Mosaic Epigenetic Inheritance as a Cause of Early-Onset Colorectal Cancer

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A ssessment of familial cancer risk is typically based on rare, highly penetrant alleles at known predisposition genes,1,2 yet many people with histories suspicious for familial cancer have no pathogenic germline mutation.3 Some of these unexplained cases result from constitutional epimutations, in which promoter hypermethylation in a specific allele causes soma-wide transcriptional gene inactivation. This is best exemplified in the epigenetic silencing of DNA mismatch repair (MMR) genes $MLH1$ or $MSH2$ in Lynch syndrome,4,5 although this syndrome usually results from a constitutional sequence mutation. Somatic loss of the other allele subsequently causes MMR defects, the development of microsatellite instability, and tumorigenesis.1

Constitutional epimutations sometimes co-segregate with a cis sequence mutation,6-9 and such epimutations in $MLH1$ produce Lynch syndrome with an autosomal-dominant inheritance pattern.7-9 However, most constitutional $MLH1$ epimutations occur without a cis genetic mutation and show non-Mendelian inheritance.5,10,11 Here, dense constitutional hemiallelic methylation occurs in individuals from successive generations, yet other family members with the same genetic allele develop neither methylation nor cancer. The mechanism(s) underlying this non-Mendelian pattern remains uncertain.5

Autosomal-dominant diseases can exist as mosaic forms, whereby a postzygotic mutation gives rise to mixed popula-tions of cells throughout the body. Mosaicism can exist in the soma alone, the germline, or both (gonosomal), with the latter 2 types potentially causing disease inheritance from asymptomatic parents.12

Herein we describe a family displaying non-Mendelian inheritance of an $MLH1$ epimutation, in which an asymptomatic mother with low-level $MLH1$ methylation produced a son who developed early-onset cancer through epigenetic silencing of his maternal $MLH1$ allele. We propose a gonosomal mosaic model that may account for transmission of the epimutation and that has important implications for the clinical assessment of individuals showing low-level constitutional epimutations.

Methods

A 29-year-old man (proband/III-7) (Figure 1A) presented with a moderately differentiated colonic adenocarcinoma. His brother (III-6) had died of acute myeloid leukemia at age 17 years. His sister (III-8) reported hyperplastic polyps and a low-grade tubular adenoma at age 24 years (colonic, normal MMR staining), whereas his maternal grandfather (I-1) had microsatellite-stable colorectal cancer at age 67 years.

Fresh samples of peripheral blood, hair, saliva, and buccal mucosa were obtained from the proband and his mother, or saliva-only from other living relatives. Archived bone
marrow aspirates (taken to evaluate candidacy for bone marrow transplantation) were available from the proband (age 13 years) and his brother (age 16 years). Formalin-fixed paraffin-embedded tumor tissues were obtained from the proband, his sister, and maternal grandfather. The study was approved by the Human Research Ethics Committee of South Eastern Sydney Local Health District. All participants in this study signed an ethics-approved informed consent form.

Relevant samples were assessed for MMR protein expression, microsatellite instability, BRAF status, extent and distribution of DNA methylation, haplotype, DNA sequence and re-

At a Glance
- A man with early-onset colorectal cancer inherited a constitutional MLH1 epimutation.
- Transgenerational inheritance of an MLH1 epimutation on the maternal allele occurred without a DNA sequence change.
- MLH1 promoter methylation level increased across 1 generation from approximately 5% in a parent to 50% in a child, resulting in transcriptional inactivation of the maternal copy of MLH1.
- Persons with low-level MLH1 promoter methylation should be considered gonosomal mosaics.
- Low-level MLH1 promoter methylation in a parent can be associated with substantial cancer risk in offspring.

Figure 1. A Stable Epimutation Causes Constitutional Loss of Expression in a Man With Early-Onset Colorectal Cancer

A, Family pedigree with haplotypes shown adjacent to each family member. The boxed nucleotide sequences indicate which family members inherited the haplotype that harbored the epimutation in the proband (III-7, arrowhead) and his mother. The boldface A in the proband’s and mother’s haplotype indicates the genotype of the methylated allele at rs3774343 and rs4647224. CRC indicates colorectal cancer; dg, age at cancer diagnosis; dashes, single-nucleotide deletion at this location; and Me, methylation. B, Mean methylation levels detected in constitutional and tumor DNA of the proband by means of pyrosequencing. C, Pyrograms showing the relative levels of MLH1 expression from each allele in the proband. The heterozygous germline genotype is shown in the bottom pyrogram. The reference sequence for the pyrosequencing assay is shown at the top.

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Results

The proband’s tumor showed loss of MLH1/PMS2 (but not MSH2/MSH6) by means of immunohistochemical analysis, high-level microsatellite instability (unstable at BAT25, BAT26, BAT40, D17S250; stable at D2S123, D5S346), and was wild type for BRAF. There were no germline mutations of MLH1 in exons or splice sites, and all exons had normal copy number. He was heterozygous for the 3’ untranslated region CTT deletion variant in MLH1 (NM_000249.2: c.*35,*37del), known to be noncausal in Lynch syndrome.13

The proband’s MLH1 promoter (eFigure 1 in the Supplement) showed evidence of a constitutional epimutation, with hypermethylation (27%-56%) in normal tissues from all 3 germ layers, as well as the tumor (Figure 1B and eFigure 2A in the Supplement). Allelic bisulfite sequencing of the

MLH1

sequencing

A
Chromosome 3: 37030000

MLH1

EPM2AIP1

SNPs

II-3

III-7

Repeats

Sequence: T/CCCTCCACA

Proband’s mother (II-3)

52%

C: 48%

51%

C: 49%

GAGYGGATAGYGATTTTTAAYGYGTAAGYGTA

B

Sequence: GACYGGATAGYGATTTTTAAYGYGTAAGYGTA

Periperal blood

Saliva

Hair

Mean, 4%

Mean, 3%

Mean, 5%

MLH1 methylation levels in proband’s mother

MLH1 methylation in family members

A, The 10-kb region sequenced in germline DNA of the proband and his mother. The pyrograms show the copy number of each allele in MLH1 intron 1. SNP indicates single-nucleotide polymorphism. B, Pyrograms showing MLH1 CpG island methylation levels in tissues derived from 3 germ layers of the proband’s mother. The inset in the top pyrogram is a magnification of 1 CpG site showing the low-level methylation. C, Levels of MLH1 CpG island methylation in various family members measured with quantitative methylation-specific polymerase chain reaction. UB indicates unmethylated DNA from the peripheral blood of a healthy control.

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**MLH1** promoter in fresh blood, saliva, hair, and archived bone marrow samples showed dense methylation limited to the maternally inherited allele (eFigure 2B in the Supplement) that was also transcriptionally silent (Figure 1C). There were no sequence alterations from the 3’ untranslated region of EPM2AIP1 to exon 2 of **MLH1** and no copy number change in intron 1 (Figure 2A). Heterozygosity found at 4 **MLH1** single-nucleotide polymorphisms (Figure 1A) and 3 sequence-tagged site markers (eFigure 2C in the Supplement) indicated no large genomic deletion incorporating this locus. His tumor showed no loss of heterozygosity at informative markers (D3S1277, D3S3685, rs1799977, rs4647224), suggesting inactivation of the second allele by point mutation or small indel. Together, these data show that the proband was predisposed to cancer by a constitutional **MLH1** epimutation on his maternally inherited allele (eFigure 2D in the Supplement).

Single-nucleotide polymorphism genotyping of family members spanning 3 generations (Figure 1A) showed that the relevant haplotype was transmitted from the grandfather (I-1) to his son (II-4) and daughter (II-3), who then passed it on to her daughter (III-8) and son (III-7). Pyrosequencing (Figure 2B) and quantitative methylation-specific polymerase chain reaction (Figure 2C) showed that the proband’s mother had approximately 2% to 5% methylation in somatic tissues derived from all germ layers. No methylation was detected in 4 other family members (Figure 2C), including the proband’s sister, who inherited the same maternal allele, and his brother, who inherited the other maternal allele. Both siblings had biallelic expression (eFigure 3A in the Supplement).

The proband’s mother showed dense methylation of only approximately 5% of **MLH1** promoter molecules (eFigure 3B in the Supplement) that was confined to the same allele transmitted to the proband (eFigures 3C and 3D in the Supplement). With the exception of a novel single-nucleotide variant in intron 1 (hg19, chr3:37,036,255), the mother showed no sequence or copy number changes (Figure 2A). Sequence-tagged site marker analysis found no recombinant event accounting for the non-Mendelian inheritance of the epimutation (eFigure 2C in the Supplement).
Discussion

This man’s early-onset colon cancer was due to a constitutional MLH1 epimutation transmitted through the maternal germline from a woman with mosaic, dense hemiallelic MLH1 methylation in only a small proportion of her cells. The epimutation occurred in a promoter without apparent genetic alteration.

Comparison of the proband’s tissues with archived bone marrow aspirate taken 16 years earlier showed no change in the level of methylation and loss of MLH1 expression from the maternally inherited allele. This supports the theory that constitutional epimutations arise early in development, and are present from birth, and are stable throughout life.

This family offers a new insight into the origin, transmission, and clinical significance of epimutations displaying non-Mendelian inheritance. We propose that the findings in this case can be explained by a de novo event occurring in an embryonic stem cell in the mother as she developed in utero. That abnormal embryonic stem cell gave rise to the dense methylation and loss of expression seen in approximately 5% of her soma and germ cells, whereas her remaining cells derived from unaffected embryonic stem cells showed normal MLH1 expression. This “gonosomal mosaic” model predicts that the proband arose from fertilization of a rare oocyte with the epimutation (Figure 3), whereas his sister inherited the same haplotype from an oocyte without the epimutation, resulting in non-Mendelian inheritance (eFigure 3E in the Supplement).

Conclusions

Mosaic disorders pose challenges to clinicians assessing familial cancer risk. Our study indicates that persons presenting with low-level MLH1 methylation may be gonosomal mosaics, and methylation screening of offspring may accurately identify (or exclude) those with constitutional epimutations and high cancer risk. Conversely, for individuals with dense hemi-allelic constitutional hypermethylation of the MLH1 promoter, it appears reasonable to evaluate their parents for evidence of gonosomal mosaicism, using appropriately sensitive assays. In terms of individual cancer risk, the significance of mosaic MLH1 promoter methylation requires further evaluation. Whereas low-level constitutional methylation occurs in other cancer predisposition genes including BRCA1 and CDKN2A, these cases show negligible cancer risk. Nevertheless, the phenomenon that we have identified has clear implications for assessing cancer risk in families in which individuals show low-level promoter hypermethylation of cancer predisposition genes in constitutional DNA.

ARTICLE INFORMATION

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REFERENCES


