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Pancreatic cancer and a novel *MSH2* germline alteration

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Abstract

Objectives—To describe a novel *MSH2* missense alteration co-segregating with pancreatic cancer.

Methods—Observational study of a kindred in which a novel *MSH2* missense alteration was identified.

Results—We report a family in which a *MSH2* P349L missense alteration is co-segregating with pancreatic cancers among three nonsmoking first degree relatives. Lynch syndrome-related tumors from individuals carrying this alteration consistently showed loss of immunohistochemical expression of *MSH2* and *in-silico* analyses support interpretation of this DNA alteration as likely pathogenic.

Conclusions—The *MSH2* P349L may increase the risk for pancreatic cancer beyond the usual mutations in DNA mismatch repair genes; however studies of additional families with the identical missense alteration are needed to confirm this initial impression.

Keywords

pancreatic cancer; HNPCC; Lynch Syndrome; hereditary; genetics

Introduction

Lynch syndrome (OMIM #s 120435, 609310) is an autosomal dominant cancer predisposition syndrome that underlies about 3–5% of all colorectal cancers.[1-5] It is caused by germline mutations in one of several DNA mismatch repair genes, including *MSH2*,

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MLH1, MSH6, and *PMS2.* The genetic heterogeneity has made diagnostic testing a challenge, such that use of tumor assessment of either DNA mismatch repair deficiency (microsatellite instability) and/or expression of the four DNA mismatch repair gene products has been widely used to screen suspected cases. The Bethesda guidelines[6] put forth recommendations based upon expert opinion for when tumor testing should be considered. Under that report, the cancers listed as Lynch Syndrome-associated included colorectal, endometrial, stomach, ovarian, pancreas, ureter and renal pelvis, biliary tract and brain (usually glioblastoma as seen in Turcot syndrome) tumors, sebaceous gland adenomas and keratoacanthomas in Muir-Torre syndrome, and carcinoma of the small bowel. Although pancreatic cancer is included in this list, risk usually appears to be only minimally increased, relative to the general population.

Materials and Methods

We have been following a family with a novel *MSH2* missense alteration in which pancreatic cancer has been more commonly observed than colon or endometrial cancer (Figure 1). The ancestry is Northern European. None of the affected individuals smoked cigarettes nor had known exposure to unusual environmental agents. There is no family history of melanoma, early onset breast cancer, ovarian cancer, or pancreatitis. Table 1 lists the cancers of all relevant family members, and, where available, the results of tumor immunohistochemical expression of the DNA mismatch repair genes. All testing was done at Mayo Clinic using standard techniques.[7–9] The *MSH2* germline change, identified by sequencing, is in exon 6, c.1046C>T, (CCT>CTT), p.Pro349Leu, hereafter called P349L. This variant co-segregates with the development of pancreatic cancer and with the loss of *MSH2* expression in tumor tissue in this family. The details of this family have not previously been published; however one aspect of this family's laboratory results was included in a prior publication that reported on use of *BRAF* screening as a strategy to simplify HNPCC genetic testing.[10, 11] No *BRAF* V600E somatic mutation was found in the MSI-high tumor tested in this family, consistent with this being a Lynch Syndrome family. The kindred is enrolled in an ongoing familial pancreatic cancer registry and an affected individual was studied and found to be negative for *CFTR* and *CDKN2A* mutations.

Results

In silico analyses. 17% of all mutations in *MSH2* are missense mutations.[12] The P349L variant is not listed in the Mismatch Repair Genes Variant Database from the Memorial University of Newfoundland (http://www.med.mun.ca/MMRvariants/search_results.aspx) nor is it included in the paper or supplemental materials in the MAPP-MMR database.[13] It is also not reported in the MMR Gene Unclassified Variants Database (www.mmruv.info), although an *MSH2* P349R mutation at the same site is reported by three *in silico* models, suggesting pathogenicity.[13] The P349L variant has not been included in functional studies of pathogenicity of *MSH2* missense variants.[14, 15] However, the P349L variant is located in the lever domain of the *MSH2* gene, a large domain that connects the ATP binding subunits to the clamp domains to mediate signals between the ATP- and the DNA-binding portions of the protein. Two of three missense substitutions studied functionally in the lever domain manifest lower stability and defective DNA mismatch repair and loss of expression in tumors, which is consistent with studies of homologous positions in yeast *MSH2*, in which half of missense alterations lead to inefficient expression of the gene.[14, 15] The Uniprot database, referring to the Domingo report of this family,[10] cites the Pro349Leu variant as possibly pathogenic (http://www.expasy.org/cgi-bin/variant_pages/get-sprot-variant.pl?VAR_043763). A BLOSUM score of -3 is reported in Uniprot.[16] This is supported by *in silico* analyses using Align-GVGD, with Grantham Variation 0 and the Grantham Deviation 97.78 resulting in a

C65 score for *MSH2* P349L.[17, 18] These findings together indicate that the residue is evolutionarily constrained, and predicts that this missense alteration is very likely to have functional consequences.

In order to derive a quantitative classification of pathogenicity, we performed Bayes factor analysis of variant segregation data using methods described previously.[19] Calculations assumed age-specific relative risks for colorectal cancer, endometrial cancer, and other Lynch Syndrome-related cancers (including pancreatic cancer) as estimated in Quehenberger et al. (2005).[20] This analysis also provided odds in favor of pathogenicity of 35.7:1, translating to a probability of pathogenicity of 0.97 for this variant. *MSH2* P349L would thus be considered class 4 (likely pathogenic), based on the IARC 5 class classification system that is linked to posterior probability estimates.[21]

Discussion

Available data suggest that, in general, penetrance for pancreatic cancer in Lynch Syndrome is low. Prior to discovery of the genetic basis of the Lynch Syndrome, Watson and Lynch (1993) studied 1,424 at-risk persons from 23 large families (with 287 colorectal cancers) who were suspected of having this disorder.[22] Six pancreatic cancers were recorded, compared with 4.1 expected, which was not statistically significantly different. In l999, Aarnio et al. had studied 360 gene carriers of 50 families with gene mutations (94% in MLH1 and 6% in *MSH2*), and found 3 pancreatic cancers, giving a Standardized Incidence Ratio of 4.5, but with a 95% CI of 1.0–14.[23] In 2008, in a study that assessed extracolonic cancer risk among 6,041 members of 261 families with documented mutations in *MLH1* (60%) or *MSH2* (40%), cancers of the biliary tract, liver and pancreas combined accounted for 1.09% of the cancers in this study, giving a hazard ratio of 1.869 and a cumulative incidence of 4.1% to age 70 years.[24] Most recently, risk of pancreatic cancer alone was addressed in 6,342 individuals from 147 families with MMR mutations (37.4% in *MLH1*, 55.1% in *MSH2*, and 7.55% in *MSH6*). A cumulative risk of pancreatic cancer was calculated as 1.31% (95% CI=0.31–2.32%) to age 50, and 3.68% (95% CI=1.45–5.88%) to age 70, which is an 8.6-fold (95% CI=4.7–15.7%) increase compared with the general population.[25] In summary, we present a family in which a novel P349L missense substitution in *MSH2* that co-segregates with disease in a Lynch Syndrome family appears particularly to be associated with a high risk for pancreatic cancer. All three cancer affected individuals carry the *MSH2* P349L missense substitution, and there is loss of expression of *MSH2/MSH6* in each of their pancreatic tumors. Together with *in silico* predictions, these data provide support that this alteration is the causative mutation. It would be of interest to learn of other families with the same missense alteration to determine if predisposition to pancreatic cancer is consistently associated with this *MSH2* change.

References

1. Hampel H, Frankel WL, Martin E, et al. Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer). N Engl J Med. 2005a; 352(18):1851–1860. [PubMed: 15872200]
2. Wijnen J, Vasen H, Kahn M, et al. Clinical findings with implications for genetic testing in families with clustering of colorectal cancer. N Engl J Med. 1998; 339:511–518. [PubMed: 9709044]
3. Vasen H, Watson P, Mecklin J, et al. New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the International Collaborative group on HNPCC. Gastroenterology. Jun; 1999 116(6):1453–1456. [PubMed: 10348829]
4. Lindor NM, Petersen GM, Hadley DW, et al. Recommendations for the Care of Individuals With an Inherited Predisposition to Lynch Syndrome. JAMA. 2006; 296:1507–1517. [PubMed: 17003399]
5. Vasen H, Moslein G, Alonso A, et al. Guidelines for the clinical management of Lynch syndrome. J Med Genet. 2007; 44(6):353–362. [PubMed: 17327285]

6. Umar A, Boland C, Terdiman J, et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst. Feb 18; 2004a 96(4):261–268. [PubMed: 14970275]
7. Lindor NM, Burgart LJ, Leontovich O, et al. Immunohistochemistry versus microsatellite instability testing in phenotyping colorectal tumors. J Clin Oncol. Feb 15; 2002a 20(4):1043–1048. [PubMed: 11844828]
8. Cunningham JM, Kim CY, Christensen ER, et al. The frequency of hereditary defective mismatch repair in a prospective series of unselected colorectal carcinomas. Am J Hum Genet. 2001; 69(4): 780–790. [PubMed: 11524701]
9. Baudhuin LM, Burgart LJ, Leontovich O, et al. Use of microsatellite instability and immunohistochemistry testing for the identification of individuals at risk for Lynch syndrome. Fam Cancer. 2005a; 4(3):255–265. [PubMed: 16136387]
10. Domingo E, Laiho P, Ollikainen M, et al. BRAF screening as a low-cost effective strategy for simplifying HNPCC genetic testing. J Med Genet. 2004; 41(9):664–668. [PubMed: 15342696]
11. Wang L, Cunningham J, Winters J, et al. BRAF mutations in colon cancer are not likely attributable to defective DNA mismatch repair. Cancer Res. Sep 1; 2003a 63(17):5209–5212. [PubMed: 14500346]
12. Woods MO, Williams P, Careen A, et al. A new variant database for mismatch repair genes associated with Lynch syndrome. Hum Mutat. 2007; 28:669–373. [PubMed: 17347989]
13. 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). Hum Mutat. 2008; 29(6):852–860. [PubMed: 18383312]
14. Gammie AE, Erdeniz N, Beaver J, et al. Functional characterization of pathogenic human MSH2 missense mutations in Saccharomyces cerevisiae. Genetics. 2007; 177(2):707–721. [PubMed: 17720936]
15. Ollila S, Dermadi Bebek D, Jiricny J, et al. Mechanisms of Pathogenicity in Human MSH2 Missense Mutants. Hum Mutat. 2008; 29(11):1355–1363. [PubMed: 18951462]
16. Henikoff S, Henikoff JG. Amino Acid Substitution Matrices from Protein Blocks. PNAS. 1992; 89:10915–10919. [PubMed: 1438297]
17. Tavtigian S, Deffenbaugh A, Yin L, et al. Comprehensive statistical study of 452 BRCA1 missense substitutions with classification of eight recurrent substitutions as neutral. J Med Genet. 2006; 43(4):295–305. [PubMed: 16014699]
18. Mathe E, Olivier M, Kato S, et al. Computational approaches for predicting the biological effect of p53 missense mutations: a comparison of three sequence analysis based methods. Nucleic Acids Res. 2006; 34(5):1317–1325. [PubMed: 16522644]
19. Arnold S, Buchanan DD, Barker M, et al. Classifying MLH1 and MSH2 variants using bioinformatic prediction, splicing assays, segregation, and tumor characteristics. Hum Genet. 2009; 30(5):757.
20. Quehenberger F, Vasen H, van Houwelingen H. Risk of colorectal and endometrial cancer for carriers of mutations of the hMLH1 and hMSH2 gene: correction for ascertainment. J Med Genet. 2005; 42:491–496. [PubMed: 15937084]
21. Plon S, Eccles D, Easton D, et al. Sequence variant classification and reporting: recommendations for improving the interpretation of cancer susceptibility genetic test results. Hum Mutat. 2008; 29(11):1282–1291. [PubMed: 18951446]
22. Watson P, Lynch H. Extracolonic cancer in hereditary nonpolyposis colorectal cancer. Cancer. Feb 1; 1993 71(3):677–685. [PubMed: 8431847]
23. Aarnio M, Sankila R, Pukkala E, et al. Cancer risk in mutation carriers of DNA- mismatch-repair genes. Int J Cancer. Apr; 1999 81(2):214–218. [PubMed: 10188721]
24. Watson P, Vasen HF, Mecklin JP, et al. The risk of extra-colonic, extra-endometrial cancer in the Lynch syndrome. International Journal of Cancer. 2008; 123(2):444–449.
25. Kastrinos F, Mukherjee B, Tayob N, et al. Risk of pancreatic cancer in families with Lynch syndrome. JAMA. 2009; 302(16):1790–1795. [PubMed: 19861671]

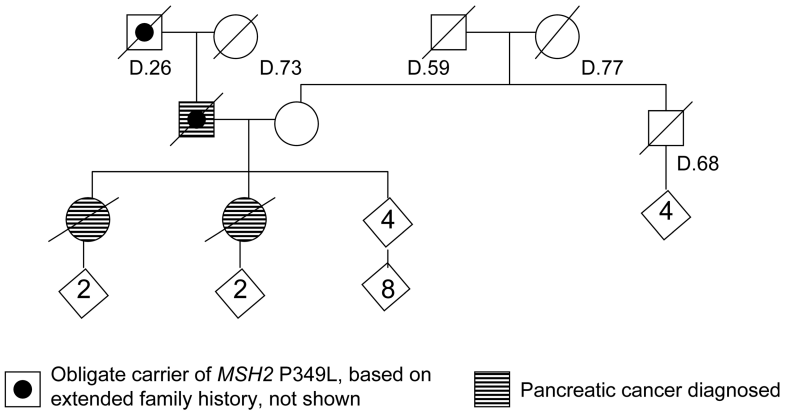

Figure 1.
Pedigree of family with *MSH2* P349L missense substitution, showing those diagnosed with pancreatic cancer.

Table 1

Results of germline and tumor molecular testing in family with three individuals with pancreatic cancer, co-segregating with an *MSH2* P349L alteration.

		Tumor immunohistochemistry expression				
Relation to proband, cancer, age at diagnosis	***MSH2* P349L germline mutation**	**MSI**	**MLH1**	**MSH2**	**MSH6**	**PMS2**
Paternal grandmother	ND					
Breast 73		ND	ND	ND	ND	ND
Father	ND (***obligate carrier***)					
Colon 43			NA	NA	NA	NA
Pancreas 50			Normal	***Loss***	***Loss***	normal
Mother	Negative					
Chronic lymphocytic leukemia		ND	ND	ND	ND	ND
Proband	***Positive***					
Endometrial 39			Normal	***Loss***	***Loss***	ND
Spindle Cell Sarcoma 39			Normal	Normal	Normal	ND
Colon 50		High	Normal	***Loss***	***Loss***	normal
Papillary bladder-58			Normal	Normal	Normal	ND
Pancreas 60			Normal	***Loss***	***Loss***	normal
Sister	***Positive***					
Pancreas 54			Normal	***Loss***	***Loss***	ND

MSI-tumor microsatellite instability; ND-not done.