Clinical Selection of Candidates for Mutational Testing for Cancer Susceptibility

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Advances in molecular genetics have evolved at such a fast pace that physicians may be bewildered about their clinical translation into patient care. However, genetic counselors, particularly those trained in cancer genetics, have been extremely helpful. The challenge to the physician, however, calls for an understanding of the natural history of hereditary cancer syndromes, which is often reflected in the pedigree. Pedigree/family history information must be compiled in sufficient detail to arrive at the most likely hereditary cancer syndrome diagnosis so that the molecular geneticist can search for the mutation. Finally, the challenge to the clinician is melding this into an accurate diagnosis, in order to provide highly targeted screening and management for high-risk patients. This article is an attempt to crystallize all of these issues in a format that will help physicians---particularly those in the oncology community---to meet this challenge effectively.

For many decades family history of malignancy has been used as a tool to identify individuals at greater than average risk of cancer.[1,2] Only recently has there been discovery of a host of genes that, when mutated, specify a markedly increased cancer risk.[3] This in turn has led to an opportunity to perform genetic susceptibility testing. This can confirm (but virtually never exclude) the presence of a mutated gene in a particular family and, when so confirmed, serve as a basis for specifying carrier or noncarrier status among those otherwise considered "at risk." This ability to discriminate carrier status enables, on the one hand, enhanced surveillance, prophylactic surgical intervention, or chemoprevention opportunities for carriers. As important, but often receiving less attention, is the ability to identify noncarriers who can be freed of the cancer worry, the need for aggressive screening, and anxiety over potential transmission of risk to their children.

With all of these advances have come a number of challenges. Among these are questions of who warrants genetic testing, how to conduct such testing, what to do with results that may or may not be definitively positive or negative, and what clinical screening to perform. Additional layers of complexity for the clinician surround the "human element": patients that are unaware of their family history, dysfunctional families that cannot work together to identify and communicate risk information to one another, and other social and psychological barriers. Finally, the economics of paying for genetic counseling, laboratory testing, and clinical screening may render most of the other considerations effectively moot. In short, comprehensive genetic counseling is central to most cases of familial cancer predisposition. The key elements of genetic counseling should be addressed; one well-considered tabulation of these elements is reproduced in Table 1.
In this article we will focus on certain of the issues that bear on the identification and evaluation of subjects who are potential carriers of colorectal cancer susceptibility. In some instances the antecedent family history is critical, such as the "worried well" patient who wants to know if he or she is at risk due to the presence of colorectal cancer in a close relative. More commonly the setting is the patient who is newly diagnosed with colorectal cancer and/or multiple colonic adenomas. In these cases the nature of the diagnosis itself is key and the family history may only provide refinement in estimating and acting on the likelihood of a genetic basis for the tumor that has been found. In still others, currently a minority, a genetic diagnosis already exists in the family and recommendations for testing and testing-based screening are quite clear. Fortunately, in all of these situations, there is now enough of a knowledge base as to allow for an
algorithmic approach. In the interest of space, the uncommon non-adenomatous polyposis disorders, Peutz-Jeghers syndrome,[4,5] juvenile polyposis,[6,7] and their variants will not be discussed. The interested reader is directed to several excellent reviews that cover the intriguing story of the historical development of these syndromes, as well as their genetic and clinical aspects.[3,8,9] Little attention will be devoted to the situation in which no genetic diagnosis can be made, a common problem. Nor will we take up the hot topic of minor susceptibility alleles that increase, or in some cases may decrease, cancer risk. There are simply too many risk-modifying alleles to consider and their individual and interactive risks too speculative for an article devoted to current clinical practice.

When Family History Is Key

Before we knew as much as we do now about cancer genetics, family history was essentially the only information that could guide management. In the case of familial adenomatous polyposis (FAP), if one's parent or sibling had hundreds to thousands of adenomas, with or without cancer, their surgeon might recommend sigmoidoscopy. A diagnosis of early polyps would lead to prophylactic colectomy or, if the rectum was heavily infiltrated, proctocolectomy with end ileostomy or ileal pouch-anal anastomosis. If no polyps were found by age 30 or so, he or she would be cautiously declared a noncarrier of the trait and screening would be relaxed.

Guillem et al[10] provide a review of the current role of risk-reducing surgery in hereditary colorectal cancer syndromes, including FAP, along with MEN type 2 syndromes, and hereditary syndromes that predispose to breast, ovarian, and endometrial cancer. Familial adenomatous polyposis was the relatively easy case. The hard case was the condition that was initially called the cancer family syndrome, later hereditary nonpolyposis colorectal cancer, and now the Lynch syndrome. Unlike the plethora of adenomas that clearly labeled the patient as having FAP, patients with Lynch syndrome exhibited no absolutely diagnostic clinical feature (with the exception of the Muir-Torre syndrome variant of Lynch syndrome, with its cutaneous stigmata[11]).
Instead, a "syndromic" diagnosis was based on a constellation of features (Table 2), several of which came to comprise the so-called "Amsterdam Criteria" (Table 3). These included early age at onset, multiple primary tumors, right-sided colon involvement, and/or the presence of these features in other family members, usually accompanied by specific extracolonic tumors, including those of the endometrium and ovary, stomach, small bowel, hepatobiliary tract, and pancreas, uroepithelial tumors, and the mentioned otherwise rare sebaceous skin tumors consonant with the Muir-Torre variant. Patients with a family history and a new personal diagnosis of colorectal cancer were sometimes offered subtotal colectomy as both treatment for the cancer and as prophylaxis against the risk of metachronous tumors in years to come. Close relatives of patients with such a syndrome diagnosis could be counseled to undergo colon surveillance by annual colonoscopy beginning at an early age (20 to 25).
Table 3

Amsterdam I and Amsterdam II Criteria and Bethesda Guidelines

Amsterdam I Criteria[30]

- At least three relatives with histologically verified colorectal cancer:
  1. One is a first-degree relative of the other two.
  2. At least two successive generations affected
  3. At least one of the relatives with colorectal cancer diagnosed at less than 50 years of age
  4. Familial adenomatous polyposis has been excluded.

Amsterdam II Criteria[31]

- At least three relatives with a hereditary nonpolyposis colorectal cancer–associated cancer (colorectal, endometrial, stomach, ovary, ureter/renal pelvis, brain, small bowel, hepatobiliary tract, and skin [sebaceous tumors]):
  1. One is a first-degree relative of the other two
  2. At least two successive generations affected
  3. At least one of the hereditary nonpolyposis colorectal cancer–associated cancers should be diagnosed at less than 50 years of age
  4. Familial adenomatous polyposis should be excluded in any colorectal cancer cases

Tumors should be verified whenever possible.

Bethesda Guidelines for Testing of Colorectal Tumors for Microsatellite Instability[32]

1. Colorectal cancer diagnosed in a patient who is less than 50 years of age
2. Presence of synchronous or metachronous colorectal, or other Lynch syndrome–associated tumors,\(^a\) regardless of age
3. Colorectal cancer with the MSI-H\(^b\) histology\(^c\) diagnosed in a patient who is less than 60 years of age\(^d\)
4. Colorectal cancer or Lynch syndrome–associated tumor\(^a\) diagnosed under age 50 years in at least one first-degree relative\(^b\)
5. Colorectal cancer or Lynch syndrome–associated tumor\(^a\) diagnosed at any age in two first- or second-degree relatives\(^a\)

\(^a\)Lynch syndrome–associated tumors include colorectal, endometrial, stomach, ovarian, pancreas, ureter or 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.

\(^b\)MSI-H indicates microsatellite instability–high in tumors refers to changes in two or more of the five National Cancer Institute–recommended panels of microsatellite markers.

\(^c\)Presence of tumor-infiltrating lymphocytes, Crohn disease–like lymphocytic reaction, mucinous/ signet-ring differentiation, or medullary growth pattern.

\(^d\)There was no consensus among the workshop participants on whether to include the age criteria in guideline 3 above; participants voted to keep less than 60 years of age in the guidelines.

\(^e\)Criteria 4 and 5 have been reworded to clarify the Revised Bethesda Guidelines.
When Tumor Characteristics Are Key
When, in 1993, the first Lynch syndrome gene was localized, then sequenced and found to be one of a family of mismatch-repair (MMR) family of genes, a new age was ushered in. It was now possible to not only achieve certainty that a given patient and family had Lynch syndrome, but it was possible to carry out predictive testing. Of equal importance to the discovery of linkage to a Lynch syndrome locus was the simultaneous recognition that the vast majority of Lynch syndrome tumors exhibited genetic instability in microsatellites (initially termed replication errors or RER phenotype). Microsatellites are stretches of nucleotide repeats that are present in a number of genes. It was later found that MSI can occur in 12% to 15% of non-Lynch syndrome colorectal cancers, but the etiology is distinct and measures have been developed to demonstrate this finding (sporadic MSI generally involves an acquired hypermethylation of the MLH1 gene for which assays have been devised). Generally, when a tumor has key presenting features (Bethesda Guidelines, see Table 3), the likelihood of Lynch syndrome-related MSI is considered high enough as to warrant clinical MSI testing.

More recently, immunohistochemical (IHC) staining to detect loss of expression of Lynch syndrome genes has come to be considered by many as a clinically useful surrogate for, or at least complement to, MSI testing. So much attention has now been devoted to MSI and IHC because of the nonspecific nature of a given clinical picture and the relative insensitivity as well as high financial cost of mutational testing.

We confess to grossly oversimplifying the story and glibly summarizing decades of elegant clinical and laboratory investigation. Nevertheless, the groundwork has been laid for the implementation of a more or less straightforward algorithmic approach to the patient suspected of having FAP or Lynch syndrome. Several professional organizations have developed practice guidelines for risk assessment, genetic testing, and clinical management for patients and families with FAP and Lynch syndrome. Among the most comprehensive of these are the guidelines of the American Cancer Society, American Society of Colorectal Surgeons, National Comprehensive Cancer Network (NCCN), and a US gastrointestinal consortium (American Gastroenterology Association and others). A key message is the degree of consensus that exists between the several groups that have considered the matter. Others groups have limited their scope to genetic testing, including the American Society of Clinical Oncology and the National Society of Genetic Counselors.

We highlight, without specifically endorsing, the NCCN guidelines. These are simply the most detailed recommendations to have been reduced to a fairly straightforward algorithm, which can be accessed in an interactive format on the organization's website (http://www.nccn.org/professionals/physician_gls/PDF/colorectal_screening...).[17]

Who Should Be Tested?
In the usual clinical setting, the patient referred or self-referred for genetic counseling and possible mutational testing will fall into one of three general categories. He or she will be clearly affected with syndromic cancer (or adenomatosis), possibly affected, or healthy and simply driven by one or more diagnoses in the immediate family. In each case, the genetic counseling process strives to identify and resolve several key issues:

• What is the subject actually seeking in the genetic counseling process? Is it simple "risk assessment," genetic testing, and/or clinical screening examination?
• What is the clinical diagnosis? Developing the family pedigree is the essential starting point. Clear documentation of affectedness by means of medical records is ideal, but such records are commonly unavailable for relatives beyond the consultand himself or herself, and perhaps selected other relatives.
• Has the educational process been conducted whereby the consultand is informed of the clinical features of the condition that is suspected, possible alternative diagnoses, pros and cons of mutational testing, and consequences of a positive, equivocal, or negative (nondiagnostic) test?
• Has the consultand indicated an understanding of the importance of sharing information from the counseling process, especially positive test results, with other family members? If not, the basis for such unwillingness should be plumbed, as awkward problems of family dynamics may exist. If not dealt with, the handling of test results could raise ethical quandaries over "duty to warn" vs maintenance of patient confidentiality.

Although most of these issues are addressed individually elsewhere, they are central to arriving at a suitable decision regarding whether to perform mutational testing and, if so, upon whom.[18] In the case of new patients with FAP or Lynch syndrome, there is general agreement that genetic testing should be offered to a clinically affected patient. The sensitivity of testing known
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FAP-affected subjects for mutations in the APC gene is approximately 80%, falling to about 60% in attenuated FAP (AFAP) and to no better than 60% for Lynch syndrome testing. The CHEK2 mutation will account for some of those that do not show an MMR mutation.[19] As noted above, affectedness is readily determined in FAP. In AFAP the diagnosis may or may not be clear, depending on how low, uncharacteristic, or late in age the adenomas occur, and depending on how extensive, characteristic of AFAP, and well-documented the family history is. In Lynch syndrome, if the clinical diagnosis is anything short of compelling, mutational testing should follow assessment of tumor tissue for MSI and/or IHC abnormalities. If there are multiple affected subjects, selection of the youngest or otherwise most clearly Lynch syndrome-affected is desired, especially if mutational testing is not preceded by informative MSI/IHC evaluation. The various practice guidelines are entirely consistent with this overall approach. The NCCN, for example, would consider the family history and presenting feature of the patient's tumor. If these meet the Bethesda Guidelines (Table 3), most cases warrant MSI testing initially. Note that the Bethesda Guidelines are rather broad, such that many indeed a majority of cases will likely not turn out to have mutations in MMR genes. If tissue is not available for MSI testing and the subject meets the more rigorous Amsterdam Criteria for Lynch syndrome, the NCCN would entertain MMR gene mutation testing directly.

It must be stated that learned opinions differ as to the circumstances in which MSI/IHC or direct mutational testing should be the initial evaluation for suspected Lynch syndrome. Clearly, the more compelling the clinical picture, as when the Amsterdam Criteria are met, for example, the stronger the case for dispensing with MSI testing. However, even when Amsterdam criteria are met, there may be some advantage to performing IHC; a case that is informative by virtue of loss of staining for a gene-specific protein points to the specific gene that is mutated, obviating the need for testing of the other MMR genes. The lower the threshold for considering Lynch syndrome testing, the more helpful the screening of tumor by means of MSI/IHC testing.

If the affected subject is deceased or unable/unwilling to undergo testing, and there is no other living affected relative, then testing of a healthy at-risk family member might be considered. The most helpful parallel is the situation with familial breast cancer (BRCA) testing. Here, in part due to the presence of well-validated predictive models, the absence of an MSI-like tumor screen, and a relatively high number of concerned healthy women whose affected relative is deceased, BRCA testing may be considered whenever the prior probability of a mutation is 10% or greater. It is clear that the yield the likelihood of a positive test cannot be greater than 40% in FAP or 30% in AFAP or Lynch syndrome, based on the sensitivities of the tests employed.

When Genetic Diagnosis Is Key
When a mutated gene has been previously detected within a family, such information can be relied upon to perform predictive testing in at-risk individuals. These include children of carriers or obligate carriers (individuals with clinically obvious syndrome features but who have not had genetic testing). The important issues are as follows: (a) Taking care that genetic counseling has been properly performed in order that the individual considering testing understands the implications of a positive or negative test. Ideally, the testing should be done no earlier and no later than the point at which clinical screening for disease would be recommended; (b) The pathologic character of the mutation has been established.

Particularly when mutational testing has been based on end-to-end sequencing, in FAP and Lynch syndrome alike there exists potential for detection of polymorphisms, which are clearly not disease-causing, as well as mutations of "unknown" or "uncertain" significance. The clinical laboratories conducting predisposition testing exercise great care in providing conservative interpretations as to the significance of sequence variances. When a mutation has been described as clearly pathologic, the genetic counselor and clinician can place great reliance on the information. In the case of mutations of uncertain significance, one could consider obtaining an opinion from a referral center. Such centers may recommend testing of additional consenting affected individuals in the family, whenever this is possible, in order to determine whether the variant at least reliably tracks with disease in the family. A more refined statistical likelihood regarding significance of the variant can then be provided, but the genetic counseling must be very careful and their limitations emphasized.

The Impact of Genetic Diagnosis on an Entire Family
A study by Watson et al[20] showed that cancer risk assessment based on personal and family history of cancer may change significantly with the use of testing for a known mutation in the family. Changes from uncertainty to certainty (that is, to carrier or noncarrier status) accounted for 89% of the risk status changes resulting from testing. Importantly, 60% of family members who had a carrier
risk status change were not tested themselves but could be reclassified based on a relative's DNA test result (carrier or noncarrier of the mutation). This is of crucial importance given the fact that such risk changes can significantly affect cancer prevention recommendations, most commonly reducing the financial and personal burden when the at-risk patient is found to be negative for the cancer-causing mutation segregating in his/her family and, conversely, often highly targeted management opportunities when positive. While these findings were originally based on families with the hereditary breast-ovarian cancer syndrome and the Lynch syndrome, they are nevertheless of extreme importance in virtually all hereditary cancer syndromes where a mutation has been identified.

Conclusions
We have outlined some of the key considerations to be addressed in evaluating the patient with known or suspected hereditary susceptibility to colorectal cancer. While there is still a tremendous role for clinical assessment, without which a syndrome diagnosis will rarely be made, an increasing role for stratified risk assessment exists. Such risk assessment is ideally conducted with the aid of a genetic counselor that is able to take the time for a comprehensive family history assessment. A genetic counselor will be able to explain the rationale for considering mutational testing and will be familiar with the tests that are available and where they are conducted. The process for ordering mutational or, in the case of Lynch syndrome, tumor testing will be a responsibility of the genetic counselor, as will be counseling regarding financial impact of testing.

The clinician, whether surgeon, gastroenterologist, oncologist, or primary care provider, now has many resources in addition to the genetic counselor. Not only are there a number of thoughtful reviews on genetic syndrome diagnosis and management, but specific guidance is also available through multiple sets of practice guidelines.

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