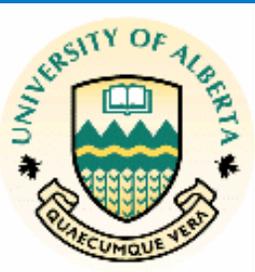


Candidate and whole genome SNP association studies of late radiation toxicity in prostate cancer patients

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Late normal tissue toxicity after radiotherapy for prostate cancer

- Gastrointestinal: fibrosis, stenosis, ulceration, telangiectasia, bleeding
- Genitourinary: fibrosis, stenosis, ulceration, telangiectasia, bleeding, erectile dysfunction

How might we reduce the burden of radiotherapy-related toxicity in our practice?

- Select, using clinical factors, patients for whom RT may be expected to most clearly benefit
- Give treatments with a high degree of conformality
- Develop pharmacologic strategies to protect normal tissues – by better characterizing the molecular targets of ionizing radiation
- Develop assays to predict which patients may display unexpectedly severe radiation toxicity and triage accordingly

Chronic toxicity grading – rectum

Scale	Gr. 0	Gr. 1	Gr. 2	Gr. 3	Gr. 4	Gr. 5
RTOG / EORTC	None	Slight rectal discharge or bleeding	Excessive rectal mucus or intermittent bleeding	Obstruction or bleeding requiring surgery	Necrosis / Perforation Fistula	Death

Chronic toxicity grading – bladder

Scale	Gr. 0	Gr. 1	Gr. 2	Gr. 3	Gr. 4	Gr. 5
RTOG / EORTC	None	Micro. hematuria	Moderate frequency Interm't Macro. hematuria	Freq. hematuria, severe frequency and dysuria	Necrosis/ contracted bladder severe hemorrh. cystitis	Death

SNP genotyping techniques can be readily performed on DNA isolated from leukocytes

1. Direct sequencing
 2. Hybridization
 3. Elution
 4. Mass spectrometry
- Emerging evidence that candidate SNPs (DNA repair/wound healing pathways) *may be* associated with late RT toxicity in breast, prostate, and gyne patients

Patient Characteristics – Cross Cancer Institute study

- 83 pts with prostate cancer underwent 3DCRT between Sept. 1996 and Dec. 2000
- Mean age 67 (range, 45 – 77)
- Mean PTV dose 77.1 Gy (range, 68.3 – 82.1)
- Number of fractions 35 – 44 (1.8-2 Gy min PTV)
- Median follow-up 28.4 mo. (range, 5-66)
- Number with >18 mo. follow-up = 81 (98%)

Choice of SNPs and Genotyping Technology

- We genotyped 49 RT-related SNPs from *ATM*, *BRCA1*, *BRCA2*, *XRCC1*, *XRCC2*, *XRCC3*, *NBS1*, *RAD51*, *RAD52*, *LIGIV*, *MLH1*, *MSH6*, *XPD*, *XPF*, *CYP1*, *CYP2*, and *TGF- β 1* genes
- Genotyping performed using the Pyrosequencing® technique.

Table 4: Multivariate analysis of factors associated with RTOG grade 2+ chronic toxicity

A:

variable	Best Subset of 4 predictors Score Chi-square = 36.6, p < 0.0001			Second Best Subset of 4 predictors Score Chi-Square = 34.9, p < 0.0001		
	p-value	Hazard	95% CI	p-value	Hazard	95% CI
LIG 4 T>C, Asp568Asp	0.0004	4.86	2.04 - 11.56	0.0034	3.56	1.52 - 8.31
CYP2D6*4 G>A, Splicing defect	-	-	-	0.0110	2.85	1.27 - 6.38
Age at diagnosis	0.0108	0.24	0.08 - 0.72	0.0002	0.13	0.04 - 0.38
Mean bladder dose	0.0111	3.02	1.29 - 7.08	-	-	-
Dose to 30% of rectal volume	0.0037	3.27	1.47 - 7.29	0.0005	4.14	1.87 - 9.17

B:

Variable	Best Subset of 5 predictors Score Chi-square = 42.0, p < 0.0001			Second Best Subset of 5 predictors Score Chi-Square = 41.7, p < 0.0001		
	p-value	Hazard	95% CI	p-value	Hazard	95% CI
LIG 4 T>C, Asp568Asp	0.0002	5.37	2.22 - 12.97	0.0003	5.22	2.14 - 12.73
ERCC2 G>A, Asp711Asp	0.0207	3.54	1.21 - 10.35	-	-	-
MSH6 T>C, Asp180Asp	-	-	-	0.0074	3.08	1.35 - 7.01
Age at diagnosis	0.0036	0.19	0.06 - 0.58	0.0057	0.22	0.07 - 0.64
Mean bladder dose	0.0086	3.19	1.34 - 7.59	0.0029	3.90	1.60 - 9.55
Dose to 30% of rectal volume	0.0105	2.89	1.28 - 6.53	0.0012	3.81	1.70 - 8.55

Candidate gene approach

Pro:

1. Based upon established pathways and reported, validated SNPs – high degree of biological plausibility
2. Usually (but not always) reflect changes in amino acid sequence

Con:

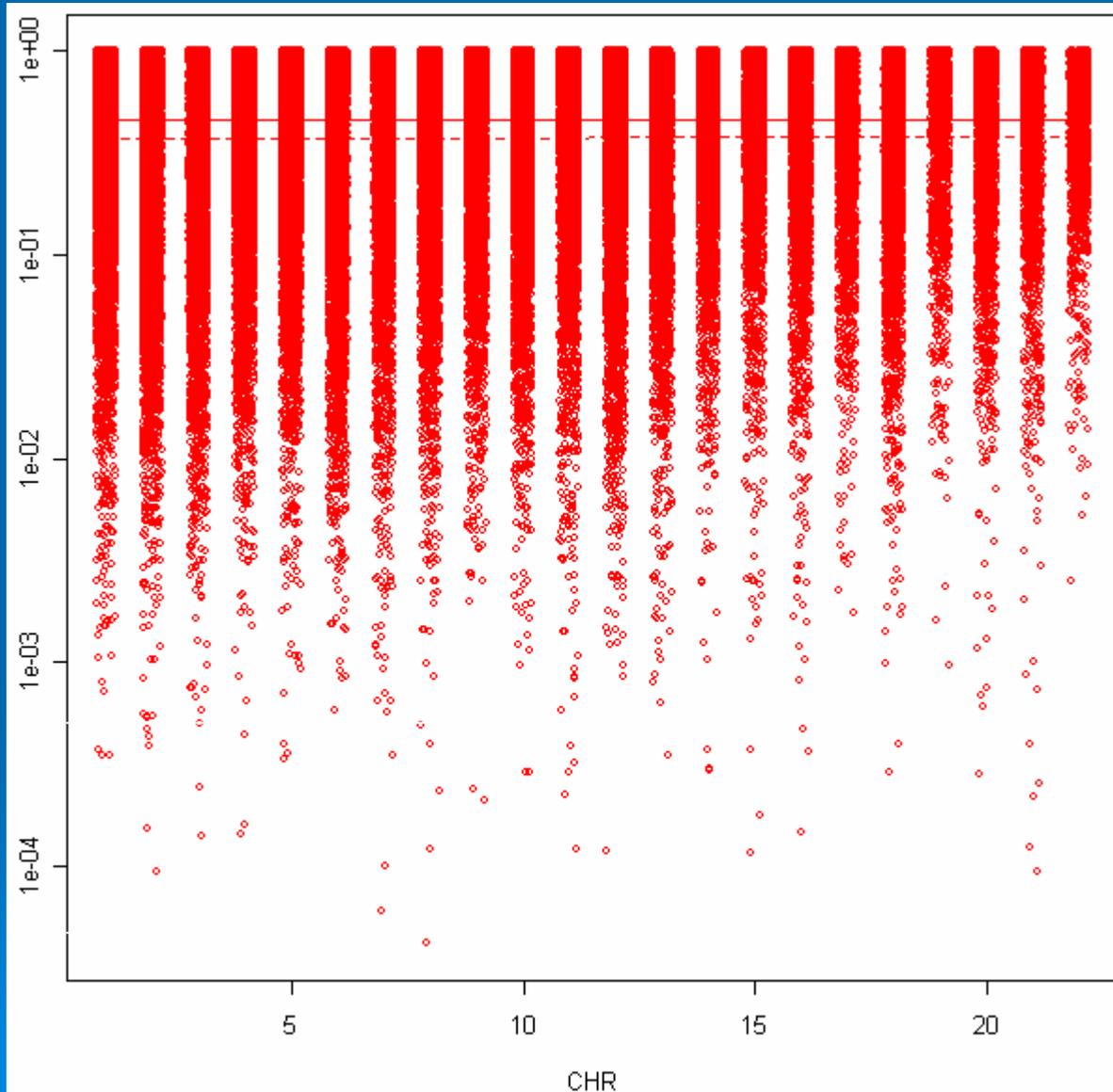
1. Unlikely to lead to discovery of new SNPs unless detailed mapping of contiguous sequences is performed
2. Will not detect intronic and nongenic SNPs - if they are important

Whole genome approach

- Affymetrix technology...250k SNP/chip
- 10^4 - 10^5 X number of variant-disease comparisons
- Pro: unbiased (“agnostic”) towards intronic, nongenic markers
- Con: false positive report probability can be difficult to estimate

Allelic Association Test (Distribution of Markers on the Genome)

Adjusted p-values

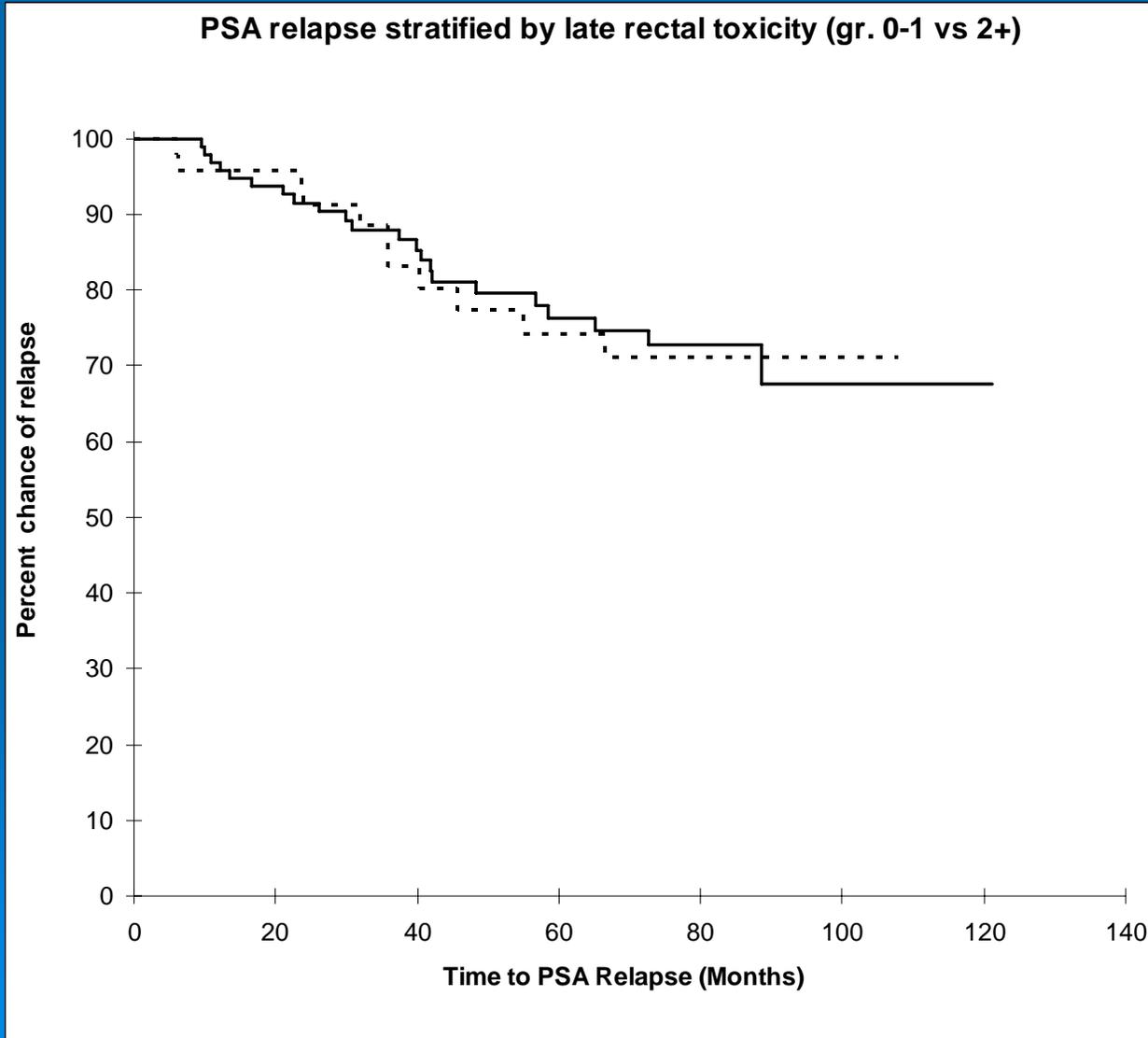


Chromosome Number

Exploratory whole genome association study for Edmonton prostate RT cohort

- Top 100 targets from Single Locus Association Tests were considered for further analysis and validation using independent cohorts and biological relevance

Does tumor radiosensitivity mirror normal tissue radiosensitivity?



RTOG prostate trial SNP initiatives

- Adam Dicker Translational Research Program chair
- Deb Citrin Normal Tissue working group
- Barry Rosenstein Normal Tissue working group

- Prospective collection of Buffy coat DNA:
 - RTOG 0126 (cohort >1500 pts)
 - RTOG 0415 (>1000 pts)
 - RTOG 0521 (600 pts)
 - RTOG 0612 (50 pts)

- Closed to accrual, collection in survivors
 - RTOG 9406 (potential 250-300 pts?)

Summary

1. Individual SNPs or those in LD may be associated with excess normal tissue radiotoxicity in some patients
2. SNPs in genes related to DNA damage recognition and repair, as well as wound healing and tissue homeostasis are potential candidates; whole genome studies in progress
3. Validation studies with comprehensive dosimetric analysis will be necessary in order to understand the independent contribution of SNPs (or haplotypes) to the expression of RT injury before this approach enters the clinic

Thank you

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