

THE USE AND INTERPRETATION OF COMMERCIAL APC GENE TESTING FOR FAMILIAL ADENOMATOUS POLYPOSIS

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ABSTRACT

Background The use of commercially available tests for genes linked to familial cancer has aroused concern about the impact of these tests on patients. Familial adenomatous polyposis is an autosomal dominant disease caused by a germ-line mutation of the adenomatous polyposis coli (*APC*) gene that causes colorectal cancer if prophylactic colectomy is not performed. We evaluated the clinical use of commercial *APC* gene testing.

Methods We assessed indications for *APC* gene testing, whether informed consent was obtained and genetic counseling was offered before testing, and the interpretation of the results through telephone interviews with physicians and genetic counselors in a nationwide sample of 177 patients from 125 families who underwent testing during 1995.

Results Of the 177 patients tested, 83.0 percent had clinical features of familial adenomatous polyposis or were at risk for the disease — both valid indications for being tested. The appropriate strategy for presymptomatic testing was used in 79.4 percent (50 of 63 patients). Only 18.6 percent (33 of 177) received genetic counseling before the test, and only 16.9 percent (28 of 166) provided written informed consent. In 31.6 percent of the cases the physicians misinterpreted the test results. Among the patients with unconventional indications for testing, the rate of positive results was only 2.3 percent (1 of 44).

Conclusions Patients who underwent genetic tests for familial adenomatous polyposis often received inadequate counseling and would have been given incorrectly interpreted results. Physicians should be prepared to offer genetic counseling if they order genetic tests. (*N Engl J Med* 1997;336:823-7.)

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OVER the past decade, numerous germ-line mutations have been discovered that increase the risk of inherited cancers. Clinical applications of these findings include genetic testing for the diagnosis of inherited syndromes in patients with cancer and testing of persons at risk for these diseases (so-called presymptomatic testing). The promises and pitfalls of gene testing have received considerable attention,¹⁻⁶ leading several professional and consumer organizations to advocate care in the use of gene tests for evaluating the risk of cancer.⁷⁻¹¹

Gene testing for familial adenomatous polyposis is

a clinically useful tool in the approach to families with this syndrome. Familial adenomatous polyposis is an autosomal dominant disease caused by a germ-line mutation of the adenomatous polyposis coli (*APC*) gene located on the long arm of chromosome 5 in band q21.¹²⁻¹⁵ The disease is characterized by the development of hundreds of colorectal adenomas in young adults,^{16,17} but in rare cases attenuated forms of the syndrome with small numbers of polyps occur.¹⁸ If prophylactic colectomy is not performed, colorectal cancer will develop by the sixth decade of life in nearly all affected people.¹⁶⁻¹⁹

Genetic tests for familial adenomatous polyposis became feasible with the development of the *in vitro* synthesized-protein assay,²⁰ which was introduced commercially in 1994. This test can identify an *APC* gene mutation in affected members in about 80 percent of families with familial adenomatous polyposis.²⁰ When the mutation in a kindred is known, direct gene testing can differentiate with essentially 100 percent accuracy affected family members from those who are unaffected by familial adenomatous polyposis. When used appropriately, *APC* gene testing can confirm the diagnosis of familial adenomatous polyposis at the molecular level, justify the surveillance with colorectal endoscopy of those at risk, and aid in surgical management and family planning.^{19,21-23} But when used inappropriately, *APC* gene testing has the potential to misinform affected patients with false negative results.²³

The purpose of this study was to evaluate the clinical use of *APC* gene testing that was performed by a commercial laboratory. Our findings identify areas for improvement in the delivery of cancer genetic services.

METHODS**Study Population**

All persons undergoing *APC* gene testing by the commercial laboratory LabCorp from January 1, 1995, to December 31,

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1995, were identified as possible study patients. During this period, 182 persons from 129 families were tested. Five persons (2.7 percent) from four families (3.1 percent) were excluded from analysis because insufficient data were available, leaving a final sample of 177 patients from 125 families. None of the patients were from Johns Hopkins Hospital.

When a request for *APC* gene testing was received at the commercial laboratory, the physician or genetic counselor requesting the test was interviewed by telephone to obtain data on the patient. The interviewer made no attempt to influence the process of testing. When the test results became available, a genetic counselor, gastroenterologist, or both discussed them by telephone with each physician to complete the collection of data and provide an explanation of the results and consultation. The physician was also sent a standardized written report with a brief interpretation of the results.

APC Gene Testing

The *APC* gene was analyzed in peripheral-blood leukocytes by the *in vitro* synthesized-protein assay.²⁰ This assay detected mutations in about 80 percent of pedigrees with familial adenomatous polyposis in initial studies. The cost of commercial testing is \$750 if the *APC* gene mutation in the pedigree is unknown and \$500 if the mutation is known. Results are reported as positive if a specific gene segment is identified with the truncating mutation, as negative when no mutation is found in a member of a family in which a mutation has been detected previously, and as "no mutation found" if the result cannot rule out the disease when a mutation has not yet been identified in the family.

Data Collection

Telephone interviews with the physicians who ordered the test or examination of medical records of the patients (or both) were conducted at the time the test was ordered (before the results were known). The data collected included information on the patients, the physicians, and the process of gene testing.

Two indications for *APC* gene testing were considered valid: testing to confirm the diagnosis of familial adenomatous polyposis in patients with typical colorectal adenomatous polyposis or multiple adenomas, as occur in attenuated adenomatous polyposis,¹⁸ and presymptomatic testing of first-degree relatives of affected patients. The approach to presymptomatic testing was deemed appropriate (coded as "yes" on the data-collection form) if an affected family member was tested before family members at risk for the disease. We ascertained whether the patient received formal genetic counseling before gene testing was performed (with the answer coded as "yes" or "no" on the data-collection form) and determined whether written informed consent for gene testing had been obtained by the person ordering the test (with the answer coded as "yes" or "no"). The patients' physicians were questioned by telephone by the genetic counselor, gastroenterologist, or both to determine whether the results of the test would be correctly interpreted and relayed to the patients (coded as "yes" or "no"). The physicians' understanding of the result was assessed on the basis of the physicians' assessment of the status of the patients — affected or not affected by familial adenomatous polyposis (coded as "correct" or "incorrect"). A correct understanding of the result required knowing that the absence of a detected mutation (a result reported as "no mutation found") could represent a false negative result unless an *APC* gene mutation had been identified previously in an affected member of the family.

Statistical Analysis

Descriptive statistics and frequency-distribution tables were used to analyze the data. The chi-square test was used to evaluate the significance of differences in the use and interpretation of *APC* gene testing among physicians of different specialties.

RESULTS

Study Population and Indications for *APC* Gene Testing

The clinical characteristics of the 177 patients and the reasons for *APC* gene testing are shown in Table 1. The most frequent indication was presymptomatic testing of at-risk members of pedigrees with familial adenomatous polyposis (35.6 percent), followed by confirmation of the diagnosis of familial adenomatous polyposis (30.5 percent). In 16.9 percent of the cases, the test was used to evaluate possible familial adenomatous polyposis in patients with multiple colorectal adenomas. Thus, in 83.0 percent of the cases the indications for the tests were appropriate according to current knowledge. In 9.6 percent of the cases tests were ordered because of a personal or family history of colorectal cancer.

Test Ordering According to Specialty

The requests for testing were made by physicians and genetic counselors in 32 states. Table 2 shows that gastroenterologists ordered the greatest proportion of tests (46.9 percent); medical geneticists and genetic counselors requested 18.1 percent of tests.

Features of the Gene-Testing Process

Among the 177 patients, 83.0 percent had a valid indication for gene testing (Table 3). When presymptomatic testing was performed in patients at risk for familial adenomatous polyposis, the correct strategy of testing an available affected family member first was used in most cases (79.4 percent of such patients and 72.9 percent of pedigrees with family members at risk). Only 18.6 percent of those tested for *APC* gene mutations received formal genetic counseling beforehand; only 17.6 percent of physicians (19 of 108) arranged this service for their patients. Written informed consent was obtained from only 16.9 percent of those tested for whom this information was available; only 12.0 percent of the physicians (13 of 108) asked their patients to provide written informed consent.

In almost one third (31.6 percent) of the cases the physicians' interpretation of the test results was incorrect and would have led to the misinforming of the patients. The physicians did not know that a test in which no mutation was detected could represent a false negative result in a pedigree in which the *APC* gene mutation had not been previously identified in an affected family member. Analysis of the use and interpretation of the *APC* gene test according to the medical specialty of the physicians (gastroenterology, surgery, medical genetics, and other specialties) showed no statistically significant differences between groups.

TABLE 1. CLINICAL CHARACTERISTICS OF 177 PATIENTS FROM 125 FAMILIES AND INDICATIONS FOR APC GENE TESTING.

VARIABLE	VALUE
Clinical characteristic	
Age — yr	
Mean ± SD	33 ± 19
Range	1–85
Sex — M/F	93/84
Indication for testing — % (no.)	
Diagnostic testing	
Affected by familial adenomatous polyposis*	30.5 (54)
Multiple colorectal adenomas	16.9 (30)
≥20*	9.0 (16)
<20*†	7.9 (14)
History of colorectal cancer‡	2.8 (5)
Presymptomatic testing	
At risk for familial adenomatous polyposis*	35.6 (63)
Family history of colorectal cancer‡	6.8 (12)
Other†‡	7.3 (13)

*This was considered a valid indication for gene testing for the purposes of this study.

†This is now considered an unconventional indication for gene testing because the rate of positive results was low.

‡Other indications were a family history of colorectal adenomas (9 patients), the presence of cancers other than colorectal cancer (3 patients), and a family history of brain tumor (1 patient).

Test Results According to Indication

Among 54 patients with clinically apparent familial adenomatous polyposis, an APC gene mutation was identified in 68.5 percent (Table 4). Of 63 at-risk patients who underwent presymptomatic testing, 56 (88.9 percent) were from kindreds with known mutations. Of the patients at risk 49.2 percent had a negative result that represented a true negative for familial adenomatous polyposis because the mutation was known in the kindred. In 11.1 percent, the result was reported as no mutation found, since no mutation had yet been identified in their pedigree. An APC gene mutation was identified in 25 percent (4 of 16) of the patients with at least 20 colorectal adenomas and no family history of familial adenomatous polyposis.

Of the 44 patients who were tested for indications other than familial adenomatous polyposis, high-risk status, or the presence of at least 20 colorectal adenomas, only 1 (2.3 percent) was found to have a mutation. She had metastatic colorectal cancer at the age of 38 years and had no family history of colorectal cancer or polyposis. Thirteen other subjects with no family history of familial adenomatous polyposis were tested for APC gene mutations because of a family history of colorectal adenomas in nine, cancers other than colorectal cancer in three, and a family history of brain tumor representing possible Turcot’s syndrome in one. No mutation was found in any of these subjects.

TABLE 2. USE OF APC GENE TESTING ACCORDING TO THE SPECIALTY OF HEALTH CARE PROVIDER.

SPECIALTY	REQUESTS FOR TESTING
	% (no.)
Gastroenterology	46.9 (83)
Surgery	13.0 (23)
Medical genetics	11.9 (21)
Internal medicine or family practice	6.8 (12)
Genetic counseling	6.2 (11)
Oncology	4.5 (8)
Pediatrics	3.4 (6)
Other*	7.3 (13)
Total	100 (177)

*Other specialties were dermatology, endocrinology, perinatology, neurology, allergy, and pathology.

TABLE 3. FEATURES OF THE GENE-TESTING PROCESS.

FEATURE	PERCENT	NO. OF PATIENTS/TOTAL
Valid indication for testing*	83.0	147/177
Appropriate strategy for presymptomatic testing among patients at risk†	79.4	50/63
Genetic counseling provided before testing	18.6	33/177
Written informed consent obtained‡	16.9	28/166
Test results interpreted correctly	68.4	121/177

*Fifty-four patients had familial adenomatous polyposis, 30 had multiple colorectal adenomas, and 63 were at risk for familial adenomatous polyposis.

†The appropriate strategy was one in which a family member with familial adenomatous polyposis, if available, was tested before a family member who was at risk for the disease.

‡There was no response to our request for documentation of written informed consent for 11 patients.

DISCUSSION

We found that 83.0 percent of 177 patients who were tested for a mutation of the APC gene had a valid indication for the test. This rate appears high in comparison with those reported in studies of the use of other diagnostic tests,²⁴⁻²⁹ but the difference may be that APC gene testing was primarily ordered by physicians with specialized knowledge of familial adenomatous polyposis. Nevertheless, nearly 20 percent of tests were ordered for indications considered unconventional according to current knowledge.

Although the majority of tests were indicated, other aspects of testing were largely ignored. Offer-

TABLE 4. INDICATION FOR *APC* GENE TESTING AND TEST RESULTS IN 177 PATIENTS.

INDICATION	No. OF PATIENTS	TEST RESULT		
		POSITIVE	NEGATIVE	NO MUTATION FOUND
		percent		
Diagnostic testing				
Affected by familial adenomatous polyposis	54	68.5	0	31.5
Multiple colorectal adenomas				
≥20*	16	25.0	0	75.0
<20*†	14	0	0	100
Personal history of colorectal cancer‡	5	20.0	0	80.0
Presymptomatic testing				
At risk for familial adenomatous polyposis	63	39.7	49.2	11.1
Family history of colorectal cancer†	12	0	0	100
Other†‡	13	0	0	100

*This was considered a valid indication for gene testing for the purposes of this study.

†This is now considered an unconventional indication for gene testing because the rate of positive results was low.

‡Other indications were a family history of colorectal adenomas (9 patients), the presence of cancers other than colorectal cancer (3 patients), and a family history of brain tumor (1 patient).

ing genetic counseling before the test and obtaining informed consent for testing are considered essential,^{1,9,10,16} but neither was done in over 80 percent of the cases. Twenty percent of clinically unaffected patients considered at risk underwent presymptomatic testing before the *APC* mutation was identified in an affected family member, which would have established the usefulness of testing. The use of genetic counseling before testing would be expected to eliminate many of these procedural errors.

Many of the physicians whom we interviewed did not recognize the limitations of the testing. Almost one third of the patients would have received an incorrect interpretation of the test. Of particular concern is that some patients at risk for familial adenomatous polyposis would have been given a false negative result. Since colorectal cancer develops in virtually all patients with this disorder, a false negative result could erroneously lead to a failure to institute endoscopic surveillance, with devastating consequences in the future. False negative results occur because the test cannot detect *APC* gene mutations in about 20 percent of patients with familial adenomatous polyposis. Hence, gene testing rules out this disorder in a person at risk only when no mutation is found in that person and a mutation has been identified in an affected family member.

When the *APC* gene test was performed for indications other than the presence of familial adenomatous polyposis, a high risk of familial adenomatous polyposis, or the possibility of attenuated familial adenomatous polyposis (in a subject with at least 20 colorectal adenomas), the rate of positive results was low (2.3 percent).

APC gene testing is just one of a large array of DNA-based tests for the identification of presymptomatic cancer or establishment of the risk of cancer. Medical, legal, and ethical issues surrounding each new genetic test will vary with the specific characteristics of the disorder. Our study supports the concept that physicians who order these tests must be prepared to offer their patients genetic counseling, either personally or through referral.

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REFERENCES

1. Wertz DC, Fanos JH, Reilly PR. Genetic testing for children and adolescents: who decides? *JAMA* 1994;272:875-81.
2. Holtzman NA. Are we ready to screen for inherited susceptibility to cancer? *Oncology* 1996;10:57-64.
3. *Idem*. Scale-up technology: moving predictive tests for inherited breast, ovarian, and colon cancers from the bench to the bedside and beyond. In: Hereditary breast, ovarian, and colon cancer. *Journal of the National Cancer Institute monographs*. No. 17. Washington, D.C.: Government Printing Office, 1995:95-7. (NIH publication no. 94-03837.)
4. Carter MA. Patient-provider relationship in the context of genetic testing for hereditary cancers. In: Hereditary breast, ovarian, and colon cancer. *Journal of the National Cancer Institute monographs*. No. 17. Washington, D.C.: Government Printing Office, 1995:119-21. (NIH publication no. 94-03837.)
5. Hubbard R, Lewontin RC. Pitfalls of genetic testing. *N Engl J Med* 1996;334:1192-4.
6. Brown ML, Kessler LG. The use of gene tests to detect hereditary predisposition to cancer: economic considerations. *J Natl Cancer Inst* 1995; 87:1131-6.

7. National Advisory Council for Human Genome Research. Statement on use of DNA testing for presymptomatic identification of cancer risk. *JAMA* 1994;271:785.
8. Statement of the American Society of Human Genetics on genetic testing for breast and ovarian cancer predisposition. *Am J Hum Genet* 1994;55:i-iv.
9. Statement of the American Society of Clinical Oncology: genetic testing for cancer susceptibility, adopted on February 20, 1996. *J Clin Oncol* 1996;14:1730-6.
10. The NIH-DOE Working Group on Ethical, Legal, and Social Implications of Human Genome Research. Interim principles of the Task Force on Genetic Testing. Baltimore: Task Force on Genetic Testing, March 1996. <<http://infonet.welch.jhu.edu/policy/genetics>>. (Also available from NAPS [document no. 05388, 33 pages], c/o Microfiche Publications, P.O. Box 3513, Grand Central Station, New York, NY 10163-3513. Remit in advance [in U.S. funds only] \$11.65 for photocopies or \$5 for microfiche. Outside the U.S. and Canada, add postage of \$4.50 for up to 20 pages, \$5.50 over 20 [\$1.50 for microfiche postage]. There is a \$15 invoicing charge for all orders filled before payment.)
11. National action plan on breast cancer position paper: hereditary susceptibility testing for breast cancer. *J Clin Oncol* 1996;14:1738-40.
12. Nishisho I, Nakamura Y, Miyoshi Y, et al. Mutations of chromosome 5q21 genes in FAP and colorectal cancer patients. *Science* 1991;253:665-9.
13. Kinzler KW, Nilbert MC, Su L-K, et al. Identification of FAP locus genes from chromosome 5q21. *Science* 1991;253:661-5.
14. Groden J, Thliveris A, Samowitz W, et al. Identification and characterization of the familial adenomatous polyposis coli gene. *Cell* 1991;66:589-600.
15. Joslyn G, Carlson M, Thliveris A, et al. Identification of deletion mutations and three new genes at the familial polyposis locus. *Cell* 1991;66:600-13.
16. Bussey HJR. Familial polyposis coli: family studies, histopathology, differential diagnosis, and results of treatment. Baltimore: Johns Hopkins University Press, 1975.
17. Bulow S. Familial polyposis coli. *Dan Med Bull* 1987;34:1-15.
18. Spirio L, Otterud B, Stauffer D, et al. Linkage of a variant or attenuated form of adenomatous polyposis coli to the adenomatous polyposis coli (APC) locus. *Am J Hum Genet* 1992;51:92-100.
19. Petersen GM, Slack J, Nakamura Y. Screening guidelines and premorbid diagnosis of familial adenomatous polyposis using linkage. *Gastroenterology* 1991;100:1658-64.
20. Powell SM, Petersen GM, Krush AJ, et al. Molecular diagnosis of familial adenomatous polyposis. *N Engl J Med* 1993;329:1982-7.
21. Petersen GM, Brensinger JD. Genetic testing and counseling in familial adenomatous polyposis. *Oncology* 1996;10:89-94.
22. Petersen GM. Genetic counseling and predictive genetic testing in familial adenomatous polyposis. *Semin Colon Rectal Surg* 1995;6:55-60.
23. Petersen GM, Boyd PA. Gene tests and counseling for colorectal cancer risk: lessons from familial polyposis. In: Hereditary breast, ovarian, and colon cancer. Journal of the National Cancer Institute monographs. No. 17. Washington, D.C.: Government Printing Office, 1995:67-71. (NIH publication no. 94-03837.)
24. Goldman L, Feinstein AR, Batsford WP, Cohen LS, Gottschalk A, Zaret BL. Ordering patterns and clinical impact of cardiovascular nuclear medicine procedures. *Circulation* 1980;62:680-7.
25. Goldman L, Cohn PF, Mudge GH Jr, et al. Clinical utility and management impact of M-mode echocardiography. *Am J Med* 1983;75:49-56.
26. Chassin MR, Kosecoff J, Solomon DH, Brook RH. How coronary angiography is used — clinical determinants of appropriateness. *JAMA* 1987;258:2543-7.
27. Kahn KL, Kosecoff J, Chassin MR, Solomon DH, Brook RH. The use and misuse of upper gastrointestinal endoscopy. *Ann Intern Med* 1988;109:664-70.
28. McDonald IG, Guyatt GH, Gutman JM, Jelinek VM, Fox P, Daly J. The contribution of a non-invasive test to clinical care — the impact of echocardiography on diagnosis, management and patient anxiety. *J Clin Epidemiol* 1988;41:151-61.
29. Bernstein SJ, Hilborne LH, Leape LL, et al. The appropriateness of use of coronary angiography in New York State. *JAMA* 1993;269:766-9.

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