Variants in DNA Repair Genes and Glioblastoma

Advances in Bioinformatics and Genomics Symposium
February 19, 2010

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Background

• Glioblastomas – most common primary BT in adults

• Etiology largely unknown with no single risk factor identified that explains a substantial number of cases.

• Ionizing radiation – established environmental factor
US adult brain tumors

• 51,410 primary benign and malignant brain tumors diagnosed in 2007.

• ~ 20,500 primary malignant brain and CNS tumors.

CBTRUS
Incidence

- Central Brain Tumor Registry of the United States (CBTRUS)

  16.5 cases per 100,000 person per year  *(2004-2007)*

    non-malignant  9.2 cases per 100,000 person per year
    malignant      7.3 cases per 100,000 person per year

  Age standardized to the 2000 US standard population.
Proportion of Brain Tumors by Histology

- Glioma: 42%
- Meningioma: 29%
- Other: 29%

CBTRUS 04-05
Proportion of Gliomas by Histology

- Glioblastoma: 50%
- Astrocytoma: 25%
- Other: 25%

CBTRUS 2004-05
Relative survival for cancer of brain and CNS, Gliomas only, in California, male, 1988-1998

- 0-19 years
- 20-54 years
- 55 + years

Relative survival (%) vs. Months
Challenges in Studying DNA Repair Genes and Glioblastoma

• Relatively rare disease
  – Sample size, power.
  – Need to combined samples from multiple centers

• Very poor survival
  – Representative sample

• Disease heterogeneity
  – Glioblastoma reasonably homogeneous group even without central neuropathology review
Brain Tumor Epidemiology Consortium (BTEC)

- open scientific forum organized to foster the development of multi-center, international and inter-disciplinary collaborations that will lead to a better understanding of the etiology, outcomes, and prevention of brain tumors.

- The Consortium formed in 2003

- Initial meeting sponsored by the National Cancer Institute’s (NCI) Division of Cancer Epidemiology and Genetics (DCEG)

- Annual meetings.

- Four working groups focusing on adult gliomas, meningiomas, pediatric brain cancers, and family studies.
Pilot Study of DNA Repair Genes and Glioblastomas
Hypothesis

Genetic variation in DNA repair pathways could predispose adults to develop a GBM by influencing susceptibility to cellular damage that occurs as part of normal biological processes or susceptibility to environmental exposures.
Approach

- Collaborative study of Glioblastoma
- 4 collaborating centers identified through BTEC
- Basis for future collaborations on grants, genetic analyses.
- Funded by NBTF
Challenges of collaborative project

- Data ascertainment. Differences in recruitment, survival, case & control definitions.

- Data Pooling. Requirements of investigators, centers. Time & effort in data preparation

- Standardization. Comparability of studies. Different data collected, different formats.


- Biospecimens. Availability, handling, specimen quality.

- Human Subjects, IRB, HIPPA. Informed consent, sharing rules.
Methods

• Assemble samples from 4 existing case-control studies in the United States
  – MD Anderson, NCI, NIOSH, UCSF

• Create central dataset of DNA SNPs and relevant Qx data.

• Genotype GBM cases and controls with DNA available at each center.
  – Taq man assays at 3 centers.

• Incorporate Coriell control standards across centers.

• Complete combined analysis of association between each DNA repair variant and GBM.
Collaborating Centers

NIOSH:  
Avima Ruder, PhD  
Mary Ann Butler, PhD

NCI:  
Pete Inskip, PhD

MD Anderson:  
Melissa Bondy, PhD

UCSF:  
Margaret Wrensch, PhD  
John Wienke, PhD
Inclusion Criteria for Genotyping Study

Glioblastoma  ICD-O code 9440

issuses of central pathology review

Ages  18 years and older

Race/ethnicity  NH White

Gender  Male and female

Location  One of 4 study centers (both population and hospital based)
## Characteristics of 4 Studies

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>MD Anderson</th>
<th>NCI</th>
<th>NIOSH</th>
<th>UCSF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Study Design</strong></td>
<td>CA center CA/CO</td>
<td>Hospital based CA/CO</td>
<td>Population-based CA/CO</td>
<td>Population-based CA/CO</td>
</tr>
<tr>
<td><strong>Control Selection Method</strong></td>
<td>Hospital and population</td>
<td>Hospital</td>
<td>Population</td>
<td>RDD</td>
</tr>
<tr>
<td><strong>Matching Factors</strong></td>
<td>Age, gender, race.</td>
<td>Age, gender, race, hospital</td>
<td>Age, gender</td>
<td>Age, gender, race</td>
</tr>
<tr>
<td><strong>Matching Type</strong></td>
<td>Frequency</td>
<td>Frequency</td>
<td>Frequency</td>
<td>Frequency</td>
</tr>
<tr>
<td><strong>Age Range</strong></td>
<td>20-60 yrs</td>
<td>18 years+</td>
<td>18-80 years</td>
<td>20 years +</td>
</tr>
</tbody>
</table>
DNA repair candidates

Direct Repair,
Base Excision (BER),
Nucleotide Excision (NER),
Double Strand Break (DSB)

• Considered relevance of each repair pathway to the types of DNA damage that result from experimental neurocarcinogens and from endogenous formation of reactive oxygen species (ROS).
  
  *(Steve Hecht, MA Butler).*

• Previous suggested associations from the literature, preliminary results from 4 collaborating centers, evidence of functional variant.
Each pathway responsible for efficient repair of specific types of DNA damage.

**Base excision repair:** multistep process for removal of small base adducts e.g. methylation or oxidation.

**Nucleotide excision repair:** corrects UV-induced lesions and bulky adducts

**Direct repair:** acts to reverse rather than excise DNA damage, typically involving methyl and other small alkyl groups.

**Doublestrand breaks** may occur following exposure to ionizing radiation or to products of cellular processes (hydrolysis, oxidation, or methylation of DNA).
Potential neurocarcinogens, associated DNA damage and relevant repair pathways.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Potential ROS formation?</th>
<th>Potential DNA damage</th>
<th>Relevant DNA repair pathways</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrosamides</td>
<td>No</td>
<td>alkyl adducts: (e.g. O&lt;sup&gt;6&lt;/sup&gt;-alkyl-thymine, O&lt;sup&gt;4&lt;/sup&gt;-methylguanine)</td>
<td>DR</td>
</tr>
<tr>
<td>Organophosphates</td>
<td>Yes</td>
<td>Does not appear to cause adducts or DNA breaks. Oxidative stress</td>
<td>BER, DSB-NHEJ, DSB-HRR</td>
</tr>
<tr>
<td>Organochlorines</td>
<td>Yes</td>
<td>Oxidative Stress</td>
<td>BER, DSB-NHEJ, DSB-HRR</td>
</tr>
<tr>
<td>Carbamates</td>
<td>No</td>
<td>alkyl adducts (form nitrosamides) O&lt;sup&gt;6&lt;/sup&gt;methylguanine Etheno (cyclic) adducts&lt;sup&gt;[41]&lt;/sup&gt; (due to derivative); DNA double-strand break&lt;sup&gt;[1]&lt;/sup&gt; (due to derivative)</td>
<td>DR, BER, DSB-NHEJ&lt;sup&gt;a&lt;/sup&gt;, DSB-HRR&lt;sup&gt;a&lt;/sup&gt;, NER&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chlorinated Hydrocarbons</td>
<td>No</td>
<td>Etheno (cyclic) adducts (e.g. 7-(2-oxoethyl)-guanine (primary))</td>
<td>BER, DSB-NHEJ, DSB-HRR, NER&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ionizing Radiation</td>
<td>Yes</td>
<td>Oxidative Stress; Double Strand Breaks</td>
<td>BER, DSB-NHEJ, DSB-HRR</td>
</tr>
</tbody>
</table>
Candidate List

Table 1. Candidate DNA repair pathway genes, Glioblastoma Collaborative Group, 2008

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Gene name</th>
<th>Gene</th>
<th>SNP ID</th>
<th>SNP</th>
<th>Base change</th>
<th>Chr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct</td>
<td>Methyl-guanine methyltransferase</td>
<td>MGMT</td>
<td>rs12917</td>
<td>Leu84Phe</td>
<td>C/T</td>
<td>10q26.3</td>
</tr>
<tr>
<td>BER</td>
<td>8-Hydroxyguanine DNA glycosylase</td>
<td>OGG1</td>
<td>rs1052133</td>
<td>Ser326Cys</td>
<td>C/G</td>
<td>3p26.2</td>
</tr>
<tr>
<td>BER</td>
<td>Apurinic endonuclease</td>
<td>APEX1</td>
<td>rs1130409</td>
<td>Asp148Glu</td>
<td>T/G</td>
<td>14q11.2</td>
</tr>
<tr>
<td>BER</td>
<td>X-ray repair, complementing defective, 1</td>
<td>XRCC1</td>
<td>rs1799782</td>
<td>Arg194Trp</td>
<td>G/A</td>
<td>19q13.2</td>
</tr>
<tr>
<td>BER</td>
<td>X-ray repair, complementing defective, 1</td>
<td>XRCC1</td>
<td>rs25487</td>
<td>Arg399Gly</td>
<td>C/T</td>
<td>19q13.2</td>
</tr>
<tr>
<td>BER</td>
<td>ADP-ribosyltransferase</td>
<td>PARP1</td>
<td>rs1136410</td>
<td>Val762Ala</td>
<td>T/C</td>
<td>1q41</td>
</tr>
<tr>
<td>NER</td>
<td>Excision repair, complementing defective, 2</td>
<td>ERCC2</td>
<td>rs13181</td>
<td>Lys751Gln</td>
<td>A/C</td>
<td>19q13.3</td>
</tr>
<tr>
<td>NER</td>
<td>RAD23</td>
<td>RAD23B</td>
<td>rs1805329</td>
<td>Ala249Val</td>
<td>C/T</td>
<td>9q31.2</td>
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<tr>
<td>NER</td>
<td>Excision repair, complementing defective, 5</td>
<td>ERCC5</td>
<td>rs17655</td>
<td>His1104Asp</td>
<td>G/C</td>
<td>13q22</td>
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<tr>
<td>NER</td>
<td>Giomma tumor suppressor candidate region</td>
<td>GLTSCR1</td>
<td>rs1085938</td>
<td>Ser387Ser</td>
<td>C/T</td>
<td>19q13.3</td>
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<tr>
<td>NER</td>
<td>Excision repair, complementing defective, 1</td>
<td>FRC1</td>
<td>rs832986</td>
<td>C8092A</td>
<td>C/A</td>
<td>19q13.2</td>
</tr>
<tr>
<td>NHEJ</td>
<td>DNA-dependent protein kinase</td>
<td>PRKDC</td>
<td>rs7003908</td>
<td>6721G&gt;T</td>
<td>G/T</td>
<td>8q11</td>
</tr>
</tbody>
</table>

Abbreviations: SNP, single-nucleotide polymorphism; ID, identification; Chr, chromosome; BER, base excision repair; NER, nucleotide excision repair; NHEJ, nonhomologous end–joining.

5 BER candidates:  *OGG1*, *APEX1*, *XRCC1*, *PARP1*

5 NER candidates:  *ERCC2*, *GLTSCR1*, *RAD23B*, *ERCC1*

1 NHEJ: **PRKDC** *(XRCC7)*

1 Direct:  *MGMT*
Characteristics of CA CO in sample

<table>
<thead>
<tr>
<th></th>
<th>Case</th>
<th></th>
<th></th>
<th>Control</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>(%)</td>
<td></td>
<td>No.</td>
<td>(%)</td>
</tr>
<tr>
<td>All sites</td>
<td>1,015</td>
<td></td>
<td></td>
<td>1,994</td>
<td></td>
</tr>
<tr>
<td>MDA</td>
<td>213</td>
<td>(20.9)</td>
<td></td>
<td>365</td>
<td>(18.3)</td>
</tr>
<tr>
<td>NCI</td>
<td>171</td>
<td>(16.8)</td>
<td></td>
<td>489</td>
<td>(24.5)</td>
</tr>
<tr>
<td>NIOSH</td>
<td>139</td>
<td>(13.7)</td>
<td></td>
<td>453</td>
<td>(22.7)</td>
</tr>
<tr>
<td>UCSF</td>
<td>492</td>
<td>(48.5)</td>
<td></td>
<td>687</td>
<td>(34.5)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>619</td>
<td>(61.0)</td>
<td></td>
<td>1,020</td>
<td>(51.1)</td>
</tr>
<tr>
<td>Female</td>
<td>396</td>
<td>(39.0)</td>
<td></td>
<td>974</td>
<td>(48.9)</td>
</tr>
<tr>
<td>Age*± SD</td>
<td>56.3 ± 12.6</td>
<td></td>
<td></td>
<td>53.6 ± 15.3</td>
<td></td>
</tr>
</tbody>
</table>

*Age at diagnosis for cases and reference age for controls.
Statistical analyses

- Logistic regression of single SNP
  - DNA repair pathways
  - By age (<50 years, 50+ years)
  - By center

- Gene x Gene analyses by DNA repair pathway
  - focused interaction testing framework
  - all 12 SNPs; SNPs in specific pathways.

- Haplotype Chr 19q genes
  - ERCC2, ERCC1, GLTSCR1
Results

• All 12 SNPs in HW equilibrium

• 5 BER candidates:  *PARP1*

• 5 NER candidates: no significant associations

• 1 NHEJ:  *PRKDC*

• 1 Direct Repair: no sig associations

• No significant gene-gene interactions

• Haplotype effect for most common haplotype compared to all others.
# Results

<table>
<thead>
<tr>
<th>Gene SNP</th>
<th>genotype</th>
<th>CA&lt;sup&gt;b&lt;/sup&gt; (%)</th>
<th>CO&lt;sup&gt;b&lt;/sup&gt; (%)</th>
<th>OR&lt;sup&gt;c&lt;/sup&gt;</th>
<th>95%CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Base Excision Repair</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PARP1</strong></td>
<td>rs1136410</td>
<td>TT</td>
<td>713 (72.2)</td>
<td>1303 (67.3)</td>
<td>ref</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CT</td>
<td>251 (25.4)</td>
<td>575 (29.7)</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC</td>
<td>23 (2.3)</td>
<td>57 (3.0)</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p-trend=0.02</td>
</tr>
<tr>
<td><strong>Non Homologous End Joining</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PRKDC</strong></td>
<td>rs7003908</td>
<td>TT</td>
<td>389 (41.8)</td>
<td>811 (42.3)</td>
<td>ref</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GT</td>
<td>397 (42.6)</td>
<td>875 (45.7)</td>
<td>1.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GG</td>
<td>145 (15.6)</td>
<td>230 (12.0)</td>
<td>1.44</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p-trend=0.009</td>
</tr>
</tbody>
</table>

<sup>a</sup>adjusted for age, gender and study center.
Table 4. Haplotypes and risk for glioblastoma for loci at ERCC2, ERCC1, GLTSCR1, Glioblastoma Collaborative Group, 2008

<table>
<thead>
<tr>
<th>Haplotype*</th>
<th>Frequency</th>
<th>OR\textsuperscript{+, \textdagger}</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1b</td>
<td>ACC</td>
<td>0.39</td>
<td>0.77</td>
<td>0.61-0.98</td>
</tr>
<tr>
<td>H2</td>
<td>ACT</td>
<td>0.152</td>
<td>1.19</td>
<td>0.85-1.68</td>
</tr>
<tr>
<td>H3</td>
<td>AAC</td>
<td>0.065</td>
<td>0.7</td>
<td>0.49-1.22</td>
</tr>
<tr>
<td>H4</td>
<td>AAT</td>
<td>0.027</td>
<td>1.04</td>
<td>0.44-2.70</td>
</tr>
<tr>
<td>H5</td>
<td>CCC</td>
<td>0.161</td>
<td>1.19</td>
<td>0.85-1.67</td>
</tr>
<tr>
<td>H6</td>
<td>CCT</td>
<td>0.052</td>
<td>1.28</td>
<td>0.69-2.37</td>
</tr>
<tr>
<td>H7</td>
<td>CAC</td>
<td>0.109</td>
<td>1.17</td>
<td>0.77-1.77</td>
</tr>
<tr>
<td>H8</td>
<td>CAT</td>
<td>0.044</td>
<td>1.34</td>
<td>0.66-2.71</td>
</tr>
</tbody>
</table>

\textsuperscript{*}Haplotypes for loci at ERCC2 rs13181, ERCC1 rs3212986, and GLTSCR1 rs1035938.
\textsuperscript{+}Odds ratio for haplotype compared with all other haplotypes.
\textsuperscript{\textdagger}Adjusted for age, gender, and center.

9q13 SNPs **ERCC1, ERCC2, GLTSCR1**
Environmental and Lifestyle data

• Examine the potential use of questionnaire data from the four study centers for G x E.

• Explore occupational and environmental exposures and risk of GBM

• Variables:
  - History of Head Injury
  - Smoking History
  - Radiation Therapy
  - Occupational History
  - Family History of Cancer
  - Demographics
Variants in the CDKN2B and RTEL1 regions are associated with high-grade glioma susceptibility

Margaret Wrensch1,2,12, Robert B Jenkins3,12, Jeffrey S Chang4,12, Ru-Fang Yeh4,12, Yuanyuan Xiao4, Paul A Decker5, Karla V Ballman5, Mitchel Berger1, Jan C Buckner6, Susan Chang1, Caterina Giannini3, Chandralekha Halder3, Thomas M Kollmeyer3, Matthew L Kosel5, Daniel H LaChance7, Lucie McCoy1, Brian P O’Neill7, Joe Patoka1, Alexander R Pico8, Michael Prados1, Charles Quesenberry9, Terri Rice1, Amanda L Rynearson3, Ivan Smirnov1, Tarik Tihan10, Joe Wiemels2,4, Ping Yang11,13 & John K Wiencke1,2,13

Discovery: Genome Wide Association study of high-grade glioma
- 692 glioma
- 3,992 controls (602 AGS and 3,390 Illumina controls)

Replication:
- 176 independent high grade glioma
- 174 controls

3 SNPs from discovery replicated
- 1 SNP near CDKN2B  (p= 3.4 x 10^{-8})
- 2 SNPs in RTEL1  (p= 3.4 x 10^{-8})

Discovery only
- TERT
Genome-wide association study identifies five susceptibility loci for glioma

Sanjay Shete, Fay J Hosking, Lindsay B Robertson, Sara E Dobbins, Marc Sanson, Beatrice Malmer, Matthias Simon, Yannick Marie, Blandine Boisselier, Jean-Yves Delattre, Khe Hoang-Xuan, Soufiane El Hallani, Ahmed Idbaïh, Diana Zelenika, Ulrika Andersson, Roger Henriksson, A Tommy Bergenheim, Maria Feychting, Stefan Lönn, Anders Ahlbom, Johannes Schramm, Michael Linnebank, Kari Hemminki, Rajiv Kumar, Sarah J Hepworth, Amy Price, Georgina Armstrong, Yanhong Liu, Xiangjun Gu, Robert Yu, Ching Lau, Minouk Schoemaker, Kenneth Muir, Anthony Swerdlow, Mark Lathrop, Melissa Bondy & Richard S Houlston

- Meta-analysis of 2 GWAS studies    Illumina 550K SNPs
- 1,878 cases
- 3,670 controls

- Replication:
- 2,545 cases
- 2,953 controls

- 5 SNPs risk loci for glioma

  **TERT** (p= 1.5 x 10^{-17})

  **CCDC26** (p=2.3 x 10^{-18})

  **RTEL1** (p= 2.5 x 10^{-12})

  **CDKN2A-2B** (p= 7.2 x 10^{-15})

  **PHLDB1** (p= 1.1 x 10^{-8})
Discovery Genes

• **TERT**
  – Encodes human telomerase
  – A polymerase that maintains telomere ends
  – Activity elevated in glioblastoma
  – Influences glioma cell growth

• **RTEL1**
  – Encodes DNA helicase
  – Critical for regulation of telomere length
  – Loss associated with shortened telomere length, chromosomal breaks, & translocations
DNA Pilot Genes

- **PARP1**
  - Potential telomere-length regulator.
  - Role in detection of DNA damage.
  - Contributes to programmed cell death and up regulation of inflammatory responses.

  - PAR inhibitors non-toxic to normal cells cytotoxic to HR-defective cancer cells.

  - Current clinical trials for gliomas.
Conclusions

• Collaborative effort to combine data for rare cancer.

• Findings suggest that DNA repair variants may play important role in etiology of GBM.

• Outside studies suggest DNA repair pathway may be important pathway to improve treatment sensitivity.

• Large collaborations essential for genetic studies of rare cancers.
• Careful planning need to assure that comparable data collected for genetic and lifestyle factors.
**MGMT** O-6-methylguanine–DNA methyltransferase

- Epigenetic silencing of the *MGMT* DNA repair gene by promoter methylation compromises DNA repair and has been associated with longer survival in patients with glioblastoma who receive alkylating agents.

- The *MGMT* gene is located on chromosome 10q26 and encodes a DNA-repair protein that removes alkyl groups from the O-6 position of guanine, an important site of DNA alkylation.

- Temozolomide is a DNA methylating agent that induces a variety of methyl adducts, and failure to repair key methylation lesions results in significantly enhanced tumor cell death.

- Patients with glioblastoma containing a methylated *MGMT* promoter benefited from temozolomide, whereas those who did not have a methylated *MGMT* promoter did not have such a benefit.