LaDuca H[1], D'Souza J[2], Witherington S[1], Gau C[1]
1) Ambry Genetics, Aliso Viejo, CA
2) Virginia G. Piper Cancer Center at Scottsdale Healthcare, Scottsdale, AZ

Proband With *MSH2* and *PTEN* Germline Alterations Identified on Multi-gene Panel Testing

BACKGROUND

- While there have been published reports of individuals with germline mutations in two highly penetrant cancer susceptibility genes, the true prevalence of individuals with mutations in more than one of these genes is unknown.
- Lynch syndrome is caused by mutations in the mismatch repair genes (*MLH1, MSH2, MSH6,* and *PMS2*) and has an estimated prevalence of one in 400. Cowden syndrome is caused by mutations in the *PTEN* tumor suppressor gene and has an estimated prevalence of one in 200,000. Cancer risks associated with these syndromes are listed in **Table I**.
- Here we present a 60-year-old Caucasian female with a personal history of two colon primaries diagnosed at ages 43 (sigmoid colon) and 56 (cecum) and moderately differentiated adenocarcinoma of the stomach diagnosed at age 52. This patient also has a history of thyroid nodules and previously underwent total abdominal hysterectomy/bilateral salpingo-oophorectomy. Her maternal family history is significant for multiple relatives with colorectal cancer (including two individuals with early-onset colorectal cancer) and one relative with lung cancer (**Figure 1**).

METHODS

- The patient's saliva sample was collected and sent to Ambry Genetics (Aliso Viejo, CA) for ColoNext™ testing.
- Next-generation sequencing/Sanger sequencing was performed for all coding domains plus at least 5 bases into the 5' and 3' ends of all the introns and untranslated regions (5'UTR and 3'UTR) of the following genes: *APC, BMPR1A, CDH1, CHEK2, MLH1, MSH2, MSH6, MUTYH, PMS2, PTEN, SMAD4, STK11,* and *TP53*. Additional Sanger sequencing was performed for any regions with insufficient read depth coverage for reliable heterozygous variant detection. Variant calls other than known non-pathogenic alterations were verified by Sanger sequencing.
- Deletion/duplication analysis was performed for the aforementioned genes, with the addition of *EPCAM*, using a targeted chromosomal microarray or MLPA (for *PMS2* due to pseudogene interference).

RESULTS

- This patient was found to carry the **p.R359T** likely pathogenic alteration in the *MSH2* gene and the **p.N356D** likely pathogenic alteration in the *PTEN* gene (**Table II**). At this point in time, it is unknown whether she inherited the *MSH2* and *PTEN* alterations from the same parent.
- These molecular results are consistent with diagnoses of both Lynch syndrome and Cowden syndrome/*PTEN* Hamartoma Tumor Syndrome, respectively.
- This patient meets Amsterdam I criteria for Lynch syndrome.
- While she has a history of colon cancer and thyroid nodules, which are minor features of Cowden syndrome, she does not meet Cowden syndrome testing criteria based on her known clinical history.

Table I. Cancer Risks and Management Recommendations For Lynch Syndrome and Cowden Syndrome

Cancer Site	Lynch syndrome		Cowden syndrome	
	Cancer Risk	**Management Recommendations** *(summarized from The NCCN Clinical Practice Guidelines in OncologyTM Genetic/ Familial High-Risk Assessment: Colorectal V1.2014. http://www.nccn.org/)*	**Cancer Risk**	**Management Recommendations** *(summarized from The NCCN Clinical Practice Guidelines in OncologyTM Genetic/Familial High-Risk Assessment: Breast and Ovarian V1.2014. http://www.nccn.org/.)*
Breast cancer	Increased risk debated	None at this time	50-85%	- Breast awareness (starting at age 18) - Clinical breast exam every 6-12 months (starting at age 25 or 5-10 years before earliest breast cancer in family) - Annual mammogram and breast MRI (starting at age 30-35 or individualized based on family history) - Discuss risk-reducing mastectomy
Endometrial cancer	25-60%	- Discuss prophylactic hysterectomy and bilateral salpingo-oophorectomy, an option that should be considered by women who have completed childbearing - No clear evidence to support endometrial cancer screening, however annual endometrial sampling is an option - Transvaginal ultrasound for endometrial/ovarian cancer screening and serum CA-125 for ovarian cancer screening may be considered at clinicians' discretion	10-28%	- Discuss risk-reducing hysterectomy - Encourage education and prompt response to symptoms - Consider annual endometrial biopsy and/or ultrasound (starting at age 30-35)
Ovarian cancer	4-12%		n/a	n/a
Thyroid cancer	n/a	n/a	10-35%	Annual thyroid ultrasound (starting at age 18 or 5 years before earliest thyroid cancer in the family)
Colorectal cancer	52-82%	Colonoscopy every 1-2 years (starting at age 25-30 or 2-5 years prior to earliest colorectal cancer if diagnosed under age 25)	9%	Colonoscopy every 5 years or more frequently if symptomatic or polyps identified (starting at age 35)
Gastric and small bowel cancer	Gastric: 6-13% Small bowel: increased	Consider EGD with extended duodenoscopy for selected individuals/families and those of Asian descent every 3-5 years (starting by age 30-35)	n/a	n/a
Urinary tract	Increased	Consider annual urinalysis (starting at age 25-30)	Renal: 34%	Consider renal ultrasound every 1-2 years (starting at age 40)
Other cancers/ recommendations	Increased risk for cancers of the hepatobiliary tract, CNS, and sebaceous glands	Annual physical/neurological exam (starting at age 25-30)	Melanoma: 6%	Annual comprehensive physical exam (starting at age 18 or 5 years before youngest age of dx in family, particular attention to thyroid exam) Dermatologic management indicated for some patients

Figure 1. Pedigree

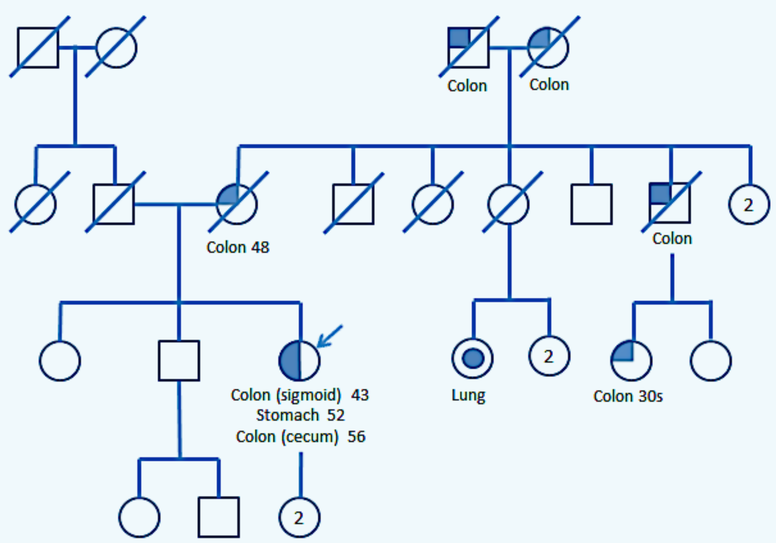

Table II. Likely Pathogenic Variants

Gene	Alteration	Evidence For Classification as Likely Pathogenic
MSH2	p.R359T (c.1076G>C)	-known mutation at same codon (p.R359S) with supporting clinical and functional data -rarity (not reported in population-based cohorts) -nucleotide and amino acid are highly conserved in available vertebrate species -predicted to decrease the efficiency of the native donor site by the BDGP and ESEfinder splice site prediction tools -predicted as damaging, deleterious, and deleterious by PolyPhen, SIFT, and MAPP-MMR *in silico* analyses, respectively
PTEN	p.N356D (c.1066A>G)	-previously reported as a pathogenic mutation -suggested that exon 9 missense mutations may result in an attenuated *PTEN* Hamartoma Tumor Syndrome (PHTS) phenotype based on the clinical histories observed in exon 9 missense mutation carriers -nearly all *PTEN* missense variants are thought to be deleterious -rarity (not reported in population-based cohorts) -amino acid is highly conserved in available vertebrate species

CONCLUSION

- Although this patient meets clinical diagnostic criteria for Lynch syndrome, a multi-gene panel was ordered to include analysis of other genes in this patient's differential diagnosis.
- If testing had been ordered only for Lynch syndrome, then the *PTEN* alteration would not likely have been detected.
- Identification of the *PTEN* likely pathogenic variant, in addition to the *MSH2* likely pathogenic variant, has significant implications for this patient's continued medical management (**Table I**).
- This case demonstrates that multi-gene panels can aid in detecting individuals with mutations in more than one highly-penetrant cancer susceptibility gene.

REFERENCES

- Chen S, Wang W, Lee S, et al. Prediction of germline mutations and cancer risk in the Lynch syndrome. *JAMA : the journal of the American Medical Association.* Sep 27 2006;296(12):1479-1487.
- Nelen MR, Kremer H, Konings IB, et al. Novel PTEN mutations in patients with Cowden disease: absence of clear genotype-phenotype correlations. *European journal of human genetics : EJHG.* Apr 1999;7(3):267-273.
- Aarnio M, Sankila R, Pukkala E, et al. Cancer risk in mutation carriers of DNA-mismatch-repair genes. *International journal of cancer. Journal international du cancer.* Apr 12 1999;81(2):214-218.
- Capelle LG, Van Grieken NC, Lingsma HF, et al. Risk and epidemiological time trends of gastric cancer in Lynch syndrome carriers in the Netherlands. *Gastroenterology.* Feb 2010;138(2):487-492.
- Hampel H, Stephens JA, Pukkala E, et al. Cancer risk in hereditary nonpolyposis colorectal cancer syndrome: later age of onset. *Gastroenterology.* Aug 2005;129(2):415-421.
- Stoffel E, Mukherjee B, Raymond VM, et al. Calculation of risk of colorectal and endometrial cancer among patients with Lynch syndrome. *Gastroenterology.* Nov 2009;137(5):1621-1627.
- Watson P, Vasen HF, Mecklin JP, et al. The risk of extra-colonic, extra-endometrial cancer in the Lynch syndrome. *International journal of cancer. Journal international du cancer.* Jul 15 2008;123(2):444-449.
- The NCCN Clinical Practice Guidelines in Oncology™ Genetic/Familial High-Risk Assessment: Colorectal V1.2014. 2014; http://www.nccn.org/.
- The NCCN Clinical Practice Guidelines in Oncology™ Genetic/Familial High-Risk Assessment: Breast and Ovarian V1.2014. *National Comprehensive Cancer Network, Inc.* 2014; http://www.nccn.org/.
- Hobert JA, Eng C. PTEN hamartoma tumor syndrome: an overview. *Genetics in medicine : official journal of the American College of Medical Genetics.* Oct 2009;11(10):687-694.
- Tan MH, Mester JL, Ngeow J, Rybicki LA, Orloff MS, Eng C. Lifetime cancer risks in individuals with germline PTEN mutations. *Clinical cancer research : an official journal of the American Association for Cancer Research.* Jan 15 2012;18(2):400-407.
- Belvederesi L, Bianchi F, Galizia E, et al. MSH2 missense mutations and HNPCC syndrome: pathogenicity assessment in a human expression system. *Human mutation.* Nov 2008;29(11):E296-309.
- Gammie AE, Erdeniz N, Beaver J, Devlin B, Nanji A, Rose MD. Functional characterization of pathogenic human MSH2 missense mutations in Saccharomyces cerevisiae. *Genetics.* Oct 2007;177(2):707-721.
- Scartozzi M, Bianchi F, Rosati S, et al. Mutations of hMLH1 and hMSH2 in patients with suspected hereditary nonpolyposis colorectal cancer: correlation with microsatellite instability and abnormalities of mismatch repair protein expression. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* Mar 1 2002;20(5):1203-1208.
- Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP). 2013. http://evs.gs.washington.edu/EVS/. Accessed June 2013.
- Abecasis GR, Auton A, Brooks LD, et al. An integrated map of genetic variation from 1,092 human genomes. *Nature.* Nov 1 2012;491(7422):56-65.
- Sherry ST, Ward MH, Kholodov M, et al. dbSNP: the NCBI database of genetic variation. *Nucleic acids research.* Jan 1 2001;29(1):308-311.
- Blanco A, de la Hoya M, Balmana J, et al. Detection of a large rearrangement in PALB2 in Spanish breast cancer families with male breast cancer. *Breast cancer research and treatment.* Feb 2012;132(1):307-315.
- Cartegni L, Wang J, Zhu Z, Zhang MQ, Krainer AR. ESEfinder: A web resource to identify exonic splicing enhancers. *Nucleic acids research.* Jul 1 2003;31(13):3568-3571.
- Reese MG, Eeckman FH, Kulp D, Haussler D. Improved splice site detection in Genie. *Journal of computational biology : a journal of computational molecular cell biology.* Fall 1997;4(3):311-323.
- Smith PJ, Zhang C, Wang J, Chew SL, Zhang MQ, Krainer AR. An increased specificity score matrix for the prediction of SF2/ASF-specific exonic splicing enhancers. *Human molecular genetics.* Aug 15 2006;15(16):2490-2508.
- Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. *Nature methods.* Apr 2010;7(4):248-249.
- Chao EC, Velasquez JL, Witherspoon MS, et al. Accurate classification of MLH1/MSH2 missense variants with multivariate analysis of protein polymorphisms-mismatch repair (MAPP-MMR). *Human mutation.* Jun 2008;29(6):852-860.
- Ng PC, Henikoff S. Predicting the effects of amino acid substitutions on protein function. *Annual review of genomics and human genetics.* 2006;7:61-80.
- Mester JL, Tilot AK, Rybicki LA, Frazier TW, 2nd, Eng C. Analysis of prevalence and degree of macrocephaly in patients with germline PTEN mutations and of brain weight in Pten knock-in murine model. *European journal of human genetics : EJHG.* Jul 2011;19(7):763-768.
- Ngeow J, Mester J, Rybicki LA, Ni Y, Milas M, Eng C. Incidence and clinical characteristics of thyroid cancer in prospective series of individuals with Cowden and Cowden-like syndrome characterized by germline PTEN, SDH, or KLLN alterations. *The Journal of clinical endocrinology and metabolism.* Dec 2011;96(12):E2063-2071.
- Eng C. PTEN Hamartoma Tumor Syndrome (PHTS). In: Pagon RA, Adam MP, Bird TD, et al., eds. *GeneReviews(R).* Seattle (WA)1993.