

Characterisation of a Portuguese origin founder missense variant in MSH6

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Abstract

In certain populations, founder pathogenic variants are a common cause of Lynch syndrome. Here, we report the identification of a novel founder variant, NM_000179.3 (*MSH6*):c.2061T > G (p.Cys687Trp). Our study examined 14 probands and 18 additional family members who carry this variant. With one exception, haplotype data are consistent with a single origin for all heterozygotes. We investigated the clinicopathological consequences of this variant and found that, as previously reported for other *MSH6* pathogenic variants a) presence of the founder pathogenic variant is not reliably associated with loss of *MSH6* expression or microsatellite instability and b) endometrial cancer is the most common associated phenotype. Based on our findings, we recommend reclassifying *MSH6* c.2061T > G from missense variant of unknown significance to likely pathogenic according to CanVIG-UK guidelines.

Introduction

Lynch syndrome (LS) is an autosomal dominant cancer predisposition syndrome which increases risk for certain cancers, most commonly colorectal (CRC) and endometrial cancer (EC). LS is most often caused by germline pathogenic variants in the DNA mismatch repair (MMR) genes *MLH1*, *MSH2*, *PMS2*, and *MSH6*; *MSH6* accounts for about 18% of cases [1, 2]. Compared with *MLH1* and *MSH2*, *MSH6*-associated LS is less penetrant and often diagnosed at later ages [3]. Notably, the risk for EC is significantly greater than the risk for CRC in female *MSH6* heterozygotes, which is not the case for other MMR genes [3].

MMR genes are tumor suppressors. Loss of MMR expression is a hallmark of LS tumors [1]. Testing for MMR deficiency through immunohistochemical (IHC) staining of MMR proteins or microsatellite instability (MSI) is standard in certain tumor types to identify patients at risk for LS. MMR status can also be used to assess germline variants in MMR genes [1]; however, the clinical utility of MMR/MSI status in screening for patients at risk for LS and subsequent variant interpretation varies. Malignancies resulting from *MLH1* or *MSH2* germline variants are more likely to demonstrate MMR deficiency than those resulting from *MSH6* or *PMS2* germline variants [1, 4]. Additionally, nearly half of reported *MSH6* variants are missense [5]. Classification of *MSH6* missense variants is further complicated by the often-attenuated LS phenotype, making co-segregation studies less convincing.

Founder variants are genetic variants present at increased frequency in certain populations, usually due to genetic drift from a common ancestor. *MSH6* pathogenic founder variants (PFVs) have been identified in the Ashkenazi Jewish and French-Canadian populations [6, 7]. Portuguese PFVs have been described in *MLH1* and in *MSH2* [8].

Historically, characterization of PFVs allowed first-line screening strategies to lower the cost of molecular diagnosis. With the decreasing costs of testing, many centers now favour full gene sequencing via multi-gene panels to avoid multiple lines of screening. Identifying and correctly interpreting germline variants remains important to ensure proper screening for LS families.

We report on the missense variant *MSH6* c.2061T > G, which we first identified in a Montreal-based woman of Portuguese descent with multiple primary cancers consistent with LS. After contacting researchers and commercial laboratories, we identified 13 additional probands with this variant. Where possible, we collected information on MSI, IHC and loss of heterozygosity as well as genealogy and segregation of the variant. We also performed haplotype analysis to investigate a potential founder origin of the variant.

Materials and Methods

Patients and Samples

This study includes 14 apparently unrelated families (Table S1). All are either of Portuguese descent (n = 10) or have origins consistent with Portuguese descent (n = 4; Table 1). Data was collected from centers in Canada, the USA, Portugal, and France. Each family includes at least one individual who tested positive for NM_000179.3(*MSH6*):c.2061T > G (p.Cys687Trp). All participants provided informed consent to their respective centres to participate in research. Ethical approval for this study was granted by the McGill University Health Centre Research Ethics Board (studies MP-37-2019-4865 and MP-37-2023-9071).

Molecular Results

Germline testing for 14 probands was performed by each centre using in-house or external laboratories. Segregation analysis was performed in 18 additional individuals from five families (Table 1). Two tumours were also analyzed for somatic mutations (Table 1).

Analysis of MMR Status

All centers used standard procedures to analyze healthy and cancerous tissues. MMR proficiency was analyzed by IHC staining for MLH1, MSH2, MSH6, and PMS2. Biocartis MSI (Mechelen, Belgium) was employed in two cases (Table 1).

Haplotype analysis

Genotyping of 15 microsatellite markers was performed using 20 DNA samples from ten families (Table 2). These markers covered the region 12.3 Mb upstream and 9.5 Mb downstream of *MSH6*. When available, segregation analysis was used to phase alleles and establish haplotypes, as previously described [6].

Results

Characterization of families with MSH6 c.2061T > G

Of the 14 families, one fulfilled Amsterdam criteria, four fulfilled Amsterdam II criteria, and six fulfilled Bethesda criteria (Table S1). No additional family history data was available for two of the remaining cases. Five of the seven families identified at centres in Portugal originate from the Leiria District of Portugal, situated north of Lisbon. One additional family has origins in a nearby region.

The variant was identified in 23 individuals (Table 1). The variant co-segregated with a LS-spectrum cancer in 15 heterozygotes with an average age of 52.2 (range: 34–81) years at first diagnosis. The variant was also identified in an individual with a clinical diagnosis of constitutional mismatch repair deficiency (CMMRD) who carries a second *MSH6* VUS *in trans* with c.2061T > G (Table 1) [9]. This variant was additionally present in two individuals with a personal history of at least one colorectal polyp (Table 1). Four individuals are, to our knowledge, unaffected to date.

Clinicopathological features

The most common diagnosis in heterozygous females (9/16) was EC with average age at diagnosis of 56.1 (range: 43–82). The most common diagnosis in heterozygous males was CRC (4/6) with average age at diagnosis of 51.3 (range: 34–69). Of the 15 tumours evaluated by IHC, six (40%) demonstrated loss of MSH6 expression. Loss or retention of MSH6 was not consistent across tumours in individuals with multiple LS-spectrum cancers (Table 1, Fig. 1). Two of the tumours that retained MSH6 were microsatellite stable (Table 1). Two tumours with loss of MSH6 had somatic genetic second hits. The proband of Family VIII demonstrated loss of MSH6 expression in non-cancerous tissue, consistent with the clinical diagnosis CMMRD [9].

Variant Interpretation

We employed the Bayesian point system described in Tavtigian et al [10] to interpret our variant according to both the ClinGen Variant Curation Expert Panel specifications for the American College of Medical Genetics (ACMG) 2015 recommendations and the MMR-specific CanVIG-UK guidelines [11, 12]. *MSH6* c.2061T > G is classified as a “Variant of Uncertain Significance” per the ACMG criteria but as “Likely Pathogenic” according to the CanVIG-UK guidelines (Table S2).

Haplotype analysis

A shared haplotype was identified in 15 heterozygous individuals from 9 families (Table 2). This conserved haplotype spans a maximum of 10.4 Mb and a minimum of 4.9 Mb. One additional affected heterozygote (Family V) carried some alleles in common with this haplotype, suggesting a possible distant connection to the other carrier families. The provided sample was confirmed to carry *MSH6* c.2061T > G by Sanger sequencing, but no additional family V members were available for genotyping and haplotyping.

Discussion

This study evaluated the clinical impact of a novel Portuguese missense founder variant causing LS, *MSH6* c.2061T > G, in 14 families. We identified a haplotype shared among nine families, suggesting a common ancestor. Based on the origins of several families, this variant likely originated in the Leiria District of Portugal.

We first identified this variant in 2008 as a VUS in a proband (Family I) with a convincing personal history of LS-spectrum tumours; however, not all tumours demonstrated loss of *MSH6* (Table 1, Fig. 1). The variant has since been identified in thirteen additional families, the majority of which (11/14) meet at least one of the clinical criteria for LS (Table S1). Despite this, it has been difficult to clarify this variant's pathogenicity. Like approximately 70% of MMR VUS, the variant results from a missense mutation, which at its foundation makes interpretation more difficult [13]. Missense variants in the MMR genes are statistically more likely to demonstrate unexpected staining patterns than truncating variants, possibly due to the presence of non-functional protein that cannot be differentiated from functional protein through IHC [5].

Adding to the challenge, the degree of MSI is lower in *MSH6*-associated CRCs and the most common cancer in female heterozygotes (EC) is more likely to have inconsistent MMR/MSI results when compared to the other MMR genes [14, 15]. This begs the question of whether MSI/IHC of tumour tissue is a sufficient and effective screening method for the less-penetrant LS genes. This may also explain why *MSH6* has the highest proportion of VUS among the MMR genes [16]. Nevertheless, MSI/IHC evidence is still considered a hallmark feature of tumours in all LS patients, and MSI/IHC results are considered important evidence of pathogenicity in both ACMG and CanVIG-UK criteria [11, 12]

We present the third known Portuguese founder variant causing LS, and the first in *MSH6*. This variant is characterized by classic *MSH6*-associated LS, including incomplete penetrance, an often-attenuated phenotype, and higher risk for EC than CRC in females. As observed for other missense variants in MMR genes and studies of *MSH6*-associated LS specifically, this variant does not reliably lead to MSI or loss of *MSH6* expression by IHC in tumours [5, 14, 15]) (Fig. 1, Figure S1, Figure S2). The emphasis of MMR proficiency as evidence against pathogenicity under the ACMG criteria is therefore in direct opposition to substantial prior research. It is plausible that similar missense variants have gone undiagnosed in several families who would benefit from recommended LS screening regimens. In fact, some are calling for the division of LS into gene-specific syndromes to avoid this very issue [17]. We hope the observations from our study help to facilitate the identification of such families and prompts a broader discussion of the limitations of IHC and MSI as a method for stratifying the risk of LS.

This study has limitations, including our inability to include allelic data (PM3) despite the variant being present in an individual with a clinical diagnosis of CMMRD, due to its being in *trans* with a second *MSH6* VUS. There are also currently no functional data available to support pathogenicity of *MSH6* c.2061T > G. Nevertheless, the variant reaches a pathogenic score using CanVIG-UK criteria.

Declarations

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Author contributions

KM prepared the manuscript, tables, and figures. LW performed variant interpretation and reviewed the manuscript. CD and NH designed and performed haplotype analyses and also contributed and reviewed the manuscript. JM, JLE, KS, SM, MRT, CA, IF, LGR, OCT, KM, and AC provided cases, data and samples for haplotype analysis where possible, and reviewed the manuscript. GC performed molecular characterization for Family I and reviewed the manuscript. VM provided expertise on IHC images and reviewed the manuscript. WDF designed study and oversaw data collection and manuscript preparation.

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Data availability statement

Data are available from the principal investigator/corresponding author (WDF) upon reasonable request.

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Tables

Table 1 and 2 are available in the Supplementary Files section.

Figures

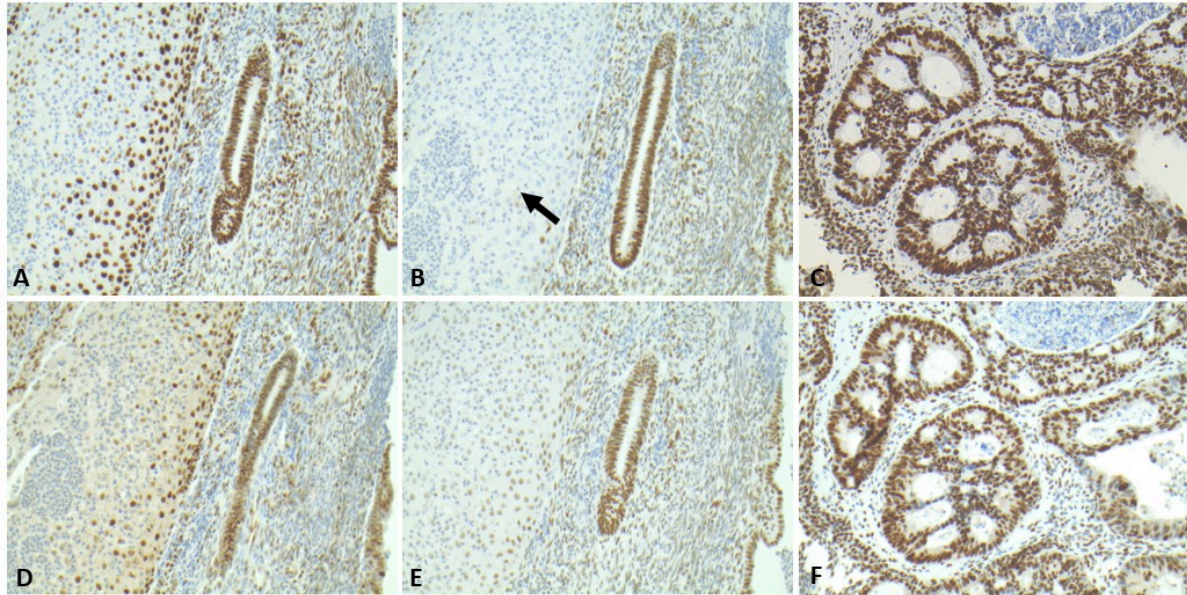


Figure 1

Immunohistochemistry of MMR proteins in cancers from proband of Family I

Female proband was diagnosed with MSH6-deficient endometrioid-type endometrial adenocarcinoma with squamous differentiation in her 40s and MMR-proficient colorectal cancer in her 50s. A) MLH1 retention in both the endometrial adenocarcinoma with squamous differentiation (left) and the non-neoplastic endometrial glands (right); B) absence MSH6 in endometrial carcinoma, arrow indicates tumor with squamous differentiation; C) retention of MSH6 in colorectal tumor; D) retention of PMS2 in endometrial tumor; E) retention of MSH2 in endometrial tumor; F) retention of MSH2 in colorectal tumor; Magnification 200x. IHC performed using antibodies G168-728 (MLH1; Cell Marque), G219-1129 (MSH2; Cell Marque), 44 (MSH6; Cell Marque), and MRQ-28 (PMS2; Cell Marque). This individual is known to carry *MSH6* c.2061T>G in the germline.

Supplementary Files

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- [Table1FINALNEWVM.xlsx](#)
- [Table2FINALNEW.xlsx](#)
- [SupplementalMaterialsFINALagerangesV2.pdf](#)