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

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BACKGROUND

- Use of microsatellite instability (MSI) and/or immunohistochemistry (IHC) for the mismatch repair (MMR) proteins are accepted methods of screening for Lynch syndrome but their sensitivity and specificity are not 100%.[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.

METHODS

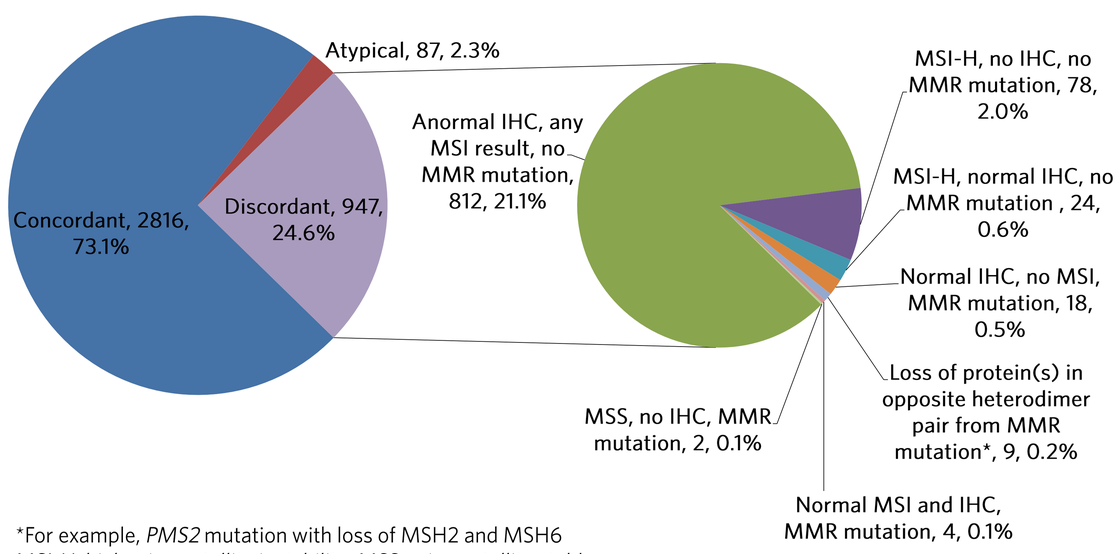
- Clinical data and previous tumor testing results were extracted from the test request forms of consecutive patients undergoing MGPT at a single laboratory between March 2012 and June 2016.
- Cases for which results of tumor testing were provided were selected and prior MSI, IHC, *BRAF*, and/or *MLH1* promoter methylation 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

MSS, microsatellite stable; MSI-L, low microsatellite instability; MSI-H, high microsatellite instability
†Includes loss of the corresponding protein and a protein from the other heterodimer pair, loss of three proteins, and loss of all four proteins

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 SUMMARY

- 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 reported.
- 3.3% of discordant cases had an MMR gene mutation with normal MSI and/or IHC or had loss of 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%).

Results by Immunohistochemistry Pattern

IHC Pattern N (% of abnormal IHC)	Positive for Corresponding MMR Mutation N (%)	Unexplained N (%)	*BRAF* Mutation and/or Methylated* N (%)
Loss of MLH1 (with or without PMS2) N = 654 (55.7%)	48 (7.3%)	457 (69.9%) • Including 1 *MSH6* carrier • 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%) • 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%) • Including 2 *PMS2* carriers	N/A
Atypical staining pattern† N = 82 (7.0%)	22 (26.8%) • All mutations corresponded with at least one of the absent proteins • 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.
- Possible explanations 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 in a patient with 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 and may not be more broadly generalizable.

REFERENCES

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