Clues From The Pathway-Driven Approach

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Overview

- **Breast Cancer:**
  - Case-control and Cohort Breast Cancer Studies
  - Breast Cancer Association Consortium

- **Bladder Cancer:**
  - Spanish Bladder Cancer Study
  - International Consortium of Case-control Studies of Bladder Cancer
Breast Cancer Etiology

High penetrance genes:
- BRCA1, BRCA2, TP53, STK11, PTEN

Environmental exposures:
- Reproductive history
- Exogenous hormones
- Alcohol intake
- Obesity
- Physical activity

“Sporadic” (95%)

Familial (5%)

Low penetrance genes:
- CHEK2, ATM, others?
Pathways of Interest in Breast Cancer

• Established or possible risk factors:
  • Hormone biosynthesis, metabolism, and action
  • Obesity
  • Alcohol metabolism
  • Carcinogen metabolism
  • Inflammation

• Carcinogenic processes:
  • DNA repair, cell cycle control, and apoptosis
  • Cell signaling pathways
  • Telomere length

• Gene expression studies

• Somatic mutations
Breast Cancer Association Consortium

20 studies:
28,000 cases
30,000 controls
Breast Cancer Association Consortium: Findings to Date

20 candidate SNPs (published + unpublished)

11 SNPs

No association
ADH1C I350V
AURKA F31I
XRCC1 R399Q
LIG4 D501D
BRCA2 N372H
XRCC3 T241M
XRCC3 5'UTR
XRCC3 IVS5
XRCC2 R188H
ERCC2 D312N
TP53 R72P
BCAC, JNCI (2006)

9 SNPs

Some evidence

Follow-up

9-15 studies: 10,783 cases, 18,312 controls

No association
SOD2 V16A
ADH1B 3'UTR
CDK1A S31R
ICAM5 V301I
NUMA1 A794G

Moderate
IGFBP3 -202 (p=0.05)
ATM S49C (p=0.09)

Reasonable
TGFB1 L10P (P=0.0001)
(ER-, PR- tumors)

Strong
CASP8 D320H (P=1x10^-7)

Cox/Dunning/Garcia-Closas for the BCAC (Under Review)
Caspase 8 (CASP8) D302H Variant Decreases Breast Cancer Risk

Overall OR (95%CI) 0.88 (0.84, 0.92) P=1x10^{-7}

Cox A/Dunning A/Garcia-Closas M* for the BCAC (Under Review)
* in alphabetical order

Studies (sorted by size)
- Kuopio
- Helsinki
- CNIO
- LSHTM
- USRTS
- ABCFS
- GENICA
- HBCS
- Mayo_Clinic
- Sheffield
- CAHRES
- PBCS
- SEARCH

Total Sample Size
- 16,423 cases
- 17,109 controls

Histidine (H) allele in 13% of controls
Caspase 8 and Breast Cancer: Plausibility and Significance of Findings

• *CASP8* D302H is the first common variant with convincing evidence of an association with breast cancer.

• Caspase 8 is a critical initiator of death receptor mediated apoptosis.

• Follow-up:
  • Fine mapping to dissect genetic variants in *CASP8*.
  • Functional significance of variants.
Bladder Cancer

Excellent model to evaluate genetic susceptibility and gene-environment interactions:

• Relatively homogenous histology.

• Well-known non-genetic causes:
  – Tobacco smoking
  – Occupational exposure to aromatic amines

• Good understanding of genetic variation in carcinogen metabolism.

• Familial association not yet explained.
Bladder Cancer Incidence Rates

Source: Globocan 2002
Spanish Bladder Cancer Study

Hospital-based case-control study in 5 areas of Spain (1998-2001)

- 1219 cases (85% of eligibles)
- 1271 controls (88% of eligibles)

Why Spain?
- Higher incidence rates
- Higher prevalence of tobacco and occupational exposures
- Higher participation rates, lower cost
Candidate Pathways for Bladder Cancer

- **Carcinogen metabolism**
  - **Detoxification**
  - **Activation**
  - **DNA damage**
  - **Oxidative damage**

- **Cell-cycle control**
  - **DNA repair**
  - **Apoptosis**
  - **Cell death**

- **VEGF**
  - **Malignant cell**
  - **Malignant tumor**
  - **Tumor growth**

**Bladder: tumor stages**

- **Normal cell**
- **Malignant cell**
- **Malignant tumor**

**Key Genes**: NAT2, GSTM1
NAT2 and GSTM1: Strong Candidate Genes for Bladder Cancer

• Metabolism of bladder carcinogens.

• Meta-analyses of previous studies:
  • Suggested associations with bladder cancer risk
  • Relatively small studies (23 to 374 cases)
  • Concerns about publication bias and heterogeneity

• Interactions with cigarette smoking:
  • Strong biological rationale for NAT2
# NAT Slow Acetylation and GSTM1 Null Genotypes Increase Bladder Cancer Risk

## Spanish Bladder Cancer Study

<table>
<thead>
<tr>
<th>Phenotypes*</th>
<th>Cases</th>
<th>Controls</th>
<th>OR</th>
<th>95%CI</th>
<th>p-value</th>
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<td><strong>NAT2</strong></td>
<td></td>
<td></td>
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<tr>
<td>Rapid/Intermediate</td>
<td>406</td>
<td>493</td>
<td>1.0</td>
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<tr>
<td>Slow</td>
<td>728</td>
<td>637</td>
<td>1.4</td>
<td>(1.2-1.7)</td>
<td>0.0002</td>
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<td><strong>GSTM1</strong></td>
<td></td>
<td></td>
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<tr>
<td>Present</td>
<td>786</td>
<td>561</td>
<td>1.0</td>
<td>1.0</td>
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<tr>
<td>Null</td>
<td>716</td>
<td>571</td>
<td>1.7</td>
<td>(1.4-2.0)</td>
<td>1x10^-8</td>
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</tbody>
</table>

* Inferred from genotype data

Stronger Effect of Smoking on Bladder Cancer Risk for NAT2 Slow Acetylators

Large-scale Evaluation of Candidate Genes for Bladder Cancer

• 1,433 SNPs within or near 386 genes.

• Most notable finding for a 5’UTR variant in the vascular endothelial growth factor (VEGF) gene:
  • Major role in angiogenesis.
  • VEGF tumor and urinary levels related to bladder cancer recurrence and progression.
  • Variants associated with VEGF plasma levels, promoter activity, bladder cancer aggressiveness.
Detailed Characterization of VEGF Variants in the Spanish Bladder Study

<table>
<thead>
<tr>
<th>Location</th>
<th>MAF</th>
<th>Heterozygous OR (95%CI)</th>
<th>Homozygous OR (95%CI)</th>
<th>P</th>
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<tr>
<td>rs833052</td>
<td>0.12</td>
<td>1.1 (0.9-1.4)</td>
<td>2.5 (1.1-6.0)</td>
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<td>rs1547651</td>
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<td>3.0 (1.4-6.6)</td>
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<td>rs25648</td>
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<td>1.1 (0.9-1.4)</td>
<td>5.1 (2.3-11.2)</td>
<td>0.00005</td>
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</tbody>
</table>

Garcia-Closas M et al. (Under Review)
20 studies:
8,391 cases
9,109 controls
Concluding Remarks

• Starting to identify associations with genetic variants unlikely to be false positives:
  – Large, good quality individual studies
  – Collaborative efforts through consortia
  – Robust and affordable genotyping technology

• From candidate pathways based on current understanding of etiology to genome-wide scans.
Collaborative Research Program

**Breast Cancer Studies**
Louise Brinton, HREB, DCEG  
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