

A Homozygous Mutation in *MSH6* Causes Turcot Syndrome

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Abstract Heterozygous mutations in one of the DNA mismatch repair genes cause hereditary nonpolyposis colorectal cancer (MIM114500). Turcot syndrome (MIM276300) has been described as the association of central nervous system malignant tumors and familial colorectal cancer and has been reported to be both a dominant and recessive disorder. Homozygous and compound heterozygous mutations in *APC*, *MLH1*, *MSH2*, and *PMS2* genes have been reported in five families. Here we describe a nonconsanguineous Pakistani family, including a son with lymphoma and colorectal cancer diagnosed at ages 5 and 8, respectively, and an 8-year-old daughter with glioblastoma multiforme. Both children had features of neurofibromatosis type 1 including atypical café au lait spots and axillary freckling without a family history consistent with neurofibromatosis type 1, familial adenomatous polyposis, or hereditary nonpolyposis colorectal cancer. Mutational analysis was done for *MLH1*, *MSH2*, and *MSH6* using denaturing high-performance liquid chromatography and sequencing of a blood sample from the daughter. A novel homozygous single base insertion mutation was identified (3634insT) resulting in a premature stop at codon 1,223 in exon 7 of the *MSH6* gene. Both parents were found to be heterozygous for the 3634insT mutation. Microsatellite instability testing showed instability in the glioblastoma sample. We report here the first identification of a homozygous mutation in *MSH6* in a family with childhood-onset brain tumor, lymphoma, colorectal cancer, and neurofibromatosis type 1 phenotype. Our findings support a role for *MSH6* in Turcot syndrome and are consistent with an autosomal recessive mode of inheritance.

Turcot syndrome is characterized by development of colorectal carcinomas and adenomas, and primary central nervous system tumors (1, 2). Two siblings with colonic polyps and brain tumors were first described in 1958 by Turcot and Payeur (3). The brain tumors described in Turcot's original report were medulloblastoma and glioblastoma. The inheritance pattern is complex, with some families demonstrating autosomal dominant inheritance and heterozygous mutations in DNA mismatch repair genes or in the *APC* gene (4, 5).

Itoh and Ohsato (6) have described three types of colonic polyposis in Turcot syndrome. Type 1 is characterized by multiple colonic polyps (20-100 polyps) which frequently undergo malignant transformation. These patients are similar to those originally described by Turcot. Type 2 is characterized by less than 10 polyps and the mode of inheritance is uncertain, whereas numerous colonic polyps occur in

type 3, resembling a classic familial adenomatous polyposis phenotype.

Turcot syndrome mutations in the *APC* gene are associated with a familial adenomatous polyposis-like phenotype and medulloblastoma (7). Adenomas usually develop in the second decade of life, with the eventual presence of hundreds to thousands of polyps (8, 9). Other Turcot syndrome cases are consistent with hereditary nonpolyposis colorectal cancer and are characterized by glioblastomas or astrocytomas (10). Hereditary nonpolyposis colon cancer, like familial adenomatous polyposis, is an autosomal dominant disorder arising from mutations in one of five DNA mismatch repair genes. The majority of hereditary nonpolyposis colorectal cancer cases (50-70%) are caused by mutations in *MSH2* and *MLH1*, but mutations also occur in *PMS1*, *PMS2*, and *MSH6* (10, 11). Mutations in *PMS2* or *MLH1* have been noted in glioblastomas of two Turcot syndrome cases, whereas *MSH2* mutations were identified in three Turcot syndrome cases studied by another group (12-14). Ninety-five percent of hereditary nonpolyposis colorectal cancer cases show microsatellite instability caused by errors in DNA mismatch repair, as compared with only 15% of unselected colon cancers showing microsatellite instability (15). Microsatellite instability has also been exhibited by gliomas in Turcot syndrome. The mutations in Turcot syndrome associated with the *APC* gene have been shown to present with medulloblastomas and astrocytomas (16). An Ashkenazi Jewish kindred with microsatellite instability in both normal and tumor tissues with a germ line missense mutation both in *APC* gene and in *MSH2* gene has been previously reported (17). There have also been several kindreds with an

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autosomal recessive pattern of inheritance and a neurofibromatosis type 1-like phenotype with homozygous or compound heterozygous mutations in the mismatch repair genes (13, 18). These kindreds presented with brain tumors in childhood, typically glioblastoma and multiple café au lait spots. In one kindred, an *MSH2* mutation was identified in the proband who presented with leukemia and multiple café au lait spots.

Here we describe two affected siblings with a neurofibromatosis type 1-like phenotype and childhood-onset malignancies. Mutation analysis of an affected child revealed a homozygous novel mutation in *MSH6*, representing the first case report to suggest a role for *MSH6* in Turcot syndrome.

Materials and Methods

Mutation analysis. Mutation analysis was done for *MLH1*, *MSH2*, and *MSH6* genes using denaturing high-performance liquid chromatography and sequencing. Analysis was done on 51 fragments (19 *MLH1*, 16 *MSH2*, and 16 *MSH6* fragments) by PCR amplification and denaturing high-performance liquid chromatography (Transgenomic WAVE). Denaturing high-performance liquid chromatography variants were sequenced on ABI3100 and sequences assembled using Sequencher software for sequence analysis. Variants were compared with the Human Gene Mutation Database (<http://archive.uwcm.ac.uk/uwcm/mg/hgmd>) and the International Collaborative Group on Hereditary Non-Polyposis Colorectal Cancer Database (<http://www.nfdht.nl>) to determine whether the mutation had been previously reported.

Microsatellite instability assay. Tumor DNA was extracted from a 20- μ m section cut from a paraffin-embedded tissue block of the glioblastoma. Extracted tumor DNA and DNA prepared from blood lymphocytes were amplified for 13 markers (*D2S123*, *D17S250*, *D5S346*, *BAT-25*, *BAT-26*, *D18S35*, *TP53-D1*, *D1S283*, *TP53-penta*, *FGA*, *NR-21*, *NR-22*, and *NR-24*) using fluorescently labeled primers in three multiplex reactions, electrophoresed on the ABI3100 Genescan, and analyzed using ABI GeneMapper software (18). DNAs prepared from blood lymphocytes from the parents were also analyzed using the same protocols. Loss of heterozygosity was scored for each locus along with microsatellite instability analysis. National Cancer Institute-

recommended guidelines were followed in scoring for microsatellite instability when comparing markers in the tumor and germ line DNAs. Briefly, high microsatellite instability was defined as >40% of markers showing instability or differences in patterns; low microsatellite instability as <40% of markers showing instability; and stable as no difference in the microsatellite patterns in normal and tumor cells (19, 20).

Case presentation. A family with two affected children both underwent a genetic evaluation by the same clinician. The son (Fig. 1, individual IV-1) was referred for a genetic evaluation due to diagnoses of lymphoblastic lymphoma at age 5 and invasive adenocarcinoma of the colon at age 8. Evaluation revealed no evidence of familial adenomatous polyposis, including a negative eye examination for congenital hypertrophy of the retinal pigment epithelium. However, eight café au lait macules (0.8-1 cm in size) with irregular borders and a few small (<5 mm) axillary freckles were noted on examination. There were no neurofibromas and no Lisch nodules were seen on ophthalmic examination. The family declined genetic testing for neurofibromatosis type 1 or to have a blood sample for DNA banking. The patient died at age 9 of metastatic adenocarcinoma of the colon.

The sister of this child (Fig. 1, individual IV-3) was referred for evaluation following diagnosis of a brain tumor. She was well until the age of 8 when she developed a right-sided limp and other gait abnormalities. A magnetic resonance imaging of the brain revealed a mass in the left temporal parietal region with lesions also noted in the left frontal lobe. An initial needle biopsy was consistent with a highly cellular infiltrating astrocytic neoplasm. Immunohistochemical studies revealed glial fibrillary acidic protein reactivity with a Mib-1 proliferation index in the 10% to 15% range. The overall impression of this limited specimen was of an infiltrating malignant glioma with features of a mixed oligoastrocytoma.

A more definitive specimen obtained at resection after ~2 months showed greater cellularity and mitotic activity. Diffuse immunostainings for glial fibrillary acidic protein and vimentin were appreciated with ~40% of tumor cells positive for p53 staining. The final pathologic diagnosis was glioblastoma multiforme. In addition to the neurologic signs, the examination was significant for six to eight café au lait spots, mostly located on the trunk, with atypical contour. Additionally, there was a solitary freckle noted in each axilla. There were no cutaneous or plexiform neurofibromas. Due to the child's dermatologic features, a fluorescence *in situ* hybridization study for possible deletion of the neurofibromatosis type 1 gene had been

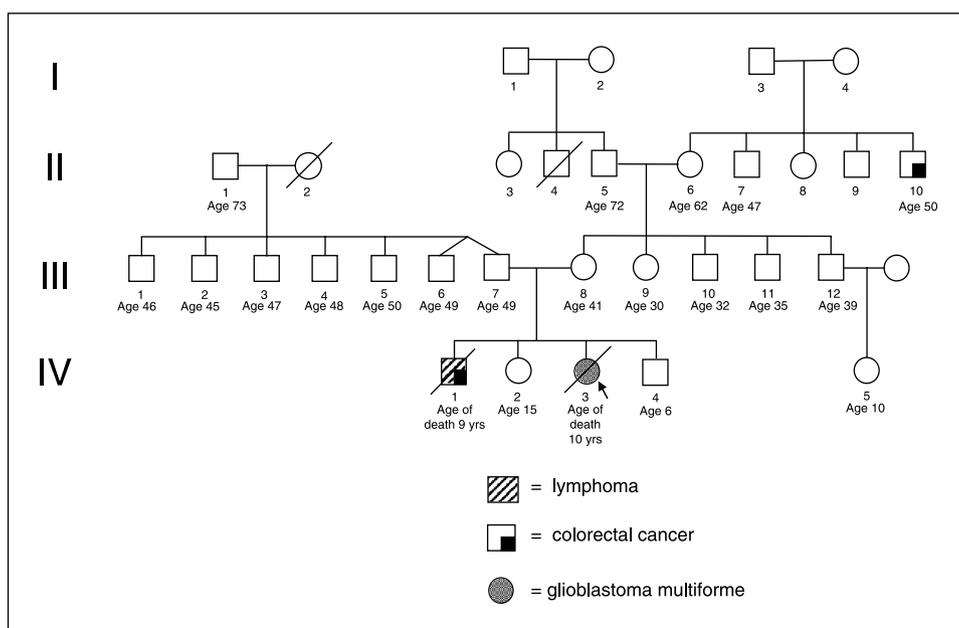
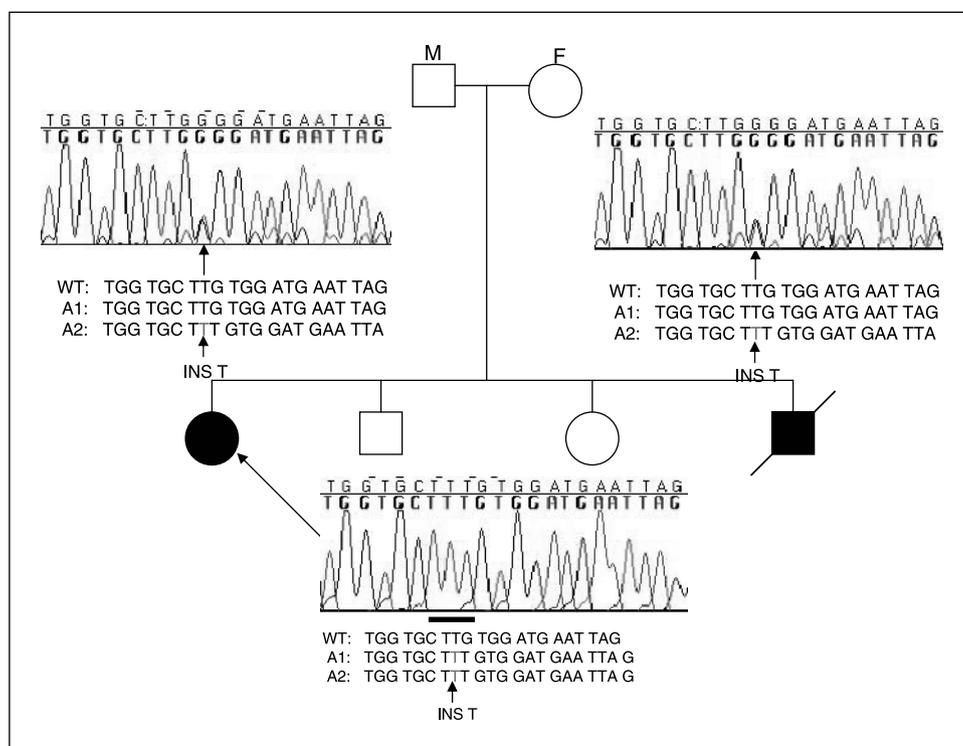


Fig. 1. Pedigree of Turcot Syndrome family showing four generations and two affected family members.

Fig. 2. Sequence analysis of exon 7 of the *MSH6* gene in a Turcot syndrome family. A novel homozygous single base pair insertion mutation was identified (3634insT) which results in a premature stop at codon 1,223 in exon 7 of the *MSH6* gene (mRNA sequence: NM.0000179). This mutation is expected to produce a truncated protein. Both parents were found to be heterozygous for the 3634insT mutation. WT, GenBank wild-type sequence; A1, allele 1; A2, allele 2.



ordered and was negative for the deletion. Karyotyping of a bone marrow specimen, done as part of the cancer work-up, revealed a 46,XX chromosome pattern. The patient died at age 10 of progressive disease from glioblastoma.

The family history is shown in the pedigree in Fig. 1. There are two other unaffected siblings. The parents are of Pakistani ancestry and deny any knowledge of consanguinity. They are alive and well, without a diagnosis of cancer. Both parents come from large families without any significant diagnoses of cancer except for a maternal great uncle to the affected children who was diagnosed with colorectal cancer at age 48. Although recommended, neither parent has had colonoscopy to look for evidence of polyps or adenocarcinoma of the colon.

A clinical diagnosis of autosomal recessive Turcot Syndrome was made based on childhood brain tumors, lymphoma, colorectal cancer, and neurofibromatosis type 1 phenotype. Molecular testing for mutations in hereditary nonpolyposis colorectal cancer-related genes was requested.

Results

Mutation analysis. By sequence analysis of DNA prepared from blood lymphocytes of the sister, we identified a novel homozygous single-base insertion mutation in exon 7 of the *MSH6* gene (3634insT; Fig. 2). This mutation creates a frameshift and is predicted to result in a stop codon at nucleotide position 3,667 in exon 7, leading to a truncated protein product missing the last 179 amino acids in the MSH6 protein. Blood samples obtained from the parents were heterozygous for this mutation, confirming the homozygosity of this mutation in the patient and ruling out a large gene deletion on one of the alleles. We anticipated that the deceased sibling was also homozygous for the 3634insT mutation, but tissue samples from this child were not available for testing.

Microsatellite instability. The microsatellite instability assay done on DNA extracted from lymphocytes and the paraffin-

embedded glioblastoma tissue (Table 1) indicated a high microsatellite instability profile in the tumor, with more than 40% of the markers tested unstable compared with lymphocyte DNA (Fig. 3). To investigate the possibility of a constitutional instability in the proband, microsatellite instability analysis was also done on DNA from both parents and compared with that from the DNA extracted from lymphocytes of the proband. Unlike the previously reported Turcot syndrome patient with a *PMS2* mutation (13), no germ line instability was identified in this patient. However, microsatellite instability of lymphocyte DNA may be missed because a clonal population was not analyzed.

Discussion

Mutations in the *APC* gene and mismatch repair genes *MLH1*, *MSH2*, and *PMS2* have been previously shown to be associated with Turcot syndrome (12–14). Mutations in the *APC* gene, which account for approximately two thirds of all cases, are usually associated with childhood cerebellar medulloblastoma, whereas mutations in the mismatch repair genes are associated with central nervous system gliomas. The clinical presentation in our family, including glioblastoma, colorectal cancer, hematologic malignancy, and neurofibromatosis phenotype, suggested a defect in the mismatch repair genes.

The first case of a homozygous germ line mutation in the *MSH2* gene was reported in 2002 by Whiteside et al. (21) in a patient with a hematologic malignancy and multiple café au lait spots. Another report by Bougeard et al. (18) in a large family of French origin described a compound heterozygous mutation in the *MSH2* gene. The father was shown to have a heterozygous deletion of exons 1 to 6, whereas the mother carried a 1 bp deletion in exon 3. No DNA from lymphocytes was available

Table 1. Microsatellite instability analysis comparing the patient's DNA extracted from blood and tumor tissue

Lab ID	Microsatellite instability assay												Final result	
	National Cancer Institute markers					Alternate markers					Quasimonomorphic markers			
	D2S123	D17S250	D5S346	BAT-25	BAT-26	D18S35	TP53-DI	D1S283	TP53-Penta	FGA	NR-21	NR-22		NR-24
	(tumor/normal)					(tumor/normal)					(tumor/normal)			
Proband- blood	210/212	147/162	110/121	122/124	117/119	102/119	120/122	162/195	122/122	195/207	102/102	139/139	130/131	Normal
Proband- tumor	MSI	MSI	110/121	MSI	117/119	MSI	MSI	MSI	MSI	MSI	MSI	MSI	MSI	MSI-high
				LOH			LOH							
Mother	212/226	147/162	108/110	122/124	117/119	104/119	116/120	162/195	122/127	193/207	102/102	139/139	130/132	Normal
Father	208/210	156/162	121/123	124/124	117/119	102/117	122/130	195/197	122/127	195/199	102/102	139/139	130/130	Normal

NOTE: Microsatellite instability was done using 13 markers. Microsatellite instability testing on tumor DNA compared with germ line DNA from the patient revealed microsatellite instability in more than 40% of the markers tested. No constitutional instability was found in this patient compared with samples from the parents.
Abbreviations: MSI, microsatellite instability; LOH, loss of heterozygosity.

from the affected children, but DNA extracted from the glioblastoma showed only a mutant allele, a 1 bp deletion in exon 3 found in the mother, strongly suggesting inheritance of the exon 1 to 6 deletion from the father as the nonamplifying allele. Further analysis using quantitative PCR confirmed this mutation. Neither parent had a personal history of cancer

although cancer was reported in distant relatives. These two reports supported biallelic inactivation of *MSH2* in Turcot syndrome.

The clinical presentation of the families reported with mismatch repair gene mutations varies widely, particularly in *PMS2* mutation families. In one Turcot family with no

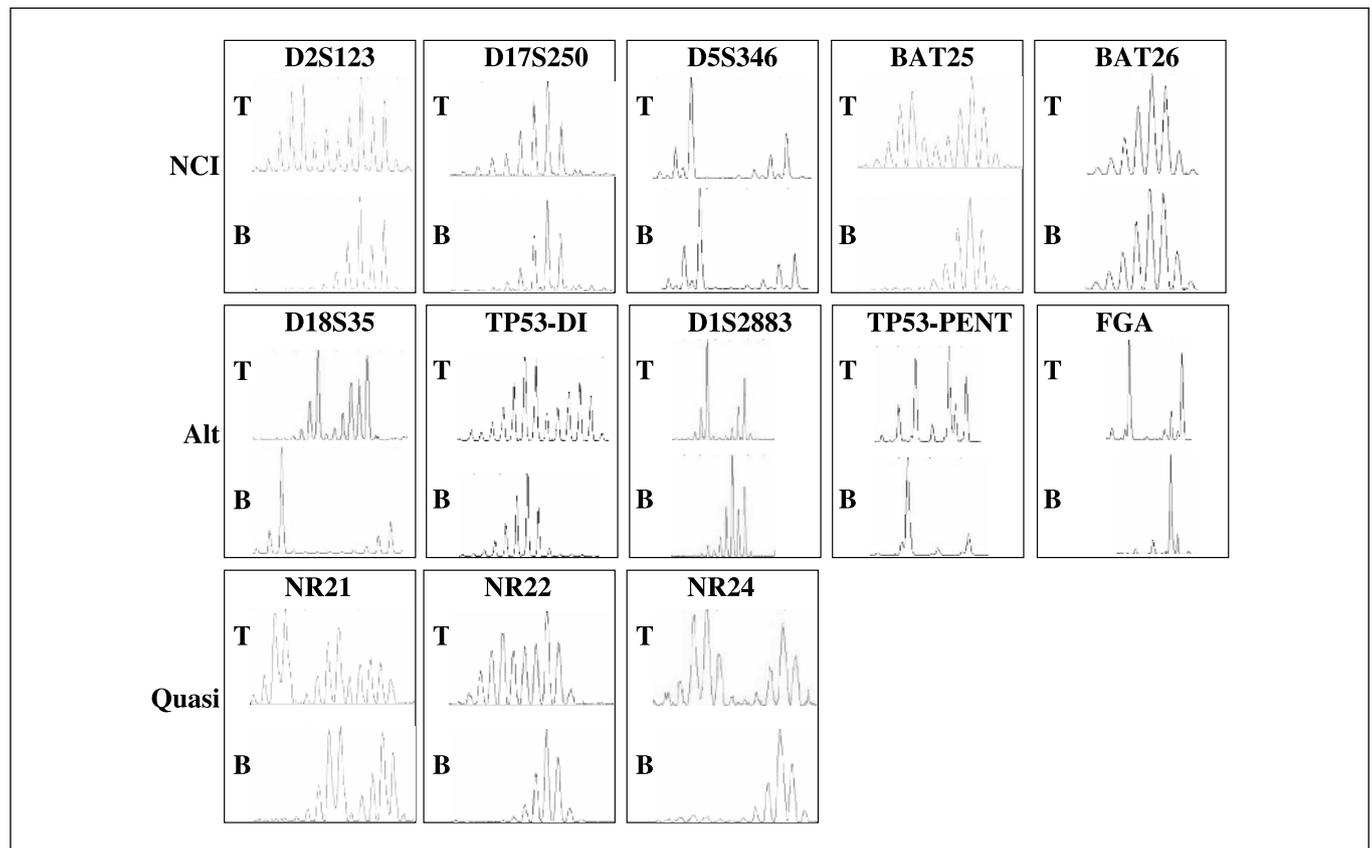


Fig. 3. Microsatellite markers using the National Cancer Institute, quasimonomorphic, and alternate markers. Each marker shows germ line and tumor DNA profiles. T, profile of DNA extracted from tumor tissue; B, profile of DNA extracted from blood.

parental consanguinity, two affected sisters, with no history of tumors in their parents, died of a brain tumor and of a colorectal tumor, respectively, at a very early age. On the other hand, some *PMS2* mutations might exert a dominant-negative effect, which was suggested by microsatellite instability in normal tissues from *PMS2* heterozygotes, as well as by the presence of café au lait spots in one patient. De Vos et al. (14) recently reported a family with an autosomal recessive inheritance with café au lait spots and childhood malignancy and biallelic mutation of *PMS2*. No cancer predisposition was noted in heterozygotes.

Our findings support a role for *MSH6* in Turcot syndrome and are consistent with an autosomal recessive mode of inheritance. *MSH6* is an integral component in the MutS complex. The first stage in the process of DNA repair is the binding of the MutS complex (*MSH6* and *MSH2*) to the mismatched bases in DNA, initiating the repair process. Thus, Turcot syndrome patients negative for *MLH1* and *MSH2* mutations should be screened for *MSH6* mutations. In our patient the tumor showed instability and loss of heterozygosity when compared with the constitutional DNA prepared from blood lymphocytes. Unlike the previous report describing constitutional instability in a patient with a *PMS2* mutation, we did not find evidence of germ line instability in our patient. However, these assays are hampered by the lack of clonal

populations in the peripheral blood sample. Thus, heterogeneous microsatellite instability would be missed in this analysis. Alternatively, these results might also be explained by the functional redundancy of *MSH6* in the MutS complex. Despite the absence of obvious microsatellite instability in the peripheral blood, the neurofibromatosis type 1 phenotype in this patient is consistent with a very high level of genetic instability in somatic tissues.

It is tempting to speculate that the *MSH6* mutation identified in this patient may represent a founder mutation in the Pakistani population because it was homozygous in this patient and the parents were nonconsanguineous. Haplotype analysis and population-based studies would be necessary to explore this possibility. Of note, no additional cancers have been reported in the extended family, with the exception of colorectal cancer in the maternal great uncle diagnosed at age 48. We interpret these findings to suggest that this mutation in the heterozygous state is expected to have relatively low penetrance. This contrasts with the very significant cancer incidence in the homozygous children. Thus, the effect of *MSH6* mutations on cancer incidence may significantly vary with the inheritance pattern. This case study of a Turcot syndrome family has firmly shown a role for *MSH6* in Turcot syndrome, thus widening the spectrum of genes involved in this disorder.

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