A Collaborative Practice Approach to Genetic Testing in Cancer: Translating Science Into Clinical Practice

Heather Hampel, MS, CGC
Associate Director, Division of Human Genetics
Professor, Department of Internal Medicine
The Ohio State University Comprehensive Cancer Center

Objectives

• Obtain a working knowledge of genetic mutations in various tumor types and how they guide treatment decisions with biomarker testing
• The role of the advanced practitioner using genetic information in determining individual patient risk for select cancers
• The use of pharmacogenetic testing to optimize cancer treatment and manage chemotoxicity
• Means to improve collaboration among oncologists and advanced practitioners in oncology

Case Study #1
A person with a triple-negative breast cancer is:

A. More likely to have a BRCA2 mutation 485971
B. More likely to have a BRCA1 mutation 485976
C. Less likely to have a BRCA2 mutation 485977
D. Less likely to have a BRCA1 mutation 485978

Case Study #2

What agent might you add to the father’s treatment regimen?

A. Sorafenib (BRAF inhibitor) 485955
B. Cetuximab (KRAS inhibitor) 485956
C. Bevacizumab (VEGF inhibitor) 485957
D. Olaparib (PARP inhibitor) 485958
What is this patient's risk for inheriting her father's BRCA2 mutation?

A. 100% 485959  
B. 50% 485960  
C. 25% 485961  
D. 0% 485962

Case Study #3

What syndrome is this patient most likely to have?

A. Cowden syndrome 485987  
B. Hereditary Breast-Ovarian Cancer syndrome 485988  
C. Li Fraumeni syndrome 485990  
D. Lynch syndrome 485991
Different Uses of Different Mutations

- **Germline**
  - Can be used to predict who is at increased risk
  - Can be used to predict who will respond better or worse to certain drugs (pharmacogenetics)
  - Not a large impact on treatment decisions at present

- **Somatic**
  - Can be used to select treatment regimens for patient
  - Can sometimes be used to rule out hereditary cancer syndromes
**Pharmacogenetics**

- **Treatment efficacy**
  - CYP2D6 and tamoxifen (breast cancer)
  - TPMT and 6-mercaptopurine (ALL)

- **Toxicity**
  - DPD deficiency and 5-FU (colon cancer)
  - UGT1A1*28 and irinotecan (colon cancer)
  - MTHFR677C>T and methotrexate (breast and ovarian cancer)
  - TPMT and 6-mercaptopurine


How Much Breast and Ovarian Cancer Is Hereditary?

Causes of Hereditary Susceptibility to Breast Cancers

- Hereditary breast-ovarian cancer syndrome
  - Breast cancer
  - Ovarian cancer
- Lynch syndrome
  - Endometrial cancer
  - Ovarian cancer
- Cowden syndrome
  - Breast cancer
  - Endometrial cancer
- Li Fraumeni syndrome
  - Breast cancer

Hereditary Breast-Ovarian Cancer Syndrome (HBOC)
Sporadic

- Later age at onset (60s or 70s)
- Little or no family history of cancer
- Single or unilateral tumors

Inherited

- Early age at onset (<50)
- Multiple generations with cancer
- Clustering of certain cancers (i.e., breast/ovarian)

Normal gene
Somatic mutation
Somatic mutation
Germline mutation

Carrier Parent
Non-carrier Parent

Aa
Aa
aa
aa
Carrier
Carrier
Non-carrier
Non-carrier

1/2
1/2

Autosomal Dominant Inheritance

Hereditary Breast-Ovarian Cancer Syndrome

- Multiple cases of early onset breast cancer
- Ovarian cancer (with family history of breast or ovarian cancer)
- Breast and ovarian cancer in the same woman
- Bilateral breast cancer
- Male breast cancer
- Ashkenazi Jewish heritage
**BRCA1-Associated Cancers: Risk by Age 70**

- Breast cancer 50%-85% (often early age at onset)
- Second primary breast cancer 40%-60%
- Ovarian cancer 15%-45%
- Possible increased risk of other cancers (e.g., prostate)

**BRCA2-Associated Cancers: Risk by Age 70**

- Breast cancer (50-85%)
- Ovarian cancer (10-20%)
- Male breast cancer (6%)
- Increased risk of prostate, laryngeal, and pancreatic cancers (magnitude unknown)

**HBOC Surveillance**

- Monthly breast self-exams (begin by age 18)
- Clinical breast exams every 6 mo beginning at age 25
- Annual mammography and breast MRI beginning at age 25
- Consider transvaginal U/S with color-Doppler & CA-125 annually beginning at age 30-35

HBOC Chemoprevention

- **Tamoxifen**
  - Limited data have shown that tamoxifen may reduce breast cancer risk by ~52% in BRCA2 mutation carriers
  - Tamoxifen may not reduce breast cancer risk among BRCA1 mutation carriers d/t low prevalence of ER+ (10%-24%)
  - Still used to reduce the risk of contralateral breast cancer in women with BRCA mutations and ER+ breast cancer
- **Oral contraceptives (OCPs)**
  - Most studies report a reduced risk of ovarian cancer among those who ever used and those who used OCPs for a longer duration (>3-6 yr)
  - Some studies suggest that OCP use may be associated with an increased breast cancer risk
  - Weigh risks and benefits of OCPs for chemoprevention and contraception with patient

HBOC Prophylactic Surgery

- **Prophylactic mastectomy**
  - Reduces breast cancer risk by 90-95%
  - Total mastectomy is recommended
  - Contralateral mastectomy should be discussed with BRCA+ women who have breast cancer d/t 43% risk of new primary in 10 years
- **Prophylactic hysterectomy and BSO**
  - Should be offered by age 40 or after completion of childbearing
  - Decreases risk of ovarian cancer by 85-90% (primary peritoneal carcinoma may still occur)
  - Reduces risk of breast cancer by 60% if done prior to age 40 and by 50% if done prior to age 50 regardless of whether HRT is used
  - Decreases overall mortality

Prophylactic Oophorectomy

- Laparoscopic procedure reduces morbidity
- Currently, consensus recommendations do not include hysterectomy, although many support this
- Inspection of the peritoneal surfaces is necessary
- Ovaries and tubes should be serial sectioned (occult tubal/ovarian malignancy in 2-15%)
- Peritoneal washings should be performed
Implications for Treatment: Chemotherapy

- PARP inhibitors showing promise for the treatment of breast and ovarian cancers due to BRCA mutations
- PARP = Poly-ADP ribose polymerase
- Enzyme involved in DNA repair
  - Base-excision (single-strand breaks)
- Ideal tumor cell target
  - Used by cancer cells
  - Homologous recombination pathways, e.g., BRCA
  - "synthetic lethality"
- PARP inhibitors have the potential to enhance the cytotoxicity of cancer treatments and reverse tumor cell resistance

PARP Inhibitors in DNA Repair

Homologous recombination

SSB
PARP
Base excision repair

DSB
Chemotherapy, Radiation

Collapsed replication fork

Alternative repair (NHEJ, SSA)

BRCA1
BRCA2

Damaged DNA

Cell death

PARP Inhibitors in DNA Repair

Phase II study of PARP inhibitor olaparib in women with a BRCA1/2 mutation and recurrent ovarian cancer

- 2 sequential cohorts: 400 mg twice daily (max tolerated dose); 100 mg twice daily (min dose with clinical response) for 168 days
- All but 1 had side effects: 52% (400 mg) and 56% (100 mg) had grade 3 or 4 events; 2 grade 5 events (determined unrelated to treatment)
- Objective response
  - 400 mg (n=33): 33%
  - 100 mg (n=24): 13%
- Stable disease
  - 400 mg: 36%
  - 100 mg: 20%
- Progressive disease
  - 400 mg: 30%
  - 100 mg: 58%

Adapted from Carden 2010 and Underhill 2010

**Phase II study of PARP inhibitor BSI-201 in triple-negative metastatic breast cancer**

- Randomized to gemcitabine/carboplatin (G/C) or G/C+PARP
- No additional side effects with BSI-201

<table>
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<tr>
<th></th>
<th>G/C (n=44)</th>
<th>G/C+BSI-201 (n=42)</th>
<th>p value</th>
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<tbody>
<tr>
<td>Clinical benefit rate</td>
<td>34%</td>
<td>52%</td>
<td>.01</td>
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<tr>
<td>Median progression-free survival</td>
<td>3.6 mo</td>
<td>5.9 mo</td>
<td>.01</td>
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<tr>
<td>Median overall survival</td>
<td>7.7 mo</td>
<td>12.3 mo</td>
<td>.01</td>
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**Histopathologic Features in BRCA+ vs. Sporadic Breast Tumors**

<table>
<thead>
<tr>
<th>Feature</th>
<th>Sporadic</th>
<th>BRCA1</th>
<th>BRCA2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medullary</td>
<td>1-3% (1.3%)</td>
<td>1-13% (12%)</td>
<td>No difference from sporadic</td>
</tr>
<tr>
<td>Triple Negative</td>
<td>10-25% (19%)</td>
<td>80%</td>
<td>No difference from sporadic</td>
</tr>
<tr>
<td>ER Negative</td>
<td>13-37% (24%)</td>
<td>53-90% (85%)</td>
<td>No difference from sporadic</td>
</tr>
<tr>
<td>PR Positive</td>
<td>58-86% (65%)</td>
<td>16-21% (20%)</td>
<td>No difference from sporadic</td>
</tr>
<tr>
<td>HER2 Positive</td>
<td>12-35% (20%)</td>
<td>0-3% (3%)</td>
<td>0-3% (3%)</td>
</tr>
<tr>
<td>ER+Grade 1</td>
<td>27-42% (30%)</td>
<td>0-2.4% (2%)</td>
<td>0-17% (7%)</td>
</tr>
<tr>
<td>ER+Grade 2</td>
<td>11-28% (27%)</td>
<td>10-17% (17%)</td>
<td>22-45% (17%)</td>
</tr>
<tr>
<td>ER+Grade 3</td>
<td>9-17% (13%)</td>
<td>12-28% (13%)</td>
<td>28-30% (29%)</td>
</tr>
<tr>
<td>ER-Grade 1</td>
<td>3-14% (5%)</td>
<td>0-1% (1%)</td>
<td>1-4% (2%)</td>
</tr>
<tr>
<td>ER-Grade 2</td>
<td>12-13% (13%)</td>
<td>9-13% (13%)</td>
<td>2-17% (9%)</td>
</tr>
<tr>
<td>ER-Grade 3</td>
<td>12-16% (16%)</td>
<td>62-91% (65%)</td>
<td>9-18% (16%)</td>
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<tr>
<td>Grade 1</td>
<td>10-55% (23%)</td>
<td>0-0% (0%)</td>
<td>11-22% (17%)</td>
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<td>Grade 2</td>
<td>23-49% (42%)</td>
<td>16-26% (23%)</td>
<td>29-49% (43%)</td>
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<tr>
<td>Grade 3</td>
<td>21-49% (36%)</td>
<td>56-100% (71%)</td>
<td>38-64% (47%)</td>
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<tr>
<td>Cyclin A5/6</td>
<td>7-11%</td>
<td>17-6%</td>
<td>8-12%</td>
</tr>
<tr>
<td>Cyclin D3/14</td>
<td>10-12%</td>
<td>39-40% (85%)</td>
<td>24-27%</td>
</tr>
</tbody>
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**Triple-Negative Breast Cancers**

- ER-, PR-, and HER2/neu- breast cancers
- Basal type breast cancers
- More likely to be due to having underlying BRCA1 mutations (should test any women under 60 with a triple-negative breast cancer regardless of family history)
- Recent evidence that these cancers respond to PARP inhibitors

[Image: JADPRO Live]
HBOC and Pancreatic Cancer

- Families with 2-3 cases of pancreatic cancer
  - 12-17% chance of having a BRCA2 mutation
  - 3% chance of having a PALB2 mutation
- NCCN guidelines now recommend BRCA testing for individuals who have:
  - Breast cancer at any age and ≥2 close relatives with breast cancer and/or pancreatic cancer at any age
  - A family member with both breast and pancreatic cancer, especially if early onset
- Insurance still often declines coverage of testing

Cowden Syndrome

- Increased risks for breast, thyroid, endometrial and possibly colon cancers
- Most affected individuals have a large head size (>60 cm in men and >58.5 cm in women)
- Also associated with benign growths such as thyroid goiter, fibrocystic breasts, endometrial fibroids, colon polyps, and skin lesions
- Skin lesions (trichilemmomas and papillomatous papules) reportedly common
- Due to mutations in the PTEN gene

Li Fraumeni Syndrome

- Increased risk for sarcoma, breast, brain, leukemia, lung, adrenocortical cancers
- 50% risk of cancer by age 30 & 90% risk of cancer by age 70
- Typical to see childhood cancers and people with multiple primary cancers
- Important to identify due to radiation sensitivity
- Whole body MRI being studied
- Due to mutations in TP53 gene
Lynch Syndrome

- Increased risk for colorectal, endometrial, ovarian and gastric cancers
- Ovarian cancer risk reported to range from 6-24%. Approaches the same risk as a BRCA2 mutation carrier
- No significant increased risk for breast cancer
- Due to mutations in MLH1, MSH2, MSH6, or PMS2 genes
- Tumor testing available to determine whether patient is more or less likely to have Lynch syndrome

Hereditary Susceptibility to CRC

Adapted from Burt RW et al. Prevention and Early Detection of CRC, 1996

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>MLH1 &amp; MSH2</th>
<th>MSH6</th>
<th>PMS2</th>
<th>General Public</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon cancer (men)</td>
<td>40-80%</td>
<td>10-22%</td>
<td>15-20%</td>
<td>5.5%</td>
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<tr>
<td>Endometrial cancer</td>
<td>25-60%</td>
<td>16-26%</td>
<td>15%</td>
<td>2.7%</td>
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<tr>
<td>Stomach</td>
<td>1-13%</td>
<td>≤3%</td>
<td>6%</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>Ovarian</td>
<td>4-24%</td>
<td>1-11%</td>
<td>6%</td>
<td>1.6%</td>
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Lynch Syndrome Surveillance Options

<table>
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<tr>
<th>Intervention</th>
<th>Recommendation</th>
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<tbody>
<tr>
<td>Colonoscopy</td>
<td>Every 1-2 yr beginning at age 20-25 (MLH1 &amp; MSH2), or 30 (MSH6 &amp; PMS2); or 2-5 yr prior to the earliest colon cancer</td>
</tr>
<tr>
<td>Endometrial sampling</td>
<td>No clear evidence to support but could consider every 1 yr beginning at age 30-35</td>
</tr>
<tr>
<td>Transvaginal U/S &amp; CA-125</td>
<td>No clear evidence to support but clinicians could consider at their discretion every 1 yr beginning at age 30-35</td>
</tr>
<tr>
<td>EGD with extended duodenoscopy</td>
<td>Every 2-3 yr beginning at age 30-35</td>
</tr>
<tr>
<td>Urinalysis</td>
<td>Every 1 yr beginning at age 28-30</td>
</tr>
<tr>
<td>History &amp; exam w/ review of systems</td>
<td>Every 1 yr beginning at age 25</td>
</tr>
</tbody>
</table>

NCCN Guidelines for Colorectal Cancer Screening 2.2012

Lynch Syndrome Prophylactic Surgery Options

- Options include subtotal colectomy, hysterectomy, and oophorectomy
- Subtotal colectomy does not eliminate cancer risk
- Hysterectomy eliminates risk of endometrial and ovarian cancer
- Expert panels made no recommendation for or against surgery due to unproven efficacy


Lynch Syndrome Implications for the Patient

- 16-30% chance of second primary CRC in the 10 years after their first diagnosis
- NCCN guidelines differ for CRC patients with LS and without LS
  - With LS, colonoscopy every 1-2 years for life
  - Without LS, colonoscopy 1 yr after dx, repeat in 1-3 yr, then every 3-5 years based on findings
- Management also changes due to the risk for other cancers
Lynch Syndrome Implications for Family

- 6 relatives tested on average per proband identified with LS
- 50% have LS and need increased cancer surveillance
  - Compliance with surveillance is good (96% for CRC and 97% for Gyn)
  - Cancer risk ratio of relatives with LS compared to relatives without LS is 5.8
  - No significant difference in cancer mortality (RR, 2.28) or overall death rates (RR, 1.26) compared to mutation-negative relatives
- 50% without LS can follow the ACS guidelines

Tumor Tests to Screen for Lynch Syndrome

- Microsatellite instability (MSI) testing
  - Performed on DNA extracted from tumor and normal tissue—requires laboratory
  - Test is positive in 15% of CRC cases
  - Test is positive in 77%–89% of LS cases
- Immunohistochemistry staining
  - Performed on thin slide of tumor—can be done in pathology department
  - 1-2 proteins are absent in 20% of CRC cases
  - 1-2 proteins are absent in 83% of LS cases

MSI Testing on Genotyper

- Results show the presence or absence of microsatellite instability in the tumor sample.
- Normal genotype is indicated by the absence of additional peaks, suggesting no MSI.
Immunohistochemistry

- Identify MMR proteins
- Normally present
- If protein is absent, gene is not being expressed (mutation or methylation)
- Helps direct gene testing by predicting likely involved gene
- If abnormal IHC (absent), MSI+

Abnormal—MLH1 and PMS2 Absent

- 15% of the time
- CRC is MSI+
- Better prognosis
- 80% acquired methylation of LH1
- 20% will be LS
- BRAF test is done to help sort this out

Sample Taken From Recent Pathology Report

Mismatch repair protein expression.
MLH1: Absent
MSH2: Absent
MSH5: Present.
MSH6: Present

Immunohistochemical stains on the colorectal adenocarcinoma demonstrate the absence of MLH1 and MSH2 protein expression and the presence of MSH5 and MSH6 protein expression.

Interpretation: Mismatch Repair Protein Panel Absent
These results indicate defective DNA mismatch repair (MMR) function within the colorectal adenocarcinoma tissue. Absence of MLH1 and MSH2 may be due to the presence of a germline (hereditary) mutation in these tumor genes. Thus, this individual and other family members may be at increased risk for having an inherited colorectal cancer syndrome due to defective DNA mismatch repair. It is important to note that these results do not discriminate between germline and somatic (tumor-specific) alterations. Additional testing is required to distinguish between these two possibilities and to provide further testing for at-risk family members. A genetic counseling session may be of benefit for this individual and their family to further discuss the implications of these findings.
Follow-up BRAF Testing

Flowchart for Hereditary Colon Cancer Differential Diagnosis

Cost of Testing for Lynch Syndrome: The Old Way

- MSI
  - $450-$1000
- IHC
  - $450-$1400
- MSI & IHC
  - $950
- Comprehensive analysis: all 4 genes + EPCAM
  - ~$4550
- Single-site analysis
  - $475
Cost of Testing for HBOC: The Old Way

- Comprehensive analysis $3340
- Follow-up large rearrangement testing $700
- Single-site analysis $475
- Multi-site 3 $575

Next-Generation Sequencing (NGS)

- Can test multiple genes at one time
  - Cancer Gene Panels available with 6–52 genes
- Costs less than traditional Sanger testing methods
  - ~$3300 for 52 genes vs. $4090 for BRCA1 and BRCA2
- Turnaround time is much longer at most labs
  - 3 months for results vs. 2-3 weeks
- Higher chance of VUS results

Breast/Ovarian Panels Currently Available

<table>
<thead>
<tr>
<th></th>
<th>Ambry</th>
<th>UW</th>
<th>Myriad</th>
<th>GeneDx</th>
<th>Baylor</th>
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<td>$3,340</td>
<td>$4,400</td>
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<td>$3,850</td>
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<td>Genes</td>
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<td>12 wk</td>
<td>12 wk</td>
<td>4 wk</td>
<td>10 wk</td>
<td>12 wk</td>
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Colon Panels Currently Available

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<td>7-18</td>
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<td>Turnaround time</td>
<td>12 wk</td>
<td>12 wk</td>
<td>4 wk</td>
<td>10 wk</td>
<td>12 wk</td>
</tr>
</tbody>
</table>

Panel Results

Courtesy of Ambry Genetics Laboratories
How Do Clinicians Decide When to Order a Cancer Gene Panel?

- In general
  - Patients with a long list of differential diagnoses
  - Patients where you want to test 2-3 different genes
- Colon Panels
  - LS patients with no living affected patient for tumor screening
  - CRC dx < 50, IHC normal, strong family history
    - Would normally consider MSI then MUTYH so why not panel
  - LS and polyposis in the differential diagnosis
- Cancer Panels
  - Patients with unusual cancer histories that are not c/w one particular syndrome
  - Patients with a history consistent with HBOC and LS

How Do Clinicians Decide When to Use a NGS Panel?

Breast panels
- Patients with a high likelihood of having a BRCA mutation:
  - Only order BRCA1/2
- Patients with breast cancer dx under 35 – Li Fraumeni is in differential: Order small panel
- Patients with lobular breast cancer and family history of gastric cancer – HDGC is in differential: Order small panel
- Patients with family history consistent with Cowden syndrome: Order small panel
- Large panels for patients with high prior likelihood of mutation who test negative on small panel
- Medicare patients where you can only get a panel covered once as the first test

Panel Test Example

Exon 12-13 deletion* PALB2
GINA

- Prevents health insurers from denying coverage, adjusting premiums, or otherwise discriminating on the basis of genetic information.
  - Group and self-insured policies
- Insurers may not request that an individual undergo a genetic test.
- Employers cannot use genetic information to make hiring, firing, compensation, or promotion decisions.
- Sharply limits a health insurer's or employer's right to request, require, or purchase someone's genetic information.

Case Study #1

A person with a triple-negative breast cancer is:

A. More likely to have a BRCA2 mutation 485979
B. More likely to have a BRCA1 mutation 485980
C. Less likely to have a BRCA2 mutation 485981
D. Less likely to have a BRCA1 mutation 485982
What agent might you add to the father’s treatment regimen?

A. Sorafenib (BRAF inhibitor) 485967
B. Cetuximab (KRAS inhibitor) 485968
C. Bevacizumab (VEGF inhibitor) 485969
D. Olaparib (PARP inhibitor) 485970

What is this patient’s risk for inheriting her father’s BRCA2 mutation?

A. 100% 485983
B. 50% 485984
C. 25% 485985
D. 0% 485986
Case Study #3

What syndrome is this patient most likely to have?
A. Cowden syndrome 486270
B. Hereditary Breast-Ovarian Cancer syndrome 486271
C. Li Fraumeni syndrome 486273
D. Lynch syndrome 486274

Resources
- Heather Hampel 614-293-7240 Heather.Hampel@osumc.edu
- Family HealthLink
  https://familyhealthlink.osumc.edu
  Free, on-line tool that assesses family history of cancer and cardiovascular disease