

Predicting and Preventing Hereditary Colorectal Cancer

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COLORECTAL CANCER (CRC) IS ONE OF THE MOST common malignancies in the United States and affects nearly 150 000 individuals per year.¹ The prognosis for patients with CRC is directly related to their stage at diagnosis, with 5-year survival greater than 90% for the rare patient diagnosed with stage I cancer but less than 5% for patients with metastatic disease.² Therefore, early diagnosis is essential for the prevention of the morbidity and mortality associated with CRC. This fact underlies the current recommendations for population-based screening for colon polyps and cancer, preferably using colonoscopy in individuals 50 years or older.³ Diet and lifestyle factors are thought to influence risk for CRC in the general population, but family history also clearly affects the individual risk for CRC, presumably due to genetic factors.

A quarter of all CRC cases occur in families containing other members with CRC, suggesting a familial basis.¹ More striking, about 3% to 4% of CRCs occur in families with a clear autosomal dominant pattern of inheritance, the most common of which is Lynch syndrome, ie, hereditary non-polyposis colorectal cancer (HNPCC).⁴ HNPCC was originally defined by the "Amsterdam" clinical criteria as a history of at least 3 affected family members involving 2 generations with at least 1 person diagnosed before age 50 years.⁵ Although this approach is fairly specific in identifying families with highly penetrant HNPCC, it is also overly restrictive and does not take into account the possibility of later-onset variants of the disease, the implications of extracolonic tumors, or the limitations imposed by small family size.⁶ Indeed, many families with known HNPCC do not meet the original Amsterdam Criteria.

In the early 1990s, the genetic basis for Lynch syndrome was uncovered, with the discovery that germline mutations in the mismatch DNA repair genes *MLH1* and *MSH2* (and, subsequently, *MSH6* and rarely *PMS2*) conferred a high susceptibility to colon and endometrial cancer and an elevated risk of other cancers, including cancers of the ovary, stomach, small bowel, hepatobiliary system, ureteral tract, brain, and other sites.⁷ This has allowed for genetic testing and counseling of

individuals in families with a clinical suspicion of HNPCC to identify those for whom early and regular cancer screening may be appropriate.³ Indeed, use of colonoscopy in known carriers of mutations in one of these genes has been proven effective for reducing the incidence and mortality of CRC^{8,9} and is recommended beginning when carriers are in their early to mid 20s, at intervals of every 1 to 2 years.³ However, genetic testing for mismatch repair gene mutations is not perfectly sensitive or specific and is expensive, and therefore methods to better identify those individuals at significant risk for Lynch syndrome are important.

Various algorithms combining family history information with molecular tumor characteristics have been developed to help in these efforts. A unique aspect of HNPCC not common to most other cancer syndromes is that specific phenotypic markers in the tumor itself can identify most cases. More than 95% of CRCs from patients with Lynch syndrome exhibit a mutational DNA pattern termed microsatellite instability (MSI), caused by the DNA repair defect intrinsic to the genetic alterations in mismatch repair genes.⁷ MSI can now be routinely detected in reference laboratories from tumor blocks, even those stored for many years. These pathologic findings have been used during the past few years to help guide genetic testing. In fact, in 1996 the Bethesda Criteria were introduced specifically to provide guidelines for selection of tumors for MSI testing, and a consensus panel of standard markers was chosen.¹⁰⁻¹² More recently, it has been shown that immunohistochemical analysis to detect loss of mismatch repair protein expression can be performed by most pathology laboratories,¹³ although with variable success, and can help further direct genetic testing to a specific gene.

These molecular pathology tests have helped greatly in identifying Lynch syndrome in individuals diagnosed with CRC, particularly when at a relatively young age.^{13,14} However, this approach is complicated by the fact that approximately 15% of sporadic CRCs that occur in patients without HNPCC also exhibit MSI and loss of *MLH1* protein expression, due to epigenetic silencing of this gene in the

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tumor.¹⁵ Furthermore, phenotypic testing is possible only if tumor tissue is available and is therefore often not helpful for the unaffected individual with a strong family history but no remaining tumor tissue from affected relatives. In addition, none of these approaches are designed to determine the likelihood of carrying a genetic mutation for an individual patient.

For all these reasons, better predictive models for assessing risk of Lynch syndrome and germline carriage rates of mutations in the most common causative genes are needed to help decide for whom germline DNA sequencing is most appropriate. In this issue of *JAMA*, Balmaña and colleagues¹⁶ and Chen and colleagues¹⁷ present 2 new algorithms for predicting the likelihood of carrying a germline mismatch repair gene mutation. In addition, a third algorithm has recently been proposed by Barnetson et al.¹⁸ The 3 rules differ in the type of algorithm used to predict carrier status, in the patient populations used to develop and validate the rule, in the genetic testing methods used to identify carriers and evaluate prediction accuracy, and in the mismatch repair genes assessed by the methods.

The algorithms developed by Balmaña et al¹⁶ and Barnetson et al¹⁸ use a multivariate logistic regression model to predict carrier status based on personal and family history of colon and endometrial cancer and of other Lynch syndrome cancers. In contrast, the algorithm used by Chen et al¹⁷ involves a detailed parametric model, invoking the Bayes rule to estimate the probability that the counselee carries a mutation, given his or her personal and family history of the Lynch syndrome malignancies. This model uses more input data (particularly from unaffected relatives of the counselee) than either of the 2 logistic regression models. This additional information may improve performance but could work against prediction accuracy when the detailed information is unknown or erroneous. The prediction rules of Barnetson et al¹⁸ and Chen et al¹⁷ allow the user to include tumor MSI data, whereas that of Balmaña et al¹⁶ does not.

The population used to develop and validate the prediction rule of Balmaña et al¹⁶ consisted of unrelated individuals whose DNA was sent for genetic testing to Myriad Genetics Inc. Chen et al¹⁷ used a combination of population-based and clinic-based data in selecting values needed for their rule (eg, carrier prevalence and carrier's cumulative cancer risks) and validated their rule using several clinic-based populations consisting of individuals presenting with CRC, a strong family history of the disease, or both. In contrast, the rule of Barnetson et al¹⁸ was developed and validated using a population-based series of CRC cases in Edinburgh, Scotland.

The results of laboratory testing form the gold standard against which the accuracy of a prediction rule is evaluated. However, the laboratory methods have imperfect sensitivity due to missed aberrations, such as large genomic deletions.¹⁹ In addition, the pathogenicity of some missense

mutations is uncertain, and subjective cut points are used to classify mutations as pathogenic or nonpathogenic. Whatever the cause, imperfect sensitivity of the laboratory methods adversely (and unfairly) affects the performance of a prediction rule. The rule of Balmaña et al¹⁶ was validated against sequencing of 19 exons and adjacent noncoding regions in *MLH1* and 16 exons and adjacent coding regions in *MSH2*. That of Barnetson et al¹⁸ involved evaluating 16 exons of *MLH1*, 10 exons of *MSH2*, and all 10 exons of *MSH6*. The rule of Chen et al¹⁷ was based on a variety of methods that cover the 3 genes with variable intensity.

The key clinical issues involve determining how these rules will perform in practice and whether there are certain patients for whom one rule is likely to predict carrier status more accurately than others. To address these issues, it is helpful to distinguish 2 yardsticks by which prediction rules are evaluated. The first, the rule's *calibration ability*, reflects the accuracy with which it predicts the actual proportion of carriers in a given population. The second, the rule's *discriminatory ability or resolution*, reflects the accuracy with which it predicts a given individual's carrier status. For example, if 1% of a given population carries a mutation of a mismatch repair gene, the rule that assigns a probability of 1% to each individual in the population has perfect calibration ability but no discriminatory ability. Clearly the clinical usefulness of a rule is determined by its discriminatory ability, as measured by its sensitivity, specificity, positive and negative predictive power, or by the area under its receiver operating characteristic (ROC) curve. Thus, it is quite noteworthy that the areas under the ROC curves reported by the 3 methods are similar: for the rules of Chen et al,¹⁷ Balmaña et al,¹⁶ and Barnetson et al,¹⁸ the areas and their 95% confidence intervals are, respectively, 0.83 (0.78-0.88), 0.80 (0.76-0.84), and 0.82 (0.72-0.91).

In summary, these prediction rules should form very useful tools for clinicians and their patients, as well as for epidemiologists who wish to assess both the magnitude of the HNPCC problem and the potential usefulness of preventive efforts. What are the next steps? Evaluation of all 3 rules using a single data set would be helpful and allow for a direct comparison of the models. Studies using population-based data would be preferable, to assess the performance of the rule among individuals with little or no family history of the Lynch syndrome malignancies. Since the rules were developed and evaluated using samples primarily composed of white individuals with European ancestry, there also is great need to evaluate the performances of these rules when applied to ethnic minorities, as the prevalence and penetrance of Lynch syndrome is poorly understood in nonwhite populations.

The clinical and genetic understanding of Lynch syndrome has progressed dramatically since Henry Lynch first described this syndrome more than 40 years ago.²⁰ Additional tools, such as molecular diagnostics and the more powerful predictive models presented in this issue of *JAMA*,^{16,17} are advancing the ability of clinicians to identify patients at risk for Lynch syndrome and hopefully to prevent cancer from

occurring using intensive surveillance techniques and prevention schemes. These tools also are making genetic testing decisions and management of hereditary cancer syndromes even more complicated, underscoring the necessity for dedicated cancer genetic counselors and cancer risk assessment clinics that can best use these evolving tools to provide appropriate and evidence-based health care consultation.

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Explaining, Predicting, and Treating HIV-Associated CD4 Cell Loss After 25 Years Still a Puzzle

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THE CLINICAL SYNDROME OF AIDS IS DUE TO INFECTION with the human immunodeficiency virus (HIV), which causes a progressive immunodeficiency characterized by the loss of CD4 T lymphocytes coupled with an immunosuppression related to global activation of the immune system. Since the seminal article by Mellors et al in 1996,¹ it has been known that as a group, individuals with a higher HIV RNA viral load tend to progress to AIDS and death at a more rapid rate than those with lower viral loads, and that different prognostic information can be derived from the CD4 cell count and the viral load. The conventional wisdom is that the CD4 cell count represents the current state of immune deficiency, whereas the viral load

reflects the rate at which the immune system will further deteriorate.²

The report by Rodríguez and colleagues³ in this issue of *JAMA* challenges the notion that, at the individual level, a limited number of HIV measurements over a short period of time provide meaningful prognostic information regarding the rate of CD4 cell decline and by extension the risk of opportunistic infections. Clinicians treating patients with HIV encounter some patients with low plasma viral levels who experience rapid progression. What mechanism is responsible for their profound and quick CD4 cell loss? On the other end of the spectrum are those patients with high-level HIV viremia who respond clinically like sooty mangabeys infected with simian immunodeficiency virus (SIV),⁴

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