Recent Developments in Cancer Genetics

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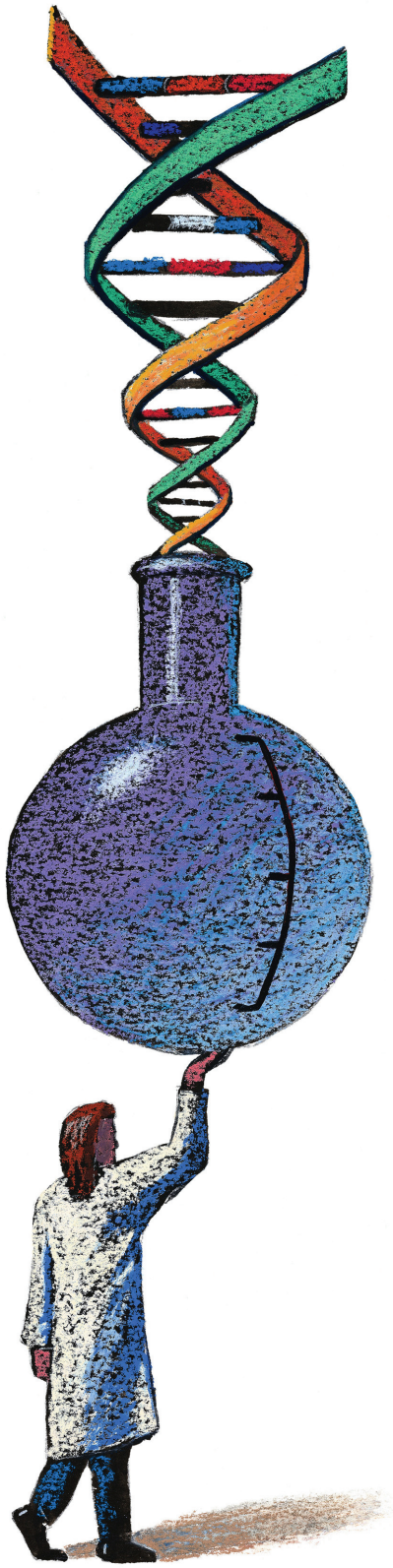
More than a decade has elapsed since the discovery of several highly penetrant (i.e., conferring high cancer risk) cancer susceptibility genes that have each had a profound impact on the current practices of genetic counseling and genetic testing for cancer risk. Today's healthcare providers recognize their importance. Comprehensive cancer centers have begun to integrate cancer risk assessment services as part of standard patient care, and many physicians are seeking out these services for referral.

The vast majority of patients currently being seen for cancer risk assessment have been referred for evaluation of hereditary breast and ovarian cancer. Approximately 1 in 400 to 1 in 800 individuals in the general population and 1 in 40 Eastern European (Ashkenazi) Jews carry a mutation in the *BRCA1* or *BRCA2* genes that result in abnormal gene function. Mutations within either the *BRCA1* or *BRCA2* genes confer up to an 87 percent lifetime risk of breast cancer and up to 54 percent and 27 percent lifetime risks of ovarian cancer, respectively. So, while mutations of these genes are rare in the general population, their effects are significant.

Clinical genetic testing for some forms of inherited colorectal cancer has also been available since the late 1990s. Hereditary nonpolyposis colorectal cancer, caused by mutations in mismatch repair genes (MMR), leads to an 80 percent lifetime risk of colorectal cancer; up to a 60 percent risk of endometrial cancer; and increased risks of ovarian, gastric, urinary tract, and other cancers.[1] Recent studies suggest that approximately 1 to 2 percent of all patients with colorectal cancer harbor mutations in mismatch repair genes.[2,3] To date, five mismatch repair genes (*MLH1*, *MSH2*, *MSH6*, *PMS1*, *PMS2*) have been associated with hereditary nonpolyposis colorectal cancer and two genes with contrasting modes of inheritance have been linked to the multiple polyp phenotype (*APC*, *MYH*). Mutations of the *APC* and *MYH* are much rarer.

Collectively, the *BRCA1*, *BRCA2*, *MLH1*, *MSH2*, *MSH6*, *PMS1*, *PMS2*, and *APC* genes are all termed "high penetrance" (because of the high cancer risk they confer) "low frequency" (because of their rare frequency in the general population) genes. Identifying individuals for whom genetic testing for these genes is indicated has proven difficult. These mutated genes have been predominantly found among individuals with an unequivocal family history of cancer. However, some are also found in individuals without any family history of cancer. These results have led to the development of new guidelines aimed at identifying potential carriers of these mutated genes.



ILLUSTRATION/GETTY IMAGES

In the past two years, the criteria for identifying families at risk for hereditary nonpolyposis colorectal cancer have been significantly altered. The criteria previously recommended for performing microsatellite instability testing on colorectal tumors to prescreen for hereditary nonpolyposis colorectal cancer have been revised (Bethesda Criteria)[4] and are significantly more inclusive than the older Amsterdam Criteria (Table 1). Only one of the Bethesda Criteria needs to be met to warrant further workup for hereditary nonpolyposis colorectal cancer. In contrast, all four Amsterdam Criteria need to be met for a diagnosis of hereditary nonpolyposis colorectal cancer.

As of 2005, testing for tumor microsatellite instability together with fulfilling the Bethesda criteria is emerging as the most sensitive method for identifying patients that harbor mutations in the mismatch repair genes and suffer from the Lynch syndrome (the most common hereditary form of colorectal cancer).[2,3] Families who meet the clinical criteria for hereditary nonpolyposis colorectal cancer but have no evidence of DNA mismatch repair deficiency in their tumors (i.e., tumors without any evidence of microsatellite instability) may not only have a lower risk of colorectal cancer com-

TABLE 1: Criteria for Identifying Families at Risk for Hereditary Nonpolyposis Colorectal Cancer

Bethesda Criteria[1]

- Diagnosed with CRC before the age of 50 years
- Synchronous or metachronous CRC or other HNPCC-related tumors: endometrial, stomach, ovarian, pancreas, ureter, renal pelvis, biliary tract, brain (glioblastoma), sebaceous gland adenoma, keratoacanthoma and adenocarcinoma of the small bowel, regardless of age
- CRC with microsatellite instability diagnosed before the age of 60
- CRC with one or more first degree relatives with CRC or other HNPCC-related tumors. One of the cancers must have been diagnosed before the age of 50
- CRC with two or more relatives with CRC or other HPNCC-related tumors

[1]Umar A , Boland CR, Terdiman JP, Syngal S, et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. *J Natl Cancer Inst*. 2004;96:261-8.

Amsterdam Criteria[2]

- Three or more relatives with verified CRC in family
- One case a first-degree relative of the other two
- Two or more generations
- One CRC by age 50
- FAP excluded

[2]Vasen HFA, Mecklin JP, Khan PM, Lynch HAT. The International Collaborative Group on Hereditary Non-polyposis Colorectal Cancer. *Dis Colon Rect* 1991;34:424–25.

pared to other hereditary nonpolyposis colorectal cancer families, but they do not appear to have increased risks for the other cancers associated with Lynch syndrome.[5] In addition, individuals with a seemingly de novo case of polyposis may have bi-allelic mutations in the *MYH* gene (rather than a single dominant mutation in the *APC* gene). In these individuals the risk of colorectal cancer can only be estimated as high at this time.[6,7] These represent just a few examples of the constantly changing practice-altering findings on diseases that had been considered well-understood in the past.

A Cohort Effect

Another important discovery is the recognition of a cohort effect among carriers of mutated *BRCA1* and *BRCA2* genes. A 2003 study showed that breast cancer risk by age 50 was 67 percent for women born after 1940 and 24 percent for women born before 1940. The authors also found that physical exercise and lack of obesity in adolescence were associated with significantly delayed breast cancer onset.[8] These findings illustrate the fact that the cancer-promoting effects of high-penetrance genes can be modified by environmental factors.

So, while the exciting discovery of highly penetrant genes and the availability of reliable sequencing methods have allowed healthcare providers to give much needed answers to many cancer patients and their families, they represent only the tip of the iceberg. Still, combined molecular techniques are unable to identify a genetic cause in the cancer history of far too many families.

Low-Penetrance Genes

There is now clear genetic evidence that a combination of genes and environment contribute to the development of a much larger fraction of common cancers than those currently attributable to high-penetrance genes.[9] This finding has led to the search for additional genes that belong to a different class altogether. These genes, which are likely to be more common in the general population, are also likely to be less penetrant (i.e., to be less likely to result in cancer development in a given individual).

While methods to identify high-penetrance genes are well established (linkage analysis and positional cloning followed by sequencing of candidate genes), methods to identify low-penetrance genes are more complex. Historically, mutations within highly penetrant genes have led to many affected individuals in one family. As a result, a relatively limited number of families were needed to identify these high-penetrance genes.

In contrast, a large proportion of individuals that carry low-penetrance genes may never develop cancer and, thus, cannot be readily identified as probable carriers of a gene mutation. Hence, linkage analysis is rarely successful in this setting, and a much larger number of

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affected individuals and unaffected individuals are needed to identify low-penetrance genes.

To further illustrate this point, 14 percent of the general population carries at least one copy of a mutated form of the *TGFBR1* gene named *TGFBR1**6A. While some initial studies were suggestive of an association of *TGFBR1**6A with cancer,[10] some subsequent studies did not confirm this association.[11] In 2004, a meta-analysis of 12 case control studies that included 4,399 cases and 3,451 controls showed that carriers of one copy of *TGFBR1**6A had a 19 percent increased cancer risk and carriers of two copies of *TGFBR1**6A had a 70 percent increased cancer risk.[12] Retrospectively, power calculations indicate that, for a gene as common as *TGFBR1**6A, a minimum of 1,824 individuals are needed to detect a 50 percent increased risk of cancer with a 90 percent power. This finding highlights the need of large case control studies to determine the true effect of candidate low-penetrance genes.

While *TGFBR1**6A appears to have a low penetrance in the general population, a recent study suggests that it may contribute to the development of colorectal cancer in patients with hereditary nonpolyposis colorectal cancer who do not carry a mutation in any of the MMR genes.[13] This alludes to the fact that high- and low-penetrance cancer-susceptibility genes may have overlapping effects in the same syndromes. Some evidence suggests that various combinations of low-penetrance genes that belong to the same pathway may have either synergistic or opposite effects on cancer risk.[14] Such evidence further supports the concept that much knowledge is likely to be gained from studies assessing not only one gene but rather the *combined* effects of several genes affecting the same pathway.

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References

[1]Aarnio M, Mecklin JP, Aaltonen LA, et al. Life-time risk of different cancers in hereditary non-polyposis colorectal cancer (HNPCC) syndrome. *Int J Cancer.* 1995;64:430-433.

[2]Pinol V, Castells A, Andreu M, et al. for the Gastrointestinal Oncology Group of the Spanish Gastroenterological Association Accuracy of revised Bethesda Guidelines, microsatellite instability, and immunohistochemistry for the identification of patients with hereditary nonpolyposis colorectal cancer. *JAMA.* 2005;293:1986-1994.

[3]Hampel H, Frankel WL, Martin E, et al. Screening for the Lynch Syndrome (hereditary nonpolyposis colorectal cancer). *N Engl J Med.* 2005;352: 1851-1860.

[4]Umar A, Boland CR, Terdiman JP, et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch Syndrome) and microsatellite instability. *J Natl Cancer Inst.* 2004;96:261-268.

[5]Lindor NM, Rabe K, Petersen GM, et al. Lower Cancer Incidence in Amsterdam-I criteria families without mismatch repair ceficiency: familial colorectal cancer type X. *JAMA.* 2005;293: 1979-1985.

[6]Croitoru ME, Cleary SP, Di Nicola N, et al. Association between biallelic and monoallelic germline MYH gene mutations and colorectal cancer risk. *J Natl Cancer Inst.* 2004;96:1631-1634.

[7]Sieber OM, Lipton L, Crabtree M, et al. Multiple colorectal adenomas, classic adenomatous polyposis, and germ-line mutations in MYH. *N Engl J Med.* 2003;348:791-799.

[8]King MC, Marks JH, Mandell JB. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. *Science.* 2003;302:643-646.

[9]Le Marchand L. The predominance of the environment over genes in cancer causation: implications for genetic epidemiology. *Cancer Epidemiol Biomarkers Prev.* 2005;14: 1037-1039.

[10]Pasche B, Kolachana P, Nafa K, et al. T beta R-I(6A) is a candidate tumor susceptibility allele. *Cancer Res.* 1999;59:5678-5682.

[11]Samowitz WS, Curtin K, Leppert MF, et al. Uncommon TGFBR1 allele is not associated with increased susceptibility to colon cancer. *Genes Chromosomes & Cancer.* 2001;32:381-383.

[12]Pasche B, Kaklamani VG, Hou N, et al. TGFBR1*6A and cancer: a meta-analysis of 12 case-control studies. *J Clin Oncol.* 2004;22:756-758.

[13]Bian Y, Caldes T, Wijnen J, et al. TGFBR1*6A may contribute to hereditary colorectal cancer. *J Clin Oncol.* 2005;23:3074-3078.

[14]Kaklamani VG, Baddi L, Liu J, et al. Combined genetic assessment of transforming growth factor-{beta} signaling pathway variants may predict breast cancer risk. *Cancer Res.* 2005;65:3454-3461.