Hi-Plex for High-Throughput Mutation Screening of BRCA1, BRCA2, TP53, and PALB2 in Breast and Ovarian Cancer Patients

Sean Wen, Lai Kah Nyin, Daniel J. Park, Tu Nguyen-Dumont, Fleur Hammet, Melissa Southey, Woo Yin Ling, Yip Cheng Har, Nur Aishah Mohd Taib, Teo Soo Hwang

Cancer Research Malaysia
Worldwide distribution of female cancers

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Breast</th>
<th>Ovary</th>
<th>Cervix</th>
<th>Endometrium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence</td>
<td>1,671,149</td>
<td>238,719</td>
<td>527,624</td>
<td>319,605</td>
</tr>
<tr>
<td>Mortality</td>
<td>521,907</td>
<td>151,917</td>
<td>265,672</td>
<td>76,160</td>
</tr>
<tr>
<td>M:I</td>
<td>31%</td>
<td>64%</td>
<td>50%</td>
<td>24%</td>
</tr>
</tbody>
</table>

Source: GLOBOCAN 2012
Breast and ovarian predisposition genes

Breast cancer
Unexplained: 50%

Ovarian cancer
Unexplained: 60%

Familial relative risk

<table>
<thead>
<tr>
<th>Genes</th>
<th>Breast cancer</th>
<th>Ovarian cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Risk estimates</td>
<td>Prevalence ¹</td>
</tr>
<tr>
<td>BRCA1</td>
<td>44-75%</td>
<td>1-3%</td>
</tr>
<tr>
<td>BRCA2</td>
<td>41-70%</td>
<td>1-3%</td>
</tr>
<tr>
<td>TP53</td>
<td>&gt; 90%</td>
<td>&lt; 1%</td>
</tr>
<tr>
<td>PALB2</td>
<td>26-46%</td>
<td>&lt; 1%</td>
</tr>
</tbody>
</table>

¹ Prevalence in unselected population-based studies
Gaps in access to genetic testing in Asia

Challenge
• Severe under-testing in Asia
• <1% of newly diagnosed breast cancer patients receive genetic testing
• Main reason: Cost

Nakamura, Public Health Genomics, 2016

Our approach
To develop a high-throughput and cost-efficient genetic testing method to increase affordability and accessibility
Hi-Plex work flow

Step-wise annealing temperature of 55, 60, 65, 70 °C

259 amplicons to cover BRCA1, BRCA2, TP53, PALB2

Hioplex primer design tool

ASP (F)  
ASP (R)

Target amplicon

ASP: Amplicon-specific primer  
UP: Universal primer

* ~275bp

Nguyen-Dumont, Biotechniques, 2015
Bioinformatics workflow

- Sequencing platform: MiSeq
- Reads alignment: Bowtie2
- Variant calling: ROVER
  - Variant present on both read-pairs
  - Variant present in at least 2 read-pairs
  - Variant present at least 15% of all read-pairs
- Variant annotation: ANNOVAR
- Variant prioritisation
  - Exclude variants with MAF >1%
    - dbSNP138
    - 1000genome
    - ESP6500
    - Japanese cohort
  - Exclude variants on intronic sites
- Candidate variants

Langmead, Nat Methods, 2012
Wang, Nucleic Acid Res, 2010
Nagasaki, Nat Commun, 2015
## Panel evaluation: Sensitivity assessment

<table>
<thead>
<tr>
<th>Genes</th>
<th>Region covered (bp)</th>
<th>Variants tested</th>
<th>Deletion</th>
<th>Insertion</th>
<th>SNV</th>
<th>Variants detected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1-3bp</td>
<td>4-6bp</td>
<td>7-9bp</td>
<td>1-3bp</td>
</tr>
<tr>
<td>BRCA1</td>
<td>7,100</td>
<td>83</td>
<td>19</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>BRCA2</td>
<td>11,900</td>
<td>121</td>
<td>13</td>
<td>8</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>TP53</td>
<td>2,000</td>
<td>7</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PALB2</td>
<td>4,900</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>25,900</td>
<td>216</td>
<td>35</td>
<td>10</td>
<td>1</td>
<td>11</td>
</tr>
</tbody>
</table>

176 patients

- 215 of 216 variants successfully detected
- Sensitivity of 99.5%
Panel evaluation: Specificity assessment

- 3,209 variants detected by our panel
- Filtered away polymorphisms (n=2,717), intronic variants (n=265), variants on homopolymer region (n=1)
- 215 variants previously reported + 11 rare coding variants previously not reported

<table>
<thead>
<tr>
<th>No.</th>
<th>Gene</th>
<th>Genomic change</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
<th>Reconfirmed to be true?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BRCA1</td>
<td>chr17:g.41245675G&gt;A</td>
<td>c.1873C&gt;T</td>
<td>p.L625L</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>BRCA1</td>
<td>chr17:g.41245465C&gt;T</td>
<td>c.2083G&gt;A</td>
<td>p.D695N</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>BRCA1</td>
<td>chr17:g.41251820A&gt;T</td>
<td>c.519T&gt;A</td>
<td>p.P173P</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>BRCA2</td>
<td>chr13:g.32912750G&gt;T</td>
<td>c.4258G&gt;T</td>
<td>p.D1420Y</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>BRCA2</td>
<td>chr13:g.32913919C&gt;T</td>
<td>c.5427C&gt;T</td>
<td>p.C1809C</td>
<td>Yes</td>
</tr>
<tr>
<td>6</td>
<td>BRCA2</td>
<td>chr13:g.32968854C&gt;T</td>
<td>c.9285C&gt;T</td>
<td>p.D3095D</td>
<td>Yes</td>
</tr>
<tr>
<td>7</td>
<td>BRCA2</td>
<td>chr13:g.32953550G&gt;A</td>
<td>c.8851G&gt;A</td>
<td>p.A2951T</td>
<td>Yes</td>
</tr>
<tr>
<td>8</td>
<td>BRCA2</td>
<td>chr13:g.32914277A&gt;G</td>
<td>c.5785A&gt;G</td>
<td>p.I1929V</td>
<td>Yes</td>
</tr>
<tr>
<td>9</td>
<td>BRCA2</td>
<td>chr13:g.32972626A&gt;T</td>
<td>c.9976A&gt;T</td>
<td>p.K3326*</td>
<td>Yes</td>
</tr>
<tr>
<td>10</td>
<td>BRCA2</td>
<td>chr13:g.32913919C&gt;T</td>
<td>c.5427C&gt;T</td>
<td>p.C1809C</td>
<td>Yes</td>
</tr>
<tr>
<td>11</td>
<td>PALB2</td>
<td>chr16:g.23647121G&gt;A</td>
<td>c.746C&gt;T</td>
<td>p.P249L</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Panel application: Breast & ovarian cancer patients

<table>
<thead>
<tr>
<th>Genes</th>
<th>Breast cancer (N = 438)</th>
<th></th>
<th>Ovarian cancer (N = 286)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Deleterious</td>
<td>VUS 3</td>
<td>Non-carriers</td>
</tr>
<tr>
<td>BRCA1</td>
<td>12 (2.7%)</td>
<td>4 (0.9%)</td>
<td>422 (96.3%)</td>
</tr>
<tr>
<td>BRCA2</td>
<td>16 (3.7%)</td>
<td>16 (3.7%)</td>
<td>406 (92.7%)</td>
</tr>
<tr>
<td>TP53</td>
<td>2 (0.5%)</td>
<td>1 (0.2%)</td>
<td>435 (99.3%)</td>
</tr>
<tr>
<td>PALB2</td>
<td>3 (0.7%)</td>
<td>3 (0.7%)</td>
<td>432 (98.6%)</td>
</tr>
<tr>
<td>Total</td>
<td>33 (7.5%)</td>
<td>24 (5.5%)</td>
<td>381 (87.0%)</td>
</tr>
</tbody>
</table>

1 High risk, hospital-based cohort
2 Unselected, hospital-based cohort
3 Non-C0 missense and inframe indels
Conclusion

• We developed a high-throughput and cost-efficient genetic testing panel for four clinically relevant breast and ovarian cancer genes
  • Sensitivity of >99%
  • Specificity of >99%

• Application of panel on high risk breast patients
  • 7.5% of patients are carriers of these genes
  • BRCA1/2 carriers were more likely to have younger age at diagnosis, have family history of breast cancers, and have triple-negative breast cancers
  • 2 TP53 carriers identified have no known family history of Li-Fraumeni Syndrome cancers but were early-onset (<35yo)
  • 3 PALB2 carriers identified have family history of breast cancer

• Application of panel on unselected ovarian cancer patients (Hasmad, Gynecol Oncol, 2016)
  • 10.8% of patients are carriers of BRCA1 and BRCA2
  • Mutation carriers were more likely to be Indian, have serous ovarian cancer, and have more relatives with breast or ovarian cancer
  • 42% of mutation carriers did not have any family history of breast or ovarian cancer
  • Offering genetic counselling and genetic testing only to women with family history would mean that 35% of BRCA1 mutation carriers and 57% of BRCA2 mutation carriers would not be offered genetic testing
  • Emphasis on genetic screening on all unselected ovarian cancer patients
Acknowledgment

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Daphne Lee
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Thank you 고맙습니다