Cancer Risk from Radiation Exposure: The Role of Genetic Susceptibility
Assays such as LD$_{50/30}$

Whole Organism

Consequence of radiotherapy
- e.g. skin erythema, lung fibrosis

Clinical radiosensitivity

Risk differences in populations
- Epidemiology studies

Susceptibility to Radiation Carcinogenesis

By specific tissues/organs
- Epidemiology/clinical studies

Tissue radiosensitivity

Cellular radiosensitivity
- e.g. cell killing, chromosomal damage, DNA damage

Adapted from Human Radiosensitivity: Report of AGIR, 2013
Genetic Susceptibility to Radiation

- Rare syndromes with extreme radiosensitivity

- Ataxia-telangiectasia (AT)
  - Rare childhood neurodegenerative disease
  - Caused by mutations in *ATM* gene

- Cultured fibroblasts from patients three times as sensitive to radiation (Taylor et al., 1975)
How Does this Affect the General Population?

Figure 1a Increasing Incidence with Radiation Dose

Figure 1b Underlying Subpopulations at Different Risk

Adapted from Hendry et al, 2006
Types of Genetic Variation

- Chromosomes, genes, RNA, DNA

- 3 billion b.p.
- 6 million diff.
Single Nucleotide Polymorphisms (SNPs)

- Most common genetic variation
- Each individual has two alleles
  - CC (common referent)
  - CT (heterozygote)
  - TT (homozygous variant)
- Much of the variation appears meaningless
- Some variation increases risk of cancer
Approaches to Study Genetic Variation - 1

1. Candidate gene approach

2. Pathways of Interest
   - DNA repair
   - Cell-cycle control
   - Apoptosis
   - Immune-related
   - Oxidative Response
Agnostic: GWAS, exome/genome-wide sequencing

- Agnostic – no assumptions about underlying biology
- GWAS approach has identified 475+ risk loci in germline DNA for cancers
- Typically 600,000 to 5 million markers across genome
Examples from Radiation

Genetic Susceptibility to Radiation-related Breast Cancer
Candidate Gene Approach: Occupational Radiation

- Nested case-control study within USRT cohort
  - 858 breast cancer, 1083 cancer-free controls
- Radiation dose
  - occupational, personal medical
- Blood samples for DNA
**Occupational Radiation, USRT**

**Breast Cancer SNP-radiation interactions:**

<table>
<thead>
<tr>
<th>Pathway (SNPs, genes examined)</th>
<th>Gene</th>
<th>SNP effect</th>
<th>Radiation Interaction</th>
<th>Replication</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA repair (61 SNPs, 21 genes)</td>
<td>PRKDC</td>
<td>✓</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>BRCA2</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apoptosis and proliferation (16 SNPs, 8 genes)</td>
<td>IL1A</td>
<td>✓</td>
<td>✓</td>
<td>?</td>
</tr>
<tr>
<td></td>
<td>CASP8</td>
<td>✓</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>Oxidative stress and inflammation (28 SNPs, 16 genes)</td>
<td>PTGS2</td>
<td>✓</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IL1B</td>
<td>✓</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>IL4</td>
<td>✓</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>GWAS Identified (38 SNPs, 35 genes)</td>
<td>MRPS30</td>
<td>✓</td>
<td>✓</td>
<td>?</td>
</tr>
</tbody>
</table>

Summary: Candidate Gene approach

- Focus on genes thought to be involved in radiation toxicity
- A few signals, but not consistent
- Limited knowledge of underlying biology; ability to query genome

**DNA Repair Damage**
- ATM, NBN, BRCA1,2
- H2AFX, RB1, XRCC1,4,5,6
- PRKDC, LIG4, Cyclins, CDKs, CDK inhibitors

**Radiation Fibrogenesis**
- TP53, BCL2, CASP3, TNF, ILIA, IL6, TGFB1,2,3
- SMADs

**Oxidative Stress**
- SOD1,2,3

**Endothelial Cell Damage**
- FGF2
- VEGF
Alternate Approach: Rare Mutations in Breast Cancer Susceptibility Genes

- \(BRCA1, BRCA2, ATM, RAD51\)

- Repair of double-strand DNA breaks by homologous recombination

- Inactivation in these genes predisposes to breast (and other) cancers

Figure source: Venkitaraman, NEJM 2003
### Diagnostic Radiation (X-rays) and Breast Cancer

#### Population Exposure Results Citation

<table>
<thead>
<tr>
<th>Population</th>
<th>Exposure</th>
<th>Citation</th>
</tr>
</thead>
</table>
| • 1,601 BRCA1/2 carriers cohort  
• UK, Canada, Netherlands, France | Chest x-rays  
- ever/never; <20yr  
- no. x-rays by age | ref never x-ray  
Higher with younger age, more reported x-rays  
Andrieu et al, JCO, 2006 |
| • 138 BRCA1 breast cancer; 158 non-mutation breast cancer  
• Poland | Chest x-rays  
- ever  
- <30yr | OR=1.7 (0.9, 3.0)  
OR=1.8 (1.2, 2.9)*  
ref non-carrier  
Gronwald et al, Br Ca Res Treat, 2008 |
| • 379 BRCA1, 611 non-mutation  
• France | Chest x-rays  
- ever, <20yr  
- no. x-rays by age | HR = 4.29 (2.1,8.8)*  
No variation by age at exposure, number x-rays  
Lecarpentier et al, Br Ca Res Treat, 2011 |
| • 454 BRCA1, 273 BRCA2 carriers  
<50yrs  
• US, Canada, Aus/NZ | Chest x-rays, ever/never | OR=1.16 (0.64-2.11) for BRCA1  
OR=1.22 (0.62-2.42) for BRCA2  
John et al, CEBP, 2013 |

### Notes
- **Self Report - misclassification - bias**
- **Overlapping populations**
- **Confounding by indication?**

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- **Overlapping populations**

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- **Self Report - misclassification - bias**

- **Overlapping populations**

- **Confounding by indication?**
## Diagnostic Radiation (Mammograms) and Breast Cancer

<table>
<thead>
<tr>
<th>Population</th>
<th>Exposure</th>
<th>Odds Ratio (95% Confidence Interval)</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,600 BRCA breast cancer cases, 1,600 non-cancer controls</td>
<td>Age at 1&lt;sup&gt;st&lt;/sup&gt; Mammography &gt;1yr before dx</td>
<td>OR=1.03 (0.85, 1.25)</td>
<td>Narod et al, Lancet Oncol, 2006</td>
</tr>
<tr>
<td>162 BRCA carriers; 34 cases</td>
<td>No. of mammograms &gt;1yr before enrollment</td>
<td>OR=0.94 (0.88, 1.00)</td>
<td>Goldfrank et al, 2006</td>
</tr>
<tr>
<td>2,346 BRCA 1/2 carriers; 238 cases</td>
<td>Any prior mammography; first mammogram age &lt;30 yrs</td>
<td>HR=0.79 (0.53, 1.19); HR 0.90 (0.35, 2.34)</td>
<td>Giannakes et al, Breast Ca Res Treat 2014</td>
</tr>
</tbody>
</table>

- Mammograms more likely to be accurately reported
- Inconsistent results

Limited Power

*Note: OR = Odds Ratio, HR = Hazard Ratio*
Risk of Breast Cancer in 1,993 BRCA 1/2 carriers

Estimated dose from any diagnostic radiation before age 30 years, HR=190 (1.20-3.00)

848 cases
Cumulative doses
Weighted Cox
Sensitivity analyses

Pipje et al, BMJ 2012
• 247 contralateral breast cancer (CBC) cases

• 51 pathogenic germline mutations in $BRCA1$, $BRCA2$, $CHEK2$, $ATM$ in 247 CBC patients

• Radiotherapy-related risk for DDRP germline mutation carriers
  – OR=2.2 (1.03, 4.62) overall
  – OR=2.51 (1.03, 6.10) for CBC five or more years after RT

Therapeutic Radiation and Breast Cancer - 2

WECARE Nested Case-Control Study

- 708 contralateral breast cancer cases; 1,397 controls
- No increase in risk with radiation dose in carriers

<table>
<thead>
<tr>
<th>Gene</th>
<th>Radiation</th>
<th>Cases/Cntr Is</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA1/2 carrier</td>
<td>--</td>
<td>96/62</td>
<td>4.5 (3.0 - 6.8)</td>
</tr>
<tr>
<td>No mutation</td>
<td>≥ 1 Gy</td>
<td>118/406</td>
<td>1.2 (1.0 - 1.6)</td>
</tr>
<tr>
<td>BRCA1/2 Carrier</td>
<td>≥ 1 Gy</td>
<td>21/26</td>
<td>1.0 (0.4 - 2.8)</td>
</tr>
</tbody>
</table>

Bernstein JB et al., EJC, 2013
<table>
<thead>
<tr>
<th>Radiation Gy</th>
<th>Case/Cntrl</th>
<th>OR (95% CI)</th>
<th>OR* (95% CI)</th>
<th>ERR/Gy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>271/480</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any rare ATM variant</td>
<td>Adj 148/264</td>
<td>1.1 (0.8-1.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Missense</td>
<td>Adj 75/129</td>
<td>1.2 (0.8-1.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Missense</td>
<td>0 26/30</td>
<td>0.6 (0.3-1.1)</td>
<td>1.0 (ref)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.01-0.99</td>
<td>1.7 (0.9-3.1)</td>
<td>2.7 (1.2-6.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥ 1.0</td>
<td>2.0 (1.1-3.9)</td>
<td>3.3 (1.4-8.0)</td>
<td>1.3 (0.1-3.9)</td>
</tr>
<tr>
<td>Deleterious Missense</td>
<td>0 14/14</td>
<td>0.6 (0.2-1.3)</td>
<td>1.0 (ref)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.01-0.99</td>
<td>2.8 (1.2-6.5)</td>
<td>5.3 (1.6-17.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥ 1.0</td>
<td>3.3 (1.4-8.0)</td>
<td>5.8 (1.8-19.0)</td>
<td>2.6 (0.0-10.6)</td>
</tr>
</tbody>
</table>

Bernstein et al, JNCI 2010
GWAS Studies of Radiation-related Cancer

1. WECARE study
   - CBC after primary breast cancer (J Bernstein)
   - Omni-1Quad

2. Childhood Cancer Survivor Study
   - Secondary malignancies after Hodgkin’s Lymphoma Survivors (K Onel)
   - All secondary malignancies after eight most common childhood cancers (L Morton)
Childhood Cancer Survivor Study

- 14,359 childhood cancer survivors
  - dx 1970-1986, age <21 yrs
  - ≥5 yr survival post-dx

- Detailed treatment data
  - RT + Chemo (49%)
  - Radiotherapy (11%)
  - Chemotherapy (22%)

- ~ 45% with biospecimens

Slide courtesy of Lindsay Morton
SMNs in Hodgkin’s Lymphoma Survivors

• HL dx before age 18yrs, treated with RT
• Genotyped on Affymetrix 6.0
• Discovery set
  - 100 SMN cases, 89 SMN-free controls
    - rs4946728, PRDM1
• Independent replication
  - 62 SMN, 71 controls
    (CCSS, MSK, USC)
• Greater number of risk haplotypes associated with lower PRDM1 mRNA expression

Best et al, Nature Medicine, 2011
Childhood Cancer Survivor Study GWAS

• 5,739 survivors
  – >1,500 subsequent malignancies in 877 persons
• Illumina Omni5M+E SNP chip (>4 million loci)
• Identify genetic variants associated with:
  1) Second cancers after childhood cancer
  2) First primary childhood cancer
  3) Other late adverse effects after childhood cancer
Parallel Investigations: Germline vs. Tissue
Tumor Sequencing: Molecular Classification

• The Cancer Genome Atlas (2005); International Cancer Genome Consortium (2008)

• Within tumors (>30 types)
  • Glioblastoma: four distinct subtypes (and specific gene mutations) with different survival and response to treatment
  • Breast cancer: confirmed four main subtypes, with distinct mutations in each. Similarity of basal-like breast cancers and serous ovarian tumors

• Across tumors
  • Common tumor-driving mutations (BAP1, FBXW7, TP53) correlated with poor survival across several cancer types
Integrating Germline and Tumor Tissue (example from Glioma)

2008: Tumor
- $IDH1/2$ (Isocitrate dehydrogenase)

2009: Germline
- 8q24.21, rs429562 CCDC26

2012: Jenkins et al, 2012
- Used fine mapping to narrow down region
- $IDH$ status more predictive of risk associated with locus than histology
The Search for “Mutational Signatures”

Alexandrov, Curr Opin Gen Dev, 2014
Sanger Institute, 7,042 tumor tissue samples

<table>
<thead>
<tr>
<th>Signature</th>
<th>Prevalence in Cancer Samples</th>
<th>Probable Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Signature 1A</td>
<td>11.7%</td>
<td>Age</td>
</tr>
<tr>
<td>Signature 1B</td>
<td>60.7%</td>
<td>Age</td>
</tr>
<tr>
<td>Signature 2</td>
<td>14.4%</td>
<td>APOBEC</td>
</tr>
<tr>
<td>Signature 3</td>
<td>9.9%</td>
<td>BRCA1/2 mutations</td>
</tr>
<tr>
<td>Signature 4</td>
<td>12.1%</td>
<td>Smoking</td>
</tr>
<tr>
<td>Signature 5</td>
<td>14.4%</td>
<td>DNA MMR deficiency</td>
</tr>
<tr>
<td>Signature 6</td>
<td>2.6%</td>
<td>HPV infection</td>
</tr>
<tr>
<td>Signature 7</td>
<td>5.0%</td>
<td>Ig gene hypermutation</td>
</tr>
<tr>
<td>Signature 8</td>
<td>0.6%</td>
<td>POLE mutations</td>
</tr>
<tr>
<td>Signature 9</td>
<td>0.5%</td>
<td>Temozolomide</td>
</tr>
<tr>
<td>Signature 10</td>
<td>1.4%</td>
<td>APOBEC</td>
</tr>
<tr>
<td>Signature 11</td>
<td>2.2%</td>
<td>APOBEC</td>
</tr>
<tr>
<td>Signature 12</td>
<td>0.1%</td>
<td>Age</td>
</tr>
<tr>
<td>Signature 13</td>
<td>0.5%</td>
<td>Smoking</td>
</tr>
<tr>
<td>Signature 14</td>
<td>1.1%</td>
<td>Age</td>
</tr>
<tr>
<td>Signature 15</td>
<td>1.8%</td>
<td>Age</td>
</tr>
<tr>
<td>Signature 16</td>
<td>2.2%</td>
<td>APOBEC</td>
</tr>
<tr>
<td>Signature 17</td>
<td>2.2%</td>
<td>POLE mutations</td>
</tr>
<tr>
<td>Signature 18</td>
<td>0.2%</td>
<td>DNA MMR deficiency</td>
</tr>
<tr>
<td>Signature 19</td>
<td>0.5%</td>
<td>HPV infection</td>
</tr>
<tr>
<td>Signature 20</td>
<td>0.3%</td>
<td>Ig gene hypermutation</td>
</tr>
<tr>
<td>Signature 21</td>
<td>13.6%</td>
<td>POLE mutations</td>
</tr>
</tbody>
</table>

22 validated signatures
Challenges: Genetic Susceptibility to Radiation

• Setting
  – High versus low dose radiation

• Outcome
  – Cancer
  – Intermediate outcomes

• Analytical challenges
  – Power
  – Volume of data, methods need to be developed

• Replication
But, exciting times ahead…

• Continue to explore genetic susceptibility
  – Germline + tumor
  – Whole genome sequencing of special populations
  – Integration of platforms: methylation, RNA, proteins

• Interaction with other risk factors
  – Age, sex, smoking
  – BMI, infection, co-morbidities
Questions and Answers

U.S. Department of Health and Human Services
National Institutes of Health | National Cancer Institute
www.dceg.cancer.gov/RadEpiCourse
1-800-4-CANCER
Produced May 2015