Genetics of radiation-related non-cancer diseases

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DCEG Radiation Epidemiology and Dosimetry Course 2019

https://dceg.cancer.gov/RadEpiCourse
Learning outcomes (1)

- Studies in patients receiving therapeutic radiation can increase understanding of the genetics of radiation-related non-cancer diseases
- Genetic studies of radiation toxicity must allow for potential confounders and modifiers
- The genetic determinants of non-cancer effects will involve rare variants with large effects, low frequency variants with moderate effects and common variants with small effects
Learning outcomes (2)

- Building cohorts with good quality data and sample collections is key and a consortium approach is required.
- Study design considerations include: retrospective/prospective, case-control/cohort, time-point for assessing toxicity, allowing for baseline toxicity, harmonizing data collected using different scoring systems, variables to include in multivariable analyses, statistical power.
- Studies should follow STROGAR reporting guidelines.
- Fine-mapping identifies the probable genetic variant (or variants) and provides mechanistic understanding.
Non-cancer effects of radiation

- Cataracts
- Circulatory disease – vascular damage
- Non-malignant respiratory and digestive diseases
- Cognitive impairment

- Radiotherapy toxicity

Studies in patients receiving therapeutic radiation can increase understanding of the genetics of radiation-related non-cancer diseases
Effects of radiation on normal tissues are tissue and time dependent.

- DNA damage
- Chromosome aberrations
- Cell kill, mutations
- Cancer

The long timescale of radiation effects:

- Physical
- Chemical
- Biological

- 10^-18 to 10^-6 seconds
- 10^-2 to 10^3 minutes
- 10^6 hours
- 1 day
- 10^3 days
- 1 month
- 10^3 months
- 1 year

- Excitations ionisations
- Enzyme reactions
- Repair
- Early effects
- Late effects
- Carcinogenic effects
- Cataracts
- Cardiac effects
Many factors affect risk of radiotherapy toxicity

- Physical factors: radiation dose, volume and type
- Treatment factors: prior surgery, use of chemotherapy or other treatments
- Patient factors: age, smoking, comorbidities
- Genetics

When we say “it’s not rocket science,” we mean it’s far more complicated.

Genetic studies of radiation toxicity must allow for potential confounders & modifiers.
The relationship between genotype and radiation effects is affected by modifiers and confounders

**Modifiers**
Variables *directly* related to risk of radiotherapy toxicity  
e.g. radiation dose, volume, type  
Potential Modifiers  
Age, surgery, smoking

**Confounders**
Variables *indirectly* related to risk of radiotherapy toxicity  
e.g. ethnicity, diabetes, collagen vascular disease, body mass index, breast size

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Genotype

Dose

Radiotherapy Toxicity

Dose
Radiogenomics aims to identify the genetic determinants of radiotherapy toxicity

- Single nucleotide variants (SNVs): mutations, **common** single nucleotide polymorphisms (SNPs)
- Copy number variation (insertions/deletions - indels)
- Epigenetics is also likely to be involved - methylation

Identifying patients with an increased risk of toxicity to personalise treatments
The genetic determinants of non-cancer effects will involve rare variants with large effects, low frequency variants with moderate effects and common variants with small effects.

Ataxia telangiectasia frequency 1/100,000
Homozygous mutations in ATM are rare associated with high relative risks

Allelic architecture: number, type, effect size and frequency of susceptibility variants of a trait
### Rare homozygote variants with large effects

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Mutated Gene(s)</th>
<th>Associated with</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ataxia telangiectasia (AT)</td>
<td>ATM</td>
<td>Radiotherapy side effects</td>
</tr>
<tr>
<td>AT-like disorder-1</td>
<td>MRE11</td>
<td>Cellular radiosensitivity</td>
</tr>
<tr>
<td>Cornelia de Lange syndrome</td>
<td>SMCL1A</td>
<td>Variable radiosensitivity</td>
</tr>
<tr>
<td>Cowden syndrome</td>
<td>PTEN</td>
<td>Radiotherapy side effects</td>
</tr>
<tr>
<td>Fanconi anemia</td>
<td>Numerous</td>
<td>Radiotherapy side effects in some</td>
</tr>
<tr>
<td>Gorlin syndrome</td>
<td>PTCH1</td>
<td>Cellular radiosensitivity, risk of second cancer</td>
</tr>
<tr>
<td>Li-Fraumeni syndrome</td>
<td>TP53</td>
<td>Risk of second malignancy</td>
</tr>
<tr>
<td>Ligase IV syndrome</td>
<td>LIG4</td>
<td>Radiotherapy toxicity</td>
</tr>
<tr>
<td>Neurofibromatosis type 1</td>
<td>NF1</td>
<td>Risk of second malignancy</td>
</tr>
<tr>
<td>Nijmegen breakage syndrome (NBS)</td>
<td>NBN</td>
<td>Radiotherapy toxicity</td>
</tr>
<tr>
<td>NBS-like syndrome</td>
<td>RAD50</td>
<td>Cellular radiosensitivity</td>
</tr>
<tr>
<td>Radiosensitive SCID</td>
<td>DCLRE1C, PRKDC</td>
<td>Cellular radiosensitivity</td>
</tr>
<tr>
<td>Retinoblastoma</td>
<td>RB1</td>
<td>Moderate radiosensitivity, risk of second cancer</td>
</tr>
<tr>
<td>RIDDLE syndrome</td>
<td>RNF168</td>
<td>Cellular radiosensitivity</td>
</tr>
</tbody>
</table>
Mechanism of action of radiation involves multiple genes/pathways

Candidate genes

- DNA repair
  - HR
    - RAD52
    - RAD51
    - RAD54
    - XRCC2
    - XRCC3
  - NHEJ
    - PRKDC
    - XRCC6
    - XRCC5
    - LIG4
    - XRCC4
    - DCLRE1C
- Ionising Radiation
  - ATM
  - ATR
  - NBN
  - RAD50
  - MRE11
  - YH2AX
- DNA double strand break repair
  - RNF8
  - MDC1
  - RNF168
- Apoptosis
  - GADD45
  - APAF1
  - BAX
- Cell cycle arrest
  - CDKN1A
  - P21
  - CDK1
  - CDK2
- Ionisation cluster
  - Multiply damaged site

Inflammation
- ROS/RNOS
  - NOS, SOD, CAT
- Cytokines, growth factors, chemokines
  - TGFB1, EGF, FGF, IL2, IL8, TNF

Microenvironment
- Fibroblasts
- Neutrophils
- Macrophages
- Endothelial cells

Target cell nucleus

ERK/MAPK signalling
- AKT
- PI3K
- RAS
- ERK
- MAPK

Smad signalling

Target cell membrane & cytoplasm
Common variants have small effects

- Strongest association for ATM rs4988023 OR 1.53; 95% CI 1.08-2.18
- Previously reported late toxicity associations were not replicated
- Individual effect sizes are small and not clinically relevant
- Large studies needed
Finding the unknown unknowns requires GWAS

I want more samples! 2000 is not enough! 5000, 10000, 20000 are not enough! I want more!

Building cohorts with good quality data and sample collections is key and a consortium approach is required
Identifying genetic variants requires careful consideration of study design

- Prospective or retrospective?
- Which toxicity data scoring system or how can data collected using different scoring systems be harmonized?
- If baseline toxicity data are available, should it be used to correct for?
- What is the best time point for assessing toxicity?
- Case-control or cohort (all patients at a particular time-point) study?
Identifying genetic variants requires careful consideration of study design

- Fixed time point or time-to-event?
- What data should be included in multivariable analyses?
- Is it possible to deal with missing data?
- Candidate gene or genome wide association study (GWAS)?
- What is the level of statistical power?

Study design considerations include: retrospective/prospective, case-control/cohort, time-point for assessing toxicity, allowing for baseline toxicity, harmonizing data collected using different scoring systems, variables to include in multivariable analyses, statistical power
Statistical power

- The power to detect a genetic variant depends on allelic effect size, marker allele frequency and toxicity endpoint prevalence.
- Common risk alleles for most complex traits confer relative risks of 1.05 - 1.2.
- Power to detect most alleles is limited.

Power of 10,000 cases GWAS to detect risk alleles of frequency 0.2 for toxicities of prevalences of 10, 20 and 30%
# A standardized total average toxicity (STAT) score to pool data from different studies

<table>
<thead>
<tr>
<th>CLINICAL INVESTIGATION</th>
<th>Normal Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>STANDARDIZED TOTAL AVERAGE TOXICITY SCORE: A SCALE- AND GRADE-INDEPENDENT MEASURE OF LATE RADIOTherAPY TOXICITY TO FACILITATE POOLING OF DATA FROM DIFFERENT STUDIES</strong></td>
<td></td>
</tr>
<tr>
<td>Gillian C. Barnett, B.M., B.Ch.,*†</td>
<td>Catherine M. L. West, Ph.D.,†‡</td>
</tr>
<tr>
<td>George A. Tanteles, M.D.,†</td>
<td>R. Paul Symonds, M.D.,‡</td>
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</tbody>
</table>

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Reporting guidelines should be followed

Studies should follow STROGAR reporting guidelines
Recent large candidate gene studies identified risk variants

**TNF rs1800629**
2,036 breast cancer patients
OR=2.4 for late effects

**XRCC1 rs2682585**
2,636 breast cancer patients
OR=0.77 for late effects

**ATM rs1801516**
5,458 breast & prostate cancer patients
OR~1.5 for early effects and 1.2 for late effects
GWAS identified novel genes

A three-stage genome-wide association study identifies a susceptibility locus for late radiotherapy toxicity at 2q24.1

Laura Fachal1,2, Antonio Gomez-caamano3, Gillian C. Barnett5, Paula Peleteiro3, Ana M. Carballo1, Patricia Calvo Crespo3, Sarah I. Kerns4, Manuel Sanchez-Garcia4, Ramon Lobato-Busto4, Leila Dorling1, Rebecca M. Elliot2, David P. Dearaley2, Matthew R. Sydes4, Emma Hall4, Neil G. Burnett5, Angel Cerracado1,2,4,12, Barry S. Rosenstein2, Catharine M. West4, Alison M. Dunming3 & Ana Vega1,2

Gene involved in muscle cell regeneration with overall toxicity

Gene involved in erectile function with erectile dysfunction

Gene involved in regulation of angiogenesis and rectal bleeding

Gene involved in smooth muscle contraction and rectal incontinence
Most recent Radiogenomics Consortium meta-analysis


- 5,705 prostate cancer patients, 3,874 analyzed
- ↑ urinary frequency, ↓ urinary stream, hematuria, rectal bleeding
- Baselines corrected late toxicity: time to first ≥grade 2 toxicity event
- Multivariable analysis: androgen deprivation therapy, prior prostatectomy, age at treatment, and total BED
### Radiogenomics Consortium Meta-analysis

**OncoArray-500K, ~7 million SNPs**  
**Time to grade 2/3 toxicity, n=3,874**

<table>
<thead>
<tr>
<th></th>
<th>RAPPER n=2,010</th>
<th>RADIOKEN n=658</th>
<th>GenePARE n=495</th>
<th>UGhent n=311</th>
<th>CCI-BT n=252</th>
<th>CCI-EBRT n=148</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Median age (yr)</strong></td>
<td>68</td>
<td>72</td>
<td>65</td>
<td>65</td>
<td>65</td>
<td>68</td>
</tr>
<tr>
<td><strong>Intermed/high risk</strong></td>
<td>60%</td>
<td>81%</td>
<td>53%</td>
<td>74%</td>
<td>38%</td>
<td>92%</td>
</tr>
<tr>
<td><strong>Prior surgery</strong></td>
<td>0%</td>
<td>20%</td>
<td>0%</td>
<td>31%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td><strong>Hormones</strong></td>
<td>100%</td>
<td>70%</td>
<td>51%</td>
<td>64%</td>
<td>22%</td>
<td>49%</td>
</tr>
<tr>
<td><strong>Median BED (Gy)</strong></td>
<td>120</td>
<td>123</td>
<td>204</td>
<td>136</td>
<td>158</td>
<td>153</td>
</tr>
</tbody>
</table>

CCI=Cross Cancer Institute; BED=biological effective dose

**Sarah Kerns et al 2019, JNCI May 16**
Three new SNPs identified

p-values must be <5 x 10^{-8}

<table>
<thead>
<tr>
<th>SNP</th>
<th>MAF</th>
<th>Toxicity</th>
<th>HR (95% CI)</th>
<th>P</th>
<th>BFDP</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs17055178</td>
<td>0.09</td>
<td>Rectal bleeding</td>
<td>1.95 (1.58, 2.43)</td>
<td>6.2x10^{-10}</td>
<td>0.09%</td>
</tr>
<tr>
<td>rs10969913</td>
<td>0.05</td>
<td>Decreased stream</td>
<td>3.92 (2.50, 5.83)</td>
<td>2.9x10^{-10}</td>
<td>1.07%</td>
</tr>
<tr>
<td>rs11122573</td>
<td>0.06</td>
<td>Hematuria</td>
<td>1.92 (1.53, 2.42)</td>
<td>1.8x10^{-8}</td>
<td>1.96%</td>
</tr>
</tbody>
</table>

MAF = minor allele frequency; BFDP = Bayesian false discovery probability

Kerns et al 2019 *JNCI* May 16
### Previously identified variants validated

<table>
<thead>
<tr>
<th>SNP</th>
<th>MAF</th>
<th>Toxicity</th>
<th>HR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs17599026 KDM3B</td>
<td>0.07</td>
<td>Urinary frequency</td>
<td>1.55 (1.23, 1.95)</td>
<td>1.8x10^-4</td>
</tr>
<tr>
<td>rs7720298 DNAH5</td>
<td>0.30</td>
<td>Decreased stream</td>
<td>1.43 (1.14, 1.78)</td>
<td>1.6x10^-3</td>
</tr>
<tr>
<td>rs1801516 ATM</td>
<td>0.22</td>
<td>Overall toxicity</td>
<td>1.29 (1.07, 1.55)</td>
<td>6.3x10^-3</td>
</tr>
<tr>
<td>rs7582141 TANC1</td>
<td>0.05</td>
<td>Overall toxicity</td>
<td>1.99 (1.33, 2.98)</td>
<td>8.3x10^-4</td>
</tr>
</tbody>
</table>

MAF = minor allele frequency

Kerns et al 2019 *JNCI*
Fine scale mapping

- GWAS find associations between a genomic region and a trait
- It is assumed at least one causal variant exists
- Fine-mapping identifies the probable genetic variant (or variants)
- Less common variants with larger effects can be found

- Second independent signal
- rs147121532 with hematuria
- MAF 0.01%
- HR 4.43, 95% CI 2.35 - 8.33
- P = 4.7x10⁻⁶
Radiogenomics can increase mechanistic understanding

- Credible causal variants (CCVs) associated with differential expression of local protein coding gene (AGT)

- AGT encoding angiotensinogen is converted to the active enzyme angiotensin II by angiotensin converting enzyme

- Prior studies suggest angiotensin signaling may influence radiation-induced blood vessel wall injury and interstitial fibrosis

- Pathway analysis identified ‘platelet adhesion to exposed collagen’
Platelet adhesion is the first step in the formation of a platelet plug, formed in response to blood vessel injury.

Collagens are involved in the process and are abundant in vascular epithelia.

Several collagen binding proteins are expressed on platelets e.g. integrins.

The integrin pathway was also associated with rectal bleeding.

Fine-mapping identifies the probable genetic variant (or variants) and provides mechanistic understanding.
Radiogenomics for particle beam therapy

Rs17599026 in *KDMB3* associated with decreased urinary stream

<table>
<thead>
<tr>
<th>Cohort</th>
<th>MAF</th>
<th>n</th>
<th>Radiation</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RGC</td>
<td>0.07</td>
<td>3,874</td>
<td>Photons</td>
<td>1.55 (1.23, 1.95)</td>
</tr>
<tr>
<td>PRRG</td>
<td>0.11</td>
<td>170</td>
<td>Photons</td>
<td>1.51 (0.56, 4.05)</td>
</tr>
<tr>
<td>NTMC</td>
<td>0.11</td>
<td>254</td>
<td>Protons</td>
<td>1.13 (0.49, 2.64)</td>
</tr>
<tr>
<td>PRRG</td>
<td>0.11</td>
<td>538</td>
<td>Carbon ions</td>
<td>0.63 (0.27, 1.49)</td>
</tr>
</tbody>
</table>

Kerns S, Rosenstein B, Tsuji H, Imai T, Saito S
Learning outcomes

- Studies in patients receiving therapeutic radiation can increase understanding of the genetics of radiation-related non-cancer diseases
- Genetic studies of radiation toxicity must allow for potential confounders and modifiers
- The genetic determinants of non-cancer effects will involve rare variants with large effects, low frequency variants with moderate effects and common variants with small effects
Learning outcomes

- Building cohorts with good quality data and sample collections is key and a consortium approach is required
- Study design considerations include: retrospective/prospective, case-control/cohort, time-point for assessing toxicity, allowing for baseline toxicity, harmonizing data collected using different scoring systems, variables to include in multivariable analyses, statistical power
- Studies should follow STROGAR reporting guidelines
- Fine-mapping identifies the probable genetic variant (or variants) and provides mechanistic understanding
Summary

- Radiogenomic studies of radiotherapy toxicity can increase understanding of the genetics of non-cancer diseases

- Collaborative work within the Radiogenomics Consortium has (and is) developing the methodology and best study designs by working with experts within and outside the radiation community

- The approaches can be applied to study the genetics of other non-cancer diseases of interest to the radiation epidemiology community
Further reading


The genetic determinants of radiotherapy toxicity involve:

A. Rare mutations with small effects
B. Common single nucleotide polymorphisms (SNPs) with large effects
C. Low frequency variants with moderate effects
D. Only mutations and SNPs
The genetic determinants of radiotherapy toxicity involve:

A. Rare mutations with small effects
B. Common single nucleotide polymorphisms (SNPs) with large effects
C. **Low frequency variants with moderate effects**
D. Only mutations and SNPs
The best radiogenomic study designs:

A. Are retrospective
B. Are case control
C. Use univariate analyses
D. Follow STROGAR guidelines
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