Identification of Novel Ovarian Cancer Susceptibility Loci using GWAS and Custom Genotyping Technologies

Catherine M. Phelan
Moffitt Cancer Center
Tampa, FL
March 2011
Identification of Novel Ovarian Cancer Susceptibility Loci

I. Overview of ovarian cancer

II. GWAS – results to date of confirmed ovarian cancer susceptibility loci

III. COGS project – custom-made iSelect Illumina OncoChip
I. Overview of Ovarian Cancer

- Symptoms and signs – vague abdominal S+S
  ‘whispering disease’ or ‘a silent killer’
- Incidence: 9th most common cancer among women
- Mortality: 5th most common cause of cancer death
- Staging at diagnosis: 30% stages I-II; 70% stages III-IV
- 5-yr relative survival: ~80% stages I-II; ~25% stages III-IV
I. Overview of Ovarian Cancer

- Histologic subtypes: Heterogeneity
  - Serous ~ 73%
  - Endometroid ~ 10%
  - Clear cell ~ 4%
  - Mucinious ~ 3%
  - Other ~ 10%

- Treatment: Surgery: curative (I-II) or debulking (III-IV) + ‘platinum’ chemo. Salvage chemo (topotecan, paclitaxel) in relapsed (platinum refractory) patients.
I. Overview of Ovarian Cancer

- Genetics
  - Autosomal dominant susceptibility (10%-40% of cases)
    - **BRCA1**: 44% lifetime risk
    - **BRCA2**: 27% lifetime risk
    - Lynch Syndrome (**MLH1**, **MSH2**, **PMS1**, **PMS2**, and **MSH6**): 12% lifetime risk

Other genes?
II. Ovarian Cancer GWAS

US – Sellers (PI)/ UK - Pharoah (PI) - Illumina 610quad chip (serous, invasive, NHW women)

Stage 1: 1,819 cases & 2,353 controls: 507,094 SNPs

Stage 2: 4,833 cases & 5,237 controls: 22,634 SNPs

Stage 3: 2,670 cases & 4,668 controls: 30 SNPs
II. Ovarian cancer GWAS: Performance of the Illumina 610quad chip

- Subjects with call rate < 95% (N=394), failed QA panel (N=15), ambiguous gender (N=7), unresolved identical genotypes (N=8), non-Caucasian (N=2), and less than 80% European ancestry (N=9) were excluded.
- 54,032 SNPs with call rate < 95% and 7,690 monomorphic SNPs were excluded from analysis, leaving 559,179 SNPs.
- Overall Call Rate for 3,715 subjects on 559,179 SNPs: **99.69%**
- Reproducibility on 81 pairs from US original 4 sites: **99.93%** (47,157,060/47,190,077)
## II. Ovarian cancer GWAS: Six confirmed susceptibility loci

<table>
<thead>
<tr>
<th>SNP</th>
<th>Locus</th>
<th>Gene</th>
<th>All cases OR</th>
<th>All cases p-value</th>
<th>Serous only OR</th>
<th>Serous only p-value</th>
<th>REF</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs3814113</td>
<td>9p22</td>
<td>BNC2</td>
<td>0.82</td>
<td>9.9 x 10^{-20}</td>
<td>0.78</td>
<td>5.4 x 10^{-22}</td>
<td>Song et al, 2010</td>
</tr>
<tr>
<td>rs10088218</td>
<td>8q24</td>
<td>Gene desert</td>
<td>0.84</td>
<td>3.2 x 10^{-9}</td>
<td>0.76</td>
<td>8.0 x 10^{-15}</td>
<td>Goode et al, 2010</td>
</tr>
<tr>
<td>rs2072590</td>
<td>2q31</td>
<td>HOXD1</td>
<td>1.16</td>
<td>4.5 x 10^{-14}</td>
<td>1.20</td>
<td>3.8 x 10^{-14}</td>
<td>Goode et al, 2010</td>
</tr>
<tr>
<td>rs2665390</td>
<td>3q25</td>
<td>TIPARP</td>
<td>1.19</td>
<td>3.2 x 10^{-7}</td>
<td>1.24</td>
<td>7.1 x 10^{-8}</td>
<td>Goode et al, 2010</td>
</tr>
<tr>
<td>rs9303542</td>
<td>17q21</td>
<td>HOXD3/ SKAP1</td>
<td>1.11</td>
<td>1.4 x 10^{-6}</td>
<td>1.14</td>
<td>1.4 x 10^{-7}</td>
<td>Goode et al, 2010</td>
</tr>
<tr>
<td>rs8170</td>
<td>19p13</td>
<td>MERIT40</td>
<td>1.12</td>
<td>3.6 x 10^{-6}</td>
<td>1.18</td>
<td>2.7 x 10^{-9}</td>
<td>Bolton et al, 2010</td>
</tr>
<tr>
<td>rs2363956</td>
<td>19p13</td>
<td>ANKLE1</td>
<td>1.10</td>
<td>1.2 x 10^{-7}</td>
<td>1.16</td>
<td>3.8 x 10^{-11}</td>
<td></td>
</tr>
</tbody>
</table>
II. NCI Post Genome-wide Association Initiative (U19)

- Five diseases:
  - Breast (DRIVE)
  - Prostate (ELLIPSE)
  - Ovary (FOCI)
  - Lung (TRICL)
  - Colorectal (CORECT)

- Each has three projects:
  1. Replication and fine-mapping
  2. Functional studies
  3. Risk prediction and clinical application
III. COGS

Collaborative Ovarian, prostate and breast cancer Gene-environment Study

Co-ordinator: Per Hall, Karolinska Institute, Stockholm, Sweden

http://www.cogseu.org/
### III. COGS – a consortium of consortia

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCAC</td>
<td>Breast cancer association consortium</td>
</tr>
<tr>
<td>OCAC</td>
<td>Ovarian cancer association consortium</td>
</tr>
<tr>
<td>PRACTICAL</td>
<td>Prostate cancer association consortium</td>
</tr>
<tr>
<td>CIMBA</td>
<td>BRCA1/2 genetic modifiers</td>
</tr>
<tr>
<td>IBCCS</td>
<td>BRCA1/2 &amp; risk factors</td>
</tr>
<tr>
<td>TRANSBIG</td>
<td>Breast cancer expression</td>
</tr>
<tr>
<td>NBAC</td>
<td>Breast cancer expression</td>
</tr>
</tbody>
</table>
III. Aims of COGS

To determine the **important common genetic variants** that underlie breast, ovarian and prostate cancer risk

To assess **gene x environmental interaction**

To assess the association between genetic and non-genetic factors and **certain tumour subtypes / clinical outcome**

To develop **comprehensive risk models** to allow the prediction of breast, ovarian and prostate cancer among individuals in the population at large

To investigate the **prevention strategies, and the associated organisational, ethical, legal and social implications** of applying the risk models
III. iSelect + COGS = iCOGS (aka Illumina OncoChip)

- Illumina Infinium Custom Array

- 220,123 SNPs attempted (target ~200,000)

- Genotype on DNA from ~200,000 subjects:
  - BCAC (95,000)
  - OCAC (40,000)
  - PRACTICAL (45,000)
  - CIMBA (23,000)

(additional sets for endometrial, testis, melanoma…)

III. iCOGS - AIMS

To genotype a large number of genetic variants in case-control studies:

• Perform comprehensive follow-up of GWAS

• Evaluate genetic associations for subsets of disease

• Evaluate associations with disease outcome

• Perform comprehensive fine-scale mapping of disease associated regions

• Evaluate functional candidate genetic variants, including rare variants

• Dr. Doug Easton, University of Cambridge
III. iCOGS - Allocation of SNPs

- BCAC (50k)
- OCAC (50k)
- PRACTICAL (50k)
- CIMBA (35k)
- COMMON (15k)
### III. iCOGS SNPs

<table>
<thead>
<tr>
<th></th>
<th>Priority</th>
<th>All SNPs Selected</th>
<th>Post synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCAC</td>
<td>59,000</td>
<td>70,862</td>
<td>67,988 (96%)</td>
</tr>
<tr>
<td>OCAC</td>
<td>58,875</td>
<td>59,402</td>
<td>57,033 (96%)</td>
</tr>
<tr>
<td>PRACTICAL</td>
<td>59,000</td>
<td>71,556</td>
<td>68,638 (96%)</td>
</tr>
<tr>
<td>CIMBA</td>
<td>41,300</td>
<td>54,034</td>
<td>51,758 (96%)</td>
</tr>
<tr>
<td>COMMON</td>
<td>17,700</td>
<td>24,282</td>
<td>23,211 (96%)</td>
</tr>
</tbody>
</table>
III. iCOGS – “common” SNPs (24,282 SNPs)

• SNPs associated with other diseases
  - all published SNP associations
  - list of hits for:
    - endometrial cancer
    - melanoma
    - testis cancer
    - lung cancer

• Dense genotyping of areas of common interest:
  - 8q24
  - CDKN2A
  - TERT
  - ESR1

• DNA repair genes (tagSNPs)
• Allelic imbalance SNPs
• Rare variants (~110)
III. iCOGS – “common” SNPs (24,282 SNPs)

- SNPs for quantitative traits
  - height, BMI, WHR (Giant)
  - menarche, menopause
  - breast density (MamGWAS)
  - telomere length
  - oestradiol, SHBG levels (♀)
  - testosterone levels (♂)
  - male pattern baldness

- population structure SNPs:
  - AIMs
  - mitochondrial SNPs
  - Y SNPs
III. iCOGS – Fine-mapping
(Cambridge Group)

• Common strategy being used by all the consortia

  • All regions at GW significance as of end March 2010:

    18 breast
    27 prostate
    6 ovary

  • plus 4 “common” regions:

    8q24
    CDK2NA
    TERT
    ESR1
III. iCOGS and OCAC SNPs (59,402)

1. US/UK meta-analysis GWAS Stage 2 - Risk and Survival SNPs (Sellers/Pharoah)

2. NIH grants (pathways approach) PIs: Chenevix-Trench; Goode; Goodman; Keleman; Moyisch; Phelan; Schildkraut

3. Supplement miRNA to US GWAS (Sellers)
III. Ovarian cancer GWAS SNPs on the iCOGS chip

**Pooled (US + UK) Overall**
UK: 4171 (1817 cs/ 2354 cn)
US: 3994 (1952 cs/ 2042 cn)

**Pooled (US + UK) Serous**
UK: 3191 (837 cs/ 2354 cn)
US: 3248 (1206 cs/ 2042 cn)

**US Overall**
3994 (1952 cs/ 2042 cn)

**US Serous**
3248 (1206 cs/ 2042 cn)

**Frequentist approach**
- Log additive model
- Pooled analysis- weighted by standard errors

**Log additive model**
- Pooled analysis- weighted by standard errors
- Logistic regression adjusting for site, age, and 1st PC
- Logistic regression adjusting for site, age, and 1st PC
III. Minimum rankings across 4 buckets using frequentist log-additive models

US/UK Pooled Overall
2,479,076 SNPs

US/UK Pooled Serous
2,479,030 SNPs

US Overall Results
2,252,643 SNPs

US Serous Results
2,252,643 SNPs

Across 4 buckets, pick the minimal ranking from the above 4 rankings

Select top 65,000 SNPs
III. iCOGS ovarian cancer GWAS SNPs

Final Process

65,000 SNPs from approach 1

51,120 SNPs from approach 1

Remove 3,881 SNPs with MAF <0.03 and pHWE <10^{-4}

(1) Remove 6,556 SNPs typed or in complete LD with UK Phase II SNPs
(2) Remove 9 SNPs overlapped with miRNA SNPs

Semi-final Significant List:
44,555 SNPs

Submit SNP List to ADT for design scores

Get Design Score information

Semi-final Significant List with design score:
44,555 SNPs

(1) Remove 13,619 SNPs highly correlated within top list \(r^2=1\)
(2) Remove 836 SNPs with poor design score or failure code

US GWAS Phase 2 List:
30,100 SNPs

Finally 24,552 GWAS risk SNPs were submitted to iCOGS of which 23,567 SNPs passed QC
III. iCOGS and BCAC (70,862 SNPs)

• SNPs for replication (61,240 SNPs):
  - Combined GWAS (9 studies)
  - Individual GWAS
  - Subsets: age<40, age<50, ER-ve
  - Interactions (GxG, GxHRT)
  - ER-ve scan (BPC3)
  - Triple negative scan (Couch)
  - AA scan (Haiman)
  - Survival (Qi/Paul)

• Fine-mapping of 17 known regions (8,978 SNPs)

• Ad-hoc candidates (2,337 SNPs)
III. iCOGS and PRACTICAL (71,556 SNPs)

- Combined GWAS (UK,CGEMS,CAPS)
  - 1df, 2df
  - age <55
- Advanced disease
  - UK/CGEMS/CAPS
  - BPC3
- Gleason score
- PSA
  - Low PSA (UK)
  - PSA level (UK2/CAPS)
- GxG interactions
- Fine-mapping of 26 known regions (14,281 SNPs)
- Survival
- Ad-hoc candidates (1,287 SNPs)
III. iCOGS and CIMBA SNPs (54,034)

- 5382insC GWAS and 185delAG OC GWAS
- BRCA1/2 interactors
- “Favourite” gene tags
- Allelic imbalance
- Associations with BC in general pop
- Hormone levels
- BRCA1/2 VUS
- BRCA1, BRCA2 tags
- Functional SNPs
- miRNA
- NMD
- RAD51/CASC5 fine mapping
The Funding to the initial genotyping has been provided through an EU FP7 grant (COGS), a grant from Cancer Research UK, and grants from the NIH to multiple investigators.
III. iCOGS Genotyping centers

CNIO – Spain
Cambridge – UK
Genome Quebec – Canada
Copenhagen – Netherlands
Mayo Clinic – Minnesota, USA
III. Design OCAC - iCOGS

**iCOGS 1:**
Organizer – Ramus, UCL, London, England (now at Univ. Southern California)
No. unique samples – 13,400
Origin: Europe, Scandinavia, Australia
Genotyping at Genome Quebec

**iCOGS 2:**
Organizer – Phelan, MCC, Tampa, FL
No. unique samples – 26,648
Origin: North America
Genotyping at Mayo Clinic, MN, USA, J. Cunningham/S. Hammer

Total samples: 40,148
III. iCOGS OCAC Eligibility Criteria

**Cases:**
Inclusions: Women aged 20 and above with a pathologically confirmed first primary diagnosis of epithelial ovarian cancer – either invasive or low malignant potential (including (adenocarcinoma, NOS), as well as fallopian tube cancer, or primary peritoneal cancer.

Exclusions: Non-epithelial ovarian cancers (sarcomas, germ-cell tumors, or sex-cord stromal tumors)

**Controls:**
Inclusions: Healthy women aged 20 and above who have at least one ovary intact at the reference date. Each study site should frequency-match cases to controls on age and race.

Exclusions: Personal history of ovarian cancer
III. OCAC General plating instructions

750ng DNA (1500ng if WGA DNA)
Plated with wells A1 and A12 empty
Two QC controls and two duplicates per plate
Cases and controls mixed on the plate
Full (96well) or half (48well) plates only
### III. OCAC iCOGS1

<table>
<thead>
<tr>
<th>STUDY</th>
<th>OCAC ID</th>
<th>DNA</th>
<th>TOT CASE</th>
<th>TOT CON</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia</td>
<td>ACS</td>
<td>G</td>
<td>181</td>
<td>189</td>
</tr>
<tr>
<td>Australian Ovarian Cancer Study and Australian Cancer Study (Ovarian Cancer)</td>
<td>AOCS</td>
<td>G</td>
<td>786</td>
<td>839</td>
</tr>
<tr>
<td>Bavarian Ovarian Cancer Cases and Controls</td>
<td>BAV</td>
<td>G</td>
<td>164</td>
<td>159</td>
</tr>
<tr>
<td>Belgian Ovarian Cancer Study</td>
<td>BEL</td>
<td>G</td>
<td>282</td>
<td>0</td>
</tr>
<tr>
<td>UKFOCR</td>
<td>FOCR</td>
<td>G</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>Gilda Radner Ovarian Register</td>
<td>GR</td>
<td>G</td>
<td>143</td>
<td>0</td>
</tr>
<tr>
<td>Hannover-Jena Ovarian Cancer Study</td>
<td>HJO</td>
<td>G</td>
<td>260</td>
<td>266</td>
</tr>
<tr>
<td>Hannover-Minsk Ovarian Cancer Study</td>
<td>HMO</td>
<td>G</td>
<td>191</td>
<td>191</td>
</tr>
<tr>
<td>Polish Ovarian Cancer Study</td>
<td>JAC</td>
<td>G</td>
<td>432</td>
<td>435</td>
</tr>
<tr>
<td>Japanese Ovarian Cancer Study</td>
<td>JAP</td>
<td>G</td>
<td>81</td>
<td>81</td>
</tr>
<tr>
<td>The Danish Malignant Ovarian Tumour Study</td>
<td>MAL</td>
<td>G</td>
<td>446</td>
<td>749</td>
</tr>
<tr>
<td>Melbourne Collaborative Cohort Study</td>
<td>MCCS/MEL</td>
<td>G</td>
<td>70</td>
<td>69</td>
</tr>
<tr>
<td>NCI Ovarian Case-Control Study in Poland</td>
<td>POL-NCI</td>
<td>G</td>
<td>230</td>
<td>231</td>
</tr>
<tr>
<td>Pelvic Mass Study</td>
<td>PVD</td>
<td>G</td>
<td>200</td>
<td>0</td>
</tr>
<tr>
<td>Royal Marsden Hospital Case Series</td>
<td>RMH</td>
<td>G</td>
<td>177</td>
<td>0</td>
</tr>
<tr>
<td>UK SEARCH Ovarian Cancer Study</td>
<td>SEA</td>
<td>G</td>
<td>1263</td>
<td>1222</td>
</tr>
<tr>
<td>Southampton Ovarian Cancer Study</td>
<td>SOC</td>
<td>G</td>
<td>319</td>
<td>282</td>
</tr>
<tr>
<td>SCOTROG</td>
<td>SRO</td>
<td>G</td>
<td>170</td>
<td>0</td>
</tr>
<tr>
<td>Genetic Epidemiology of Ovarian Cancer</td>
<td>STA</td>
<td>G</td>
<td>282</td>
<td>427</td>
</tr>
<tr>
<td>UC Irvine Ovarian Cancer Study</td>
<td>UCI</td>
<td>G</td>
<td>241</td>
<td>227</td>
</tr>
<tr>
<td>UK Ovarian Cancer Population Study</td>
<td>UKO</td>
<td>G</td>
<td>821</td>
<td>852</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td></td>
<td></td>
<td>6789</td>
<td>6219</td>
</tr>
</tbody>
</table>
## III. OCAC iCOGS2

<table>
<thead>
<tr>
<th>STUDY</th>
<th>OCAC ID</th>
<th>DNA</th>
<th>TOT CASE</th>
<th>TOT CON</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diseases of the Ovary and their Evaluation Study</td>
<td>DOV</td>
<td>G</td>
<td>1363</td>
<td>1627</td>
</tr>
<tr>
<td>Finnish Study of Ovarian Cancer</td>
<td>FIN/HOC</td>
<td>G</td>
<td>368</td>
<td>0</td>
</tr>
<tr>
<td>German Ovarian Cancer Study</td>
<td>GER</td>
<td>G</td>
<td>219</td>
<td>424</td>
</tr>
<tr>
<td>Hawaii Ovarian Cancer Study</td>
<td>HAW</td>
<td>W, G</td>
<td>437</td>
<td>650</td>
</tr>
<tr>
<td>Hormones and Ovarian Cancer Prediction</td>
<td>HOP</td>
<td>G</td>
<td>1247</td>
<td>1513</td>
</tr>
<tr>
<td>Women’s Cancer Research Institute (Cedars-Sinai Medical Center)</td>
<td>LAX</td>
<td>G</td>
<td>368</td>
<td>0</td>
</tr>
<tr>
<td>Mayo Clinic Ovarian Cancer Case Control Study</td>
<td>MAY</td>
<td>G</td>
<td>799</td>
<td>764</td>
</tr>
<tr>
<td>MD Anderson</td>
<td>MDA</td>
<td>G</td>
<td>397</td>
<td>396</td>
</tr>
<tr>
<td>Memorial Sloan Kettering Cancer Center Study</td>
<td>MSK</td>
<td>G</td>
<td>823</td>
<td>600</td>
</tr>
<tr>
<td>North Carolina Ovarian Cancer Study</td>
<td>NCO</td>
<td>G</td>
<td>1094</td>
<td>1086</td>
</tr>
<tr>
<td>New England-based Case-Control Study of Ovarian Cancer</td>
<td>NEC</td>
<td>W</td>
<td>978</td>
<td>1071</td>
</tr>
<tr>
<td>Nurses Health Study</td>
<td>NHS</td>
<td>W</td>
<td>193</td>
<td>576</td>
</tr>
<tr>
<td>New Jersey Ovarian Cancer Study</td>
<td>NJO</td>
<td>W</td>
<td>205</td>
<td>205</td>
</tr>
<tr>
<td>Nijmegen Polygene Study &amp; Nijmegen Biomedical Study</td>
<td>NTH</td>
<td>G</td>
<td>307</td>
<td>333</td>
</tr>
<tr>
<td>Ovarian Cancer in Alberta and British Columbia Study</td>
<td>OVA</td>
<td>G</td>
<td>914</td>
<td>820</td>
</tr>
<tr>
<td>Roswell Park Cancer Institute Cases</td>
<td>RPX</td>
<td>G</td>
<td>208</td>
<td>0</td>
</tr>
<tr>
<td>Familial Ovarian Tumour Study</td>
<td>TOR</td>
<td>G</td>
<td>644</td>
<td>644</td>
</tr>
<tr>
<td>Los Angeles County Case-Control Studies of Ovarian Cancer</td>
<td>USC</td>
<td>W,G</td>
<td>1587</td>
<td>1587</td>
</tr>
<tr>
<td>Additional LMP cases and controls</td>
<td>MAL/UCI LMP</td>
<td>G</td>
<td>230</td>
<td>230</td>
</tr>
<tr>
<td>Wiesbaden, germany</td>
<td>HSK</td>
<td>G</td>
<td>175</td>
<td>0</td>
</tr>
<tr>
<td>Oregon</td>
<td>ORE</td>
<td>G</td>
<td>129</td>
<td>129</td>
</tr>
<tr>
<td>Warsaw Ovarian Cancer Study</td>
<td>WOC</td>
<td>G</td>
<td>450</td>
<td>450</td>
</tr>
<tr>
<td>RPXp3 + NECp23 (30) + NHSp9 (37)</td>
<td>mixed plate</td>
<td>W,G</td>
<td>46</td>
<td>46</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td></td>
<td></td>
<td>13181</td>
<td>13151</td>
</tr>
</tbody>
</table>
III, iCOGS Data Management and Preliminary Analysis

Data Management – covariates
Duke University Medical Center – Schildkraut, Berchuck, Palmieri, Catoe

Data Analysis – genotype QC etc
Cambridge University – Pharoah, Song, Tyrer, Dennis
III. Performance of the iCOGS chip (Illumina OncoChip)

- 211,155 SNPs manufactured
- ~205,000 SNPs callable
- >120,000 samples now genotyped

- Genotyping will be complete by end May 2011
- Preliminary analysis Stockholm June 2011
- Final datasets ready for analysis in August 2011
Acknowledgements

OCAC members
COGS consortia members

Funding agencies:
COGS (EU)
NIH
CR-UK