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Prevalence of germline cancer susceptibility gene mutations in a clinic-based series of colorectal cancer patients

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BACKGROUND

- Hereditary factors play a key role in colorectal cancer (CRC) risk
- Existing prevalence estimates of hereditary CRC syndromes calculated by syndrome-specific genetic testing in CRC patients with high-risk features:
  - 2-4% Lynch syndrome prevalence among CRC patients when universal MSI and/or mismatch repair immunohistochemistry (MMR IHC) testing used to guide germline testing
  - <1% combined prevalence of germline *APC* and biallelic *MUTYH* mutations among CRC patients, using pre-selection for polyposis phenotypes
  - Prevalence of other hereditary syndromes in CRC patients unknown (presumed to be rare)

AIM

- To determine the prevalence of germline cancer susceptibility gene mutations among colorectal cancer patients without selection for high-risk features (e.g. age at diagnosis, personal/family history, or MMR-D / MSI-H tumor testing results)

METHODS

- Eligibility: Individuals seen at Dana-Farber Cancer Institute for CRC diagnosis
- Frozen whole blood sent to commercial laboratory (Myriad Genetic Laboratories, Inc.) for testing (sequencing, deletion/duplication analysis) with a 25-gene hereditary cancer panel.
  - All sequence variations classified for pathogenicity
  - Deleterious, Suspected Deleterious (Pathogenic); Variant of uncertain significance (VUS); Favor polymorphism, Polymorphism (Benign)
- 1100 consented participants provided blood sample and consented to enrollment from December, 2008 through March, 2014
  - 41 excluded (33 failed sequencing; 8 with non-CRC diagnoses)
  - Study population: 1059 participants

25 Cancer Susceptibility Genes Analyzed

- High-Penetrance Genes: *MLH1*†, *MSH2*†, *MSH6*†, *PMS2*†, *EPCAM*†, *APC*†, biallelic *MUTYH*†, *BMPR1A*†, *PTEN*†, *SMAD4*†, *STK11*†, *CDKN2A*, *TP53*†, *CDH1*†, *BRCA1*†, *BRCA2*†, *PALB2*†
- Moderate Penetrance Genes:
  - Linked to CRC risk - *APC*I1307K*, *CHEK2*†, monallelic *MUTYH*
  - Not linked to CRC risk - *ATM*†, *BARD1*, *BRIP1*†, *CDK4*, *NBN*, *RAD51C*†, *RAD51D*†

†Indicates genes for which NCCN guidelines recommend specialized screening / risk-reducing interventions

METHODS

Clinical Data

- Demographics
- Personal medical history, family history of cancer
- Details of CRC diagnosis
  - Age at 1st CRC diagnosis, Stage, Location, # prior CRC diagnoses, KRAS / NRAS and BRAF mutation analysis, MSI and MMR IHC results
- Numerical estimate of likelihood of underlying Lynch syndrome mutation calculated using $PREMM_{1,2,6}$ scores (http://premm.dfci.harvard.edu)

RESULTS

Table 1. Clinical features of cohort (N=1059)

Characteristic	N (%)	Characteristic	N (%)
Male/Female	588 (56%) / 471 (44%)	Right-sided CRC	353 (33%)
		Left-sided CRC	362 (34%)
Non-Hispanic white	940 (89%)	Rectal/rectosigmoid CRC	342 (32%)
Non-Hispanic black	50 (5%)		
Hispanic/Latino	27 (3%)	Personal history of >1 CRC	29 (3%)
Asian	22 (2%)	Personal history of other non-CRC cancer†	160 (15%)
Median age at 1st CRC, years	55.7 (SD ±12.6) range 21-92	Family history any cancer†	871 (82%)
Age <50 years	337 (32%)	Family history CRC (any)	337 (32%)
		Family history CRC (1st degree relative)	138 (13%)
Stage 0/I	130 (12%)	Family history breast cancer	285 (27%)
Stage II	202 (19%)		
Stage III	404 (38%)	Median $PREMM_{1,2,6}$ score (IQR)	3.93% (2.58%-6.67%)
Stage IV	321 (30%)	$PREMM_{1,2,6}$ score ≥5%	415 (39%)

† Excluding cutaneous basal / squamous cell carcinomas

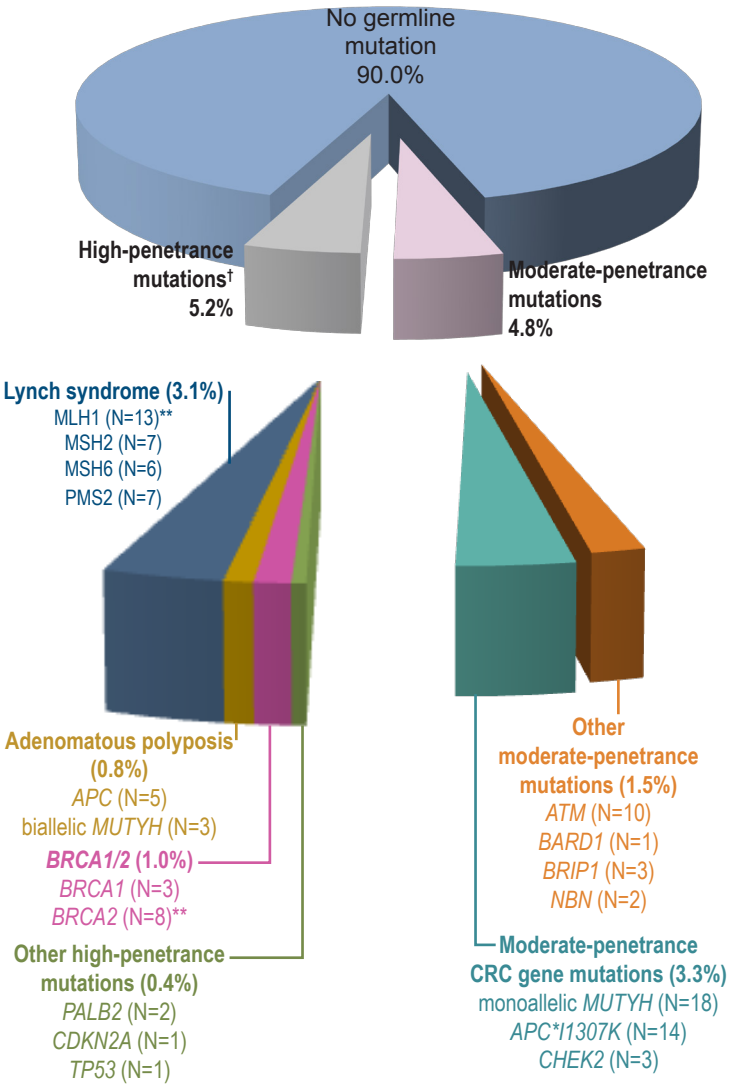
Tumor testing results (N=1059)

- MSI / MMR IHC testing: 566 (53%) with results
  - 85% MSS / MMR-P; 12% MSI-H and/or MMR-D; 2% MSI-L / MMR-P
- KRAS / NRAS mutation status: 741 (70%) with results
  - 57% wildtype; 43% mutation
- BRAF mutation status: 648 (61%) with results
  - 93% wildtype; 7% mutation

Germline testing results

- 106 / 1059 (10.0%; 95% CI 8.3-12.0%) with ≥1 pathogenic mutation(s)
  - 33 (3.1%) patients with Lynch syndrome
  - 75 (7.1%) patients with non-Lynch syndrome mutations
  - 5 patients with multiple mutations
- 416 VUS detected among 336 patients (31.8% of cohort)
  - Most common: *ATM* (52), *APC* (36), *PMS2* (34), *NBN* (32)

RESULTS



**1 patient had both an MLH1 and BRCA2 mutation
†Includes 4 patients with both a high- and a moderate-penetrance mutation

Table 2. Factors associated with presence of a non-Lynch mutation (versus non-mutation carriers)

Characteristic	Odds ratio (95% CI)
>1 CRC diagnosis (ref: 1 CRC diagnosis)	3.94 (1.38, 11.22)
Personal history of other cancer diagnosis†	1.80 (0.98, 3.29)
# 1st/2nd degree relatives with CRC (ref: 0 relatives)	1 relative: 0.98 (0.53, 1.84) ≥2 relatives: 1.50 (0.61, 3.73)
Family history ovarian cancer	2.86 (1.26, 6.52)
CRC stage (ref: Stage 0/I)	Stage II/III: 0.38 (0.19, 0.77) Stage IV: 0.66 (0.32, 1.37)
KRAS mutation status	KRAS G12C mutation: 5.43 (2.12, 13.92) Unknown KRAS status: 0.81 (0.45, 1.46) Other KRAS status: 1.0 (reference)

CI: confidence interval

Lynch syndrome

- 3.1% of overall cohort of CRC patients
- 28/29 (97%) had tumors with MSI-H and/or MMR-D (4 missing data)
- 28/33 (85%) had $PREMM_{1,2,6}$ scores ≥5%
- 32/33 (97%) met NCCN criteria for Lynch syndrome evaluation

BRCA1/2 Mutations (N=11) – 1.0% of cohort

- Most common non-Lynch high-penetrance finding
- Only 3 (27%) Ashkenazi founder mutations
- Higher than estimated (0.2-0.3%) prevalence in the general population
- 8 (73%) did not meet NCCN criteria for BRCA1/2 testing based on personal/family history
  - Only 2 (18%) w/ personal history of BRCA-associated cancer (breast cancer and melanoma)
- 5 (45%) with CRC at age <50 (range 31-69 yrs)
- 7 (64%) BRCA1/2 carriers were male

Other high-penetrance non-Lynch mutations

- Adenomatous polyposis genes: APC (N=5) and biallelic MUTYH (N=3)
- 0.8% of cohort
  - 3 (38%) failed to meet NCCN criteria for testing (≥20 lifetime adenomas or known mutation in family)
    - 53F (APC mutation) with 3 adenomas; numerous healthy siblings; no family history of FAP-related cancer/polyposis
    - 62F (APC mutation) with 15 adenomas; no family history of FAP-related cancer/ polyposis
    - 43F (biallelic MUTYH) with 3 adenomas and 1 sessile serrated polyp; no family history of cancer/polyposis
- Other high-penetrance genes: PALB2 (N=2), CDKN2A (N=1), TP53 (N=1)
- 0.4% of cohort
  - None had personal / family histories suggestive of their syndrome
  - Age at 1st CRC diagnosis: range 64-72 yrs

SUMMARY

- 10.0% of CRC patients with cancer susceptibility gene mutations
- 5.2% with high-penetrance gene mutations
  - 3.1% with Lynch syndrome → virtually all detected by MSI / MMR IHC testing
  - 0.8% with adenomatous polyposis syndromes (APC or biallelic MUTYH mutations)
  - 1.0% with BRCA1/2 mutations → only 27% met NCCN criteria for BRCA1/2 testing
    - Estimated 0.2-0.3% prevalence in general population
  - 0.4% with other high-penetrance mutations (PALB2, CDKN2A, TP53)
- 3.3% with moderate-penetrance gene mutations linked to CRC risk
  - Monoallelic MUTYH, APC*I1307K, and CHEK2
- 1.5% with moderate-penetrance gene mutations not linked to CRC risk

STRENGTHS AND LIMITATIONS

Strengths

- Large cohort without pre-selection for high-risk features
- Extensive clinical data
  - Personal history, Family history, Tumor testing results
- Testing done through CLIA-approved commercial laboratory
  - Extensive experience in variant classification

Limitations

- Clinic-based cohort
- Some details limited
  - Incomplete tumor testing data
  - Many patients enrolled prior to universal MMR IHC testing
  - Lack of data on Ashkenazi ancestry
  - Incomplete data on polyp #/histology
  - Unable to verify personal/family history data beyond medical record review

CONCLUSIONS

- Although universal MSI / MMR IHC identifies almost all Lynch probands, multigene germline testing identifies an additional 7% of CRC patients with inherited cancer risk
  - Most non-Lynch gene mutations have specific management recommended by NCCN guidelines
- Spectrum of genetic factors in CRC more diverse than traditionally appreciated and cannot be reliably predicted based on personal/family histories
- Further studies needed to investigate:
  - Phenotypes of recently discovered cancer risk genes
  - Implications for cancer risk conferred by high-penetrance gene mutations ascertained through non-classic clinical histories