Identification of Susceptibility Genes for Peritoneal, Ovarian, and Deep Infiltrating Endometriosis Using a Pooled Sample-Based Genome-Wide Association Study

Bruno Borghese, Jörg Tost, Magalie de Surville, Florence Busato, Frank Letourneur, Françoise Mondon, Daniel Vaiman & Charles Chapron
Complex heritable trait
additive genetic effects for ~**50%** of the variance
A genome-wide association study identifies genetic variants in the CDKN2BAS locus associated with endometriosis in Japanese

Satoko Uno1,2, Hitoshi Zembutsu1, Akira Hirasawa3, Atsushi Takahashi4, Michiaki Kubo5, Tomoko Akahane3, Daisuke Aoki3, Naoyuki Kamatani4, Koichi Hirata2 & Yusuke Nakamura1
Genome-wide association study identifies a locus at 7p15.2 associated with endometriosis

Jodie N Painter\textsuperscript{1,13}, Carl A Anderson\textsuperscript{2,3,13}, Dale R Nyholt\textsuperscript{4,13}, Stuart Macgregor\textsuperscript{5}, Jianghai Lin\textsuperscript{6}, Sang Hong Lee\textsuperscript{5}, Ann Lambert\textsuperscript{6}, Zhen Z Zhao\textsuperscript{1}, Fenella Roseman\textsuperscript{6}, Qun Guo\textsuperscript{7}, Scott D Gordon\textsuperscript{8}, Leanne Wallace\textsuperscript{1}, Anjali K Henders\textsuperscript{1}, Peter M Visscher\textsuperscript{5}, Peter Kraft\textsuperscript{9,10}, Nicholas G Martin\textsuperscript{8}, Andrew P Morris\textsuperscript{2}, Susan A Treloar\textsuperscript{1,11,14}, Stephen H Kennedy\textsuperscript{6,14}, Stacey A Missmer\textsuperscript{7,9,12,14}, Grant W Montgomery\textsuperscript{1,14} & Krina T Zondervan\textsuperscript{2,6,14}
Genome-Wide Association Study Link Novel Loci to Endometriosis

Hans M. Albertsen, Rakesh Chettier, Pamela Farrington, Kenneth Ward

An Italian association study and meta-analysis with previous GWAS confirm WNT4, CDKN2BAS and FN1 as the first identified susceptibility loci for endometriosis

Luca Pagliardini, Davide Gentilini, Paola Vigano', Paola Panina-Bordignon, Mauro Busacca, Massimo Candiani and Anna Maria Di Blasio
odds ratios < 1.5

different genetic backgrounds across populations

heterogeneity of endometriosis
SUP, OMA, DIE

different responsiveness to progesterone,
differential gene expressions,
specific variants influencing one form

Borghese B | *Am J Pathol* 2012
Borghese B | *Hum Reprod* 2008
Pooled Sample-Based GWAS
with a distinction between SUP, OMA, and DIE

**initial discovery step**
10-individual pools
biological duplicates

QC filtering
SNPs ranking

**replication step**
individual genotyping
independent cohort
259 endometriosis
288 controls

Endometriosis was confirmed histologically in cases and invalidated surgically in controls
Initial discovery step
10-individual pools
biological duplicates

280 top-ranked SNPs

32 (11.4%)
common to two subtypes or more

in OMA, 108 SNPs
might affect gene expression
25.0% of modified corresponding genes

Borghese B | Mol Endocrinol 2008
### Replication step

**individual genotyping**

**independent cohort of 259 endometriosis and 288 controls**

<table>
<thead>
<tr>
<th>SNP (gene) [chromosome]</th>
<th>Genotypes</th>
<th>Alleles</th>
<th>OR (95% CI)</th>
<th>Phet</th>
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</thead>
<tbody>
<tr>
<td><strong>Rs227849</strong> <em>(RUNX2, SUPT3H, CDC5L)</em> [6]</td>
<td>AA AG GG AA AG GG</td>
<td>A G A G</td>
<td>2.31 (1.41–3.78)</td>
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<tr>
<td></td>
<td>39 39 17 7 27 16</td>
<td>61.6 38.4 41.0 59.0</td>
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<tr>
<td>*<em>Rs4703908 (ZNF366) [5]</em></td>
<td>CC CG GG CC CG GG</td>
<td>C G C G</td>
<td>2.22 (1.26–3.92)</td>
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<td>64 30 1 24 21 5</td>
<td>83.2 16.8 69.0 31.0</td>
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<tr>
<td><strong>Rs2479037 (VTI1A) [10]</strong>*</td>
<td>CC CT TT CC CT TT</td>
<td>C T C T</td>
<td>4.36 (1.32–14.43)</td>
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<tr>
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<td>5 25 160 0 3 63</td>
<td>9.2 90.8 2.3 97.7</td>
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<td><strong>Rs966674 [5]</strong>*</td>
<td>CC CG GG CC CG GG</td>
<td>C G C G</td>
<td>2.95 (1.60–5.42)</td>
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<td>169 22 1 50 17 3</td>
<td>93.7 6.3 83.6 16.4</td>
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</tr>
</tbody>
</table>

Borghese B | *Biomed Res Int* 2015
Pooled Sample-Based GWAS with a distinction between SUP, OMA, and DIE

cost-effective alternative to classic GWAS

limitations
associated with the pooling strategy (small number of DNA samples)

minimized by biological duplicates and appropriate QC filtering (Monte-Carlo simulation)

no replication of classic GWAS
rs4703908 associated with OMA and intestinal DIE

**ZNF366**
transcription factor for target genes in response to *estrogen*

independent prognostic factor for breast tumors
Annems M | *JNCI* 2010

novel regulator of **CDKN2B**
induces cell cycle arrest in breast cancer cells
Annems M | *Breast Cancer Res Treat* 2015

plausible candidate gene for endometriosis