

THE MOLECULAR BASIS OF TURCOT'S SYNDROME

STANLEY R. HAMILTON, M.D., BO LIU, PH.D., RAMON E. PARSONS, M.D., PH.D.,
NICKOLAS PAPADOPOULOS, PH.D., JIN JEN, PH.D., STEVEN M. POWELL, M.D., ANNE J. KRUSH, M.S.,
THERESA BERK, M.S.S.A., ZANE COHEN, M.D., BERNARD TETU, M.D., PETER C. BURGER, M.D.,
PATRICIA A. WOOD, M.D., PH.D., FOWZIA TAQI, M.D., SUSAN V. BOOKER, B.A., GLORIA M. PETERSEN, PH.D.,
G. JOHAN A. OFFERHAUS, M.D., ANNE C. TERSMETTE, PH.D., FRANCIS M. GIARDIELLO, M.D.,
BERT VOGELSTEIN, M.D., AND KENNETH W. KINZLER, PH.D.

Abstract Background. Turcot's syndrome is characterized clinically by the concurrence of a primary brain tumor and multiple colorectal adenomas. We attempted to define the syndrome at the molecular level.

Methods. Fourteen families with Turcot's syndrome identified in two registries and the family originally described by Turcot and colleagues were studied. Germ-line mutations in the adenomatous polyposis coli (*APC*) gene characteristic of familial adenomatous polyposis were evaluated, as well as DNA replication errors and germ-line mutations in nucleotide mismatch-repair genes characteristic of hereditary nonpolyposis colorectal cancer. In addition, a formal risk analysis for brain tumors in familial adenomatous polyposis was performed with a registry data base.

Results. Genetic abnormalities were identified in 13 of the 14 registry families. Germ-line *APC* mutations were detected in 10. The predominant brain tumor in these 10

families was medulloblastoma (11 of 14 patients, or 79 percent), and the relative risk of cerebellar medulloblastoma in patients with familial adenomatous polyposis was 92 times that in the general population (95 percent confidence interval, 29 to 269; $P < 0.001$). In contrast, the type of brain tumor in the other four families was glioblastoma multiforme. The glioblastomas and colorectal tumors in three of these families and in the original family studied by Turcot had replication errors characteristic of hereditary nonpolyposis colorectal cancer. In addition, germ-line mutations in the mismatch-repair gene *hMLH1* or *hPMS2* were found in two families.

Conclusions. The association between brain tumors and multiple colorectal adenomas can result from two distinct types of germ-line defects: mutation of the *APC* gene or mutation of a mismatch-repair gene. Molecular diagnosis may contribute to the appropriate care of affected patients. (N Engl J Med 1995;332:839-47.)

IN 1959, Turcot and colleagues described two teen-aged siblings with numerous adenomatous polyps of the colorectum in whom malignant tumors of the central nervous system developed.¹ One patient had a medulloblastoma involving the spinal cord (the brain was not examined at autopsy) and adenocarcinomas of the sigmoid colon and rectum. His sister had a cerebral glioblastoma multiforme and a pituitary adenoma. Ten years earlier, Crail had described a patient with adenomatous polyposis, medulloblastoma of the brain stem, and papillary carcinoma of the thyroid gland,² but the eponym "Turcot's syndrome" denotes the syndrome of colorectal polyposis and a primary tumor of the central nervous system. More than 120 cases resembling those of Turcot and Crail have been reported.^{3,4} They encompass a broad spectrum of colorectal findings, from a single adenoma to typical adenomatous polyposis, as well as various histopathologic types of central nervous system tumors. The mode of inheritance of Turcot's syndrome is controversial; some au-

thors support autosomal recessive inheritance, and others an autosomal dominant pattern.³⁻⁹

Two major inherited syndromes with colorectal neoplasia can now be characterized at the molecular genetic level: familial adenomatous polyposis and hereditary nonpolyposis colorectal cancer (or Lynch syndrome). In familial adenomatous polyposis there is a germ-line alteration of the adenomatous polyposis coli (*APC*) gene¹⁰⁻¹⁸; mutations of the gene can be detected in about 80 percent of families with familial adenomatous polyposis.¹⁷ The condition has an autosomal dominant inheritance, but up to one third of cases appear without a positive family history.¹⁹ Typically, hundreds to thousands of colorectal adenomas develop in familial adenomatous polyposis, although in some families only small numbers occur.²⁰ A variety of benign and malignant extracolonic manifestations have been reported in this condition.^{7,21-30}

In hereditary nonpolyposis colorectal cancer, a germ-line mutation occurs in one of a group of genes involved in DNA nucleotide mismatch repair, including *hMSH2* (human mutS homologue 2),³¹⁻³⁴ *hMLH1* (human mutL homologue 1),³⁵⁻³⁷ and *hPMS1* and *hPMS2* (human postmeiotic segregation 1 and 2).³⁸ The DNA in cancers of patients with hereditary nonpolyposis colorectal cancer has characteristic errors of replication, also termed microsatellite instability, that are due to the uncorrected mispairing of nucleotides and resultant misalignment of DNA strands.³⁹⁻⁴² This condition, like familial adenomatous polyposis, is autosomal dominant, but it is difficult to recognize clinically because the colorectal and extracolonic cancers are not distinctive.^{43,44}

Previous genetic analysis of a few patients with Tur-

From the Division of Gastrointestinal and Liver Pathology, Department of Pathology (S.R.H.); the Oncology Center (S.R.H., B.L., R.E.P., N.P., J.J., S.M.P., S.V.B., B.V., K.W.K.); the Division of Medical Genetics, Department of Medicine (A.J.K.); the Division of Neuropathology, Department of Pathology (P.C.B.); the Department of Pathology (F.T., G.J.A.O.); and the Division of Gastroenterology, Department of Medicine (F.M.G.); School of Medicine, and the Department of Epidemiology, the School of Hygiene and Public Health (G.M.P., A.C.T.), Johns Hopkins University, Baltimore; the Familial Gastrointestinal Cancer Registry, Mount Sinai Hospital, Toronto (T.B., Z.C.); the Laboratoire d'Anatomie Pathologique, Hôtel-Dieu de Québec, Québec, Canada (B.T.); and the Division of Medical Oncology, Department of Medicine, Stratton Veterans Affairs Medical Center and Albany Medical College, Albany, N.Y. (P.A.W.). Address reprint requests to Dr. Hamilton at the Department of Pathology, Ross Bldg., Rm. 632, Johns Hopkins University School of Medicine, 720 Rutland Ave., Baltimore, MD 21205-2196.

Supported by the Clayton Fund and by grants (CA47527, CA45831, CA57345, CA35494, and CA62924) from the National Cancer Institute.

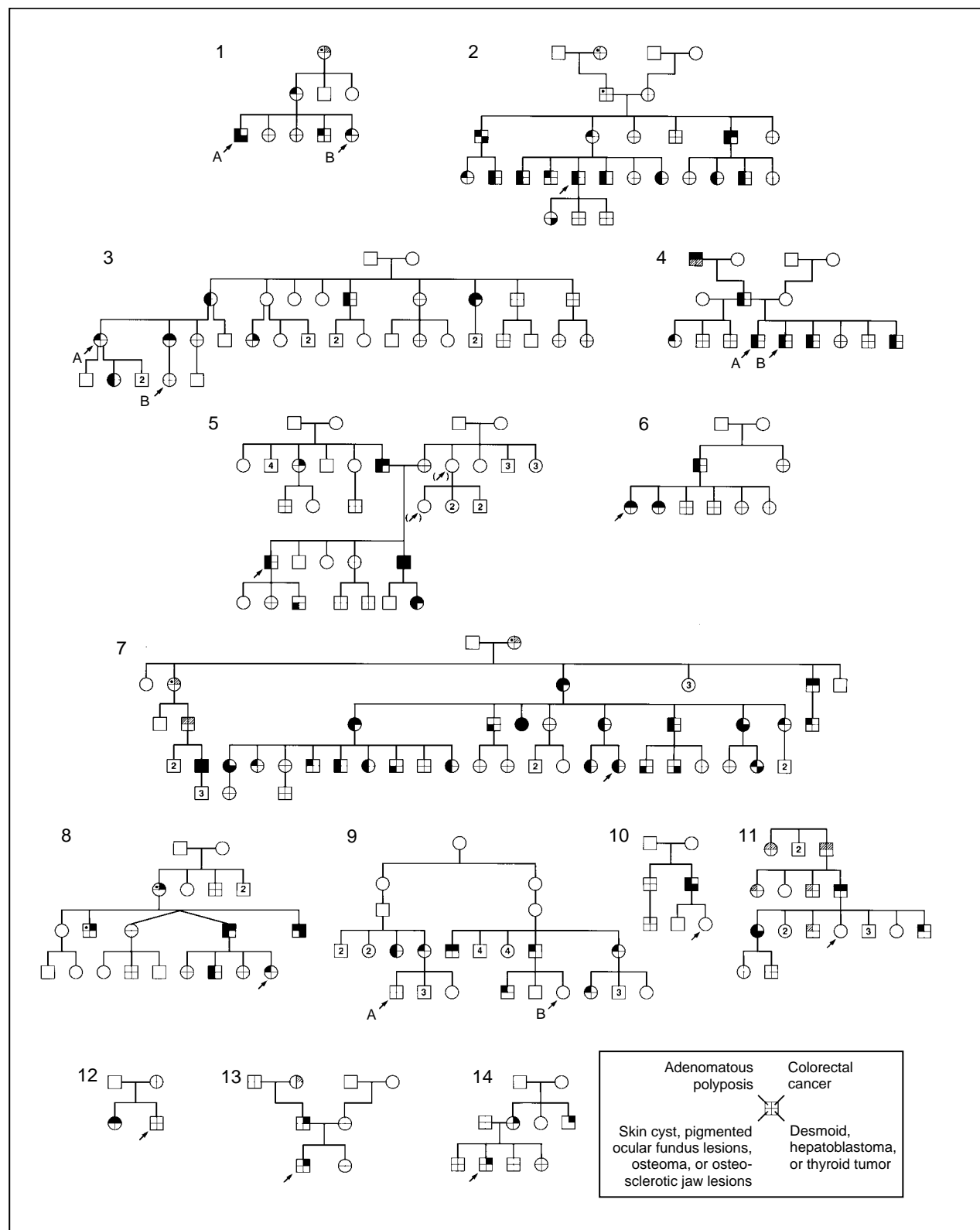


Figure 1. Abbreviated Pedigrees of the 14 Families Included in the Study.

Patients with brain tumors are indicated by arrows. When two members of the same family had verified brain tumors, as in Families 1, 3, 4, and 9, the patients are designated A and B. Solid quadrants indicate the presence of the findings indicated in the key. Open symbols without quadrant delineations indicate that the patient's clinical status is unknown, and numbers within symbols indicate the number of siblings of unknown clinical status. A hatched quadrant or parentheses around an arrow indicates a reported history unverified by medical records, and a dot in a quadrant indicates an uncertain history, as reported. Twelve families (Families 1 through 12) had the colorectal phenotype of adenomatous polyposis,⁵⁴ whereas two families (Families 13 and 14) had colorectal carcinoma with multiple adenomas but did not have the phenotype of polyposis.

cot's syndrome has not revealed the molecular pathogenesis.⁴⁵⁻⁵³ We therefore evaluated 14 families and one of the patients originally described by Turcot and colleagues¹ for genetic evidence of familial adenomatous polyposis and hereditary nonpolyposis colorectal cancer. We also carried out a formal risk analysis for brain tumors in a registry of patients with familial adenomatous polyposis. Our findings have implications for the care of patients as well as the classification of the diseases.

METHODS

Study Patients

We studied 14 families that each included a patient with a primary brain tumor and either multiple colorectal adenomas or a family his-

tory of adenomatous polyposis (Fig. 1 and Table 1). (When two members of the same family had brain tumors, they were designated A and B, as shown in Fig. 1 and Table 1.) A 15th family was identified, but specimens were not available for study. The families were identified through 1993 from the Bowel Tumor Working Group Registry at Johns Hopkins Hospital in Baltimore and the Familial Gastrointestinal Cancer Registry at Mount Sinai Hospital in Toronto. Adenomatous polyposis was defined by the presence of more than 100 colorectal adenomas.⁵⁴ Hereditary nonpolyposis colorectal cancer was defined according to the criteria of the International Collaborative Group.⁵⁵

The Hopkins registry contained 368 families with adenomatous polyposis, 102 with hereditary nonpolyposis colorectal cancer, and 517 with familial aggregation of colorectal adenomas and carcinomas. Eleven families with Turcot's syndrome were identified (Families 1 through 8, 11, 13, and 14). Peripheral-blood leukocytes were obtained for the analysis of mutations by venipuncture from the family member with a brain tumor or from another affected member

Table 1. Clinical Characteristics of the Study Patients with Turcot's Syndrome.

PATIENT No.*	AGE (YR) AT LAST FOLLOW-UP/ SEX	VITAL STATUS	FAMILY HISTORY		GASTROINTESTINAL TRACT		CENTRAL NERVOUS SYSTEM			OTHER EXTRAINTESTINAL LESIONS
			ADENOMATOUS POLYPOSIS	COLORECTAL CANCER, NO POLYPOSIS	ADENOMATOUS POLYPOSIS PHENOTYPE	COLORECTAL CANCER, NO POLYPOSIS	MEDULLO- BLASTOMA	GLIOBLAS- TOMA	OTHER PRIMARY BRAIN TUMOR	
patient's age at detection of condition (yr)										
1A	36/M	Alive	Yes	No	8	—	14	—	—	Papillary carcinoma of thy- roid, pigmented ocular- fundus lesions
1B	26/F	Alive	Yes	No	16	—	24†	—	—	None reported
2	39/M	Alive	Yes	No	19	—	—	—	37‡	Epidermal inclusion cyst
3A	49/F	Dead	Yes	Yes	27	—	—	—	48‡	None reported
3B	6/F	Dead	Yes	Yes	—§	—	6†	—	—	None reported
4A	23/M	Alive	Yes	No	20	—	19†	—	—	Skin cysts, osteosclerotic jaw lesions, pigmented ocular-fundus lesions
4B	22/M	Alive	Yes	No	19	—	16†	—	—	Skin cysts, osteosclerotic jaw lesions, pigmented ocular-fundus lesions
5	58/M	Dead	Yes	Yes	58§	—	—	54†	—	Osteoma, skin cysts, follicu- lar adenoma of thyroid
6	30/F	Dead	Yes	No	30	—	25	—	—	None reported
7	30/F	Alive	Yes	No	23	—	—	—	16	Pigmented ocular-fundus lesions
8	5/F	Dead	Yes	No	5**	—	5†	—	—	None reported
9A	16/M	Dead	Yes	No	—§	—	16†	—	—	None reported
9B	8/F	Dead	Yes	No	Not examined	—	6†	—	—	None reported
10	7/F	Dead	Yes	No	Not examined	—	6	—	—	None reported
11	13/F	Dead	Yes	No	Not examined	—	10††	—	—	None reported
12	18/M	Alive	Yes‡‡	Yes‡‡	—	13§§	—	4†	—	Café au lait spots
13	39/M	Dead	No	Yes¶¶	—	37	—	35	—	None reported
14	39/M	Alive	No	Yes***	—	30†††	—	33†	—	Transitional-cell carcinoma of ureter
15‡‡‡	32/F	Dead	Unknown	Unknown	20	—	29†	—	—	Epidermal inclusion cyst, desmoid, pigmented ocular-fundus lesions

*A and B denote siblings.
†This tumor was identified by a neuropathologist as an anaplastic astrocytoma.
‡This tumor was identified by a neuropathologist as a calcified ependymoma.
§Only two adenomas were found on colonoscopy at the age of 49 years.
¶Focal adenomatous epithelial proliferation was found in colonic mucosa at autopsy.
‡‡The patient's sister had rectal carcinoma at the age of 11 with only three adenomas of the sigmoid colon and rectum. Phenotypic adenomatous polyposis developed at the age of 14, for which the sister underwent total colectomy. She had cutaneous café au lait spots. The patient's mother had no manifestations, and the father was unavailable for examination.
§§Patient 12 had two colonic adenomas and one hyperplastic polyp by the time of total abdominal colectomy at the age of 13. He had non-Hodgkin's lymphoma of the rectum at the age of 17.
¶¶The father of Patient 13 had synchronous adenocarcinomas of the descending and sigmoid colon at the age of 45.
|||Patient 13 had 10 colonic and rectal adenomas, adenocarcinomas of the sigmoid colon and rectum, and a hyperplastic polyp by the time of total abdominal colectomy at the age of 37.
***Hereditary nonpolyposis colorectal cancer according to the criteria of the International Collaborative Group.
†††Patient 14 had adenocarcinomas of the ascending and transverse colon at the age of 30, adenomas of the descending and sigmoid colon at the ages of 32 and 33, and an ileal adenocarcinoma at the age of 33.
‡‡‡This patient had no specimens available for molecular genetic analysis.

after informed consent was given. Six of the patients (Patients 1A, 3B, 4A, 4B, 8, and 11) have been described previously.^{53,56,57} The Toronto registry included 230 families, among which 4 with Turcot's syndrome were identified. A sample of peripheral blood was obtained from three (Families 9, 10, and 12), but not from Family 15. Two families (Families 9 and 12) have been described previously.^{58,59}

The Family Described by Turcot et Al.

The two siblings described by Turcot et al. were identified in the pathology records of l'Hôtel-Dieu de Québec and l'Hôpital de l'Enfant-Jésus in Quebec from the dates reported in the original publication.¹ A review of the histopathologic sections confirmed the published findings, except for the presence at autopsy in Subject 2 of a 3-cm hepatic adenoma not described in the case report. The photographs of the colorectal specimens¹ were subjected to image processing (Adobe Photoshop, Mountain View, Calif.). Subject 1 had 8 large polyps (2 to 5 cm) and about 33 small polyps (0.5 to 1 cm) in the descending and sigmoid colon. Subject 2 had 6 large polyps and about 17 small polyps in the cecum, ascending colon, and proximal transverse colon. The total number of colorectal polyps could not be ascertained. No "microadenomas" of the type seen in familial adenomatous polyposis were identified histopathologically in the colorectal mucosa of either subject. No affected member of the family was known to be living.

Molecular Genetic Analysis

The germ-line status of the APC gene, which is mutated in familial adenomatous polyposis, was determined in peripheral-blood-leukocyte DNA from at least one affected member of all 14 study families. The APC gene was analyzed by a ribonuclease (RNase) protection assay (reported previously for Families 2, 5, 6, 7, and 10),¹⁶ by an in vitro synthesized-protein assay,¹⁷ or by cloning and sequencing of the entire coding region of the APC gene.¹¹

The APC gene in the medulloblastoma of Patient 1B was analyzed with DNA from cryostat sections to minimize contamination by nonneoplastic cells.⁶⁰ We used an in vitro synthesized-protein assay in this analysis.¹⁷

Replication errors in DNA from brain and colorectal tumors were identified by the analysis of simple repeated genomic sequences (microsatellites) isolated from routine histopathological sections (Table 2).⁶¹ DNA from the surgical specimens of the subjects originally described with Turcot's syndrome¹ was unsatisfactory because the specimens had been fixed in Bouin's solution; only the slides of specimens obtained at autopsy from Subject 2 could be analyzed. Polymerase-chain-reaction (PCR) methods for the amplification of microsatellite sequences were used as described.^{39,61} The minimal criterion for an error in replication was that at least one of the five markers tested contain a band in the tumor PCR product that was not found in the nonneoplastic PCR product. Because sporadic tumors positive for replication errors do occur,^{39,62-65} the finding of such an error in more than one tumor from a single patient was taken as evidence of a germ-line, rather than a somatic, mutation of a mismatch-repair gene.

The germ-line status of the hMSH2, hMLH1, hPMS1, and hPMS2 genes, which are found to be mutated in hereditary nonpolyposis colorectal cancer, was determined from RNA of peripheral-blood lym-

Table 2. Molecular Genetic Characterization of Turcot's Syndrome.

FAMILY NO.	GERM-LINE STATUS OF APC AND MISMATCH-REPAIR GENES	TUMORS STUDIED FOR DNA REPLICATION ERRORS*
1	Protein truncation in APC† segment 2 (codons 686–1217)	Negative medulloblastoma with missing full-length APC gene product from second allele in segment 2 (Patient 1B)
2	Truncating point mutation in APC‡ codon 215, nonsense mutation (CAG→TAG, gln→stop)	Negative astrocytoma
3	Protein truncation in APC† segment 2 (codons 686–1217)	Negative medulloblastoma (Patient 3B)
4	Protein truncation in APC† segment 1 (codons 1–804)	Negative medulloblastomas (Patients 4A and 4B)
5	No mutation in APC identified‡	Negative glioblastoma; negative cecal and rectal carcinomas and colonic adenomas in father, paternal aunt, brother, and fraternal niece
6	Truncating frame-shift mutation in APC‡ codon 1191, 1-nucleotide deletion (CAGA→CAA)	Not done
7	Truncating point mutation in APC‡ codon 541, nonsense mutation (CAG→TAG, gln→stop)	Not done
8	Protein truncation in APC† segment 3 (codons 1099–1693)	Negative medulloblastoma
9	Protein truncation in APC† segment 2 (codons 686–1217)	Not done
10	Truncating frame-shift mutation in APC‡ codon 1061, 5-nucleotide deletion (AAACAAAG→AAG)	Not done
11	Protein truncation in APC† segment 2 (codons 686–1217)	Not done
12	Truncating point mutation in hPMS2§ codon 134, nonsense mutation (CGA→TGA, arg→stop)	Positive glioblastoma and colonic adenoma; positive rectal carcinoma in sister
13	No mutation in APC identified‡¶ No mutation identified in APC, hMSH2, hMLH1, hPMS1, or hPMS2‡§¶	Positive glioblastoma, sigmoid and rectal carcinomas, and sigmoid adenoma, and negative sigmoid adenomas
14	Amino acid loss in hMLH1§ codon 618, 3-nucleotide deletion (AAG AAG GCT→AAG GCT, deletion of lys) No mutation in APC identified† Original Turcot family Not available	Positive glioblastoma, colonic carcinomas, and ileal carcinoma Positive glioblastoma and rectal adenoma (Turcot's Subject 2)

*Positive and negative indicate that replication errors were present and absent, respectively.
†According to an in vitro synthesized-protein assay for APC.¹⁷
‡According to an RNase protection assay for APC as previously reported.¹⁶
§According to direct sequencing of the products of reverse transcription and PCR amplification for hMSH2,^{33,34} hMLH1,³⁷ hPMS1,³⁸ and hPMS2.³⁸
¶According to sequencing of the entire coding region of the APC gene.¹¹

phocytes. An in vitro synthesized-protein assay and direct sequencing of the products of reverse transcription and PCR amplification were used as described.^{33,34,37,38}

Risk Analysis

To determine whether familial adenomatous polyposis predisposes patients to brain tumors, patients and their first-degree relatives enrolled in the Hopkins registry were compared with the general U.S. population, as represented in the Surveillance, Epidemiology, and End Results (SEER) data.^{26,29,30} None of the families in the registry were initially identified because of a proband who presented with a brain tumor. With a computer program for cohort analysis,⁶⁶ person-years at risk were calculated according to age-, race-, and sex-specific categories, from birth to 89 years of age, in subsequent five-year periods of observation to account for any trends over time. The expected numbers of brain tumors (classifications 1910 through 1919 of the International Classification of Diseases, 9th Revision [ICD-9]) and cerebellar medulloblastomas (ICD-9 classifications 1916 and M94703) were calculated by multiplying the number of patient-years by the corresponding incidence rates obtained from the SEER data.⁶⁷ The ratio of the number of observed cases to the expected number of cas-

es was computed with a test of significance and a calculation of the 95 percent confidence intervals with the assumption of a Poisson distribution.

RESULTS

Phenotypes of the Study Families

Twelve families met the clinical criteria for familial adenomatous polyposis⁵⁴ (Families 1 through 12 in Fig. 1 and Table 1). In all but one family (Family 12), more than one member was affected. Extraintestinal lesions included those typical of familial adenomatous polyposis, but one patient (Patient 12) and his affected sister had café au lait spots, which are not associated with familial adenomatous polyposis but have been reported with Turcot's syndrome.³ Two families (Families 13 and 14) had colorectal adenomas and carcinoma without evidence of polyposis, and Family 14 met the criteria for hereditary nonpolyposis colorectal cancer.⁵⁵

Germ-Line Status of the APC Gene

We studied the *APC* gene in 14 families that included at least one affected member (Table 2). Among the 12 families classified as having polyposis, mutations were found in 10 (83 percent). All the mutated genes encoded truncated variants of the APC protein (Fig. 2), as is true of the vast majority of patients with familial adenomatous polyposis.^{15,17} The mutations were heterogeneous in type and location (Table 2), and there was no association between specific mutations and the development of brain tumors. Two families with polyposis (Families 5 and 12) and both families without polyposis (Families 13 and 14) had no identifiable germ-line *APC* mutations.

Somatic Status of the APC Gene in a Brain Tumor

The medulloblastoma from Patient 1B, who had a germ-line mutation of the *APC* gene, was studied for a somatic mutation in the allele inherited from her unaffected parent. The in vitro synthesized-protein assay showed no wild-type *APC* gene product (Fig. 2, lane M1B). This finding, which indicated that both copies of the gene had been inactivated, favored a direct role for the *APC* alterations in the pathogenesis of the brain tumor.

Phenotypic Characteristics of Patients with Germ-Line APC Mutations

In the 10 families with identified germ-line *APC* mutations, medulloblastoma predominated (in 11 patients, or 79 percent). Two patients had anaplastic astrocytomas, and one had an ependymoma. In 4 of the 10 families, two members had brain tumors. The clinical presentations of the patients varied. The brain tumor presented after the diagnosis of polyposis in four patients, but in six patients it was identified before polyposis was found. It is noteworthy that the brain tumors in Patients 3A and 3B in the same family were dramatically different: Patient 3A had an anaplastic astrocytoma at the age of 48, and Patient 3B had a medulloblas-

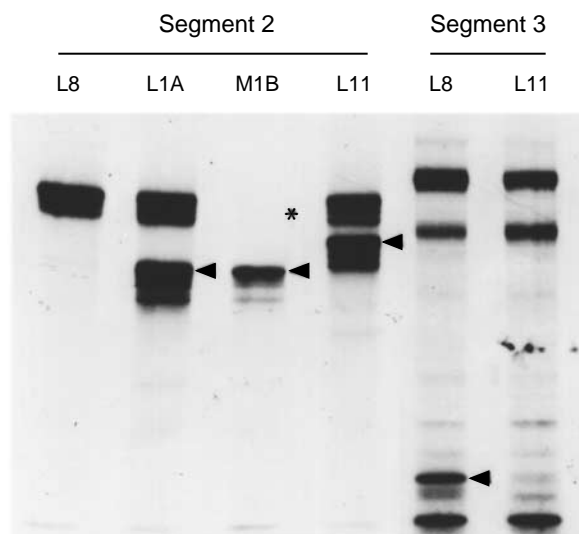


Figure 2. Germ-Line and Somatic Alterations in the APC Gene. In this example of analysis by an in vitro synthesized-protein assay,¹⁷ five overlapping segments encompassing the entire coding region of *APC* were amplified with specifically designed PCR primers that place the transcriptional and translational regulatory sequences at the 5' end of the PCR product. Radiolabeled protein was synthesized in vitro from these surrogate genes in a simple one-step coupled transcription-translation reaction. The truncating mutations could then be identified as smaller protein products after gel electrophoresis and autoradiography. The protein products from segment 2 (codons 686 to 1217) and segment 3 (codons 1099 to 1693) are shown. The lanes labeled L8 and L11 are from lymphoblastoid cell lines prepared from peripheral-blood lymphocytes of affected members of Families 8 and 11, respectively, whereas lane L1A is from the lymphoblastoid cell line of Patient 1A. The lane labeled M1B was prepared from the cryostat-dissected medulloblastoma of Patient 1B, the sister of Patient 1A. Truncated APC proteins are indicated by arrowheads.

Affected members of Families 1 and 11 have germ-line mutations in segment 2 (arrowheads, lanes L1A and L11 of segment 2), whereas the affected member of Family 8 has a germ-line mutation in segment 3 (arrowhead, lane L8 of segment 3). The medulloblastoma from Patient 1B shows truncated protein (arrowhead, lane M1B) representing the same germ-line mutation as in her brother (arrowhead, lane L1A), but the brain tumor also lacks the full-length *APC* gene product (asterisk in lane M1B), indicating the somatic loss of the wild-type allele.

toma at the age of 6. The brain tumor was the cause of death in seven of the eight patients who died.

Risk Analysis for Brain Tumors

To determine whether familial adenomatous polyposis predisposes patients to brain tumors, we used risk analysis to compare families with familial adenomatous polyposis with the general population. Of the 1390 subjects in the Hopkins registry (with 18,673 patient-years of follow-up), there were 604 white male subjects, 645 white female subjects, 76 black male subjects, and 65 black female subjects. The registry revealed five brain tumors in patients with familial adenomatous polyposis and their at-risk relatives during the study period: three medulloblastomas (in Patients 1A, 3B, and 6), one glioblastoma multiforme (in Patient 5), and one

ependymoma (in Patient 7). No brain tumors were found among the black subjects.

The relative risk of brain tumor (Table 3) was increased by a factor of 23 among the families with familial adenomatous polyposis in the group from birth to 29 years of age ($P<0.001$), and by a factor of 7 in the group as a whole ($P<0.001$). The corresponding relative risks of cerebellar medulloblastoma were 99 ($P<0.001$) and 92 ($P<0.001$), respectively. The absolute lifetime risk of brain tumor, however, was low: 1 in 3735 patient-years.

Replication Errors in Brain and Colorectal Tumors

DNA replication errors characteristic of the abnormal mismatch repair in tumors of patients with hereditary nonpolyposis colorectal cancer were sought in 25 tumors from 10 patients with familial adenomatous polyposis and 5 affected relatives, representing nine families in the registry (Table 2 and Fig. 3). In three patients the tumors had replication errors (Patients 12, 13, and 14), and in each there were at least two such tumors. Intertumoral heterogeneity of replication errors was found in two patients (Patients 13 and 14): they had colonic adenomas without replication errors, as well as colorectal tumors with such errors (an adenoma and four carcinomas). The microsatellite instability was less pronounced in the glioblastomas than in the colorectal neoplasms. None of the tumors from families with germ-line *APC* mutations had DNA replication errors.

Both the glioblastoma multiforme and a rectal adenoma from the autopsy of Subject 2 described by Turcot et al.¹ showed errors in DNA replication characteristic of hereditary nonpolyposis colorectal cancer.

Germ-Line Status of DNA Mismatch-Repair Genes

Analysis of the *hMSH2*, *hMLH1*, *hPMS1*, and *hPMS2* mismatch-repair genes, which are mutated in hereditary nonpolyposis colorectal cancer, was carried out in the three patients with tumors that contained replication errors. Two had germ-line alterations: the *hPMS2* gene was mutated in Patient 12, and *hMLH1* was mutated in Patient 14 (Table 2 and Fig. 3). No germ-line *APC* mutations were detected in these three patients.

Phenotypes of Patients with Brain and Colorectal Tumors Containing Replication Errors

The brain tumors in the three patients with tumors containing replication errors differed histopathologically from the brain tumors in patients with germ-line

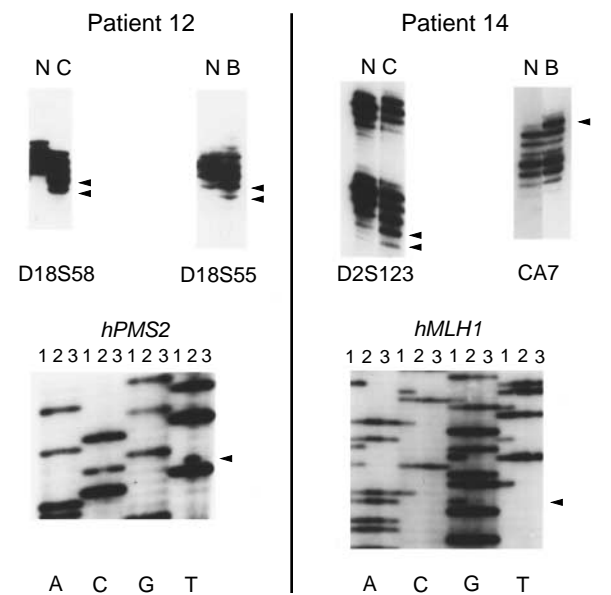


Figure 3. Replication Errors in Brain and Colorectal Tumors (Upper Panels) and Germ-Line Mutations in Mismatch-Repair Genes (Lower Panels).

Replication errors in tumors were identified by PCR amplification of repeated sequences on chromosome 18 (D18S58 and D18S55) or chromosome 2 (D2S123 and CA7). At the top, the lanes marked N show the results obtained after amplification of DNA isolated from nonneoplastic tissue. The lanes marked C represent colorectal tumors (a colorectal adenoma from Patient 12 and a colorectal carcinoma from Patient 14). The lanes marked B show PCR products from the brain tumors in these patients (glioblastoma multiforme in both). Shifted bands, indicating replication errors in the tumors, are indicated by arrowheads.

The lower panels show the results of nucleotide-sequence analysis of the *hPMS2* gene from Patient 12 and the *hMLH1* gene from Patient 14. For Patient 12, the arrowhead indicates a C-to-T change (a new band in lane 2 of the T lanes and a less intense band in lane 2 of the C lanes) in codon 134 (C₆G_A), which results in the creation of a premature termination codon (TGA). For Patient 14, the arrowhead indicates the first nucleotide of a three-nucleotide deletion that removes codon 618 (the deletion of AAG produces a downward shift by three positions for all bands in lane 1 above the arrowhead). In both cases, the sequence from the normal allele is also present, as expected.

APC mutations. All three patients had glioblastoma multiforme, which was not found in any of the 10 families with identified *APC* gene mutations ($P=0.004$). The glioblastoma presented before the colorectal neoplasms in two patients and afterward in one. The colorectal phenotypes and family histories of all three patients were atypical for familial adenomatous polyposis (Table 1). Patient 12 had café au lait spots.

One patient died of glioblastoma, but all three patients with DNA-replication errors had unusually long survival: Patient 13 lived for 36 months after diagnosis, and the other two remained alive 14 and 6 years after diagnosis. This long survival stands in contrast to the two-year survival rate of about 5 percent and the median survival of

Table 3. Risk Analysis for Brain Tumors in Familial Adenomatous Polyposis.

AGE RANGE (YR)	NO. OF BRAIN TUMORS		RELATIVE RISK	95 PERCENT CONFIDENCE INTERVAL	NO. OF MEDULLOBLASTOMAS		RELATIVE RISK	95 PERCENT CONFIDENCE INTERVAL
	OBSERVED	EXPECTED			OBSERVED	EXPECTED		
0-29	4	0.2	23*	6-58	3	0.02	99*	20-288
30-59	1	0.4	3	0.1-15	0	0	—	—
60-89	0	0.2	—	—	0	0	—	—
0-89	5	0.7	7*	2-17	3	0.03	92*	29-269

* $P<0.001$.

about eight months in the usual patients with glioblastoma multiforme.⁶⁸

DISCUSSION

Our results indicate that the syndrome of primary brain tumors and multiple colorectal adenomas, which has previously been termed Turcot's syndrome, can be associated with two different types of germ-line genetic defects: mutation of the *APC* gene that is usually found in familial adenomatous polyposis, or mutation of a mismatch-repair gene that is usually found in hereditary nonpolyposis colorectal cancer. Moreover, we found molecular evidence of hereditary nonpolyposis colorectal cancer in the family originally described by Turcot and colleagues.

The phenotypes of familial adenomatous polyposis and hereditary nonpolyposis colorectal cancer have been regarded as distinctive, hence the descriptor "nonpolyposis" in hereditary nonpolyposis colorectal cancer. However, attenuated forms of familial adenomatous polyposis with small numbers of adenomas have been reported in families with mutations in the 5' portion of the *APC* gene.²⁰ Our findings also demonstrate the difficulty of distinguishing between these two genetically distinctive diseases on purely clinical grounds.³ Large numbers of colorectal adenomas, characteristic of familial adenomatous polyposis, occurred in two families that did not have the clinical criteria of hereditary nonpolyposis colorectal cancer (Family 12 and the family originally described by Turcot et al.). Nonetheless, in both families there was evidence of a germ-line mutation in a DNA mismatch-repair gene, which is characteristic of hereditary nonpolyposis colorectal cancer, rather than the *APC* mutation typical of familial adenomatous polyposis.

Thus, patients with Turcot's syndrome can be classified by testing for mutations of the *APC* gene and for mutant DNA mismatch-repair genes in peripheral-blood lymphocytes, and by evaluating tumor DNA for replication errors. The vast majority of cases should be definable by molecular genetics (13 of 14 families in our series; 95 percent confidence interval, 66 to 100 percent). This molecular approach will clarify the phenotypic spectrums of familial adenomatous polyposis and hereditary nonpolyposis colorectal cancer, which have been uncertain because of variations in the number and size of colonic adenomas.³ Our findings indicate that most patients with Turcot's syndrome who have small numbers of colorectal neoplasms, colorectal carcinoma in childhood or adolescence, glioblastoma, or café au lait spots^{3,48,69} have hereditary nonpolyposis colorectal cancer rather than familial adenomatous polyposis.

This study sheds light on the mechanisms of tumorigenesis associated with the two different types of germ-line alterations. In patients with a germ-line mutation of *APC*, inactivation of the second copy of the gene appears to be a factor in brain tumorigenesis, as evidenced by Patient 1B. In a previous study, no mutation in the second copy of *APC* was found in the brain tumors of three patients with familial adenomatous polyposis,⁵² but this result could have been due to the tech-

nical difficulty of identifying alterations in the large *APC* gene. In addition, the markedly increased risk of brain tumor in our pedigrees with familial adenomatous polyposis (increased by a factor of 23 in the age range of birth to 29 years) is further strong evidence that the occurrence of brain tumors in familial adenomatous polyposis is not merely coincidental.⁷⁰ Thus, brain tumors, especially medulloblastoma, for which the relative risk was 92, represent a pleiotropic manifestation of the germ-line *APC* mutation. Specific germ-line *APC* mutations do not appear to be associated with the development of brain tumors, as evidenced by the striking heterogeneity of the *APC* mutations in our patients (Table 2).

Genetic characterization of the two inherited conditions has implications for patient care. Symptoms or signs suggesting central nervous system tumor require prompt and careful investigation in patients with familial adenomatous polyposis, hereditary nonpolyposis colorectal cancer, or multiple tumors with replication errors, and in at-risk offspring or siblings. At-risk members of families with familial adenomatous polyposis who present with brain tumors can be tested for a mutant *APC* gene^{17,18}; they will require sigmoidoscopic surveillance for colorectal adenomas and colectomy if adenomas develop. We recommend vigilant neurologic evaluation of families with familial adenomatous polyposis in which a member has a brain tumor, because of familial clustering: 40 percent of the families we studied had two members with brain tumors.

Patients with brain tumors who have hereditary nonpolyposis colorectal cancer or multiple tumors with replication errors require a different type of care than do patients with familial adenomatous polyposis. Data supporting firm recommendations are incomplete. The relative and absolute risks of brain tumors in patients with germ-line mutations of the various mismatch-repair genes are unknown.⁴⁴ Routine genetic testing is not yet feasible. Nonetheless, complete colonoscopy, rather than sigmoidoscopy, should be used for surveillance, because of the predisposition to right-sided cancers in hereditary nonpolyposis colorectal cancer.⁴³ Surveillance of high-risk extracolonic sites in hereditary nonpolyposis colorectal cancer (i.e., the endometrium in women⁴⁴) may also be advisable.

The underlying germ-line genetic alteration may favorably influence the prognosis of patients with glioblastoma multiforme and colorectal neoplasia. Our series was too small to permit us to draw firm conclusions, but the length of survival of the four patients with glioblastoma was exceptional, with a minimum of three years until death. Long survival in similar cases has been reported.⁷¹ Improved stage-specific survival from colorectal cancer may be a feature of hereditary nonpolyposis colorectal cancer.⁴³ The extensive genomic alterations due to microsatellite instability may modify the behavior of various neoplasms and also elicit an improved host resistance and response.

We are indebted to Dr. Mark Redston and Dr. Lawrence Seidenstein for assistance in obtaining pathological specimens, to Chris Kelley for assistance in data management, to Dr. Naomi Rance for

reviewing some of the brain tumors, to Dr. John H. Yardley for reviewing the manuscript, and to Mrs. Nancy Folker for assistance in the preparation of the manuscript.

REFERENCES

- Turcot J, Després J-P, St Pierre F. Malignant tumors of the central nervous system associated with familial polyposis of the colon: report of two cases. *Dis Colon Rectum* 1959;2:465-8.
- Crail HW. Multiple primary malignancies arising in rectum, brain, and thyroid: report of a case. *US Naval Med Bull* 1949;49:123-8.
- Itoh H, Hirata K, Ohsato K. Turcot's syndrome and familial adenomatous polyposis associated with brain tumor: review of related literature. *Int J Colorectal Dis* 1993;8:87-94.
- Jarvis L, Bathurst N, Mohan D, Beckly D. Turcot's syndrome: a review. *Dis Colon Rectum* 1988;31:907-14.
- McKusick VA. Genetic factors in intestinal polyposis. *JAMA* 1962;182:271-7.
- Baughman FA Jr, List CF, Williams JR, Muldoon JP, Segarra JM, Volk JS. The glioma-polyposis syndrome. *N Engl J Med* 1969;281:1345-6.
- Rustgi AK. Hereditary gastrointestinal polyposis and nonpolyposis syndromes. *N Engl J Med* 1994;331:1694-702.
- Lewis JH, Ginsberg AL, Toomey KE. Turcot's syndrome: evidence for autosomal dominant inheritance. *Cancer* 1983;51:524-8.
- Costa OL, Silva DM, Colnago FA, Vieira MS, Musso C. Turcot syndrome: autosomal dominant or recessive transmission? *Dis Colon Rectum* 1987;30:391-4.
- Kinzler KW, Nilbert MC, Su L-K, et al. Identification of FAP locus genes from chromosome 5q21. *Science* 1991;253:661-5.
- Nishisho I, Nakamura Y, Miyoshi Y, et al. Mutations of chromosome 5q21 genes in FAP and colorectal cancer patients. *Science* 1991;253:665-9.
- Groden J, Thliveris A, Samowitz W, et al. Identification and characterization of the familial adenomatous polyposis coli gene. *Cell* 1991;66:589-600.
- Joslyn G, Carlson M, Thliveris A, et al. Identification of deletion mutations and three new genes at the familial polyposis locus. *Cell* 1991;66:601-13.
- Groden J, Gelbert L, Thliveris A, et al. Mutational analysis of patients with adenomatous polyposis: identical inactivating mutations in unrelated individuals. *Am J Hum Genet* 1993;52:263-72.
- Nagase H, Nakamura Y. Mutations of the APC (adenomatous polyposis coli) gene. *Human Mut* 1993;2:425-34.
- Miyoshi Y, Ando H, Nagase H, et al. Germ-line mutations of the APC gene in 53 familial adenomatous polyposis patients. *Proc Natl Acad Sci U S A* 1992;89:4452-6.
- Powell SM, Petersen GM, Krush AJ, et al. Molecular diagnosis of familial adenomatous polyposis. *N Engl J Med* 1993;329:1982-7.
- van der Luijt R, Khan PM, Vasen H, et al. Rapid detection of translation-terminating mutations at the adenomatous polyposis coli (APC) gene by direct protein truncation test. *Genomics* 1994;20:1-4.
- Bussey HJR. Familial polyposis coli: family studies, histopathology, differential diagnosis, and results of treatment. Baltimore: Johns Hopkins University Press, 1975:10.
- Spirio L, Olschwang S, Groden J, et al. Alleles of the APC gene: an attenuated form of familial polyposis. *Cell* 1993;75:951-7.
- Haggitt RC, Reid BJ. Hereditary gastrointestinal polyposis syndromes. *Am J Surg Pathol* 1986;10:871-87.
- Blair NP, Trempe CL. Hypertrophy of the retinal pigment epithelium associated with Gardner's syndrome. *Am J Ophthalmol* 1980;90:661-7.
- Traboulsi EI, Krush AJ, Gardner EJ, et al. Prevalence and importance of pigmented ocular fundus lesions in Gardner's syndrome. *N Engl J Med* 1987;316:661-7.
- Giardiello FM, Offerhaus GJA, Traboulsi EI, et al. Value of combined phenotypic markers in identifying inheritance of familial adenomatous polyposis. *Gut* 1991;32:1170-4.
- Giardiello FM, Hamilton SR, Krush AJ, Offerhaus JA, Booker SV, Petersen GM. Nasopharyngeal angiofibroma in patients with familial adenomatous polyposis. *Gastroenterology* 1993;105:1550-2.
- Offerhaus GJA, Giardiello FM, Krush AJ, et al. The risk of upper gastrointestinal cancer in familial adenomatous polyposis. *Gastroenterology* 1992;102:1980-2.
- Gurbuz AK, Giardiello FM, Petersen GM, et al. Desmoid tumors in familial adenomatous polyposis. *Gut* 1994;35:377-81.
- Kingston JE, Herbert A, Draper GJ, Mann JR. Association of hepatoblastoma and polyposis coli. *Arch Dis Child* 1983;58:959-62.
- Giardiello FM, Offerhaus GJA, Krush AJ, et al. Risk of hepatoblastoma in familial adenomatous polyposis. *J Pediatr* 1991;119:766-8.
- Giardiello FM, Offerhaus GJA, Lee DH, et al. Increased risk of thyroid and pancreatic carcinoma in familial adenomatous polyposis. *Gut* 1993;34:1394-6.
- Peltomäki P, Aaltonen LA, Sistonen P, et al. Genetic mapping of a locus predisposing to human colorectal cancer. *Science* 1993;260:810-2.
- Fishel R, Lescoe MK, Rao MRS, et al. The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. *Cell* 1993;75:1027-38. [Erratum, *Cell* 1994;77:166a.]
- Leach FS, Nicolaides NC, Papadopoulos N, et al. Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. *Cell* 1993;75:1215-25.
- Liu B, Parsons RE, Hamilton SR, et al. hMSH2 mutations in hereditary nonpolyposis colorectal cancer kindreds. *Cancer Res* 1994;54:4590-4.
- Lindblom A, Tannergard P, Werelius B, Nordenskjöld M. Genetic mapping of a second locus predisposing to hereditary non-polyposis colon cancer. *Nat Genet* 1993;5:279-82.
- Bronner CE, Baker SM, Morrison PT, et al. Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary non-polyposis colon cancer. *Nature* 1994;368:258-61.
- Papadopoulos N, Nicolaides NC, Wei Y-F, et al. Mutation of a mutL homolog in hereditary colon cancer. *Science* 1994;263:1625-9.
- Nicolaides NC, Papadopoulos N, Liu B, et al. Mutations of two PMS homologs in hereditary nonpolyposis colon cancer. *Nature* 1994;371:75-80.
- Aaltonen LA, Peltomäki P, Leach FS, et al. Clues to the pathogenesis of familial colorectal cancer. *Science* 1993;260:812-6.
- Parsons R, Li G-M, Longley MJ, et al. Hypermutability and mismatch repair deficiency in RER+ tumor cells. *Cell* 1993;75:1227-36.
- Lothe RA, Peltomäki P, Meling GI, et al. Genomic instability in colorectal cancer: relationship to clinicopathological variables and family history. *Cancer Res* 1993;53:5849-52.
- Aaltonen LA, Peltomäki P, Mecklin J-P, et al. Replication errors in benign and malignant tumors from hereditary nonpolyposis colorectal cancer patients. *Cancer Res* 1994;54:1645-8.
- Lynch HT, Smyrk TC, Watson P, et al. Genetics, natural history, tumor spectrum, and pathology of hereditary nonpolyposis colorectal cancer: an updated view. *Gastroenterology* 1993;104:1535-49.
- Watson P, Lynch HT. Extracolonic cancer in hereditary nonpolyposis colorectal cancer. *Cancer* 1993;71:677-85.
- Minami T, Himeno S, Shinomura Y, Kariya Y, Tarui S, Sakurai M. Rearrangement, amplification and overexpression of c-Ha Ras gene in premalignant lesion of Turcot's syndrome. *Jpn J Med* 1990;29:391-6.
- Newton HB, Rosenblum MK, Malkin MG. Turcot's syndrome: flow cytometric analysis. *Cancer* 1991;68:1633-9.
- Rosenfeld M, Schlegel U, Malkin M, et al. p53 Mutations in glioma polyposis (Turcot syndrome). *Neurology* 1991;41:Suppl 1:304. abstract.
- Tops CMJ, Vasen HFA, van Berge Henegouwen G, et al. Genetic evidence that Turcot syndrome is not allelic to familial adenomatous polyposis. *Am J Med Genet* 1992;43:888-93.
- Kikuchi T, Rempel SA, Rutz H-P, et al. Turcot's syndrome of glioma and polyposis occurs in the absence of germ line mutations of exons 5 to 9 of the p53 gene. *Cancer Res* 1993;53:957-61.
- Rochlitz CF, Heide I, de Kant E, et al. Molecular alterations in a patient with Turcot's syndrome. *Br J Cancer* 1993;68:519-23.
- Paraf F. Syndrome de Turcot: évolution des connaissances. *Gastroenterol Clin Biol* 1994;18:297-9.
- Mori T, Nagase H, Horii A, et al. Germ-line and somatic mutations of the APC gene in patients with Turcot syndrome and analysis of APC mutations in brain tumors. *Genes Chromosomes Cancer* 1994;9:168-72.
- Lasser DM, DeVivo DC, Garvin J, Wilhelmsen KC. Turcot's syndrome: evidence for linkage to the adenomatous polyposis coli (APC) locus. *Neurology* 1994;44:1083-6.
- Bussey HJR. Familial polyposis coli: family studies, histopathology, differential diagnosis, and results of treatment. Baltimore: Johns Hopkins University Press, 1975:23.
- Vasen HFA, Mecklin J-P, Khan PM, Lynch HT. Hereditary non-polyposis colorectal cancer. *Lancet* 1991;338:877.
- Cohen SB. Familial polyposis coli and its extracolonic manifestations. *J Med Genet* 1982;19:193-203.
- Hamilton SR, Bussey HJR, Mendelsohn G, et al. Ileal adenomas after colectomy in nine patients with adenomatous polyposis coli/Gardner's syndrome. *Gastroenterology* 1979;77:1252-7.
- Pierce ER, Weisbord T, McKusick VA. Gardner's syndrome: formal genetics and statistical analysis of a large Canadian kindred. *Clin Genet* 1970;1:65-80.
- Tithecott GA, Filler R, Sherman PM. Turcot's syndrome: a diagnostic consideration in a child with primary adenocarcinoma of the colon. *J Pediatr Surg* 1989;24:1189-91.
- Vogelstein B, Fearon ER, Hamilton SR, et al. Genetic alterations during colorectal-tumor development. *N Engl J Med* 1988;319:525-32.
- Jen J, Kim H, Piantadosi S, et al. Allelic loss of chromosome 18q and prognosis of colorectal cancer. *N Engl J Med* 1994;331:213-21.
- Peinado MA, Malkhosyan S, Velazquez A, Perucho M. Isolation and characterization of allelic losses and gains in colorectal tumors by arbitrarily primed polymerase chain reaction. *Proc Natl Acad Sci U S A* 1992;89:10065-9.
- Thibodeau SN, Bren G, Schaid D. Microsatellite instability in cancer of the proximal colon. *Science* 1993;260:816-9.
- Ionov Y, Peinado MA, Malkhosyan S, Shibata D, Perucho M. Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. *Nature* 1993;363:558-61.

65. Kim H, Jen J, Vogelstein B, Hamilton SR. Clinical and pathological characteristics of sporadic colorectal carcinomas with DNA replication errors in microsatellite sequences. *Am J Pathol* 1994;145:148-56.
 66. Coleman M, Douglas A, Hermon C, Peto J. Cohort study analysis with a FORTRAN computer program. *Int J Epidemiol* 1986;15:134-7.
 67. Breslow NE, Day NE. Statistical methods in cancer research. Vol. 2. The design and analysis of cohort studies. Lyon, France: International Agency for Research on Cancer, 1987. (IARC scientific publications no. 82.)
 68. Black PM. Brain tumors. *N Engl J Med* 1991;324:1555-64.
 69. Radin DR, Fortgang KC, Zee C-S, Mikity VG, Halls JM. Turcot syndrome: a case with spinal cord and colonic neoplasms. *AJR Am J Roentgenol* 1984;142:475-6.
 70. Kropilak M, Jagelman DG, Fazio VW, Lavery IL, McGannon E. Brain tumors in familial adenomatous polyposis. *Dis Colon Rectum* 1989;32:778-82.
 71. Rutz HP, de Tribolet N, Calmes JM, Chapuis G. Long-time survival of a patient with glioblastoma and Turcot's syndrome. *J Neurosurg* 1991;74:813-5.
-