American Journal of Gastroenterology
© 2005 by Am. Coll. of Gastroenterology
Published by Blackwell Publishing

ISSN 0002-9270
doi: 10.1111/j.1572-0241.2005.50441.x

## CASE REPORT

# Two PMS2 Mutations in a Turcot Syndrome Family with Small Bowel Cancers

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**We report the clinicopathological, genetic, and immunohistochemical characterization of an atypical Turcot syndrome (TS) family with small bowel cancer. The tumor family history of a patient with cafè-au-lait spots (CALS) and early onset adenomas, duodenal cancer, and glioblastoma was positive for colonic adenoma (mother), jejunal (maternal grandfather), lung (father), and colorectal (paternal uncle) cancers. *PMS2* genetic testing identified the nonsense 1951C>T (Q643X) and the missense 161C>T (S46I) mutations. PMS2 expression was absent in the proband's duodenal cancer with high microsatellite instability. The normal cells also displayed no PMS2 expression and some degree of instability. Our findings point out the association between *PMS2* and TS, and support the hypothesis that patients with a few polyps, small bowel tumors with a very early onset, glioblastoma, and CALS should be considered as a variant of hereditary nonpolyposis colorectal cancer. A recessive model of inheritance caused by compound heterozygous mutations was consistent with the observed severe clinical phenotype and has important implications for predicting cancer risk in both the proband and his relatives.**

(Am J Gastroenterol 2005;100:1886–1891)

## INTRODUCTION

Turcot syndrome (TS) is a rare, heritable disorder characterized clinically by the simultaneous presence of a primary tumor of the central nervous system and multiple colorectal adenomas and/or carcinomas, usually at early ages (1). However, there is a wide variation in reporting the diagnosis of TS and single occurrences of brain neoplasias are sometimes present in hereditary nonpolyposis colorectal cancer (HNPCC) pedigrees not usually labeled as TS.

Both autosomal dominant and recessive modes of transmission were described for TS, and sporadic patients were reported (1, 2). Genetic heterogeneity is partially responsible of this controversial mode of inheritance. In fact, TS can result not only from mutation of *APC* gene that is usually found in familial adenomatous polyposis, but also from mutations of one of the mismatch-repair (*MMR*) genes that are usually found in HNPCC (3). The proteins encoded by *MMR* genes (*MSH2*, *MSH6, MLH1*, *PMS1*, and *PMS2*) recognize and repair errors created during DNA replication and loss of MMR activity leads to microsatellite instability (MSI). Mutations of *MSH2*, *MLH1*, and *PMS2* genes were identified in patients for whom a diagnosis of TS was formally reported (3–7), but involvement of *PMS2* in TS is especially striking because its mutations are very rarely a cause of HNPCC (8–10).

In this study, we report the case of a suspected TS family with a young patient with early-onset duodenal and brain tumors, colonic adenomas, and cafè-au-lait spots (CALS).

## CASE REPORT

Genetic counseling was offered to the A-VA17 family on December 2000, after the 17-yr-old son had undergone surgery for an infiltrating G2 adenocarcinoma of the ampullary region, arisen on a villous adenoma with severe dysplasia and metastatic to some regional lymph nodes, and four tubulo-villous adenomas with moderate dysplasia of the transverse and sigmoid colon. Cutaneous CALS were also removed and the histological diagnosis was dysplastic compound nevi (dorsal region) and compound nevi (forearm and leg). On October 2001, the patient underwent a neurosurgical operation because of giant cell glioblastoma (grade IV, WHO) in the right frontal lobe.

His family history was suggestive of HNPCC (Figure 1): his mother had a tubulo-villous adenoma with moderate dysplasia in the rectum at 44 yr and his maternal grandfather had a large G2 infiltrative jejunal adenocarcinoma at 52 yr. His father died of lung cancer at 55 yr and in the paternal lineage, there was a referred diagnosis of colorectal cancer in an uncle. His maternal aunt had only a small colonic

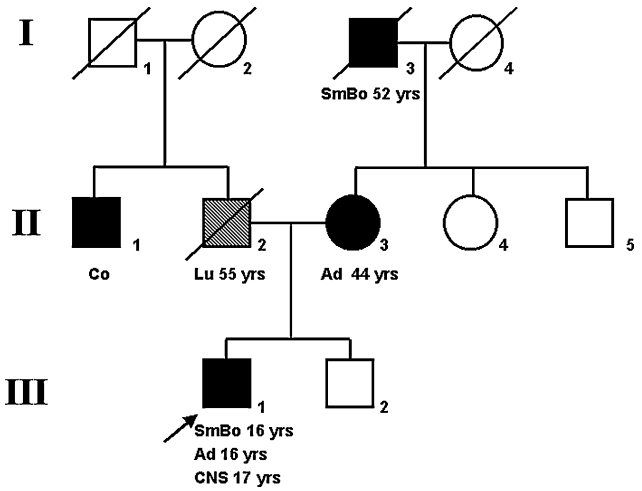


**Figure 1.** Pedigree of family A-VA17. Tumors and age at onset are reported. Black symbols indicate HNPCC-related tumors (Co, colon; CNS, central nervous system; SmBo, small bowel; Ad, colonic adenoma); diagonal shaded symbols indicate non-HNPCC-related tumors (Lu, lung).

hyperplastic polyp, whereas a "clean colon" was verified in his maternal uncle.

Standard MSI analysis was performed on paired tumor-normal tissue DNA samples according to the international criteria (11). The duodenal cancer, lymph node metastases, and adenomas of the proband were extensively unstable, showing also instability of additional target genes. However, MSI was not observed in the glioblastoma, or in the normal tissues, blood, and intestinal mucosa. The adenoma of the mother and the jejunal adenocarcinoma of the maternal grandfather were also MSI-high (Table 1).

At first, IHC was performed for MLH1, MSH2, and MSH6 proteins. Along with a positive expression of the first two proteins, a reduced immunoreaction for MSH6 was evidenced in the proband's intestinal tumors and in the adenoma of his mother. On the contrary, glioblastoma displayed a normal expression pattern of all three proteins. Concerning the PMS2 protein (12), lack of expression was observed in all proband's tumors both in neoplastic and normal cells. However, along with a positive normal internal control, adenoma cells of his mother were PMS2 negative and the grandfather's jejunal adenocarcinoma had focal expression of PMS2 (Table 1, Figure 2).

Since MSI and IHC data were suggestive of a *MMR*-related family, but involvement of *MLH1* and *MSH2* appeared less probable, we focused our attention on *MSH6* and *PMS2* genes, that were entirely sequenced. Whereas no significant *MSH6* sequence variants were detected, two constitutional *PMS2* mutations were identified: a truncating nonsense mutation in exon 11 (1951C>T; Q643X) and a missense mutation affecting exon 2 (161G>T; S46I) (Figure 3). Both mutations were detected on constitutional DNA isolated from peripheral blood and normal mucosa of the proband, as well as in the duodenal tumor and metastasis, with no evidence of loss of heterozygosity. His mother and his brother carried only the nonsense mutation, suggesting that the two mutations were located in two different alleles of the proband, with the nonsense mutation inherited from his mother and the missense mutation probably inherited from his father. Unfortunately, presence of the truncating mutation could not be confirmed in the maternal grandfather due to the difficulty of obtaining a *PMS2*-specific PCR from DNA extracted from very old paraffin embedded tissue blocks.

A quantitative mutation analysis was performed in order to evaluate whether microsatellite mutations, expected to occur with biallelic MMR inactivation, can accumulate also in the absence of tumorigenesis. The *D5S436* di-nucleotide repeat was amplified using extensively diluted DNA (5 pg/reaction or less) from normal tissues in order to obtain PCR products in about 50% of reactions, from single copy templates (5,

**Table 1.** Standard MSI, IHC, CG, and *MMR* Mutation Analyses of Normal and Tumor Tissues of A-VA17 Family Members

| | | III-1[a] | | | | | II-3[a] | | I-3[a] | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | N | T1 | T2 | T3 | T4 | N | T1 | N | T1 |
| MSI[b] | NCI panel | S | + | + | + | S | S | + | S | + |
| | *MSH6* | S | + | + | nt | nt | nt | nt | S | S |
| | *MSH3* | S | + | + | nt | nt | nt | nt | nt | nt |
| | *TGF-βRII* | S | + | + | nt | nt | nt | nt | S | S |
| IHC[c] | MSH2 | + | + | + | + | + | + | + | + | + |
| | MLH1 | + | + | + | + | + | + | + | + | ± |
| | MSH6 | + | ± | ± | ± | + | + | ± | + | + |
| | PMS2 | − | − | nt | − | − | + | − | + | ± |
| CG | Chromosome anomalies | no | del(15)(q21qter) der(2)t(2;X)(p25;?) | del(15)(q21qter) add(1q) trisomy 2 monosomy 8 | nt | nt | nt | nt | nt | nt |
| *MMR*[d] | *MSH6* | | | wt | | | | nt | | nt |
| | *PMS2* | | | Q603X - S46I | | | | Q603X - wt | | nt |

[a]III-1, proband: N, normal tissue; T1, duodenal adenocarcinoma; T2, lymph node metastasis; T3, colonic adenoma; T4, glioblastoma. II-3, mother: N, normal tissue; T1, colonic adenoma. I-3, maternal grandfather: N, normal tissue; T1, jejunal adenocarcinoma.
[b]NCI panel: *BAT26, BAT25, D2S123, D5S346, D17S250.* S, stability; +, instability; nt, not tested.
[c]+, positive expression; −, no expression; ±, reduced or focal expression; nt, not tested.
[d]wt, wild type; nt, not tested.

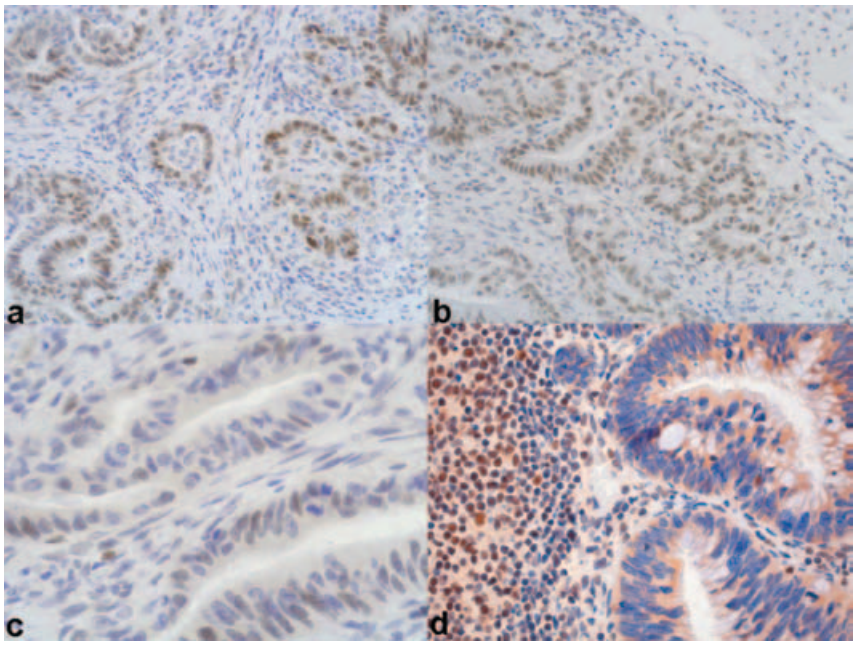


**Figure 2.** Intense IHC expression of MSH2 (*A*) and MLH1 (*B*) in duodenal carcinoma of the proband (×200); (*C*) Reduced MSH6 expression in the proband's duodenal carcinoma (×400); (*D*) Absent PMS2 expression in the mother's adenoma. The positive internal control cells are lymph node follicular center lymphocytes and stromal lymphocytes. The adenomatous cells stain negative in the nuclei (×300). The immunostainings were performed with the mouse monoclonal antibodies G168-15 (hMLH1 protein), FE11 (hMSH2), Clone 44 (hMSH6), and clone A16-4 (PMS2 protein).[12]

13). Data obtained with six normal DNAs are reported in Table 2. Instability of normal DNAs of the proband was about twofold the instabilty of blood DNA of his mother and about threefold the instability of normal mucosa control DNA from an unrelated heterozygous *MSH2* mutated carrier.

Conventional cytogenetics (CG) showed diploid karyotype of the duodenal cancer, with few chromosome aberrations: an unbalanced translocation involving chromosomes 2 and X, and 15q deletion. The deletion was constantly present in the lymph node metastasis, displaying chromosome instability

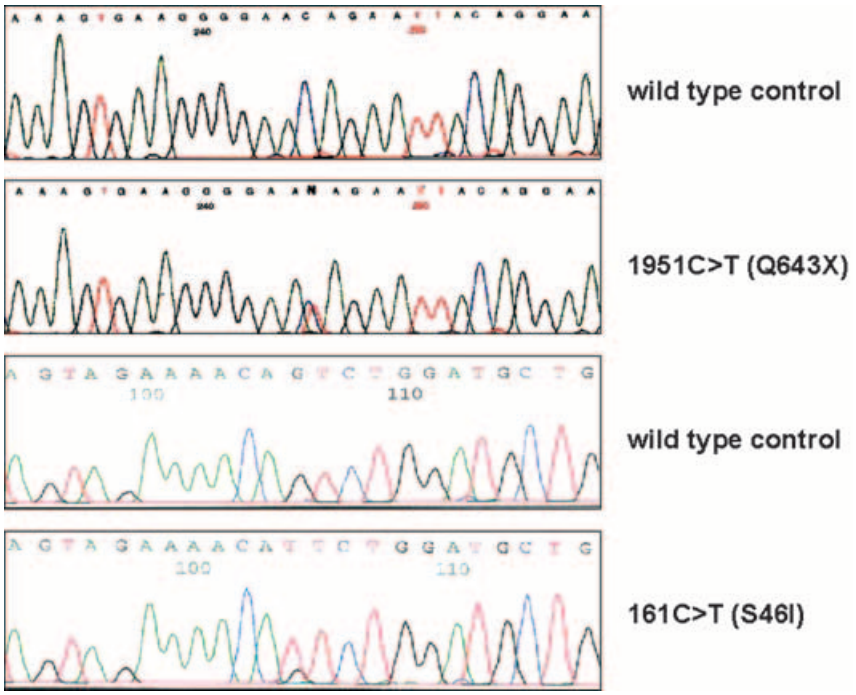


**Figure 3.** *PMS2* sequences of constitutional DNA of proband showing the 1951C>T nonsense (second panel) and the 161C>T missense (fourth panel) mutations. Reference *PMS2* sequences of a healthy control are also shown (first and third panels).

**Table 2.** Frequency of Microsatellite *D5S346* Mutations in Extensively Diluted DNA from Normal Cells

| Individual/Tissue | *MMR* Status | Mutations (%)[a] |
|---|---|---|
| III-1/mucosa | *PMS2* mut/mut | 10/35 (29%) |
| III-1/blood | *PMS2* mut/mut | 5/18 (28%) |
| II-3/blood | *PMS2* mut/wt | 3/21 (14%) |
| I-3/mucosa | *PMS2* mut?/wt | 4/12 (33%) |
| Control A/mucosa | *MSH2* mut/wt | 2/19 (10%) |
| Control B/mucosa | *MMR* wt/wt | 1/23 (4%) |

[a]Ratios between the number of mutant alleles and number of the valuable PCRs are reported.

and showing different cell populations with diploid and polyploid chromosome complements (Table 1). FISH analysis performed on duodenal cancer mataphases with probes mapping on 2p25 and 2p16.3-22.1 revealed that these portions of 2p arm were retained on normal and derivative chromosome 2 (data not shown), suggesting that the unbalanced translocation did not affect the *MSH2* and *MSH6* genes located in these regions.

## DISCUSSION

The personal and family histories of our proband were consistent with a TS, typically characterized by the association of malignant tumor of the central nervous system, gastrointestinal polyposis, and colon cancer. The absence of diffuse polyposis in this patient and his relatives was suggestive of the involvement of an *MMR*, rather than *APC* gene. Actually, this hypothesis was soon confirmed by the MSI analysis on multiple tumors of this family. We also obtained evidences in favor of a *PMS2* defect in this family, since two constitutional *PMS2* mutations were identified in the proband: the Q643X truncating mutation, inherited from the maternal branch of the family, and the S46I missense mutation, probably inherited from his father.

Although a comprehensive *PMS2* mutation screening may be affected by the existence of several pseudogenes (10, 14, 15), we are confident about the good quality of our mutation data and the real existence of both identified mutations, since a *PMS2*-specific strategy was adopted.

The involvement of *PMS2* gene in this family was in agreement with the IHC data, showing absence of PMS2 protein both in tumor and in normal proband's cells. The lack of PMS2 expression in mother's adenomatous cells, along with the focal PMS2 pattern in grandfather's jejunal adenocarcinoma, also supports the existence of a *PMS2* defect in this family.

While the pathogeneity of the Q643X mutation is unquestionable, some doubts may concern the S46I variant, which was recently reported also in a sporadic patient with an early-onset colorectal cancer not expressing the PMS2 protein (10). Several points are in favor of its pathogenetic role: first, Serine 46 maps on an evolutionary conserved domain and is an evolutionary conserved aminoacid; second, the aminoacid change is not conservative and is predicted to be "probably damaging" (PolyPhen: http://tux.embl-heidelberg.de/ramensky/); third, this variant was absent in 118 normal control chromosomes from healthy blood donors. The observation that S46I occurs outside the putative MLH1 interaction domain of PMS2 (amino acids 675-850) is not sufficient for rejecting the pathogenetic hypothesis. It is worth noting that the two mutations located in different alleles were both retained in the duodenal tumor and metastasis, without evidence of allelic loss and with the presence of both intact chromosome 7 homologues at CG investigation, suggesting that the presence of both mutated alleles may be critical in developing instability and cancer.

In light of these considerations, patient III-1 is a compound heterozygous individual and his TS phenotype may be caused by two mutations with a recessive mode of transmission.

However, despite the extensive instability displayed by his intestinal tumor, normal tissues were stable in a standard MSI molecular test, apparently not supporting the hypothesis of a complete *PMS2* inactivation at constitutional level. Therefore, additional MSI tests were performed with diluted DNA, to ascertain if individual cells of normal colon mucosa or blood could exhibit a mutator phenotype. Tumors usually have greater number of microsatellite mutations, reflecting greater number of divisions and abnormal clonal expansion due to other advantageous hits in singular cells carrying a specific microsatellite defect. As a result, microsatellite mutations present in a large fraction of tumor cells appear evident in a standard MSI test. However, somatic microsatellite mutations should be possible in normal cells and still compatible with nontumorigenic life (5, 13). The fewer number of mutations in the normal tissues are consistent with their lower mitotic activity and absence of clonal expansion. Because of their heterogeneity and lack of stepwise mutations, these alterations may not be detectable in undiluted DNA samples. As expected, we found some degree of instability in diluted DNAs from both constitutional tissues of proband, with evidence of CA-repeat mutations in about 30% of alleles. This frequency was higher than those of his mother, of an *MSH2* heterozygous carrier and of an additional *MMR*-wild-type patient. The observation of MSI in normal mucosa of the TS proband is quite stricking and suggests that this instability contributed to the early development of duodenal tumor. Surprisingly, microsatellite mutations were also found abundant in the normal intestine of the maternal grandfather, another probable heterozygous *PMS2* patient, but this analysis could be partially invalidated by the poor quality of DNA extracted from a 24-yr-old paraffin tissue block.

An additional critical point is the absence of MSI in the glioblastoma of the proband, but we can speculate that, at least in certain tissues, a PMS2 deficiency could lead to tumorigenesis mainly through a mechanism distinct from a defect in the postreplicative mismatch repair of repetitive sequences (16). According to this, chromosome instability and p53

inactivation seem to be required for genesis of glioblastoma but not for colorectal cancer in patients with germline *MMR* gene mutation (17).

Karyotype of the primary duodenal cancer was essentially diploid, with few chromosome aberrations. Although an inverse relationship between MSI and chromosome instability can be usually demonstrated, karyotype of the MSI metastatic cells displayed several chromosome anomalies, indicating that both instability pathways may act together for the progression of *PMS2*-related tumors.

*MMR* mutations were previously identified in patients with a formal diagnosis of TS or in families segregating both intestinal and brain tumors. In addition to our patient, there were four other reports of *PMS2* gene mutation (3–5, 7); other five cases had mutations of the *MLH1* or *MSH2* genes (6, 18); thus *PMS2*, never mutated in classical HNPCC (8–10), appears to be the *MMR* gene most frequently involved in TS. Additional clinical manifestations were described in some TS families, mainly hematological malignancies and CALS. CALS were present in our TS proband since birth. Although CALS were not always reported in *MMR*-related TS cases (5–7), their finding should be considered as a possible early clinical manifestation and a warning signal for syndromic conditions with increased risk for several malignancies, including those of the TS spectrum. Notably, in some of the previously published TS patients two mutations causing complete inactivation of an *MMR* gene were reported (3–7, 14, 18, this study). Autosomic recessive inheritance was also demonstrated for other homozygous or compound heterozygous carriers of *PMS2* (14), *MSH2* (16, 19), or *MLH1* (20, 21) mutations, who had a TS-negative personal or family history, but were affected in childhood by different types of tumors, sometimes in association with CALS. However, in the context of recessive transmission, early onset colorectal cancer or other HNPCC-related tumors were also developed in the adult life by several relatives, ascertained or presumed to be heterozygous carriers of one of the two *MMR* mutations (3, 4, 16, 18, 20, 21, this study). Thus, in some families *MMR* mutations apparently act with two overlapping modalities in conferring genetic predisposition to cancer: autosomic recessive for some childhood tumors and autosomic dominant for the classical HNPCC-related tumors in adults.

Although this behavior was not a constant finding, but apparently restricted to a subset of mutations, it has important implications for genetic counseling, in particular for predicting cancer risk of mutation carriers. In family A-VA17 MSI tumors were also developed by the heterozygous Q603X carriers (colonic adenoma and jejunal cancer), thus the heterozygous brother should also be considered at increased risk of HNPCC-related cancers, though, possibly, not in infanthood. Moreover, the presence of two cases of small bowel cancer in this family is intriguing, and it could suggest that individuals with *PMS2* mutations are more likely to develop duodenal and jejunal cancers than those with mutations of other *MMR* genes.

## ACKNOWLEDGMENTS

The study was partially supported by *Alleanza Contro il Cancro* (to M.B.) and by the *Italian Health Ministery*, Special Project ex. Art. 12 (to S.P.). The authors thank S. Jagmohan and Professor R. Fodde (Rotterdam, NE) for their useful suggestions in approaching the study of *PMS2*-related families, Professor M. Roncalli (Rozzano, MI, Italy), and Dr. S. Sulfaro (Pordenone, Italy) for providing paraffin-embedded materials.

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*Received March 21, 2005; accepted March 31, 2005.*

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