Secondary findings in hereditary cancer genes from multigene panel data: A new frontier unanticipated by the ACMG
Overview

1. Background on incidental/secondary findings
2. Utilization of virtual panels
3. Objectives of this study
4. Prevalence of secondary findings on a virtual panel
5. Summary
Background

• ACMG recommendations for reporting secondary findings in diagnostic whole-exome or genome sequencing (WES/WGS), independent of indication:

  - Various studies have estimated the prevalence of secondary findings in apparently unaffected individuals using WES/WGS
    - Published estimates range between 1.0% and 6%
Background

• It is now possible to perform diagnostic multigene panel testing on assay platforms that cover hundreds of genes
  
  - These are used to generate customized panels based on clinician indication

 Hundreds of genes sequenced on a single platform

 Cardiovascular disease
  
  - Focused panel
  - Comprehensive panel
  - Customized panels

 Cancer syndromes
  
  - Focused panel

Clinician indicates genes analyzed
Study objectives

• Use a large multigene panel strategy
  - Estimate the overall prevalence of cancer gene pathogenic variants
  - In a multi-ethnic population of patients with no known cancer history

• Determine the number of secondary findings by gene

• Assess the clinical actionability of identified gene variants

• Estimate the difference in prevalence between multiple ethnic populations and Caucasians
Cancer gene panel selection

**47-gene customized panel**

<table>
<thead>
<tr>
<th>ACMG genes</th>
<th>Cancer-risk genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>APC</td>
<td>CDKN2A</td>
</tr>
<tr>
<td>BRCA1</td>
<td>SMAD4</td>
</tr>
<tr>
<td>BRCA2</td>
<td>BMPR1A</td>
</tr>
<tr>
<td>MEN1</td>
<td>SMARCB1</td>
</tr>
<tr>
<td>MLH1</td>
<td>CDC73</td>
</tr>
<tr>
<td>MSH2</td>
<td>ATM</td>
</tr>
<tr>
<td>MSH6</td>
<td>CDH1</td>
</tr>
<tr>
<td>MUTYH</td>
<td>BAP1</td>
</tr>
<tr>
<td>NF2</td>
<td>FH</td>
</tr>
<tr>
<td>PMS2</td>
<td>CHEK2</td>
</tr>
<tr>
<td>PTEN</td>
<td>BRIP1</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>APC</td>
<td>CDKN2A</td>
</tr>
<tr>
<td>BRCA1</td>
<td>SMAD4</td>
</tr>
<tr>
<td>BRCA2</td>
<td>BMPR1A</td>
</tr>
<tr>
<td>MEN1</td>
<td>SMARCB1</td>
</tr>
<tr>
<td>MLH1</td>
<td>CDC73</td>
</tr>
<tr>
<td>MSH2</td>
<td>ATM</td>
</tr>
<tr>
<td>MSH6</td>
<td>CDH1</td>
</tr>
<tr>
<td>MUTYH</td>
<td>BAP1</td>
</tr>
<tr>
<td>NF2</td>
<td>FH</td>
</tr>
<tr>
<td>PMS2</td>
<td>CHEK2</td>
</tr>
<tr>
<td>PTEN</td>
<td>BRIP1</td>
</tr>
</tbody>
</table>

- Inclusive cancer gene selection strategy (benefit > risk for gene-variant clinical management)
  - ACMG56 cancer-risk genes (23)
  - Reviewed literature for cancer-risk genes with:
    - Strong evidence of gene-condition association
    - Clinical management recommendations
      - Surveillance
      - Family cascade testing
      - Circumstances to avoid
  - 24 additional genes deemed clinically actionable by a panel of Clinical Geneticists, Genetic Counselors & PhD Scientists
Methods

- 3,679 patients referred for hereditary cardiovascular multigene panel testing
  - No known personal/family history of cancer
- Reviewed de-identified sequence data, under an IRB-approved protocol, for the 47-gene customized cancer-risk panel
- Classification of variants from these 47 cancer-risk genes:
  - Pathogenic if previously classified at Invitae as pathogenic
  - Novel variants predicted to be pathogenic if variant resulted in a frameshift, nonsense, or splice-site disruption predicted to cause loss of function (LOF)
  - Removed known non-pathogenic LOF variants
- Predicted pathogenic variants (PVs)
Cancer-risk gene PV prevalence

- Prevalence of ALL cancer gene PVs in 3,769 cardiovascular patients
  - 6% of patients with PV on customized panel
    - Includes 7 patients with P/LP CNVs
  - 2.7% of patients with PV when limited to ACMG cancer genes
- Positive patients after excluding lower risk variants
  - MUTYH heterozygotes (hets)
  - Low penetrance PVs in CHEK2, MITF, FH

Cardiovascular patients with cancer PVs

- 6% of patients with PV on customized panel
  - Includes 7 patients with P/LP CNVs
  - 2.7% of patients with PV when limited to ACMG cancer genes
- Positive patients after excluding lower risk variants
  - MUTYH heterozygotes (hets)
  - Low penetrance PVs in CHEK2, MITF, FH
Cancer-risk gene PV prevalence by ethnicity

• Prevalence of cancer gene PVs in patients by ethnicity
  - African-American 1.73%
  - Asian 8%
  - Hispanic 4.98%
  - Caucasian 6.43%

• When limited to ACMG cancer genes
  - African-American 0.87%
  - Asian 6%
  - Hispanic 2.3%
  - Caucasian 2.69%

• Spectrum of variants by ethnicity
  - Broad variant spectrum contributes to high Caucasian prevalence
  - Asian prevalence is dominated by a single MUTYH variant
Positive secondary findings by gene

- 82% of the identified PVs were in:
  - ATM
  - BRCA1
  - BRCA2
  - CHEK2
  - FH
  - MITF
  - MUTYH
  - NBN
  - PALB2
  - PMS2

- 7 patients (3% of positives) had PVs in two cancer-risk genes.
# Management guidelines for cancer genes with most PVs

<table>
<thead>
<tr>
<th>Gene</th>
<th>Cancer risk</th>
<th>Management recommendations$^{1,2,3,4}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATM</td>
<td>Breast cancer</td>
<td>Annual mammogram and consider breast MRI starting at 40 years</td>
</tr>
<tr>
<td>BRCA1/BRCA2</td>
<td>Breast, ovarian, and prostate cancer</td>
<td>Breast screening, RRM, RRSO</td>
</tr>
<tr>
<td>CHEK2</td>
<td>Breast and colon cancer</td>
<td>Annual mammogram and consider breast MRI starting at 40 years old</td>
</tr>
<tr>
<td>FH</td>
<td>Renal cell cancer</td>
<td>Annual abdominal MRI</td>
</tr>
<tr>
<td>MITF</td>
<td>Melanoma, renal cell cancer</td>
<td>Monthly skin exams, renal ultrasound</td>
</tr>
<tr>
<td>MUTYH (het)</td>
<td>Colon cancer (moderate at most)</td>
<td>Colonoscopy at 40 years old for unaffected proband with colon cancer in 1st-degree relative</td>
</tr>
<tr>
<td>NBN</td>
<td>Breast cancer</td>
<td>Annual mammogram and consider breast MRI starting at 40 years old</td>
</tr>
<tr>
<td>PALB2</td>
<td>Breast cancer</td>
<td>Annual mammogram and consider breast MRI starting at 30 years old</td>
</tr>
<tr>
<td>PMS2</td>
<td>Colon and ovarian cancer</td>
<td>Colonoscopy every 1-2 years starting at 20-25 years of age</td>
</tr>
</tbody>
</table>

RRM – risk reducing mastectomy; RRSO – risk reducing salpingo-oophorectomy; het – heterozygote.  
Factors impacting PV prevalence estimate

• Secondary findings in only cancer-risk genes estimated at up to 6%
  - Possibly impacted by analyzing larger number of cancer-risk genes
  - Inclusion of variants conferring moderate risk (e.g., MUTYH heterozygotes)

• Prevalence is likely underestimated
  - We did not include novel missense or copy number variants

• Lower prevalence in certain ethnicities
  - Suggests pathogenic missense variants (e.g., low penetrance founder mutations) are underrepresented in current databases
Summary

• Using a customized panel strategy, we estimate the prevalence of secondary findings for cancer risk at up to 6% in individuals undergoing hereditary cardiovascular multigene testing.

• True prevalence of secondary findings in certain ethnicities is likely underreported, highlighting the need for sequencing research in these populations.

• Each of the identified secondary finding PVs is associated with published management guidelines with the potential to impact the clinical care of patients and their family members.

• This study suggests that secondary findings of potential clinical utility could be gleaned from customized multigene panels, a situation not currently addressed by the ACMG 2016 recommendations.
Acknowledgement of colleagues

• Co-authors (Invitae)
  - Shan Yang, PhD
  - Eden Haverfield, PhD, FACMG
  - Swaroop Aradhya, PhD, FACMG
  - Robert Nussbaum, MD, FACMG, FACP

• Poster – Emilie Zoltick, PeopleSeq Consortium early findings
• Poster – Eden Haverfield, Genetic screening for healthy individuals