Multi-Gene Panel Testing in Patients Suspected to Have Lynch Syndrome

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**Background – Genetic Testing for Hereditary Cancer Syndromes**

**Traditional model**
- Analyze specific genes for patients who fulfill clinical criteria for a specific syndrome
  - Per NCCN guidelines, Lynch syndrome testing recommended for patients whose histories fulfill Bethesda guidelines or Amsterdam criteria

**Emerging model → Multi-gene panel testing**
- Next generation sequencing of numerous cancer susceptibility genes in parallel
  - Advantages:
    - Analyze multiple genes simultaneously
    - Cost is dropping
  - Concerns:
    - Identification of uninformative variants of uncertain significance (VUS)
    - Identification of mutations in moderate-penetrance cancer susceptibility genes
  - Does panel testing offer meaningful advantages over targeted, criteria-based testing strategies?

**Study Aims**
- Using 25-gene panel:
  - Determine prevalence of non-Lynch mutations in patients undergoing testing for Lynch syndrome
  - Describe clinical phenotype of mutation carriers

**Methods: Study Population**
- 3057 consecutive subjects
  - Personal history of Lynch-associated cancer and/or polyps
  - DNA submitted in 2012-13 for clinical Lynch testing
  - Subjects undergoing testing for <5 Lynch syndrome genes were not included
  - After completion of clinical Lynch testing, samples anonymized for research-based testing
- 1797 subjects excluded
  - Testing originated from one of 10 states that mandate destruction of samples after clinical genetic testing (N=1615)
  - Technical factors (insufficient remaining DNA, non-blood sample) N=182
- Final study population:
  - 1260 subjects
  - All with personal history of Lynch-associated cancer and/or polyps

**Methods: Clinical Characteristics**
- As part of routine clinical testing, clinicians completed standard test request forms
  - Ancestry
  - Personal history of cancer and/or polyps
    - Age at diagnosis
  - Family history of cancer
  - Personal/family history data broadly categorized to protect anonymization
  - “Lynch-associated” cancers included:
    - Colorectal, endometrial, ovarian, gastric, pancreatic, small bowel, urinary tract, hepatobiliary, and brain cancers, and sebaceous adenomas/carcinomas
    - Breast cancer not considered Lynch-associated, but data on personal/family histories of breast cancer were tracked
  - Fulfillment of NCCN criteria for Lynch testing and hereditary breast ovarian cancer (HBOC) testing
    - Determined based on reported personal/family history data

**Methods: 25-Gene Hereditary Cancer Panel**

**High-penetrance genes**
- Lynch syndrome
  - MLH1
  - MSH2
  - MSH6
  - PMS2
  - EPCAM
  - BRCA1/2

**Other high-penetrance genes**
- APC
- BMPR1A
- CDH1
- CDKN2A
- MUTYH
- PTEN
- SMAD4
- STK11
- TP53

**Moderate-penetrance**
- ATM
- BARD1
- BRIP1
- CDK4
- CHEK2
- NBN
- PALB2
- RAD51C
- RAD51D

*All sequence variations and large rearrangements classified for pathogenicity*
Results: Subject Characteristics (N=1260)
- 73% female
- 41% Western/Northern European ancestry
- Median age 1st cancer diagnosis: 47 years (IQR 39-55.5)
- 63% with history of colorectal cancer
  - 34% with colorectal cancer age<50
- 23% with endometrial cancer
- 7% with ovarian cancer
- 5% with breast cancer
- 14% with multiple primary cancers
- 74% with family history of any Lynch-associated cancer
- 23% with family history of breast cancer
- 88% met NCCN criteria for Lynch testing
- 25% met NCCN criteria for hereditary breast/ovarian cancer (HBOC) testing

Results: Germline Testing (N=1260)
- 155 (12.3%) subjects with ≥1 pathogenic mutation on the 25-gene panel
  - 114 (9.0%) subjects with a Lynch mutation
  - 43 (3.4%) with a non-Lynch mutation
    - Including 2 subjects with both Lynch and non-Lynch mutations
      - One with MSH6 and STK11 mutations
      - One with MSH2 and ATM mutations

Pathogenic mutations identified by multi-gene panel testing

Lynch syndrome mutations identified by multi-gene panel testing

Non-Lynch mutations identified by multi-gene panel testing

Pathogenic mutations identified by multi-gene panel testing
Multi-Gene Panel Testing in Patients Suspected to Have Lynch Syndrome

BRCA1/2 carriers (N=15) 10% of all mutations identified

- Personal history
  - 53% female
  - 60% colorectal cancer
    - 33% colorectal cancer age <50
  - 27% endometrial cancer
  - 7% ovarian cancer
  - 0 with breast cancer
  - 0 with pancreatic cancer

- Family history
  - 67% any Lynch cancer
  - 47% colorectal cancer
  - 13% endometrial cancer
  - 47% breast cancer
  - 13% ovarian cancer

- 93% fulfilled NCCN Lynch testing criteria
  - versus 95% of Lynch carriers $P=0.59$

- 33% fulfilled NCCN HBOC testing criteria
  - versus 16% Lynch carriers $P=0.15$

Other high-penetration mutation carriers (N=8)

- APC (N=5) and biallelic MUTYH (N=2)
  - 5 (71%) with colorectal cancer
    - 2 at age <50
  - 3 (43%) with history of colorectal polyps
  - 1 (14%) with history of breast cancer
  - 100% with family history colorectal cancer
  - 100% met NCCN Lynch criteria

- STK11 (N=1); same patient also carried pathogenic MSH6 mutation
  - Personal history of 3 primary cancers
    - Colorectal, endometrial, and breast cancers
  - Met NCCN Lynch criteria

- Note: 28 subjects (2% of study cohort) with monoallelic MUTYH mutations
  - Significance unclear
  - 23/28 were G396D or Y179C

Variants of Uncertain Significance (VUS)

- Of the 20 non-Lynch genes, 594 VUS were seen in 433 (34%) subjects

- Most common genes to have a VUS:
  - ATM (N=114 subjects)
  - APC (N=50)
  - NBN (N=50)
  - BRIP1 (N=50)
  - CDKN2A (N=32)
  - CHEK2 (N=31)

Results Summary

- 1260 subjects with a Lynch-associated cancer referred for clinical Lynch testing
  - 9% with Lynch mutation
  - 12.3% with ≥1 mutation on 25-gene panel
    - 3.4% with a non-Lynch mutation
    - 34% with ≥1 VUS in a non-Lynch gene

- 28% of mutation carriers had mutations in non-Lynch cancer susceptibility genes
  - 54% of non-Lynch mutations in high-penetration genes

- 10% of mutation carriers had BRCA1/2 mutations
  - Clinically appear more "Lynch-like" than "BRCA-like"

Strengths/Limitations

- Strengths
  - Large cohort of consecutive individuals
  - Representative sample of patients referred for clinical Lynch testing

- Limitations
  - Clinical data obtained via clinician report
    - Unable to verify accuracy or completeness
  - No data on other non-Lynch genetic testing done clinically
  - No data on tumor testing (MSI, mismatch repair IHC)
  - Do the identified mutations explain clinical phenotype?

Conclusions: Multi-Gene Panel Testing in Suspected Lynch Patients

- Identification of unexpected actionable mutations in high-penetration non-Lynch genes
  - BRCA1/2 mutations in “Lynch-like” patients who do not fulfill clinical criteria for HBOC

- Increased yield comes at the cost of VUS identification and discovery of mutations in moderate-penetration genes