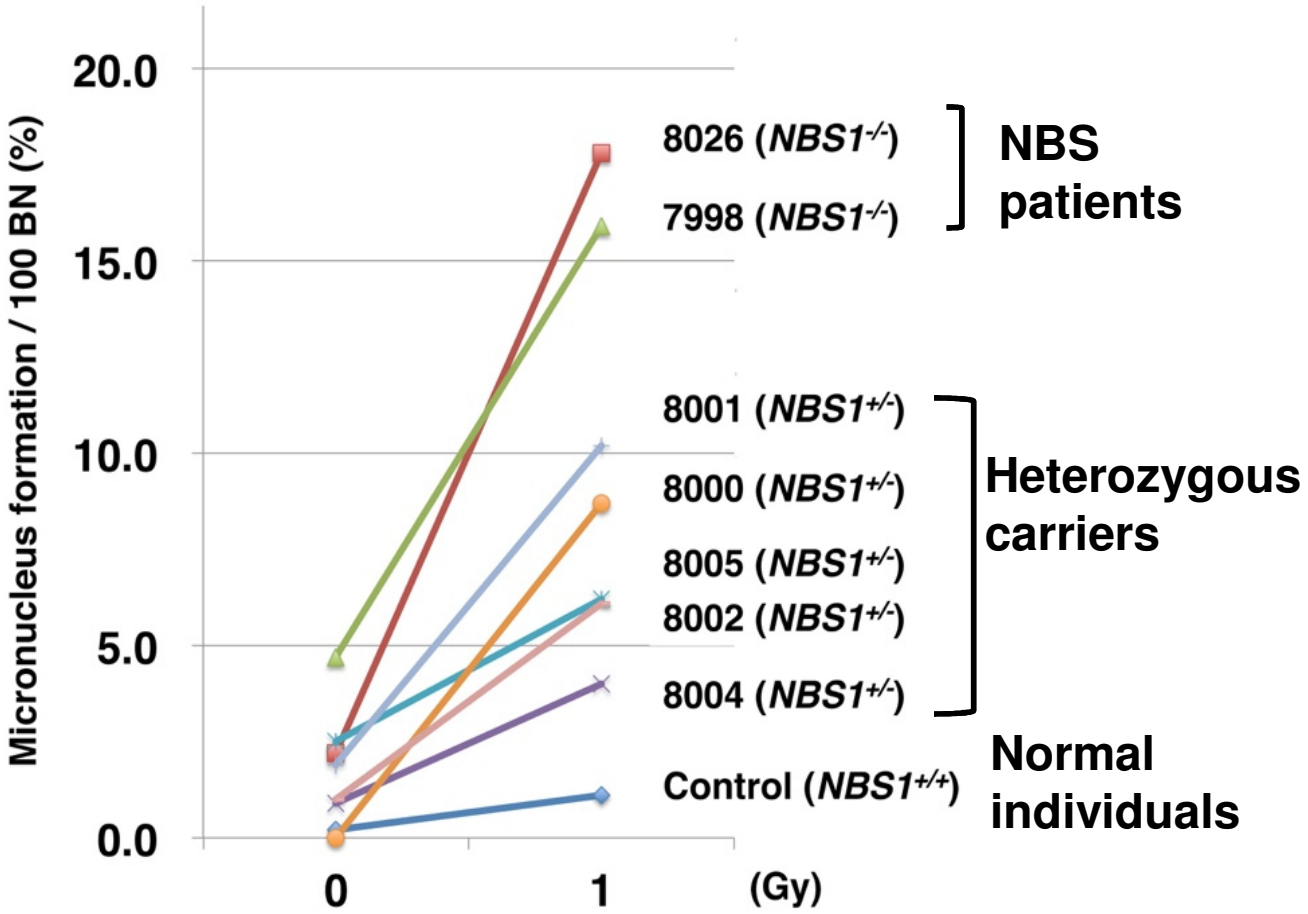


Nijmegen breakage syndrome (NBS) heterozygous carries showed increased frequency of MN formation

EB-transformed lymphocytes from NBS family members were analyzed by CBMN assay



A founder mutation
***NBS1* 657del5**



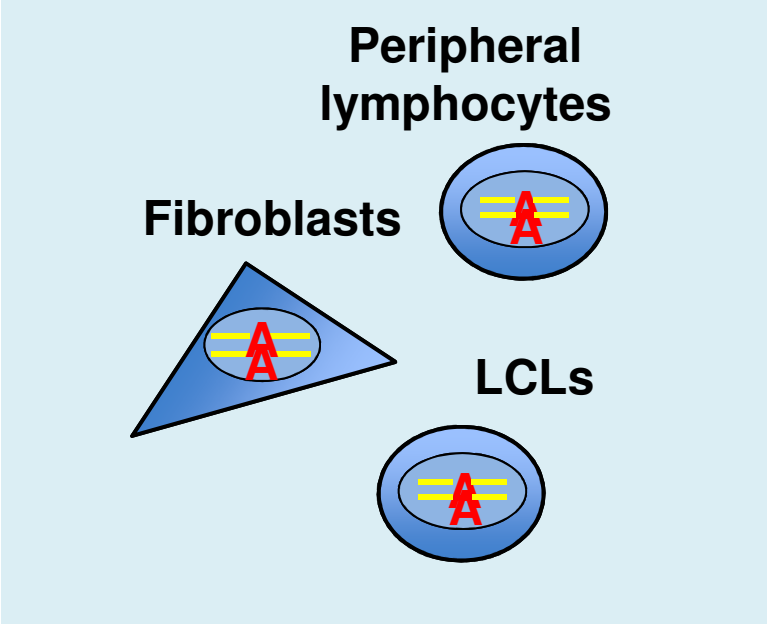
A relationship between heterozygous mutations of familial hyper-radiosensitive diseases and mild radiosensitivity

Individual radiosensitivity may be attributed to SNPs in DNA repair genes

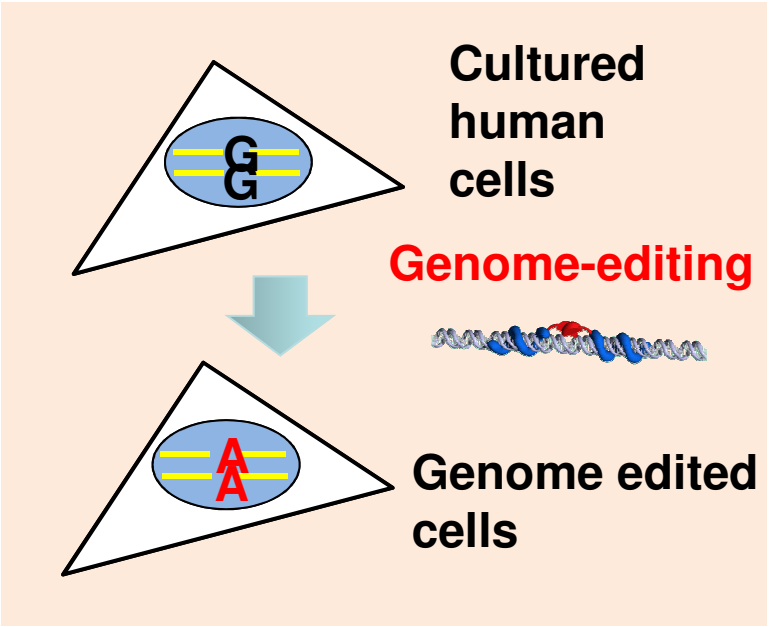
Gene	Amino acid change	Change of base	Phenotype
XRCC1	Q399R	c.1196A>G	Acute/Late radiation reaction
XRCC1	R194W	c.580C>T	Acute/Late radiation reaction
XRCC1	R280H	c.839G>A	Cancer risk, late radiation reaction
XRCC3	Y241M	c.722C>T	Late radiation reaction
LIG4	A3V	c.8C>T	Lung cancer risk
LIG4	T9I	c.26C>T	Lung cancer risk
ATM		c.8850+60A>G	Late radiation reaction
ATM		c.5674+1518T>A	Breast cancer risk
XPD/ERCC2	D711D	c.2133C>T	Late radiation reaction
MDC1	A1657A	c.4971C>G	Acute/Late radiation reaction
CHEK1		c.1233+35G>A	Pancreatic cancer risk
XRCC6/Ku70	G593G	c.1779G>T	Breast cancer risk
XRCC5/Ku80		c..2110-2408G>A	Breast cancer risk
RAD51C		c.-98G>C	Head/neck cancer risk
MRE11		c.*2501A>G	Bladder cancer risk
NBS1	I171V	c.511A>G	Breast cancer risk
RAD50		c.3390-1922T>G	Non-Hodgkin lymphoma risk

Two strategy to evaluate the DNA repair variants

Cells from individuals carrying candidate SNPs



Evaluation system in a uniform genetic background

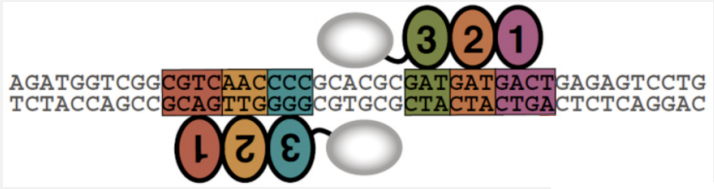


Evaluation of such variants proved difficult

1. smaller size effects
2. confounding factors
3. diverse genetic background

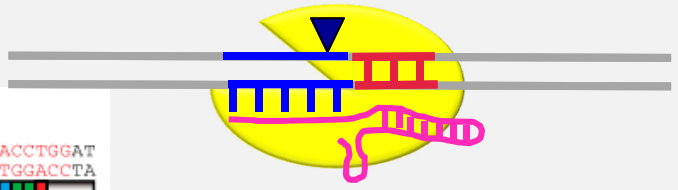
Artificial nucleases and genome editing

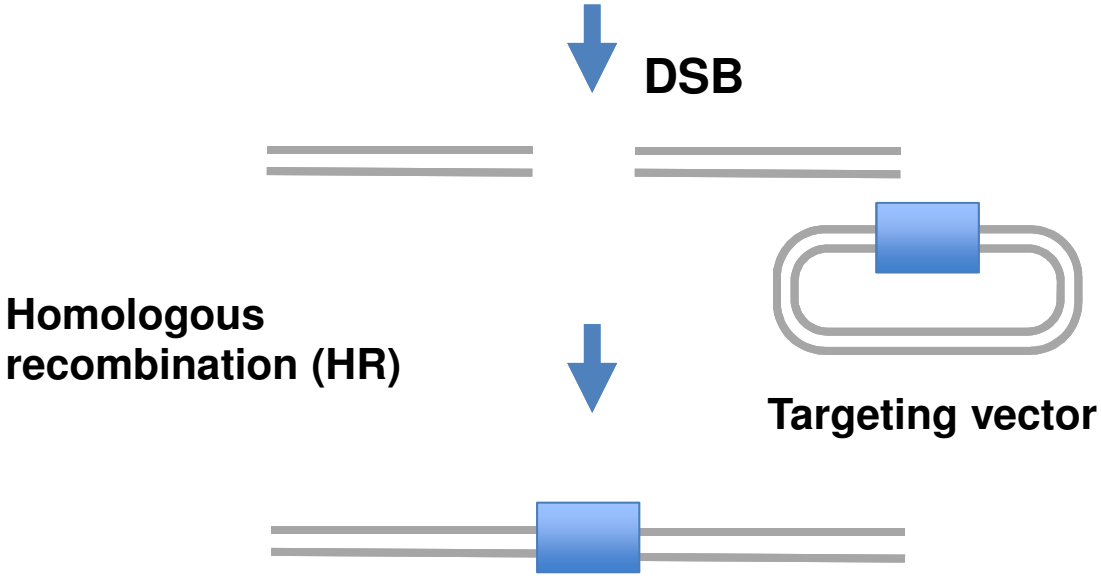
Zinc finger nuclease (ZFN)



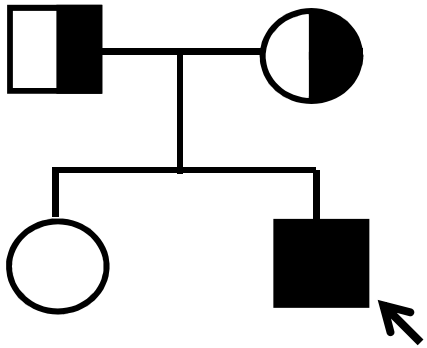
Transcription Activator-like Effector Nuclease (TALEN)

Clustered Regularly Interspaced Short Palindromic Repeat /Cas9 based RNA-guided DNA endonuclease (CRISPR/CAS9)





Genome editing identification of an intergenic mutation as causative of genetic disorder

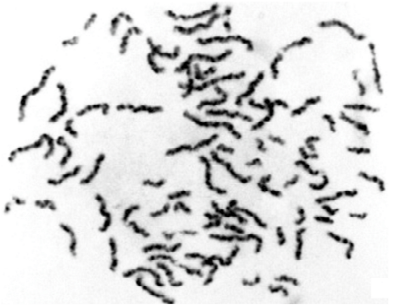


One-year-old boy with a severe disease

Wilms tumor, seizures, and nonverbal.
His parents expected to have a third healthy child.
However, prenatal DNA diagnosis was difficult because no coding mutation in *BUBR1* was found, suggesting that causative mutation is a non-coding one.

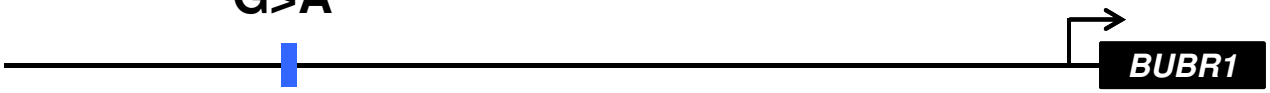
Premature chromatid separation (PCS) syndrome

Autosomal recessive disorder
Loss-of–function mutations in a gene encoding BUBR1, a spindle assembly checkpoint protein



Premature chromatid separation (PCS)

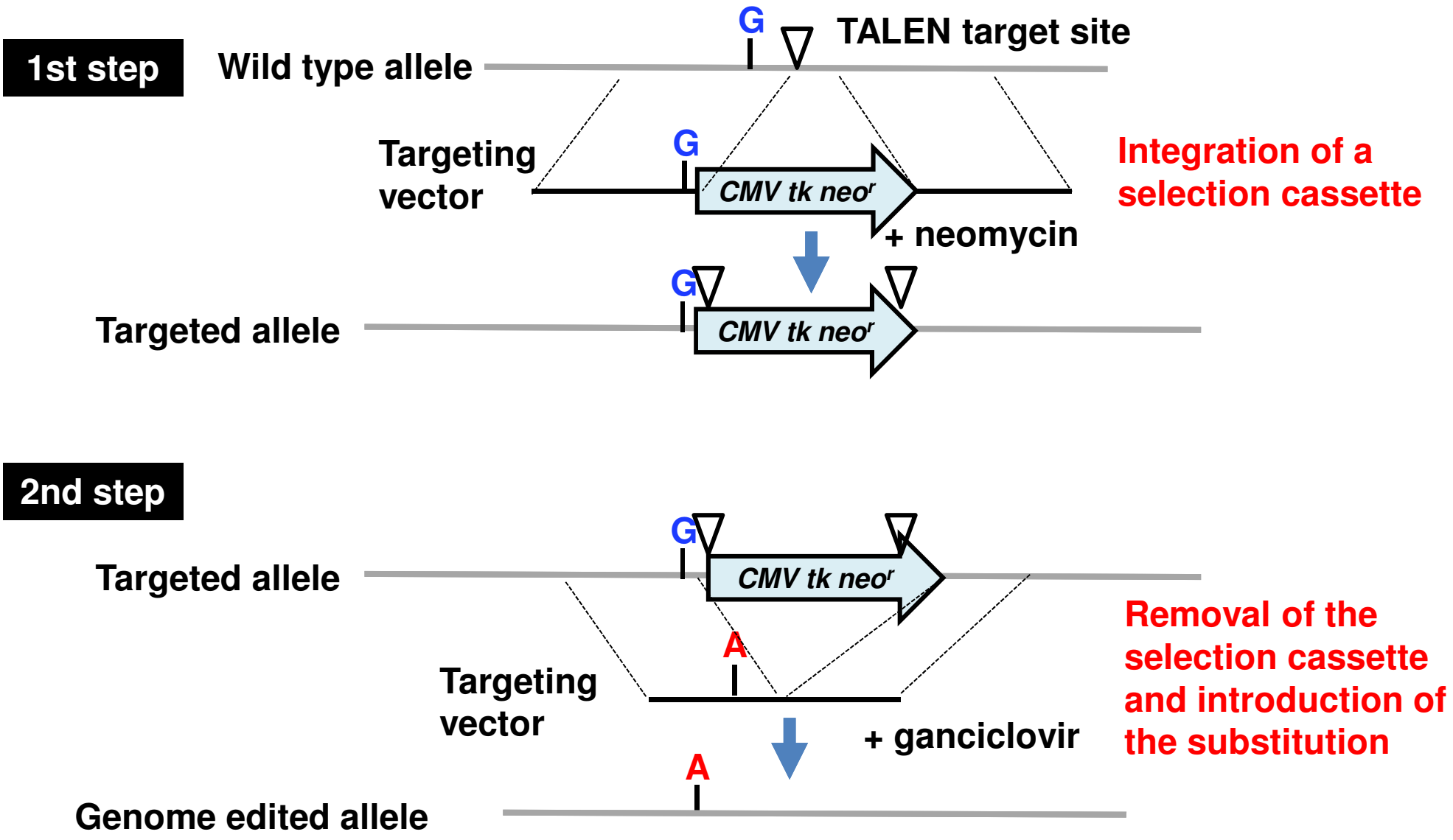
A single base substitution (G>A) in an intergenic region 44-kb upstream of *BUBR1* was identified as potentially causative



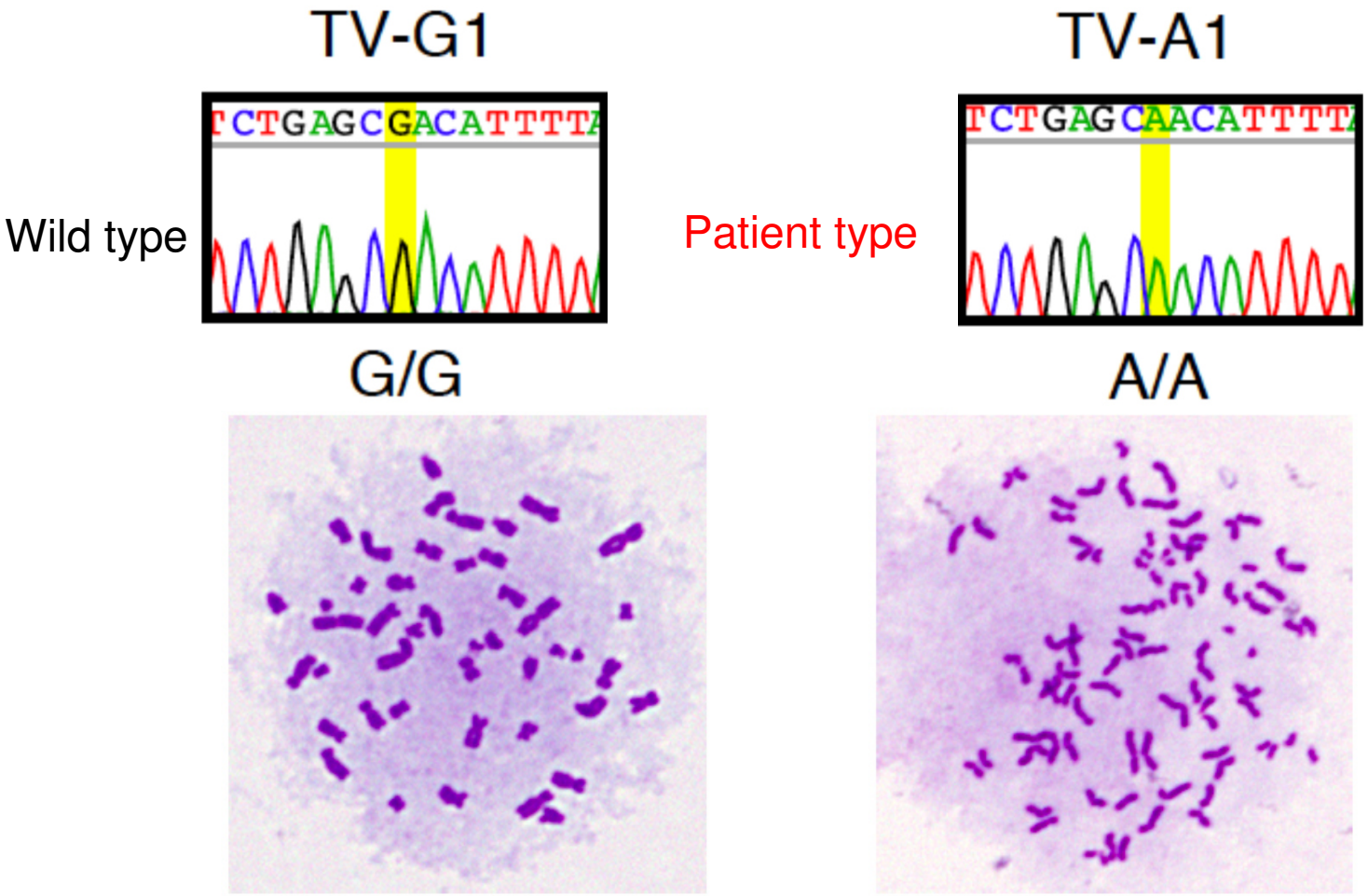
Is this the causal mutation or merely correlates with the syndrome ?

To answer this question, we used genome editing

Two-step single-base-pair editing strategy



The nucleotide substitution identified was the causal mutation of the syndrome



Ochiai et al.,
PNAS 2014

The parents performed amniocentesis during the third pregnancy.
It was found to be heterozygous. A healthy baby boy was born.

NBS1 I171V polymorphism (511A>G)

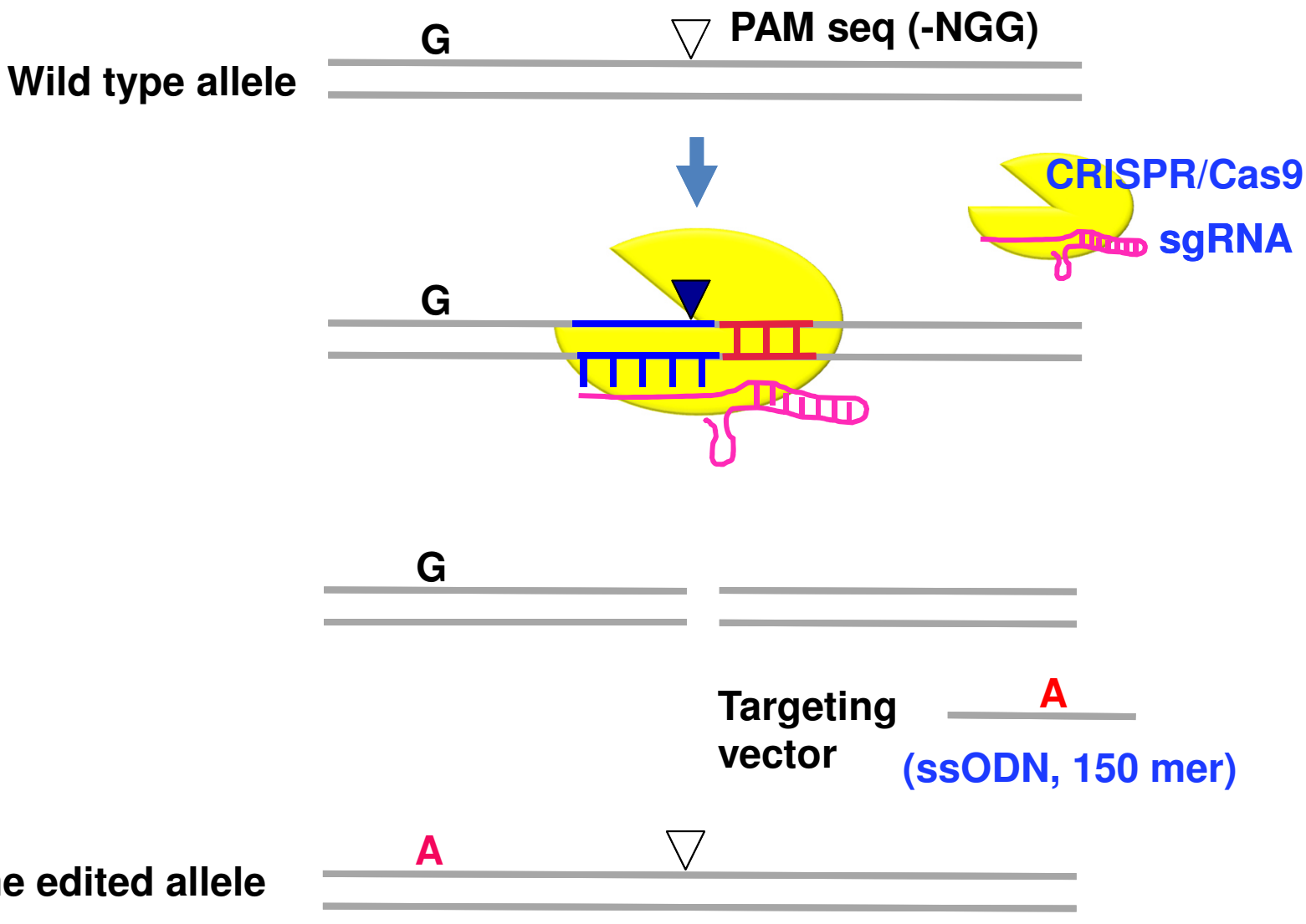
1. **Association with an increased breast cancer risk (Roznowski et al 2007).**

2. **2.58% of cancer patients are I171V carriers, compared to the 0.17% in the control group, suggesting that the I171V may be susceptibility factor in cancer (Nowak et al 2008)**

Genome editing was used to verify that this SNP is indeed involved in cellular radiosensitivity

One-step genome editing strategy

One-step



Restriction enzyme and sequence analysis of genome edited cells

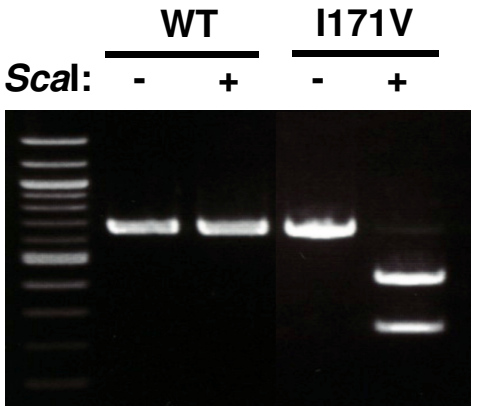
Targeting vector

5’-AGGTCAACAcTTCGGcCTcATgAAAATGA-(150mer)-3’

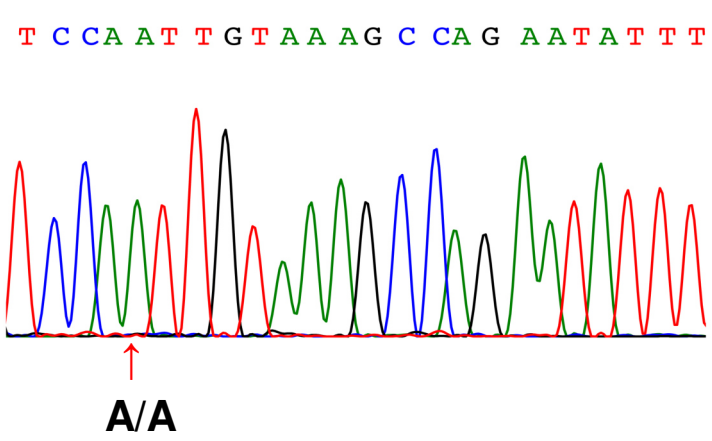
T→C

Sca I

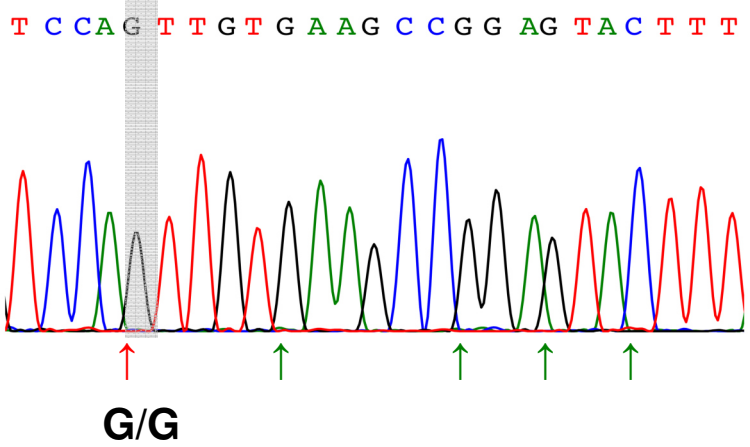
number of clones analysed	*Sca*I-digested clones
96	3 (3.15%)



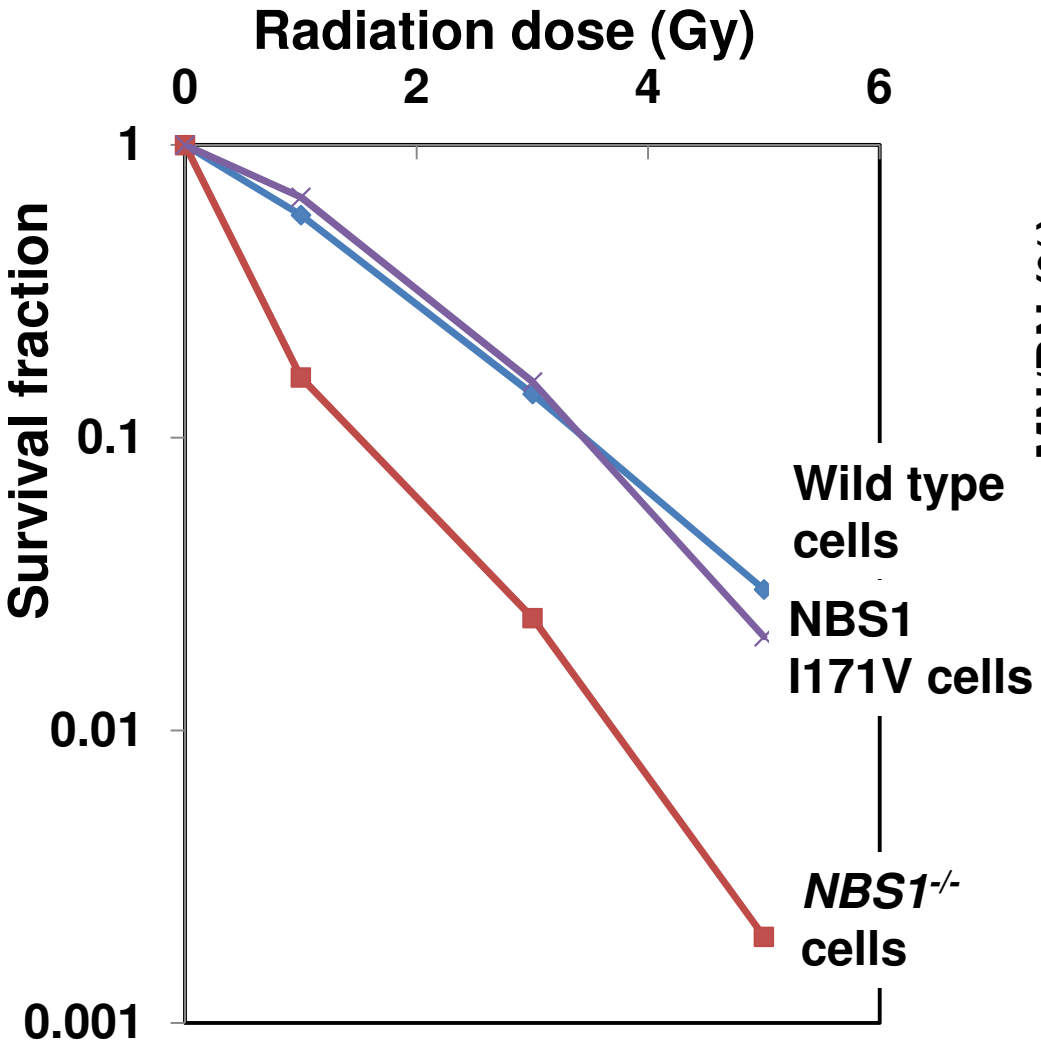
***NBS1* wild type clone**

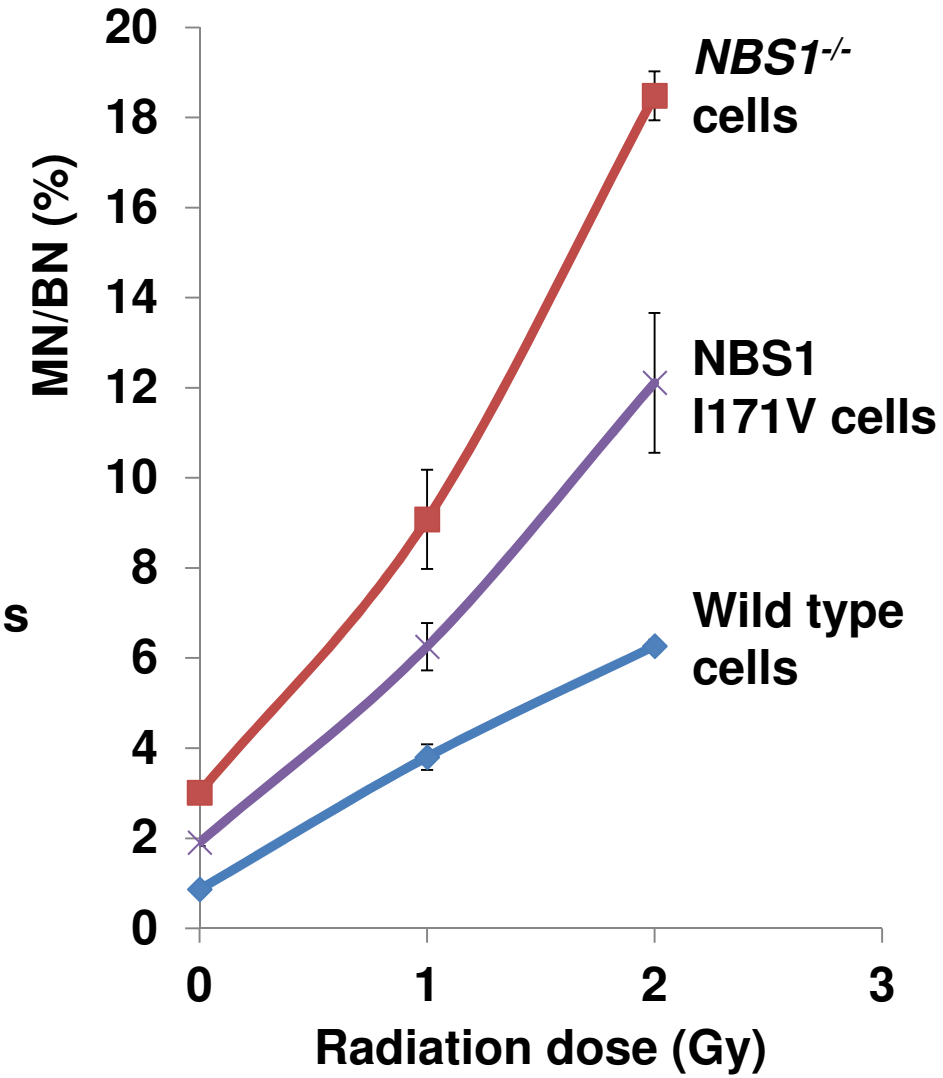


***NBS1* I171V homozygous clone**



Genome edited cells showed increased frequency of MN formation





Conclusion

1. **Individual differences in radiosensitivity exist in human populations.**
2. **We designed TALEN-mediated two-step single-base-pair editing, which we used to introduce a nucleotide variant associated with a chromosomal instability syndrome into human cultured cells to demonstrate that it is the causative mutation.**
3. **We designed CRISPR/CAS9-based one-step genome editing and applied it to the evaluation of NBS1 I171V polymorphism for cellular radiosensitivity.**
4. **Genome editing is now widely used and become a valuable tool to investigate individual radiosensitivity.**

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