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Advances in Brief

A Homozygous Germ-Line Mutation in the Human *MSH2* Gene Predisposes to Hematological Malignancy and Multiple Café-au-Lait Spots[1]

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Abstract

Individuals with a germ-line mutation in one of the DNA mismatch repair (MMR) genes are at significant risk for colorectal cancer and other tumors. Three families have previously been reported with individuals homozygous for mutations in the MMR gene *MLH1* that are predicted to compromise MMR. These individuals develop hematological malignancies and/or neurofibromatosis type 1 at an early age. Here, in an individual, we demonstrate that a homozygous novel mutation in the MMR gene *MSH2* is associated with leukemia and multiple café-au-lait spots, a feature of neurofibromatosis type 1. Because the hematological malignancies observed in the individuals homozygous for the loss of MMR are reflective of the lymphomas seen in mice lacking MMR, the mice may provide a useful model for human neoplasia.

Introduction

DNA MMR[3] plays an essential role in the maintenance of genome stability. It is a postreplicative repair system that repairs normal or damaged single-base mismatches as well as insertions and deletions in the DNA (reviewed in Ref. 1). Individuals with germ-line mutations in one of the MMR genes (*MSH2*, *MSH6*, *MLH1*, *PMS2*, and *PMS1*) are at risk of developing HNPCC on loss of the normal allele. An absence of MMR results in a strong "mutator" phenotype with somatic mutation frequencies several hundredfold to a thousandfold higher than those of wild type (2), which is most readily visible as MSI. Microsatellites are good indicators of repair proficiency because their repetitive nature makes them prone to replicative slippages in the absence of MMR. It is hypothesized that acquisition of subsequent mutations within key tumor suppressor genes or genes involved in cell growth occurs at an increased rate in the absence of MMR, resulting in malignancy (3).

An association does exist between loss of MMR in some human lymphomas and leukemias (4–10). Although the development of lymphoma in human HNPCC kindreds is not common, it has been observed in the HNPCC variant Muir-Torre syndrome and in Turcot's syndrome with MMR mutations (11, 12). One patient with a dominant negative mutation in *PMS2* presented with early-onset non-Hodgkin's lymphoma, further strengthening the connection between MMR gene mutations and the path to hematological malignancy (13).

Most recently, three HNPCC families have been reported with consanguinity resulting in homozygosity of two inherited *MLH1* mutant alleles predicted to result in a lack of MMR activity (14–16). Notably, five homozygous children in two of the families developed leukemia or lymphoma. The homozygous offspring in all families also were diagnosed with NF-1 with no family history of the disorder. The other published example of constitutional loss of MMR is a heterozygous *MLH1* mutation (13). However, this mutation is proposed to have dominant negative activity and may have had additional effects on the cell besides inactivating MMR.

Here we report a fourth example of inherited homozygous deficiency of MMR. A novel *MSH2* mutation that results in exon skipping and lack of MSH2 protein was inherited by a child presenting with ALL and features of NF-1. This individual case therefore suggests that it is the general lack of MMR from conception that underlies hematological malignancy in the rare MMR-deficient individuals and that the occurrence of sporadic NF-1 in these individuals may also be associated with a constitutive lack of MMR.

Materials and Methods

Patient Samples. The proband was referred at 24 months of age with a height of 86 cm (50th centile), a weight of 9.4 kg (well below the 3rd centile), and an occipito-frontal circumference of 46.5 cm (2nd centile). The patient presented with failure to thrive and a gastrointestinal infection that led to the diagnosis of ALL (T-cell ALL) and IgA deficiency. He was also noted to have multiple café-au-lait spots of a size and number sufficient to satisfy one of the criteria for the diagnosis of NF-1 that had been present from birth. However, he had no neurofibromas, axillary or inguinal freckling, Lisch nodules, optic glioma, sphenoid wing dysplasia, pseudoarthrosis, or previous history of malignancy. In the absence of a second diagnostic criterion, the diagnosis of NF-1 was not completely met at the proband's young age. There was no family history of NF-1 or cancers indicative of HNPCC. Parents are nonconsanguineous but are from the same ethnic, religious, and geographic background.

DNA Analysis. Genomic DNA was isolated from peripheral blood using standard phenol chloroform extraction and precipitation with 4 M ammonium acetate and isopropanol. DNA was dissolved in low TE buffer [10 mM Tris and 1 mM EDTA (pH 8.0)]. Exonic and intronic sequences flanking each *MSH2* exon were amplified by PCR using primers and conditions as described previously.[4] PCR products were sequenced by ^{33}P cycle sequencing (thermosequenase radiolabeled terminator cycle sequencing kit; United States Biochemical). Sequencing reactions were run on 6% polyacrylamide gels.

Semiquantitative PCR was carried out with primers from the *MSH2*, *ATRX*, and *CF* genes using 8 ng of template and the following primers: (*a*) MSH2 Ex11F, CATTGCTTCTAGTACATTT; (*b*) MSH2 Ex11R, CAGGTGACATTCAGAACATTA; (*c*) ATRX Di, GGATTATGAGGTATTTCATGTCT; (*d*) ATRX DRi, CTCAAAGGCCTGGTATATGG; (*e*) M1101KF, CAACACTGCGCTGGTTCCATA; and (*f*) M1101KR, ATAACCTATAGAATGCAGCA. PCR conditions for all reactions were 95°C 5 min; (95°C 45 s, 60°C 45 s, 72°C 45 s) 26, 27, 28 and 29 cycles; 72°C 2 min. Samples were run on an Applied Biosystems Prism 377 automated sequencer.

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[3] The abbreviations used are: MMR, mismatch repair; HNPCC, hereditary nonpolyposis colorectal cancer; NF-1, neurofibromatosis type 1; ALL, acute lymphocytic leukemia; MSI, microsatellite instability.

[4] www.hgu.mrc.uk/Users/Malcolm.Dunlop/MMRprim.htm.

Microsatellites D2S123, BAT26, BAT25, D5S346, and D17S250 were amplified using previously published primers (IRD700 labeled) and conditions (17). PCR products were run on a Licor automated sequencer. The causative CAG repeat and adjacent 3′ polymorphic CCG repeat within the Huntington disease gene (*IT-15*) were amplified using primers and conditions published previously (18) labeled with 4,7,2′,7′-tetrachloro-6-carboxyfluorescein. PCR was performed using 5 cycles of 99.9°C for 5 s, 55°C for 30 s, and 72°C for 1 min; 30 cycles of 99.9°C for 3 s, 55°C for 30 s, and 72°C for 1 min; and 72°C for 10 min. The CGG repeat in the 5′-untranslated region of the *FMR-1* gene was amplified (19) using primers labeled with 4,7,2′,7′-tetrachloro-6-carboxyfluorescein. Amplified products were analyzed on an Applied Biosystems 310 Genetic Analyzer with GS500 size standard (Applied Biosystems) and GeneScan and GenoTyper analysis software (Applied Biosystems). The TG variable repeat in tandem with a poly(T) tract from the cystic fibrosis transmembrane conductance regulator gene (*CFTR*) exon 9 (20) was analyzed using a multiplex Amplification Refractory Mutation System approach using primers and PCR conditions described elsewhere.[5]

RNA and Immunoblotting. RNA and total protein extracts were isolated from a lymphoblastoid cell line established from the patient, and cDNA was generated using the SuperScript First Strand Synthesis System for reverse transcription-PCR (Life Technologies, Inc.) according to the manufacturer's instructions. Primers within exon 10 (5′-TATTACTTTCGTGTAACCTG-3′) and exon 12 (5′-ACAGGTGCTCCATTTGACACG-3′) were used to amplify across the predicted splice junction using the following PCR conditions: 95°C for 3 min; 10 cycles of 95°C for 30 s, 65°C for 30 s, and 72°C for 30 s; 39 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s; and 72°C for 1 min. The PCR product was sequenced.

Whole cell lysates were resolved by discontinuous 8% SDS-PAGE and transferred to polyvinylidene difluoride membranes. Proteins were detected using the following antibodies: (*a*) purified mouse antihuman MSH2 monoclonal antibody (PharMingen); (*b*) purified mouse antihuman PMS2 monoclonal antibody (PharMingen); and (*c*) horseradish peroxidase-conjugated secondary antibody (goat antimouse; BD Biosciences). Antibody binding was visualized using enhanced chemiluminescence (NEN Western Blot Chemiluminescence Reagent Plus Kit; Life Science Product).

Results and Discussion

The exons of the MMR genes *MSH2* and *MLH1* were sequenced in the proband presenting with ALL and multiple café-au-lait spots (Fig. 1). This was undertaken despite an absence of cancer in the family inconsistent with inheritance of a germ-line MMR mutation because *MLH1* mutations were previously identified in individuals with a similar phenotype (14–16). A novel, homozygous G to A transition mutation was found in the proband in the invariant G of the intron 10 splice acceptor of the *MSH2* gene (Fig. 2). This mutation at position 1662–1 bp (relative to the ATG translational start site) was predicted to result in skipping of exon 11 to exon 12, with out-of-frame translation of the mutant mRNA resulting in a truncated, nonfunctional protein (Fig. 3*A*). This was confirmed by sequencing cDNA from the proband using primers in exon 10 and 12 (Fig. 3*B*) and demonstrating a lack of MSH2 protein by Western blot (Fig. 3*C*).

The parents are both heterozygous for the G to A transition mutation at position 1662–1 bp. To demonstrate that the proband is homozygous and not hemizygous for the *MSH2* mutation, semiquantitative PCR was performed using primers for *MSH2* exon 11, a region within the cystic fibrosis gene (marker M1101; chromosome 7), and for the *ATRX* gene, an X-linked gene. The signal strength for the markers in the proband (male) suggest that the *MSH2* exon is present in two copies, similar to the autosomal marker M1101, rather than the half-strength signal observed for the X-linked gene *ATRX* (Fig. 4).

This particular *MSH2* mutation has not been reported previously in HNPCC individuals.[6] Because the parents share the same ethnic, religious, and geographic background and the mutation is unique, this is likely an ancestral mutation, and the parents are probably related. Interestingly, no cancers have been identified in this family, despite confirmed germ-line inheritance of an inactivating MMR gene in both parents (Fig. 1). The mutation results in the loss of a *Bbv*I restriction enzyme site that was used to determine that the mutation was not seen in 83 normal control chromosomes.

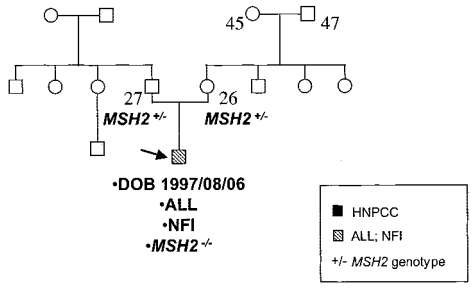


Fig. 1. Pedigree of the family containing the proband, who presented with ALL and multiple café-au-lait spots. Genotypes for *MSH2* and the ages of the individuals are shown.

Because MSH2 is the key MMR protein required for recognition of a DNA mismatch and subsequent repair, the proband is predicted to completely lack any MMR activity. The heterozygous parents are at risk of developing HNPCC and require counseling and screening. The young age of the parents and grandparents or an occult malignancy may explain the lack of observed cancer in this pedigree at the time of ascertainment. Environmental factors play an important role in loss of the wild-type allele and in risk of tumor development in HNPCC carriers, therefore the diet and/or lifestyle of this particular family may be protective of the occurrence of such a mutational event. The family will be followed closely for development of malignancies.

A lack of MMR in neoplastic cells is associated with instability at repetitive DNA sequences. We used microsatellite markers BAT26, BAT25, D5S346, D2S123, and D17S250 as well as a TG variable repeat in tandem with a poly(T) tract at the splice acceptor site of the cystic fibrosis transmembrane conductance regulator gene (*CFTR*) exon 9 to test instability in lymphocyte DNA from the MMR-deficient patient. Interestingly, and in contrast to other *MLH1* homozygotes (14, 16), no instabilities were observed in the proband (data not shown). However, because leukemic cells cannot be tested alone for MSI, we cannot exclude the fact that MSI may be present in tumorigenic cells from this patient. However, this is consistent with the results from *MSH2*-deficient mice, in which instability was seen in thymic lymphomas but not in the adjacent normal tissue (21). Trinucleotide repeats can also be unstable; therefore, we determined the trinucleotide repeat length for the Huntington disease and fragile X repeats in the proband. No instability was seen in the MMR-deficient proband (data not shown), supporting other evidence that MMR does not play a role in expansion of trinucleotide repeats (22).

The role of MSH2 in hematological malignancy is not clear. It is

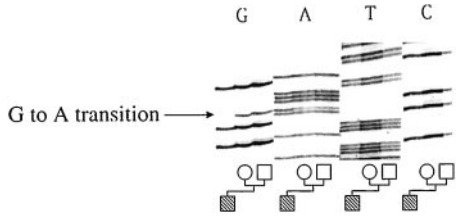
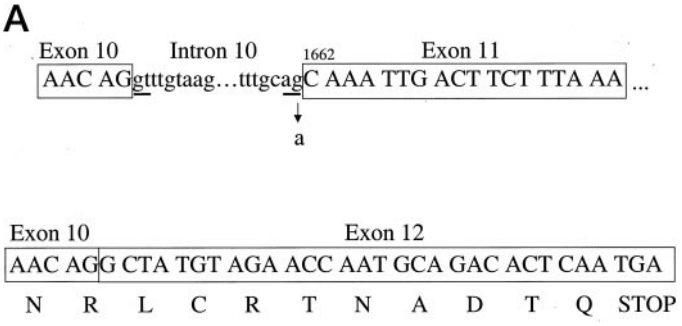


Fig. 2. Partial sequence of *MSH2* exon 11 and flanking intronic sequence in the proband and parents, demonstrating the heterozygous and homozygous inheritance of the G to A transition at 1662–1 bp.

[5] M. J. Somerville, A. V. Ng, S. M. Hasse, M. Hicks, K. A. Sprysak, B. G. Elyas, R. Tomaszewski, L. M. Vicen-Wyhony. High throughput screening of both components of the CFTR intron 8 polyvariant locus, manuscript in preparation.

[6] www.nfdht.nl.

possible that genes involved in T- and B-cell development contain targets for mutation that remain unrepaired in the absence of MMR. Several such genes (such as *TGFβRII*, *IGFRII*, *BAX*, and *PTEN*) have successfully been identified in HNPCC colon tumors (23). These genes contain mononucleotide repeats within coding regions that result in a truncated, nonfunctional protein after polymerase frameshift errors remain uncorrected in the absence of MMR. Alternatively, a role for MSH2 in signaling apoptosis has recently been suggested (24, 25), and lack of MSH2 may hinder initiation of cell death pathways and contribute to neoplasia.



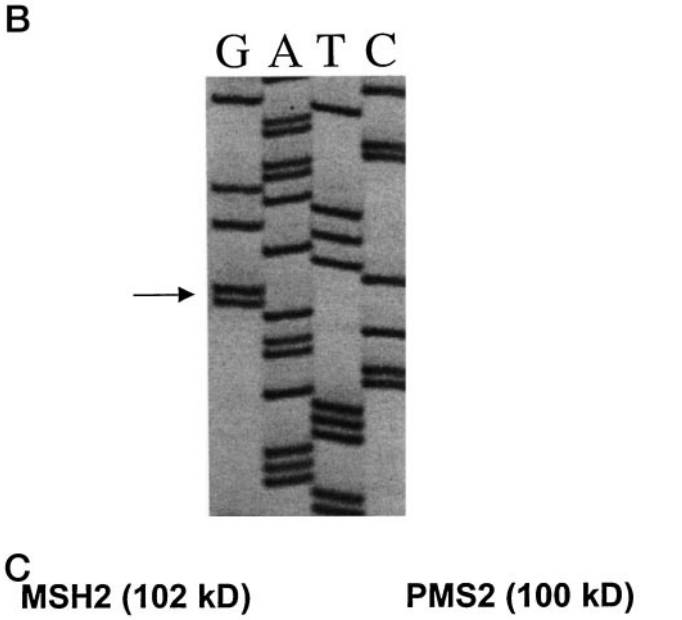


Fig. 3. *A*, schematic demonstrating the position of the novel *MSH2* mutation inherited by the proband. The *MSH2* exons are *boxed*, and the ends of the intron 10 sequences are shown, including the G to A transition at position 1662 (relative to the ATG translation start site)-1 bp. The predicted effect of such a mutation on splicing and the resulting mRNA and truncated, nonfunctional protein is shown below. *B*, sequence of cDNA across exon 10 and 12, demonstrating the skipping of exon 11. An *arrow* marks the junction between exon 10 and exon 12. *C*, Western blotting using MSH2 antibody demonstrates a lack of MSH2 protein (M_r 102,000) in the proband (*Lane 1*) compared with a normal control (*Lane 2*). PMS2 was used as a control antibody on the same blot.

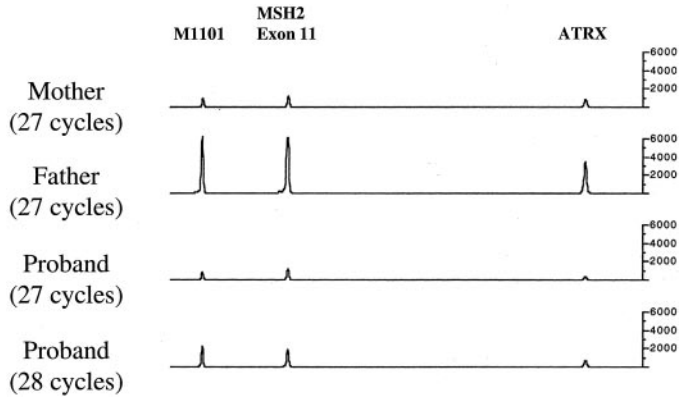


Fig. 4. Semiquantitative PCR using primer sets for MSH2 exon 11, an autosomal locus (*M1101*), and an X-linked locus (*ATRX*) for parents and proband demonstrate homozygosity for the MSH2 exon 11 locus in the proband (male) *versus* hemizygosity.

Mouse models for MMR deficiency have been generated (26). More than two thirds of the *Msh2*$^{-/-}$ mice succumb to thymic lymphomas, with 50% of the colony developing tumors by 5 months of age (27). The development of hematological malignancy in MMR-deficient individuals, similar to that seen in the homozygous knockout mouse, suggests that the mouse may be an excellent model to study human lymphomagenesis.

We describe the fourth known example of MMR deficiency in a homozygous state that is associated with hematological malignancy and multiple café-au-lait spots. We suspect that the proband may develop other features that allow the clinical diagnosis of NF-1 to be confirmed in the future. If so, our patient's phenotype would support previous claims that lack of MMR and the development of NF-1 are likely a consequence of the mutator phenotype in the absence of MMR. This is the first patient described with a complete loss of MSH2. This case demonstrates that hematological malignancy can arise in the absence of MLH1 and MSH2 and is therefore likely a consequence of the lack of activity of the MMR complex rather than due to specific individual roles of MLH1 or MSH2.

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