Identification of heritable genetic risk factors for bladder cancer through genome-wide association studies (GWAS)

Ludmila Prokunina-Olsson, PhD

Investigator
Laboratory of Translational Genomics
Division of Cancer Epidemiology and Genetics
NCI/NIH

BCAN 2014
August 9, 2014
## Somatic vs. germline genetics

<table>
<thead>
<tr>
<th><strong>Somatic mutations</strong></th>
<th><strong>Germline variants</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Present only in some cells (tumors)</td>
<td>Present in all cells of the body</td>
</tr>
<tr>
<td>Not heritable</td>
<td>Don’t change</td>
</tr>
<tr>
<td>Induce new mutations</td>
<td>Heritable</td>
</tr>
<tr>
<td>Heterogeneity</td>
<td>Susceptibility and predisposition factors</td>
</tr>
<tr>
<td>Driving and passenger mutations</td>
<td>Possibility of long-term prediction of events</td>
</tr>
<tr>
<td>Drug targets</td>
<td>Combination with many “if” factors – environment, somatic changes</td>
</tr>
<tr>
<td>Clinical predictors</td>
<td></td>
</tr>
</tbody>
</table>
Is it all in the (genetic) cards?

Environment:
- smoking, drugs, pollutants,
- occupational exposures,
- infections

Genetic predisposition

- Increased risk
- Decreased or no risk
Germline genetic studies in families

Pros:

Informative

Can identify family-specific causes

Cons:

Need information and DNA from family members

Hard for late-onset diseases

There might be multiple causes within families

Need many families with the same cause
The search for most common causes: genome-wide association studies (GWAS)

Patients → DNA → PHASE 1 → DNA → PHASE 2 → PHASE 3 → Controls

Number of samples

Number SNPs tested
Catalog of GWAS results, March 2013

http://www.genome.gov/gwastudies/

all SNP-trait associations with p-value $\leq 5 \times 10^{-8}$
Cancer GWAS signals

Cancer associations with p-value ≤ 5×10^-8

http://www.genome.gov/gwastudies/
Bladder cancer GWAS in Europeans

Stage 1
- 591,637 SNPs
- 3,532 cases & 5,120 controls

Stage 2a
- 100 SNPs
- 969 cases & 957 controls

Stage 2b
- 5 SNPs
- 1,274 cases & 1,832 controls

Stage 3
- 4 SNPs
- 6,139 cases & 45,486 controls

6 known and 5 novel signals

Wu, 2009
Rothman, 2010
5 years of NCI bladder cancer GWAS in Europeans

- NCI-GWAS1
  3,532 cases / 5,120 controls and follow-up studies
  (Rothman et al, NatGen, 2010),

- NCI-GWAS2
  2,422 cases / 5,751 controls and follow-up studies
  (Figueroa, HumMolGen, 2014)

In total – 13 significant GWAS signals
Utility of GWAS results

- Biological mechanisms
- Correlations with biomarkers – mRNA/protein expression
- Prediction of disease course, clinical decisions
- Targets for existing drugs
- Leads for future drugs
### Bladder cancer GWAS regions

<table>
<thead>
<tr>
<th>Signal</th>
<th>Gene</th>
<th>Work done</th>
<th>Mechanism</th>
<th>Translational applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1p13.3</td>
<td>GSTM1</td>
<td>Prior candidate gene</td>
<td>Carcinogen detoxification</td>
<td>No</td>
</tr>
<tr>
<td>8p22</td>
<td>NAT2</td>
<td>Prior candidate gene</td>
<td>Carcinogen detoxification</td>
<td>No</td>
</tr>
<tr>
<td>2q37.1</td>
<td>UGT1A6</td>
<td>Tang, et al, HMG, 2012</td>
<td>Carcinogen detoxification</td>
<td>No</td>
</tr>
<tr>
<td>8q24.3</td>
<td>PSCA</td>
<td>Fu, et al, PNAS, 2012 Kohaar et al, JNCI, 2013</td>
<td>Leader peptide Protein mislocalization</td>
<td>Yes</td>
</tr>
<tr>
<td>18q12.3</td>
<td>SLC14A2</td>
<td>Koutrus et al, IJC, 2013</td>
<td>Urea transporter Urinary specific gravity</td>
<td>No</td>
</tr>
<tr>
<td>19q12</td>
<td>CCNE1</td>
<td>Kohaar et al, Can Res in press</td>
<td>Cell cycle regulation</td>
<td>Yes</td>
</tr>
<tr>
<td>3q28</td>
<td>TP63</td>
<td>Banday et al, (in preparation)</td>
<td>???</td>
<td>??</td>
</tr>
</tbody>
</table>

In work: TMEM129-TACC3-FGFR3, TERT-CLPTM, CBX6-APOBEC3A, 8q24.21 New regions: 3q26.2 (TERC)/MYNN, 11p15.5 (LSP1)
Prostate stem cell antigen (PSCA), 8q24.3

- 12.5 Mb from the 8q24.2 ‘gene desert’ region
- Neither prostate nor stem cell specific
- Bladder cancer GWAS signal: rs2294008, OR = 1.13 (95% CI = 1.09–1.17, p = 4.4x10^{-11}) in 10,196 cases and 44,705 controls (Wu, 2009, Rothman, 2010)

A representative maker (Fu et al, PNAS, 2012)
PSCA mRNA expression in bladder tissue

Expression in 3 data sets

- **Microarrays**
  - Tumors: n=36, p=0.0007

- **TaqMan**
  - Tumors: n=34, p=0.0054
  - Normal: n=34, p=0.015

- rs2294008, T=risk

39 pairs

Fu, et al, PNAS, 2012
rs2294008 and PSCA protein

Extended leader peptide

9 aa

Exon 1

Exon 2

Exon 3

Non-risk = C
acg aag gct gtg ctg ctt gcc ctg ttg atg gca ggc ttg gcc

M A G L A...

Risk = T
atg aag gct gtg ctg ctt gcc ctg ttg atg gca ggc ttg gcc

M K A V L L A L L M A G L A...

11 aa

20 aa

Lumen of the ER cell surface
PSCA with risk T allele is expressed on cell surface

- Mock, empty vector
- PSCA-T, long (123 aa)
- PSCA-C, short (114 aa)

**Figure a**
- 11 aa leader
- 20 aa leader

**Figure b**
- Mock
- PSCA-C
- PSCA-T

Kohar et al, JNCI, 2013
Bladder PSCA expression by rs2294008
rs2294008 – predictor of PSCA protein expression

Kohaar et. al., JNCI, 2013
PSCA as a drug target

- Humanized version of the anti-PSCA antibody **WAS** in phase I and II clinical trials for prostate and pancreatic cancer, no bladder cancer trials

- 75% of bladder cancer patients carry the risk T allele - express PSCA and could benefit from anti-PSCA treatment

- Anti-PSCA antibody was dropped as a candidate drug, was never tested on bladder cancer
No germline genetic markers available to predict the course of disease

- Low-grade NMIBC
- High-grade NMIBC
- MIBC

NCI GWAS1+2
5,942 patients

31.5%
17.4%
9.1%

Incomplete data – 42%
Incomplete data – 36%
The 19q12 region, OR=1.13 (1.09-1.17), p=1.22x10^{-11} in 12,257 cases and 55,019 controls

Total OR=1.13 (1.07-1.19), p=8.6x10^{-6}

Non-aggressive:
1,855 cases vs 10,773 controls

OR=1.01 (0.93-1.10) P = 0.79

Aggressive:
1,916 cases vs 10,773 controls

OR =1.18 (1.09-1.27) P = 4.7x10^{-5}

Difference: p=0.0015

Fu et al, Can Res, in press
Cyclin E – essential regulator of cell cycle

- Cyclin E – regulates cell cycle, G1-S transition
- Often overexpressed in bladder tumors
- Reported by TCGA for bladder cancer
Higher expression of Cyclin E in aggressive tumors and in carriers of risk allele

IHC analysis

265 bladder tumors

Fu et al, Can Res, in press
Predicting of aggressive bladder cancer, based just on one germline marker, age, sex and smoking status

NCI GWAS1+2
5,942 patients
risk based on rs7257330

Increased risk of aggressive cancer:
18% per allele
No association with risk of non-aggressive cancer

Higher Cyclin E expression
Higher mRNA expression of alternative splicing form
Accelerated cell cycle
Increased genomic instability

Rs7257330 - first germline genetic markers to predict development of aggressive disease

Fu et al, Can Res, in press
Contribution of GWAS markers

Genetic fine-mapping and functional work

GWAS locus 1  Locus 2  Locus 3  Locus 4  Locus 5  Locus 6

Non-modifiable risk factors: germline genetic markers, gender
Changing risk factors: age, somatic changes
Modifiable risk factors: smoking, drinking, drugs, occupation

Disease course prediction ??
Bladder cancer risk prediction model based on 12 SNPs and smoking status

Cumulative 30-year absolute risk for bladder cancer in a 50 y.o. white American male, overall and by quartiles of the polygenic risk score

**UGT1A region:** Original GWAS rs11892031, $p_{\text{int}}=0.103$; fine mapping - functional UGT1A6 rs17863783, $p_{\text{int}}=7 \times 10^{-4}$ (Tang, HMG, 2012)

Garcia-Closas et al., Can Res, 2013
Exploration of GWAS regions with TCGA

- GWAS regions – imputation, validation by genotyping
- GWAS region in TCGA – imputation based on germline variants (blood DNA)
- Analysis of RNA-seq – mRNA expression total and splicing-form specific. Does it cover all splicing forms? Does it make sense?
- miRNA expression analysis
- Analysis of mRNA and miRNA expression in relation to associated variants
- Validation in an additional set of tissue samples
- Biological assays to explore mechanism
Conclusions

- 5 years of bladder cancer GWAS

- 13 significant regions – 2 previous candidates, 4 regions worked through and published; 2 regions in preparation

- GWAS signals – lead to further discoveries of biological mechanisms and useful markers

- In combination with TCGA – more opportunities

- Clinical predictions – based on a **combination** of germline genetic markers and additional factors
Bladder cancer study:  
DCEG/NCI:  
Nathaniel Rothman, Debra Silverman, Lee Moore, Stephen Chanock, Jonine Figueroa, Nilanjan Chatterjee  

CCR/NCI:  
Andrea Apolo, Stephen Hewitt  

NCI-GWAS Collaborative Studies:  
Spanish Bladder Cancer Study, New England Bladder Cancer Study, MD Anderson Bladder Scan, PLCO, ATBC, American Cancer Society, follow-up studies  

ICR UK: Montserrat Garcia-Closas  

Prokunina-Olsson group:  
Yi-Ping Fu, Indu Kohaar, Patricia Porter-Gill, Wei Tang, Rouf Banday  

ICR UK: Montserrat Garcia-Closas  

Acknowledgements

prokuninal@mail.nih.gov