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# Concordance of Multi-Gene Panel Testing with Prior Microsatellite Instability and Immunohistochemistry Analyses

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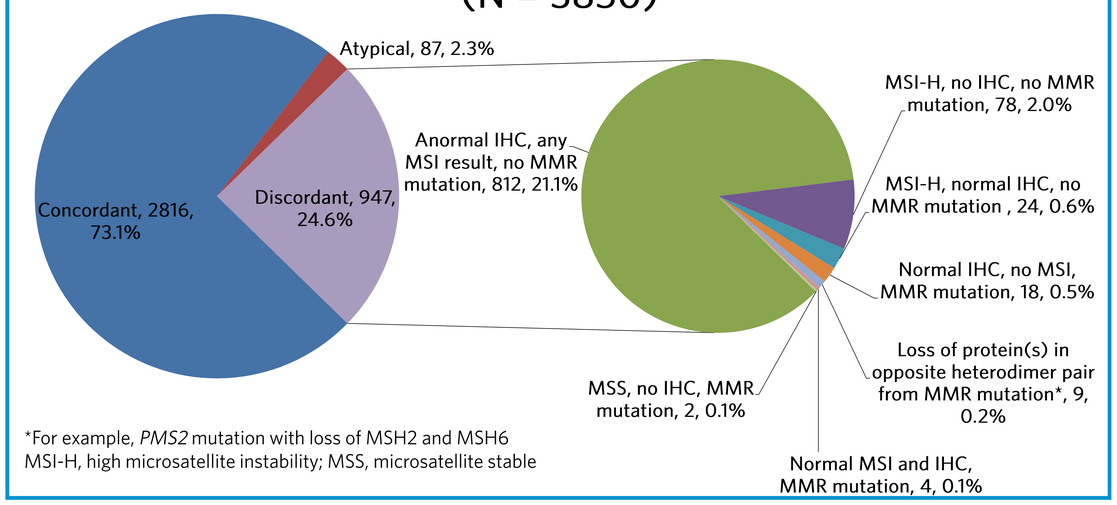
## Background

- Use of microsatellite instability (MSI) testing or immunohistochemistry (IHC) analysis for the mismatch repair (MMR) proteins are recommended methods for screening tumors for Lynch syndrome and for identify eligibility for targeted therapy, however their sensitivity and specificity are each less than 90%.[1-3]
- A recent study showed 8.1% of MSI and/or IHC results in MMR mutation carriers found by multi-gene panel testing (MGPT) to be discordant,[4] but the concordance of MSI and IHC with germline MMR gene testing overall is not well understood.
- We aimed to describe MGPT results in a cohort of individuals with prior MSI and/or IHC.
- We also aimed to assess the concordance of MSI and IHC with MGPT results.

## Concordance Rate and Types of Discordant Results (N = 3850)




*For example, *PMS2* mutation with loss of MSH2 and MSH6
MSI-H, high microsatellite instability; MSS, microsatellite stable

## Results

- Of 3850 eligible cases, 73.1% were concordant, 24.6% were discordant, and 2.3% were atypical.
- The most frequent discordant results had abnormal IHC, with or without MSI, but no MMR mutation (N = 812, 85.7% of discordant results).
  - The majority of these (N = 530) had loss of *MLH1*, some of which may be explained by *MLH1* promoter methylation, not available to us.
- 3.3% of discordant cases had a MMR gene mutation with normal MSI and/or IHC, or had loss of a different protein(s).
- Concordance was high among ovarian (92.1%), breast (79.4%) and colorectal cancers (79.1%), but only moderate in endometrial cancer (54.0%).

## Methods

- Clinical data and previous tumor testing results were extracted from the test request forms of consecutive patients undergoing MGPT at a single laboratory between March 2012 and June 2016.
- Cases for which results of tumor testing were provided were selected and prior MSI, IHC, *BRAF*, and/or *MLH1* promoter hypermethylation results were reviewed and compared with MGPT results.
- Cases were classified as concordant if prior tumor testing matched MGPT results, discordant if they did not match, and atypical if they had both concordant and discordant features

## Concordance Categories

| Description | Category |
|---|---|
| Normal IHC, MSS or no MSI, no MMR mutation | Concordant |
| Normal IHC, MSS/MSI-L, no MMR mutation | Concordant |
| Abnormal IHC, MSI-H, MMR mutation corresponding to protein(s) absent | Concordant |
| Abnormal IHC, no MSI, MMR mutation corresponding to protein(s) absent | Concordant |
| MSI-H, no IHC, any MMR mutation | Concordant |
| MSS no IHC, and no MMR mutation | Concordant |
| MSS no IHC, any MMR mutation | Discordant |
| Loss of protein(s) in opposite heterodimer pair from MMR mutation (i.e. *PMS2* mutation with loss of MSH2/MSH6) | Discordant |
| Abnormal IHC (including loss of all 4 proteins), any MSI result, no MMR mutation | Discordant |
| MSI-H, no IHC, no MMR mutation | Discordant |
| MSI-H, normal IHC, no MMR mutation | Discordant |
| Normal MSI and IHC, MMR mutation | Discordant |
| Normal IHC, no MSI, MMR mutation | Discordant |
| MSI-H, normal IHC, MMR mutation | Atypical |
| Equivocal, weak, or focal staining | Atypical |
| Loss of corresponding protein and other non-corresponding protein(s)† | Atypical |
| Loss of corresponding protein, but MSS | Atypical |
| Loss of opposite protein (i.e. loss of MSH2 only with *MSH6* mutation) | Atypical |

†Includes loss of the corresponding protein and a protein from the other heterodimer pair, loss of three proteins, and loss of all four proteins

## Results by Immunohistochemistry Pattern

| IHC Pattern N (% of abnormal IHC) | Positive for Corresponding MMR Mutation N (%) | Unexplained N (%) | *BRAF* + and/or Hypermethylated* N (%) |
|---|---|---|---|
| Loss of MLH1 (with or without PMS2) N = 654 (55.7%) | 48 (7.3%) | 457 (69.9%)<br>• Including 1 *MSH6* carrier<br>• May include cases that were not tested for *BRAF* mutations or *MLH1* promoter methylation or if performed and positive, results were not reported | 149 (22.8%) |
| Loss of PMS2 only N = 97 (8.2%) | 34 (35.1%) | 63 (64.9%) | N/A |
| Loss of MSH2 (with or without MSH6) N = 226 (19.2%) | 89 (39.4%) | 137 (60.6%)<br>• Including 2 *PMS2* carriers, 1 biallelic *PMS2* carrier, & 1 *MLH1* carrier | N/A |
| Loss of MSH6 only N = 116 (9.9%) | 39 (33.6%) | 77 (66.4%)<br>• Including 2 *PMS2* carriers | N/A |
| Atypical staining pattern† N = 82 (7.0%) | 22 (26.8%)<br>• All mutations corresponded with at least one of the absent proteins<br>• Including 1 *MSH6* carrier with reported *BRAF* mutation and loss of MLH1 and MSH6 | 56 (68.3%) | 4 (4.9%) |

**BRAF* p.V600E and or *MLH1* promoter hypermethylation analysis performed previously at another laboratory, some results were by report only
†Includes loss of the corresponding protein and a protein from the other heterodimer pair, loss of three proteins, loss of all four proteins, and equivocal, weak, or focal staining

## Concordance by Cancer Type

| Cancer Type and Concordance | N | % |
|---|---|---|
| **Colorectal Cancer** (no endometrial) | 2609 | |
| Concordant | 2064 | 79.1% |
| Discordant | 492 | 18.9% |
| Atypical | 53 | 2.0% |
| **Endometrial Cancer** (no colorectal) | 794 | |
| Concordant | 429 | 54.0% |
| Discordant | 344 | 43.3% |
| Atypical | 21 | 2.7% |
| **Ovarian Cancer** (no colorectal or endometrial) | 89 | |
| Concordant | 82 | 92.1% |
| Discordant | 6 | 6.8% |
| Atypical | 1 | 1.1% |
| **Breast Cancer** (no colorectal, endometrial, or ovarian) | 34 | |
| Concordant | 27 | 79.4% |
| Discordant | 7 | 20.6% |
| Atypical | 0 | 0% |

## Conclusions

- Nearly 25% of MSI and/or IHC results were discordant with MMR gene test results and possible explanations for these include:
  - *MLH1* promoter methylation not ruled out
  - Two somatic MMR mutations
  - Unclassified variants that are pathogenic
  - Inaccurate MSI/IHC
  - Mutations not detectable by current technology
- These results provide guidance on what to expect from MGPT given MSI and/or IHC results, suggest scenarios where somatic gene testing may be useful, and highlight several opportunities for further study.
- Our findings are representative of a MGPT cohort.

## References

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