Application of chromosomal radiosensitivity assays to temporary nuclear power plant workers

Research group University Ghent in collaboration with Dr. M. Barbé and the Occupational Medicine Service Nuclear Power Plant Doel, Belgium

Introduction

- **External temporary** radiation workers involved in the revision and cleaning activities in the hot zone receive nowadays the highest dose at a relatively short period in the nuclear industry: up to **10 mSv in one month**
- For their activities at the reactors these external workers are followed up by the Occupational Medical Service of the Nuclear Power Plant CBMT. The dose is continuously followed by electronic dosemeters apart from the legal personnel filmbadge dosemeter.

Study population

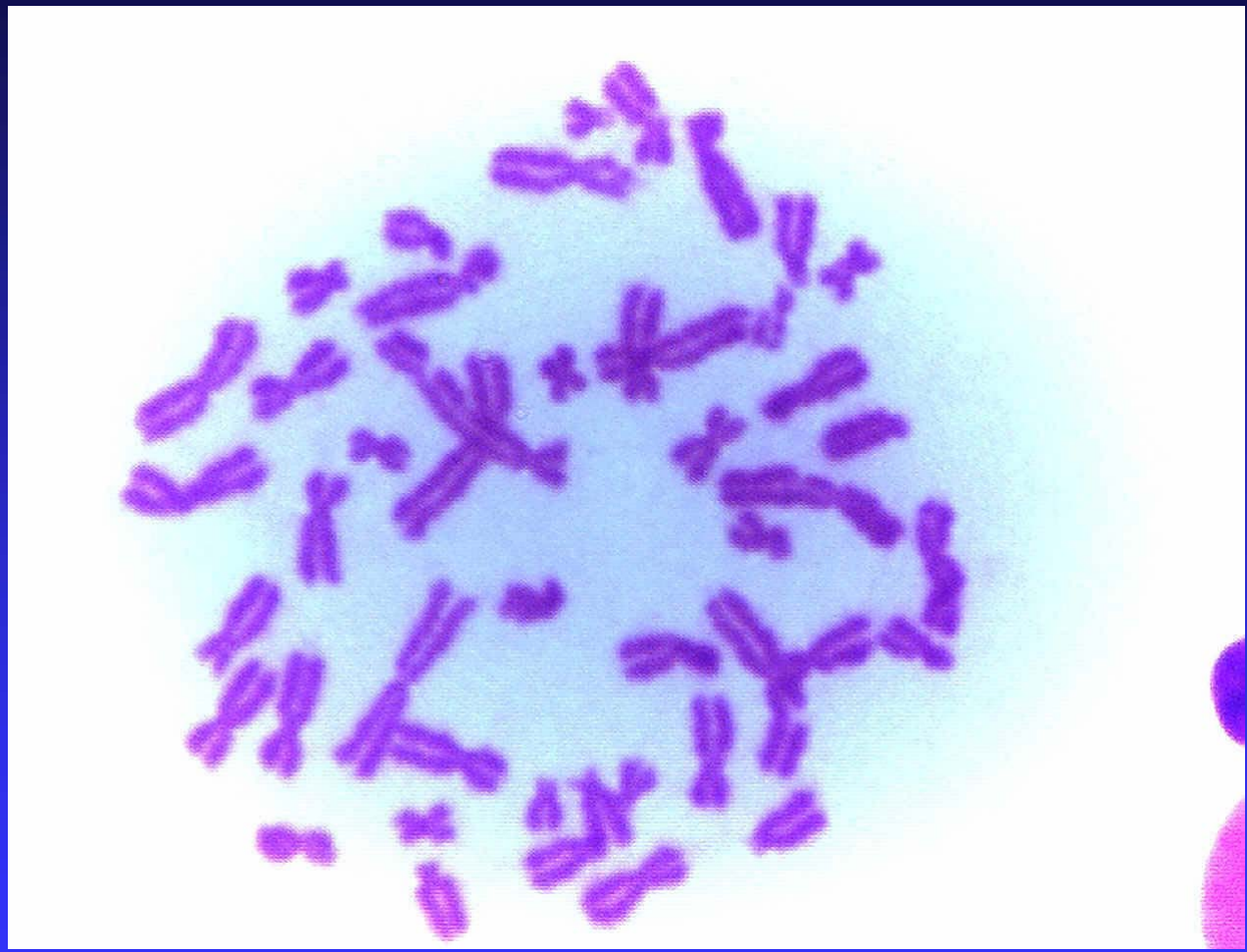
- Consists of 41 male nuclear workers involved in revision and cleaning activities in the hot zone of the reactors Doel I-IV during April-August2000.
  - **Age**: mean 30.6 years range 18-55 years
  - **Smoking habits**: 13 ind. were non-smokers, smoking population mean 208 CY range 40-750 CY
  - **Dose accumulated before the revision** extracted from official personnel dosimetry records : mean 13.9 mSv range 0-68.7 mSv.

Assessment of chrom. radiosensitivity

- **Chromosomal radiosensitivity assessment** on blood samples with (1) **G2 assay** and (2) **micronucleus assay**
- Of each worker a **blood sample** was taken **before** and **directly after the activities in the hot zone**, a few weeks later. Dose was read-out on electronic personnel dosemeters.
- The blood samples were coded by the Occupational Medicine Service allowing blind scoring of the samples at the laboratory

G2 assay

- A lymphocyte culture with RPMI-1640 and PHA as mitogen was started up of each bloodsample. After 71 h of incubation the cultures were irradiated *in vitro* with a 0.4 Gy ^{60}Co dose, 30 min later colcemid was added and the cultures were arrested 60 min after the irradiation.
- Fifty metaphases were analysed for the appearance of chromatid breaks and gaps.



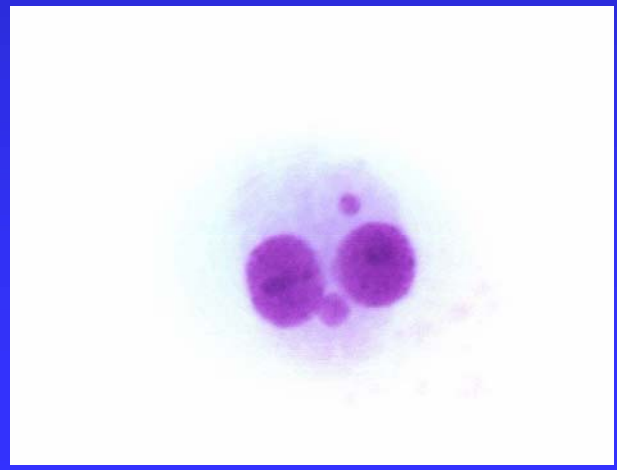
Micronucleus assay

- For the micronucleus **assay as** radiosensitivity test lymphocytes from each blood sample were stimulated with PHA after an *in vitro* dose of 3.5 Gy and harvested 70 h later.
- Micronucleus frequencies were scored in 1000 binucleated (BN) cells.
- An irradiation was performed at high dose rate **MN-HDR** (1Gy/min) and at low dose rate **MN-LDR** (0.25 Gy/h).

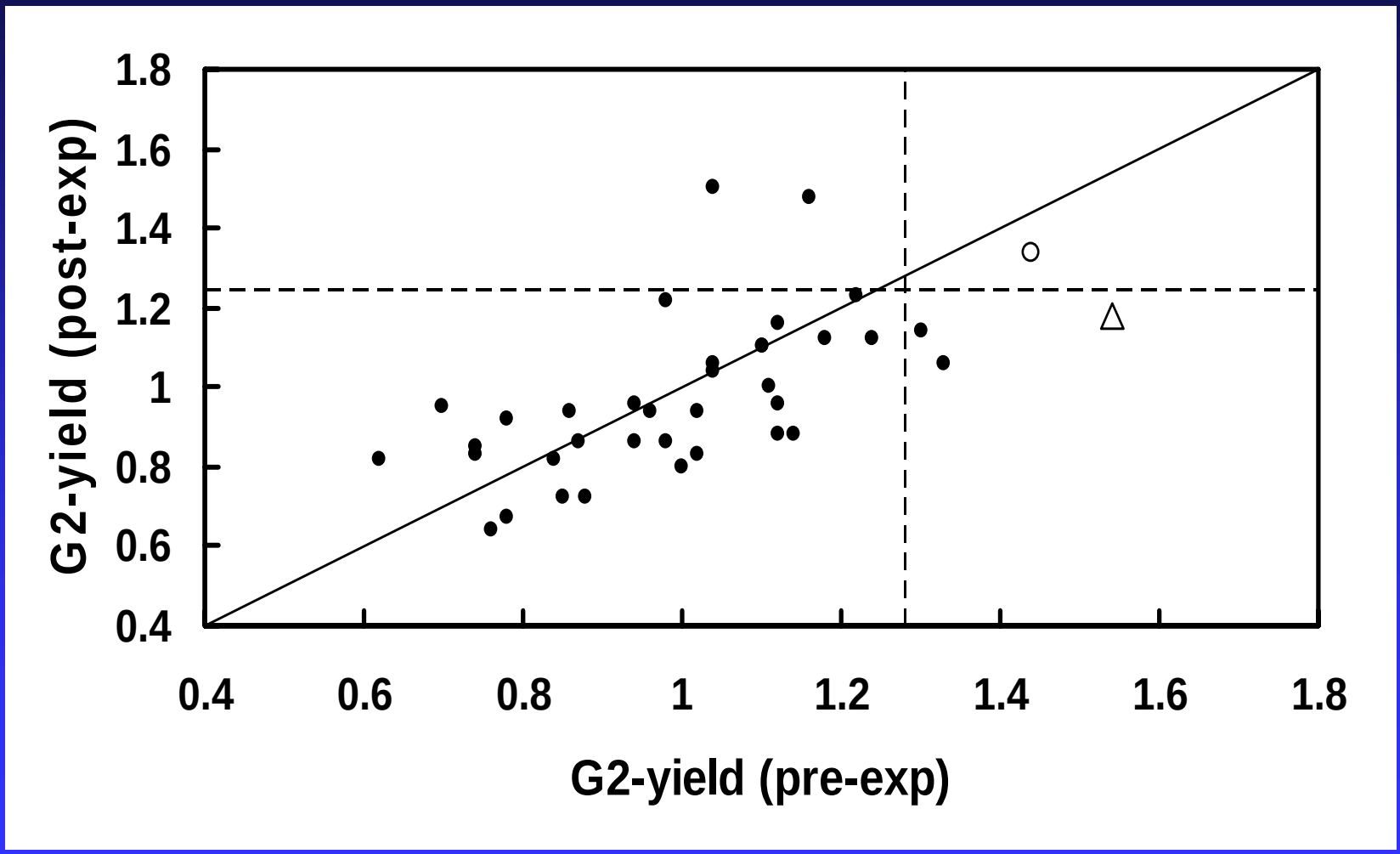
- The **dose rate sparing factor (DRS)** was calculated from the difference in micronucleus yields between the HDR (Y_{HDR}) and LDR (Y_{LDR}) irradiation using the expression:

$$DRS = (1-Y_{LDR}/Y_{HDR}) 100$$

- The DRS is representative for the individual **DNA repair capacity**



Results: G2 assay

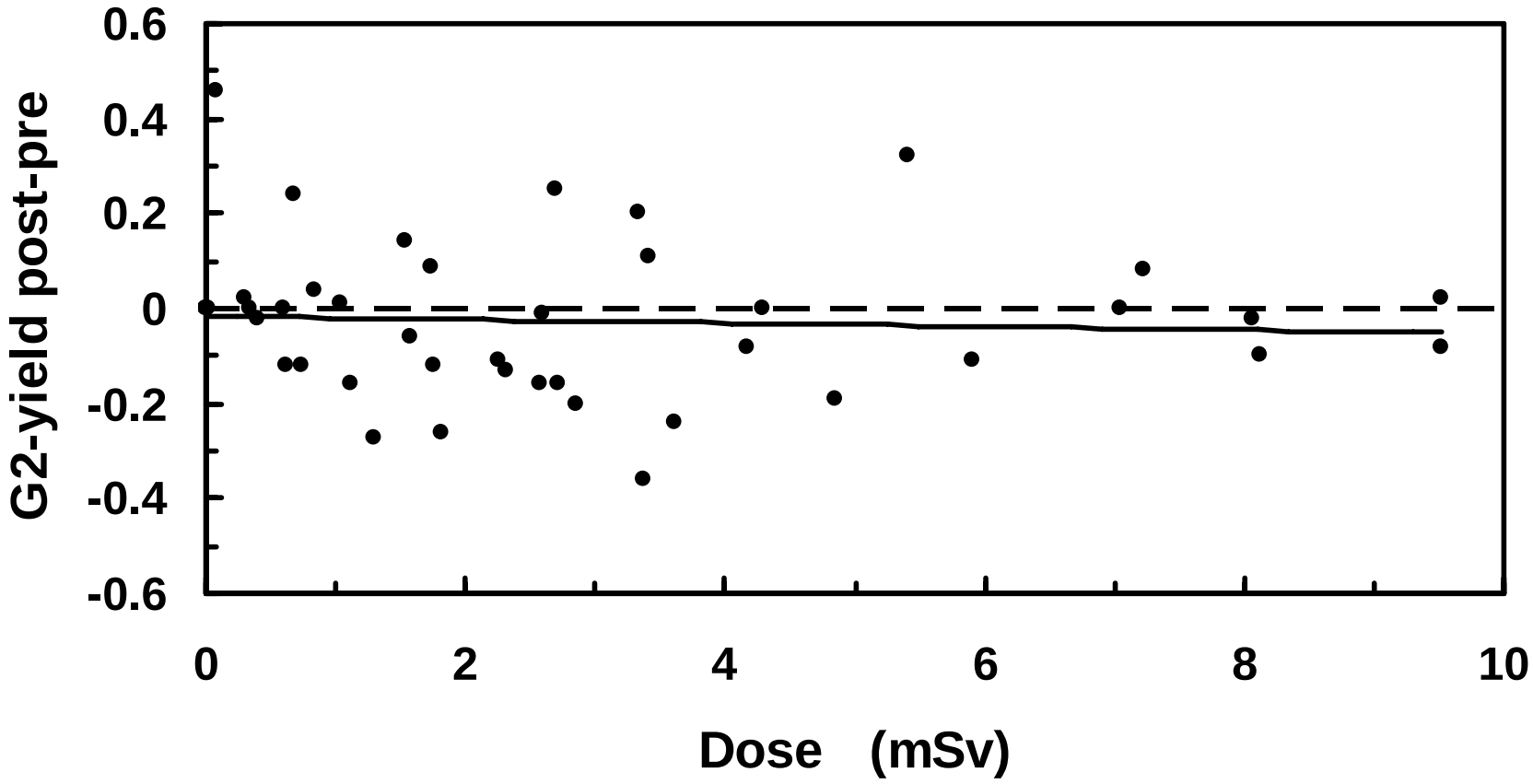


Results: G2 assay

- A **significant correlation** exists between the G2 data **pre- and post exposure** (r = 0.74)

- Applying the 90th percentile as cut-off **one radiation worker** (○) shows an **elevated chromosomal radiosensitivity** status as well before as after the revision activities.

Results: G2 assay

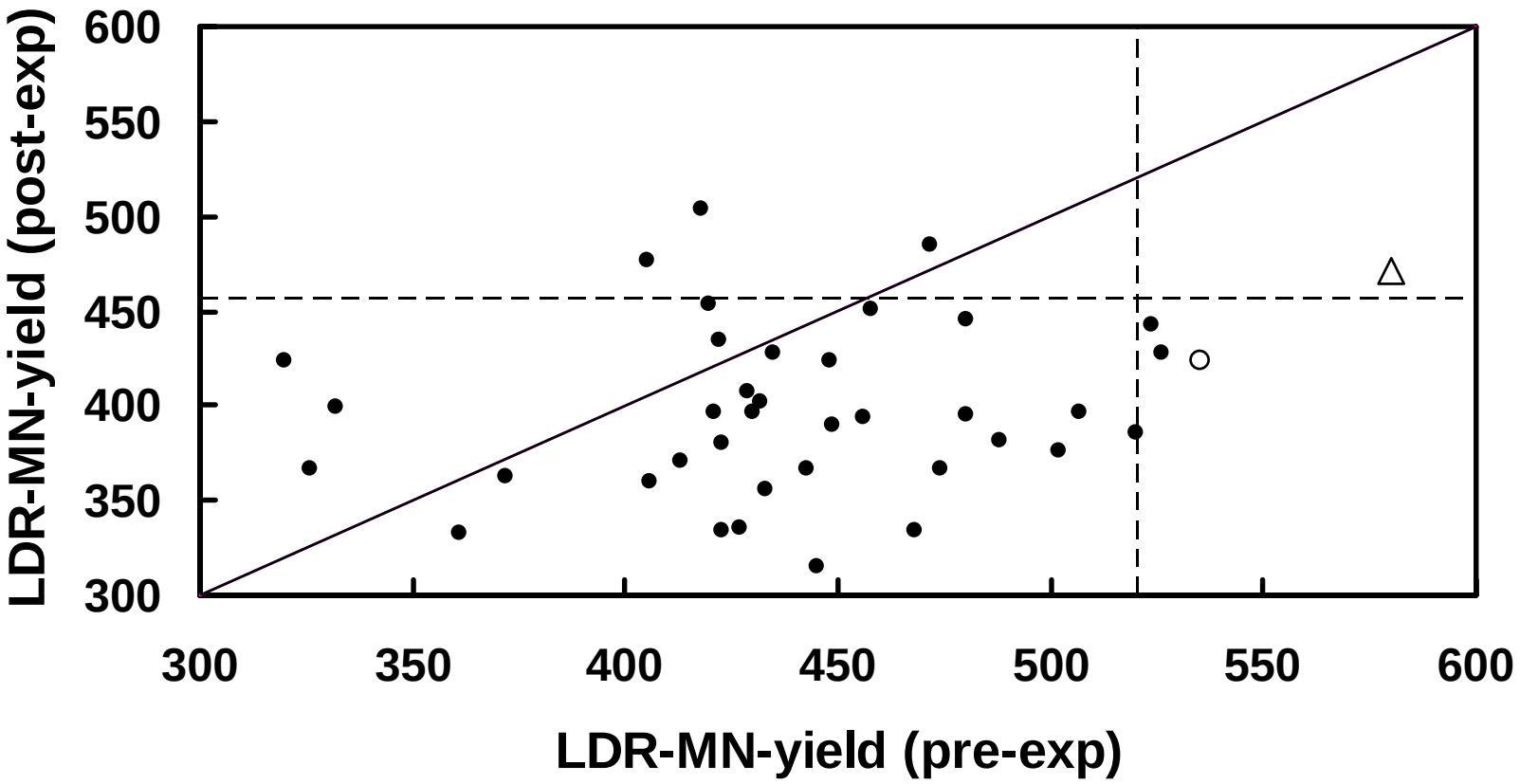


Results: G2 assay

- A plot of the individual differences between the number of chromatid breaks post minus pre exposure versus the dose shows **no effect of the dose**.

- An analysis of the data sorted into dose groups (0-2 mSv, 2-4 mSv, 4-10 mSv) shows also **no effect of the exposure**.

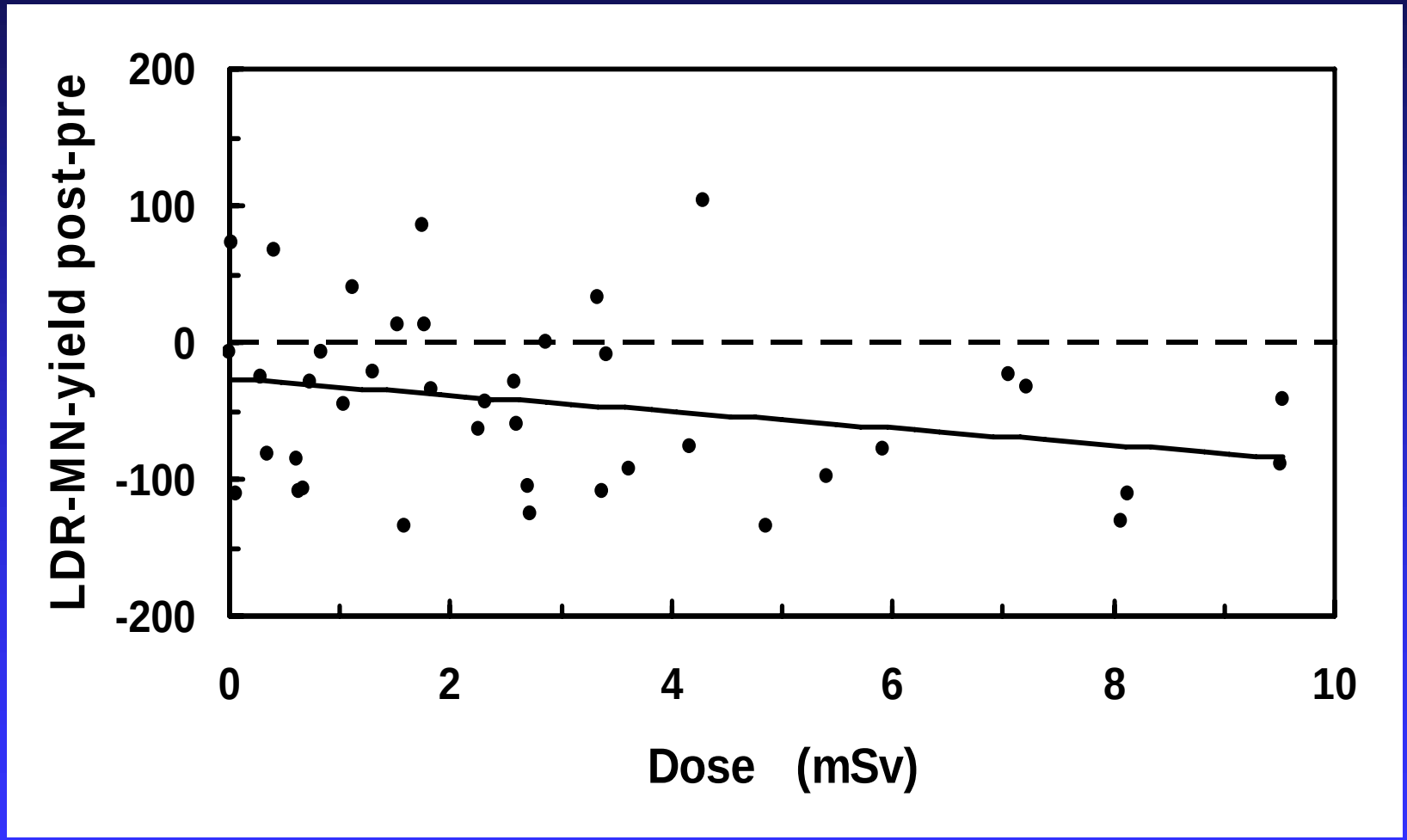
Results: LDR-MN assay



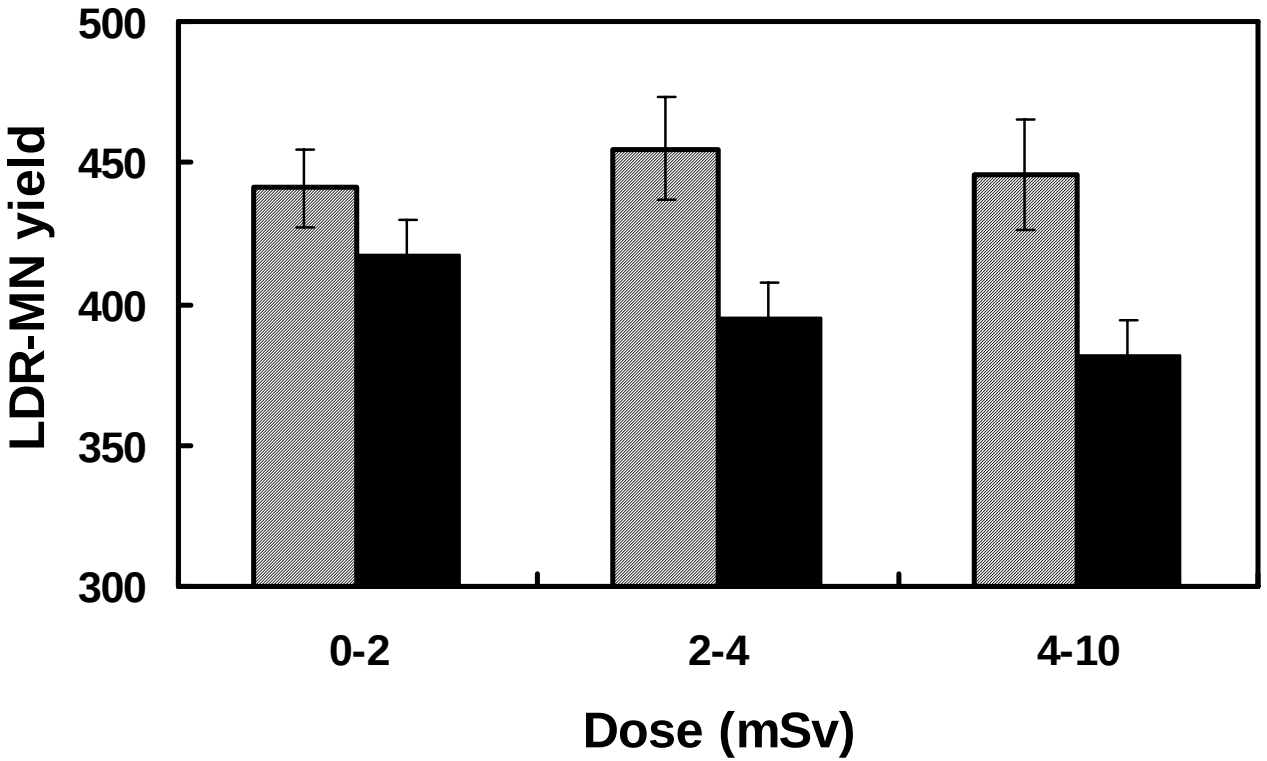
Results: LDR-MN assay

- A **significant**(p=0.0002)decrease of the LDR-MN frequency post-exposure 400±45 versus pre-exposure 445±58
- The correlation between the G0-MN data pre- and post-exposure is poor: r = 0.20.
- **One individual** (Δ) shows an **elevated radiosensitivity status** as well before as after the revision activities. The worker with the enhanced G2 radiosensitivity (○) scores also high with respect to the LDR-MN assay.

Results: LDR-MN assay



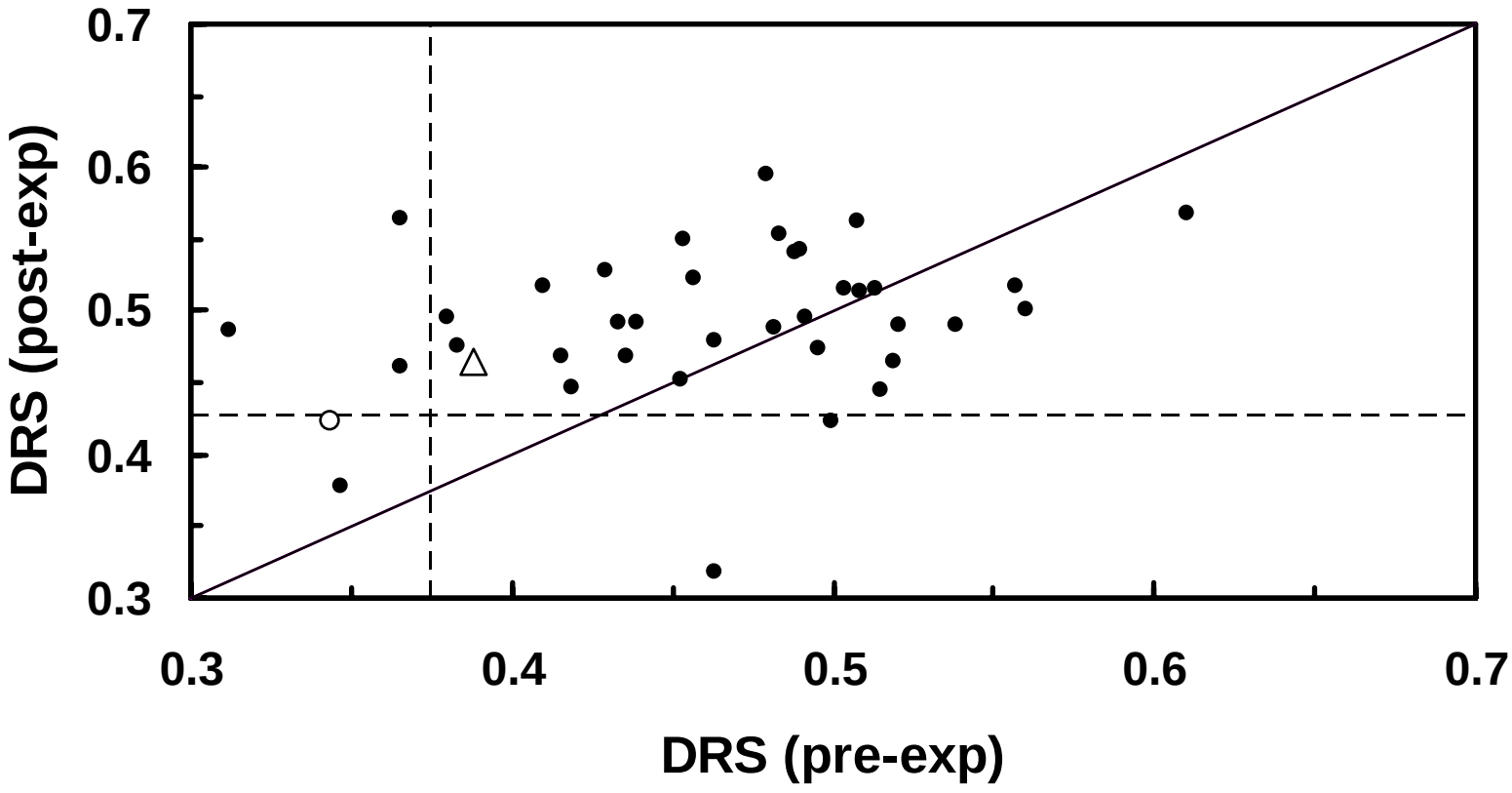
Results: LDR-MN assay



Results: LDR-MN assay

- A plot of the individual differences between the LDR-MN freq. post minus pre exposure versus dose shows **systematic drop with the dose**.
- An analysis of the data into dose groups (0-2 mSv, 2-4 mSv, 4-10 mSv) shows also a **significant decrease** ($p<0.05$)for the **highest class**.

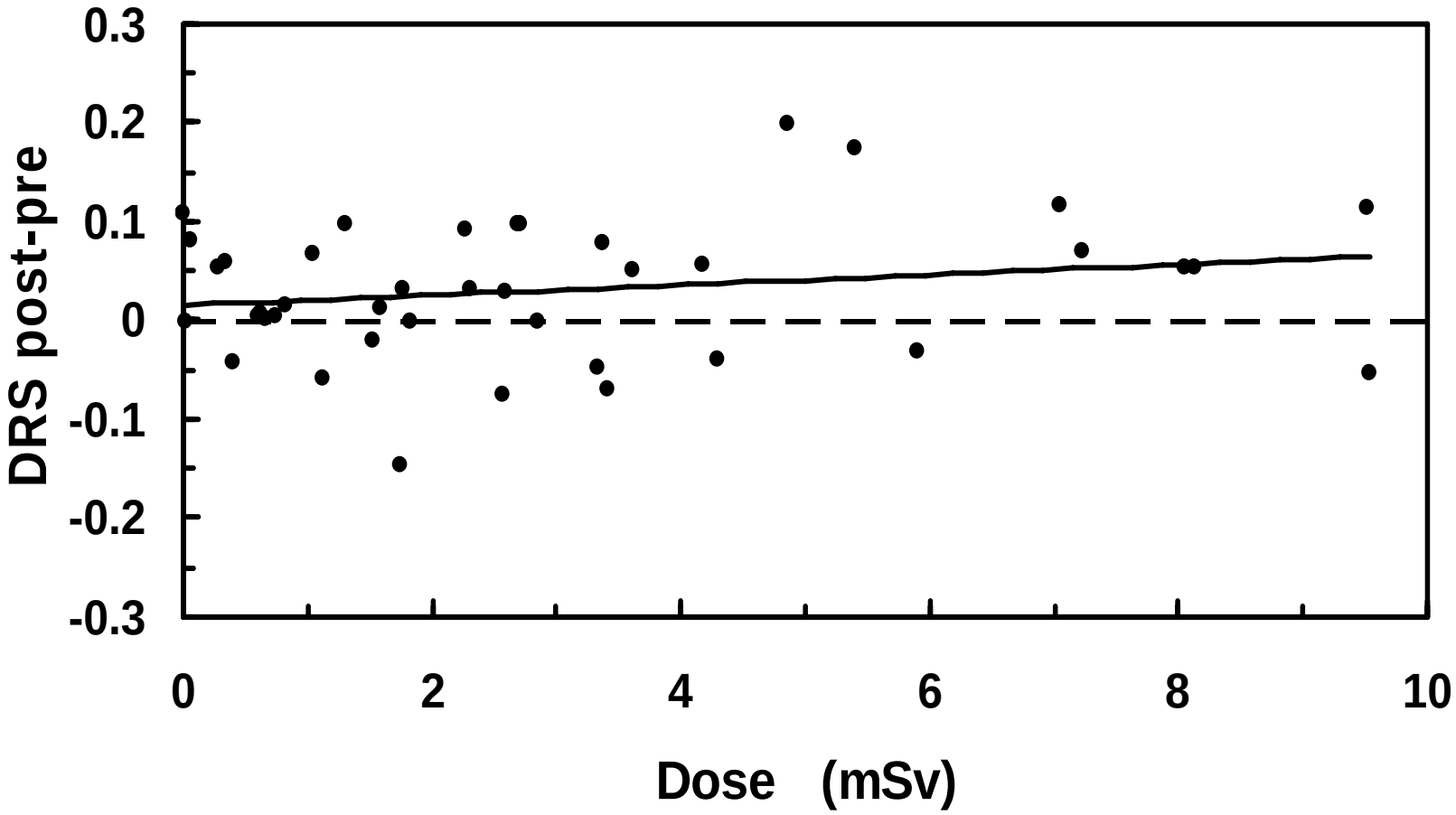
Results: Dose rate sparing



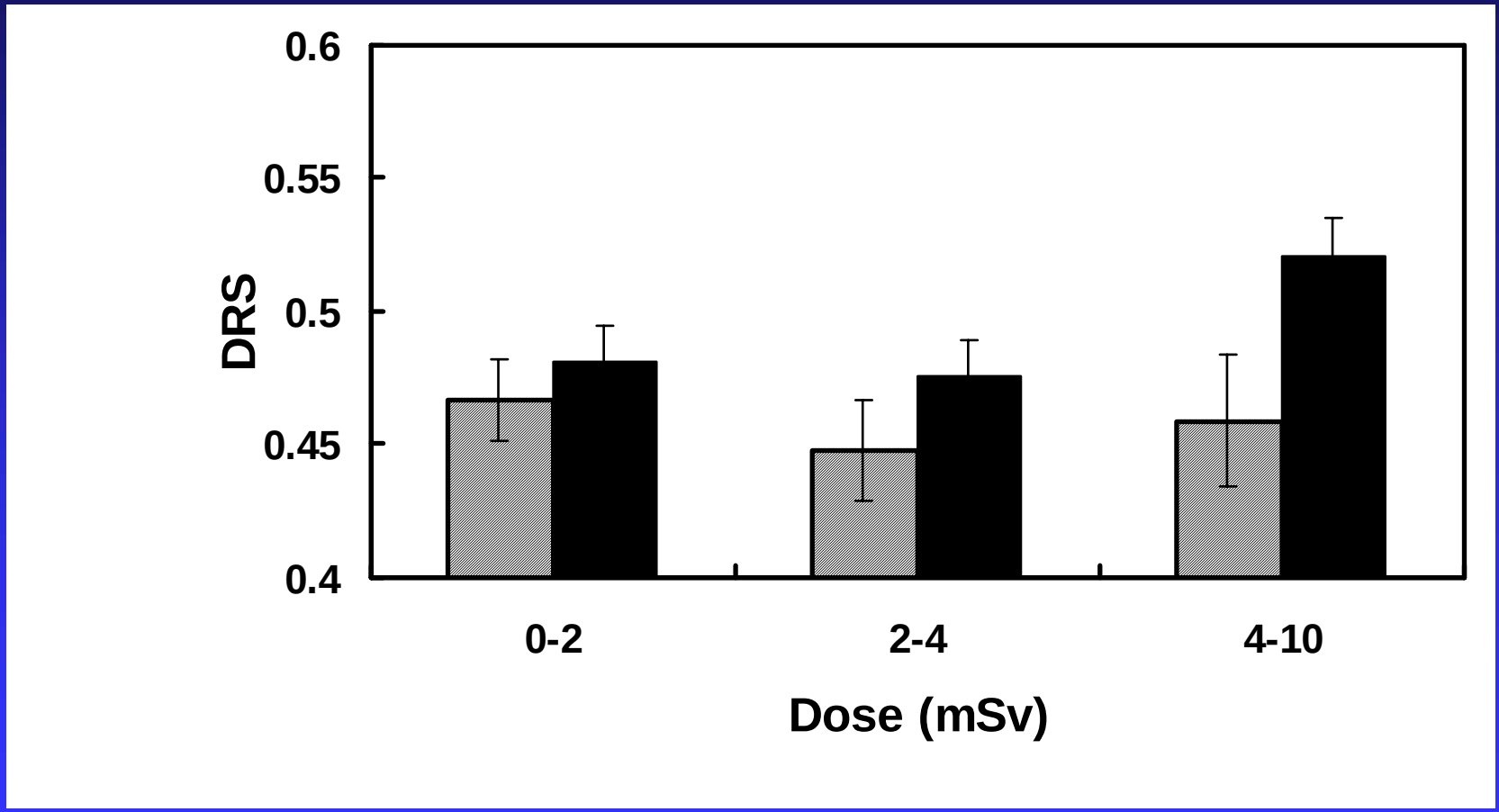
Results: Dose rate sparing

- A **significant**(p=0.006) increase of the dose rate sparing post-exposure 0.49±0.05 versus pre-exposure 0.46±0.07
- The correlation between the DRS values pre- and post-exposure is poor: r = 0.28.
- The **two radiation workers** showing an enhanced chromosomal radiosensitivity have a **reduced DNA repair** within the population.

Results: Dose rate sparing



Results: Dose rate sparing



Results: Dose rate sparing

- A plot of the individual differences between the DRS post minus pre exposure versus dose shows **systematic increase with dose**.
- An analysis of the data into dose groups (0-2 mSv, 2-4 mSv, 4-10 mSv) shows a **significant increase in DNA repair** ($p<0.05$)for the **highest class**.

Discussion

- Using the 90th percentile as cut-off, **two radiation workers** show **an elevated radio-sensitivity status** and reduced DNA repair within the population, pre- and post-exposure with the used chromosomal assays.
- ⇒ **Possible candidates** for **further investigation** of **underlying mechanisms** and **enhanced cancer risk**

Discussion

- The **MNLDR** and the **dose rate sparing** data show a **significant decrease** of the radiosensitivity and increase of repair in 8 out of 11 workers after a short term in vivo exposure with doses exceeding 4 mSv:

➔ **in vivo adaptive response phenomenon**

- Present study confirms conclusions of Barquinero et al.(1995) that **occupational dose** can act as an ***in vivo* conditioning dose**

- However, **no effect** on the **G2 data** and of previous **exposures before the revision exposure** (pre-exp. data) is observed on the chromosomal radiosensitivity

- Can be explained by the **time span** the effect remains effective (a few days) or **differences** in the **DNA DSB repair mechanisms** in G0 (non homologous end-joining) and G2 (homogous recombination) with different time kinetics .

Conclusions : further studies necessary

- **Impact** of **in vivo adaptive response** on the **cancer risk** induced by occupational exposure to mutagens and carcinogens.

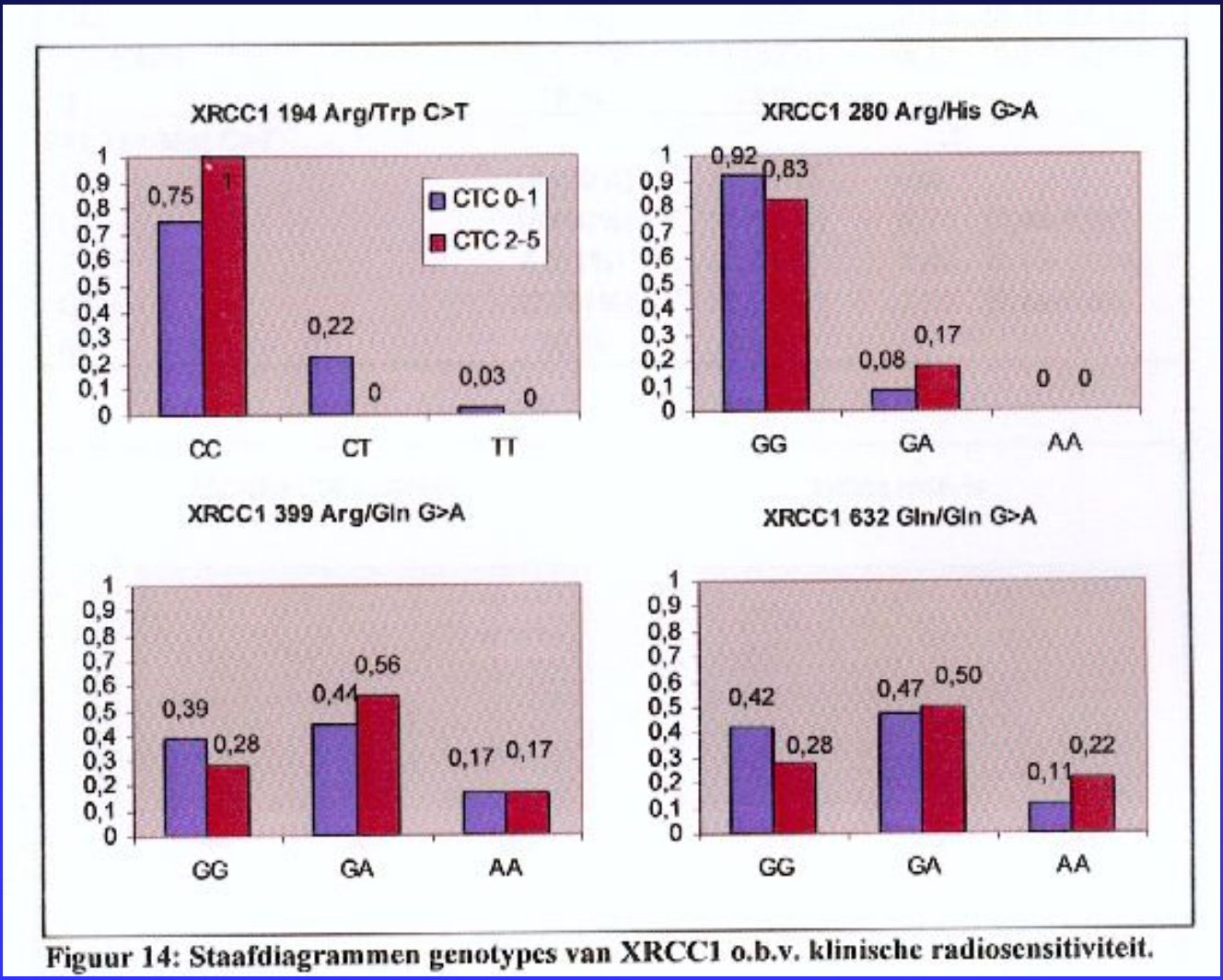
- Study of **genomics** to investigate mechanisms underlying enhanced radiosensitivity and cancer risk.

  $\Rightarrow$ Radiogenomics study radiotherapy patients

Radiogenomics in radiotherapy patients

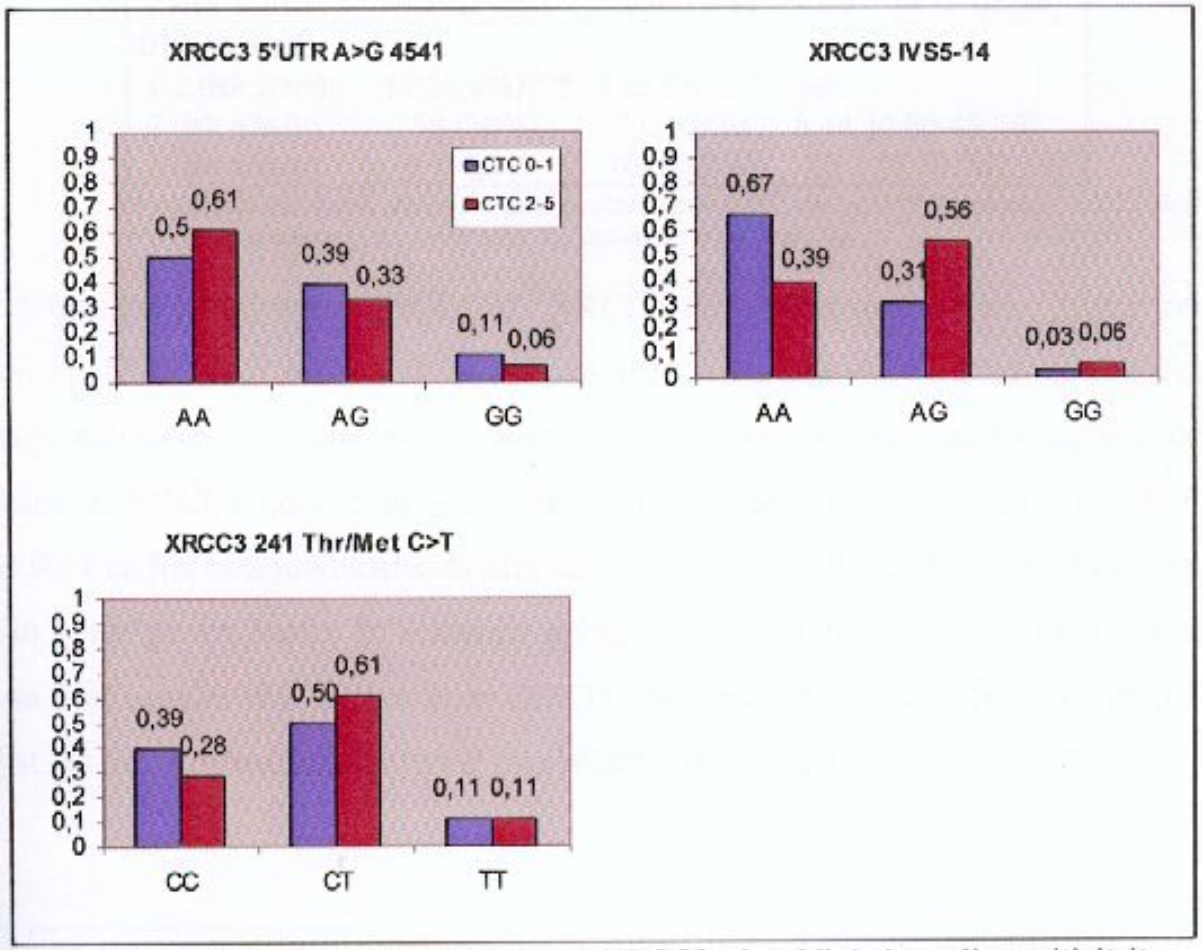
- Analysis of **single nucleotide polymorphisms** in DNA repair genes for 62 patients treated with radiotherapy for gynecological cancer
- Radiopathological follow up of these patients for complications: **CTC score**
- Comparison of genotype of group of patients with no of very low radiotoxic effects (CTC 0-1) with group of patients with moderate and severe complications (CTC 2-5)

Radiogenomics in radiotherapy patients: BER pathway XRCC1



Figuur 14: Staafdiagrammen genotypes van XRCC1 o.b.v. klinische radiosensitiviteit.

Radiogenomics in radiotherapy patients: HR pathway XRCC3



Figuur 15: Staafdiagrammen genotypes van XRCC3 o.b.v. klinische radiosensitiviteit.

Radiogenomics in radiotherapy patients

- The 280 Arg/His, 399 Arg/Gln and 632Gln/Gln XRCC1 polymorphisms, involved in cancer susceptibility, correlate slightly positive with an increased clinical radiosensitivity
- The XRCC3 (IVS5-14A/G) genotype is associated with an increased risk for radiotherapy reactions. The same holds but to a minor extent for the 241 Thr/Met XRCC3 polymorphism.
- These XRCC1 and XRCC3 polymorphisms can be considered as risk alleles.

Radiogenomics in radiotherapy patients

- An analysis of the data according to the number of XRCC1 and XRCC3 risk alleles in relation to the clinical radiosensitivity
-

Gene(s)	# risk alleles	CTC 0-1	CTC 2-5	Odd Ratio (95%CI)	p
XRCC1	2	64	89	9.0(1.1-75)	0.045
	3	0	6	- -	
XRRC3	1	51	37	2.2(0.23-21)	0.824
	2	33	58	5.5(0.60-52)	0.234
XRCC1	3	51	39	4.9(0.54-44)	0.266
&XRCC3	4	13	56	28.0(2.8-278)	0.004

Radiogenomics in radiotherapy patients

Conclusion: SNP analysis shows that the combined effect of different risk alleles in the XRCC1 and XRCC3 genes may result in an increased risk for radiotherapy complications.

⇒ Further studies necessary