Analysis of a rare *PMS1* variant identified in Discordant Sibling Pairs From Hereditary Breast Cancer Families

Kara K Landry, MD[1], Julie A. Dragon, PhD[2], David Seward, MD, PhD[3], Wendy C McKinnon, MS[1], Laura Colello, MS[1], Susan S. Wallace, PhD[2], Melissa Cuke, MS[1], Marie E Wood, MD[1]

[1] Hematology and Oncology, University of Vermont Medical Center; [2]Department of Microbiology and Molecular Genetics, University of Vermont, Burlington, VT; [3]Department of Pathology and Laboratory Medicine, University of Vermont, Burlington, VT

Background

- Additional genes associated with hereditary breast cancer likely exist.
- We have identified a rare and potentially pathogenic *PMS1* variant in two affected sisters (and no unaffected sisters) from hereditary breast cancer families.
- Published in silico analyses previously predicted that this variant may have functional clinical significance.

Objective

1.) To determine the genotype-phenotype correlation within the two families with the rare PMS1 variant.

2.) To sequence PMS1 mRNA from cell lines with and without the c.605G>A variant to test the hypothesis that the PMS1 c.605G>A germline variant disrupts PMS1 mRNA splicing.

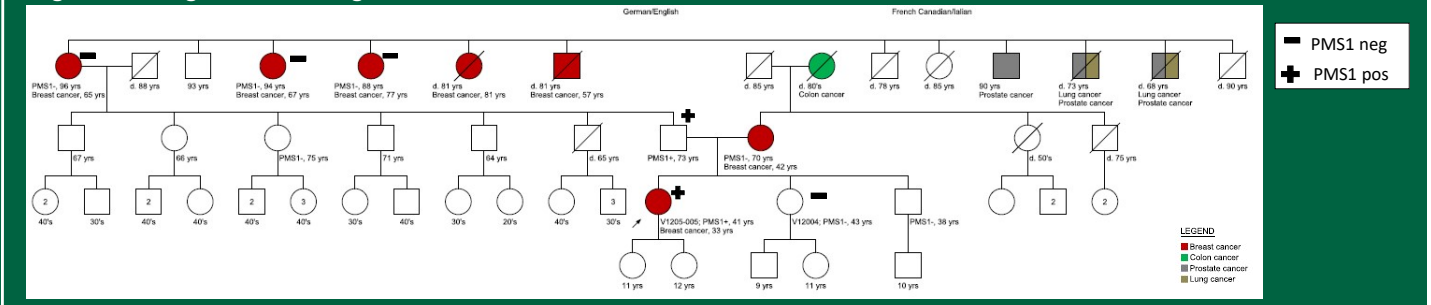
Methods

1.) Germline DNA from extended family members were collected and analyzed to determine if the PMS1 c.605G>A variant tracks with affected family members.

2.) Using NCI-H441 lung cancer cells heterozygous for the PMS1 c.605G>A variant we sequenced PMS1 mRNA and compared the results with cell lines harboring WT PMS1 to determine whether PMS1 c.605G>A impacts exon 6 utilization.

Results

Figure 1. Pedigrees of two high-risk breast cancer families with same PMS1 variant seen in two affected sisters.



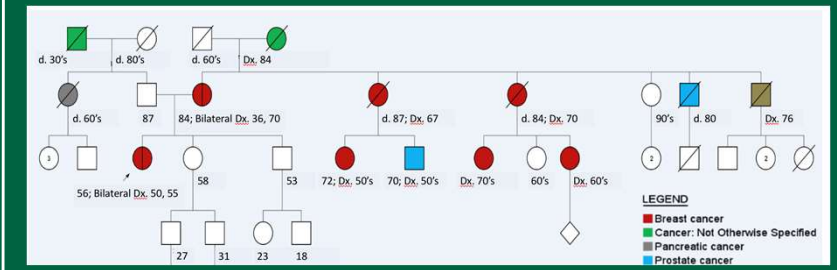
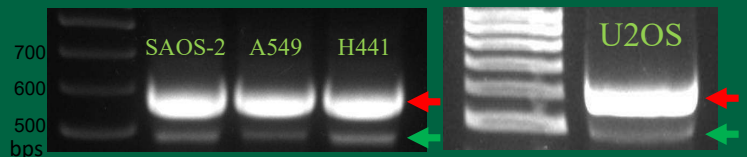


Figure 2. RT-PCR targeting PMS1 to evaluate mRNA splicing



All cell lines tested expressed the predicted full length PMS1 mRNA fragment (RED arrow on gel and minor smaller bad (GREEN arrow on gel) representing PMS1 mRNA that skips exon 6.

Figure 3. Sequence traces for the lower band RT-PCR PMS1 products showed exon 5 spliced to exon 7.

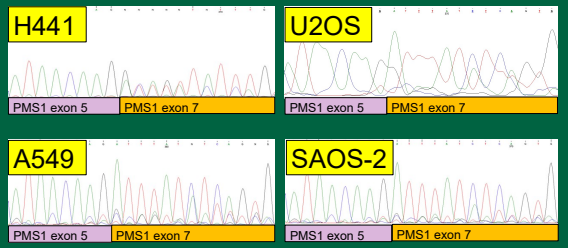
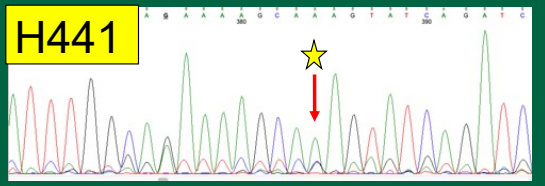


Figure 4. Full length PMS1 mRNA from H441 contains the variant "A", (yellow star) proving variant does <u>not</u> prevent splicing of exon 6.



Conclusions

- PMS1 c.605G>A variant did not segregate with disease
- There was no variant-dependent impact on splicing. This was unexpected based upon the published in silico predictions and demonstrates the necessity of functional testing.
- These findings taken together suggest that **<u>the PMS1 c.605G>A variant is likely benign</u>**.
- This information can help others investigating functional significance of either somatic or germline mutations.