Molecular Cytogenetics

BRIEF REPORT Open Access

Novel MSH6 exon 5–6 skipping variant in a Taiwanese family with Lynch syndrome: implications for genetic testing and cancer management

Ting-Yao Wang[1], Chao-Yu Chen[2,3], Huei-Chieh Chuang[4], Yuan-Yuan Jiang[5] and Jrhau Lung[6,7,8*]

Abstract

Lynch syndrome is an autosomal dominant disorder predisposing individuals to colorectal and other cancers, primarily caused by variants in mismatch repair genes. This study describes a novel *MSH6* variant affecting transcript structure in a Taiwanese family meeting the Amsterdam II criteria for Lynch syndrome. A 67-year-old male presented with jejunal adenocarcinoma and a strong family history of colorectal cancer. Immunohistochemistry revealed loss of MSH6 expression, while next-generation sequencing performed on tumor tissue failed to detect any *MSH6* variants. Comprehensive genetic analysis, including RT-PCR and Sanger sequencing of both cDNA and genomic DNA, identified a novel exon 5–6 skipping variant in the *MSH6* gene transcript (NM_000179.3:r.3262_3645del), linked to a 2268 bp deletion from the 3′ portion of intron 4 to the middle of intron 6 of the *MSH6* gene (NC_000002.12:g.47803007_47805274del). This variant was also detected in two of the patient's asymptomatic sons, highlighting its heritability and potential cancer predisposition. The study emphasizes the limitations of capture-enrichment NGS panels in detecting certain types of variants and underscores the value of orthogonal confirmation using cDNA analysis for transcript aberrations. The identification of this novel variant expands our understanding of Lynch syndrome's mutational spectrum and has implications for genetic diagnosis and counseling. Based on these findings, the patient was treated with pembrolizumab, resulting in stable disease for 8 months. This case highlights the importance of comprehensive genetic approaches in suspected Lynch syndrome cases and the potential utility of mRNA-based screening as an additional method when NGS analysis is negative and the clinical presentation strongly suggests Lynch syndrome.

Keywords Lynch syndrome, *MSH6* gene, Exon skipping, Small bowel cancer, Next-generation sequencing, Hereditary cancer

*Correspondence:
Jrhau Lung
yukishirochy@cgmh.org.tw
[1]Division of Hematology and Oncology, Department of Internal Medicine, Chang Gung Memorial Hospital Chiayi, Chiayi 613, Taiwan
[2]Department of Obstetrics and Gynecology, Chang Gung Memorial Hospital Chiayi, Chiayi 613, Taiwan
[3]Graduate Institute of Clinical Medical Sciences, College of Medicine, Chang Gung University, Taoyuan 333, Taiwan
[4]Department of Anatomic Pathology, Chang Gung Memorial Hospital Chiayi, Chiayi 613, Taiwan
[5]Department of Pulmonary and Critical Care Medicine, Chiayi Branch, Chang Gung Memorial Hospital, Chiayi 613, Taiwan
[6]Department of Medical Research and Development, Chiayi Branch, Chang Gung Memorial Hospital Chiayi, Chiayi 613, Taiwan
[7]Department of Medicine, College of Medicine, Chang Gung University, Taoyuan 333, Taiwan
[8]Chang Gung University of Science and Technology, Chiayi 613, Taiwan

Introduction

Lynch syndrome, also known as hereditary non-polyposis colorectal cancer (HNPCC), is an autosomal dominant disorder characterized by a significantly increased risk of colorectal cancer and other malignancies. This syndrome primarily results from (likely) pathogenic germline variants in key mismatch repair (MMR) genes, including *MLH1*, *MSH2*, *MSH6*, and *PMS2* [1]. The lifetime risk of colorectal cancer varies significantly by genotype - approximately 46–61% for *MLH1*, 33–52% for *MSH2*, and 10–44% for *MSH6* variants [2]. Affected individuals also face elevated risks of endometrial, ovarian, gastric, and other extracolonic cancers, with the specific cancer spectrum varying by the underlying gene variant [3].

The identification of Lynch syndrome is crucial for implementing appropriate cancer surveillance and prevention strategies. The Amsterdam II criteria, which consider family history of Lynch-associated cancers across generations, serve as a clinical tool for identifying potential Lynch syndrome families [4]. These criteria include: (1) at least three relatives with a Lynch syndrome-associated cancer; (2) one affected individual should be a first-degree relative of the other two; (3) at least two successive generations should be affected; (4) at least one relative should be diagnosed before age 50; and (5) familial adenomatous polyposis should be excluded.

Current strategies for detecting Lynch syndrome typically involve tumor testing for microsatellite instability and immunohistochemistry for MMR protein expression, followed by targeted germline variant analysis in candidates [5]. However, the increasing use of next-generation sequencing (NGS) panels in clinical practice has introduced new opportunities and challenges in identifying pathogenic variants [6].

Despite these advances, some variants remain difficult to detect using standard NGS approaches. Intronic variants affecting splicing can be particularly challenging to identify and interpret when they occur, especially with capture-based NGS panels that may not thoroughly cover intronic regions. The discovery of novel variants and unusual mutational mechanisms continues to expand our understanding of Lynch syndrome genetics and improve our ability to diagnose and manage this condition.

In this report, we describe a case of suspected Lynch syndrome in a Taiwanese family, where comprehensive genetic analysis revealed a novel *MSH6* aberrant transcript variant. This case highlights the importance of thorough genetic investigation in families meeting clinical criteria for Lynch syndrome, even when initial genetic testing using a comprehensive targeted sequencing panel yields negative results. It also underscores the potential limitations of certain NGS approaches and the value of complementary genetic testing methods in identifying complex variants.

Case presentation

A 67-year-old Taiwanese male presented to our gastroenterology clinic with a two-week history of hematochezia. The patient had no significant past medical history and did not report any previous gastrointestinal symptoms. Upon initial evaluation, his vital signs were stable, and physical examination revealed mild abdominal tenderness in the periumbilical region.

Given the presenting symptoms, the patient underwent a colonoscopy, which revealed no significant findings in the colon. Subsequently, an upper endoscopy with push enteroscopy was performed, revealing an ulcerative lesion in the proximal jejunum. Biopsy of this lesion confirmed a diagnosis of moderately differentiated adenocarcinoma.

Further imaging studies, including computed tomography (CT) of the abdomen and pelvis, suggested localized disease without evident metastases. The patient underwent surgical resection of the jejunal tumor. Histopathological examination of the resected specimen revealed tumor perforation through the visceral peritoneum, one nodal metastasis, and peritoneal metastasis. The final pathological staging was pT4N1M1.

A detailed family history was obtained, revealing a significant pattern of early-onset colorectal cancers:

- The patient's mother was diagnosed with colon cancer at age 58.
- The patient's maternal aunt developed colorectal cancer at age 51.
- The patient's maternal cousin was diagnosed with colon cancer at age 36.

This family history fulfilled the Amsterdam II criteria, raising strong suspicion for Lynch syndrome.

Given the metastatic nature of the disease and the suspicion of Lynch syndrome, both next-generation sequencing (NGS) using the TruSight Oncology 500 assay® (TSO500) and immunohistochemistry (IHC) were performed on the tumor tissue. The TSO500 assay enables comprehensive genomic profiling from FFPE tissue and targets 523 genes to assess various DNA variant types, including copy number variants (CNVs) through read depth analysis. The IHC results showed a loss of MSH6 expression (Fig. 1), while retaining normal expression of MLH1, MSH2, and PMS2. The tumor was found to be microsatellite stable (MSS) based on NGS analysis using the TSO500 assay, with a borderline high Tumor Mutation Burden of 12.5 mut/Mb (cutoff 10 mut/Mb). Surprisingly, our NGS analysis did not detect any pathogenic variants in the *MSH6* gene or any other mismatch repair genes in the tumor tissue.

Given these inconclusive findings and the strong clinical suspicion of Lynch syndrome, we proceeded with a

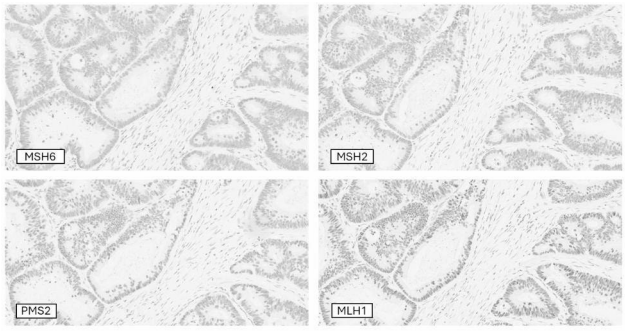

Fig. 1 Immunohistochemistry testing for the proband's cancer tissue specimen showed negative staining for MSH6, but positive staining for MSH2, PMS2 and MLH1

more comprehensive genetic analysis. Genomic DNA and RNA were extracted from peripheral blood leukocytes, and RT-PCR was performed using primer sets (set 1 to set 4) designed to amplify the entire coding region of the *MSH6* cDNA in overlapping fragments (Fig. 2A). This approach revealed an additional 0.8 kb band in one of the amplified regions (Fig. 2B, left panel). Subsequent Sanger sequencing of this fragment identified a novel exon 5–6 skipping variant in the *MSH6* transcript (NM_000179.3:r.3262_3645del) resulting in an in-frame deletion [NP_000170.1:(p.Asp1058_Ser1185del)] in the ATPase domain of MSH6 protein (Fig. 2C, upper panel). To exclude the possibility of naturally occurring alternative *MSH6* transcripts lacking exon 5–6, we performed the same RT-PCR analysis on multiple healthy control

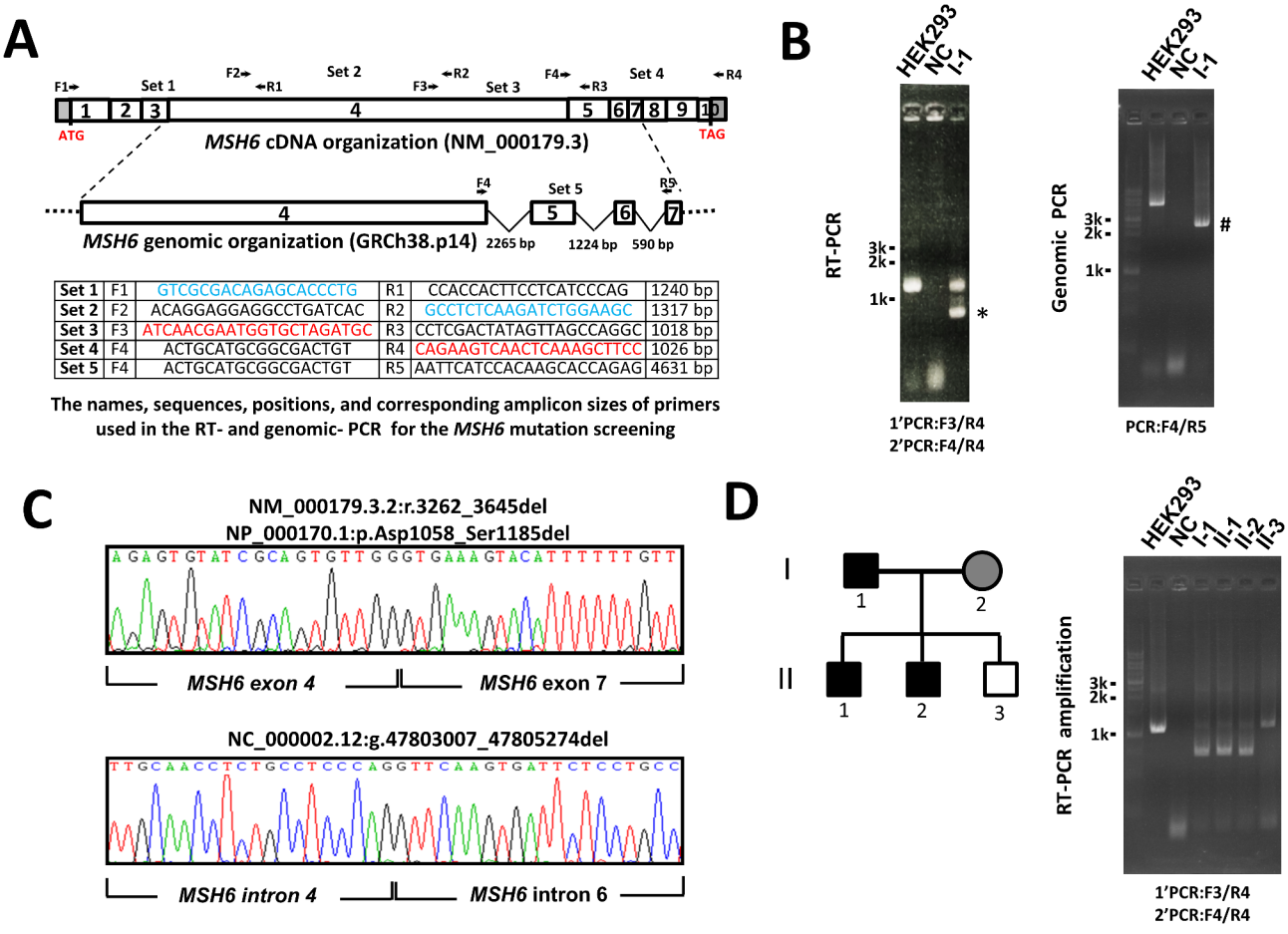

Fig. 2 Molecular analysis of a patient family carrying the novel *MSH6* exon 5–6 skipping variant. (**A**) The names, sequences, positions, and corresponding amplicon sizes of primers used in the RT- and genomic PCR analysis for the *MSH6* variant screening. (**B**) Mutant variant detection by RT- and genomic PCR and agarose gel electrophoresis. The RT- and genomic PCR reactions were performed with primers spanning the skipping exons listed below the gel images and compared between the patient (I-1), normal control (HEK293 cell line), and negative control (NC, distilled water). The asterisk (*) labels the mutant variant amplicon in RT-PCR, while the pound sign (#) indicates the mutant variant amplicon in genomic PCR. (**C**) The Sanger sequencing results of variant fragments amplified from RT- or genomic PCR. The variant is described in relation to the reference sequence following the HGVS nomenclature (version 20.05). (**D**) Pedigree and RT-PCR analysis of the patient family with the novel *MSH6* variant. In the pedigree, filled symbols represent affected individuals, and the arrow indicates the proband. The RT-PCR gel shows the presence of the variant (indicated by the lower band) in the proband and two of his sons

samples, which did not show the aberrant transcript (data not shown).

To determine the underlying genomic alteration causing this variant, we amplified and sequenced the genomic region spanning exons 4 to 7 of the *MSH6* gene (Fig. 2A), and revealed a 2268 bp deletion from the 3' portion of intron 4 to the middle of intron 6 of *MSH6* gene (NC_000002.12:g.47803007_47805274del) (Fig. 2B, right panel and Fig. 2C, bottom panel).

Given the potential hereditary nature of this variant, we extended our genetic analysis to the patient's offspring. RT-PCR analysis revealed that two of the patient's asymptomatic sons also carried this novel aberrant transcript variant (Fig. 2D).

Based on the tumor's mismatch repair deficiency (shown by loss of MSH6 expression on IHC) and the patient's metastatic disease status, treatment with pembrolizumab was initiated. The patient demonstrated stable disease for 8 months following the commencement of immunotherapy.

Discussion

This case report describes a novel *MSH6* aberrant transcript variant in a Taiwanese family meeting the Amsterdam II criteria for Lynch syndrome. The proband presented with jejunal adenocarcinoma, an uncommon manifestation of Lynch syndrome, highlighting the diverse spectrum of cancers associated with this condition [1]. The identification of this variant through comprehensive genetic analysis, despite initial negative findings from next-generation sequencing (NGS), underscores the importance of pursuing thorough genetic investigations in suspected Lynch syndrome cases.

The novel variant we identified - exon 5–6 skipping in the *MSH6* gene transcript (NM_000179.3:r.3262_3645del) - is linked to a 2268 bp deletion from the 3' portion of intron 4 to the middle of intron 6 of *MSH6* gene (NC_000002.12:g.47803007_47805274del). This finding aligns with previous research highlighting the significance of intronic variants affecting splicing in elevating the risk of Lynch-associated cancers [7]. The exon skipping event results in an in-frame deletion [NP_000170.1:(p.Asp1058_Ser1185del)] in the ATPase domain of MSH6 protein, likely compromising its mismatch repair function.

Notably, the family history in this case demonstrates an unusually strong phenotype for an *MSH6* variant. While pathogenic *MSH6* variants typically show lower penetrance (10–44% lifetime risk for colorectal cancer) and later age of onset compared to *MLH1* and *MSH2* mutations, this family presents multiple early-onset colorectal cancers. This discordance raises interesting questions about potential genetic modifiers or the possibility of additional pathogenic variants. A significant limitation of our study is that genetic testing was not performed on the affected maternal relatives (mother, aunt, and cousin) to confirm the presence of this *MSH6* variant. Therefore, we cannot rule out the possibility that a different mismatch repair gene mutation might be responsible for the early-onset colorectal cancers in the maternal lineage. This underscores the importance of comprehensive genetic testing of all affected family members when possible, to fully understand the genetic basis of cancer predisposition in high-risk families.

To our knowledge, this specific variant has not been previously reported in public databases such as gnomAD or ClinVar. However, other intronic variants in *MSH6* affecting splicing have been described in the literature [8]. The identification of this novel variant expands our understanding of the mutational spectrum in Lynch syndrome and highlights the importance of investigating intronic regions when standard genetic testing yields negative results.

Interestingly, while loss of MSH6 protein expression in the tumor suggests a second hit affecting the wild-type allele, our NGS analysis did not detect the event of loss heterozygosity (LOH). The loss of MSH6 expression in the patient's tumor could potentially result from another intronic variant not readily detectable by our exon-focused NGS panel, particularly given that we identified the germline variant through comprehensive analysis of intronic regions. Alternatively, it could relate to epigenetic silencing like that of *MLH1* in MMR deficiency, albeit less frequent as a second hit for the *MSH6* gene. Other possibilities include mutations in regulatory regions or complex structural changes affecting MSH6 expression without altering exon copy number. Future investigations, such as whole-genome or long-read sequencing, or LOH detection using *MSH6* germline polymorphisms (e.g., rs1042821, identified in this patient) with high-purity tumor DNA obtained via microdissection, could clarify the specific mechanism behind the MSH6 expression loss in this case.

Importantly, our study reveals a limitation of using capture-enrichment NGS panels in detecting certain types of variants in diagnostic routines, particularly those occurring in introns and affecting splicing, if the sequencing panel is not thoroughly optimized [9]. Our findings emphasize the value of orthogonal confirmation using cDNA analysis of blood to identify splice aberrations followed by genomic sequencing, especially when clinical suspicion remains high despite negative NGS results [5]. It should be noted, however, that exon skipping transcripts of MMR genes are frequently observed in cDNA analysis due to alternative splicing [10] or may result from a loss of transcript amplification or degradation of the aberrantly spliced transcript when premature stop codons are generated [11]. Further analyses using

methodologies such as genomic sequencing or precluding nonsense-mediated mRNA decay (NMD) with puromycin treatment prior to RNA isolation, should be performed to confirm the link between a newly found splice variant and Lynch syndrome. The identification of this variant in two of the patient's asymptomatic sons underscores the hereditary nature of Lynch syndrome and the importance of cascade genetic testing in families. This finding necessitates appropriate clinical intervention for these individuals, including regular colonoscopic monitoring to reduce the likelihood of hereditary cancer [6]. Moreover, it highlights the potential utility of mRNA-based screening approaches as an additional method when NGS analysis is negative, and the clinical presentation strongly suggests Lynch syndrome [7].

From a therapeutic perspective, the decision to treat the patient with pembrolizumab, resulting in stable disease for 8 months, aligns with emerging evidence supporting the use of immune checkpoint inhibitors in dMMR tumors, regardless of the tissue of origin [8]. While our patient's tumor was found to be microsatellite stable, the loss of MSH6 expression suggests a deficiency in mismatch repair that could potentially benefit from immunotherapy. This outcome suggests that identifying such variants can have direct implications for personalized treatment strategies in Lynch syndrome-associated cancers.

Our case expands the repertoire of known Lynch syndrome mutational mechanisms to include splicing disruption based on a large intronic deletion. This has implications for genetic diagnosis and counseling, potentially necessitating adjustments in genetic screening strategies for Lynch syndrome. Furthermore, it underscores the need for ongoing optimization of NGS probe and primer design configurations, as well as the use of multiple enrichment technologies to enhance detection capabilities across the full mutational spectrum [12].

Conclusion

In summary, we identified an exon skipping variant in *MSH6* traced to a splicing-disrupting intronic deletion in a male patient presenting with jejunal adenocarcinoma and family history meeting the Amsterdam criteria for Lynch syndrome. Detection of this variant in the patient's offspring indicated heritability and cancer predisposition conferred by this novel genetic mechanism. Submission for inclusion in public genomic databases is recommended to enable risk assessment, genetic counseling efforts and early surveillance interventions for at-risk relatives, and to advance understanding of genotype-phenotype correlations across the allelic spectrum of Lynch syndrome.

Abbreviations

IHC	Immunohistochemical
MMR	Mismatch repair
NGS	Next-generation sequencing RT-PCR
RT-PCR	Reverse transcription-polymerase chain reaction MSS
MSS	Microsatellite stable

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13039-025-00708-5.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Acknowledgements

The manuscript was edited by ATS Medical Editing.

Author contributions

T.W. and C.C. conceived and designed the study. H.C. carried out the IHC analysis. Y.J. and J.L. carried out the genetic analysis. All authors discussed the results and contributed to the final manuscript.

Funding

None.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

This study was approved by the Institutional Review Board of Chang Gung Memorial Hospital, Chiayi (IRB number: 202400504B0). All participants signed consent forms.

Consent for publication

Written informed consent for publication of their details was obtained from the participants.

Competing interests

The authors declare no competing interests.

Received: 1 November 2024 / Accepted: 25 February 2025
Published online: 11 March 2025

References

1. Yurgelun MB, Allen B, Kaldate RR, Bowles KR, Judkins T, Kaushik P, et al. Identification of a variety of mutations in Cancer predisposition genes in patients with suspected Lynch syndrome. Gastroenterology. 2015;149(3):604–13. e20.
2. Network NCC. Genetic/Familial High-Risk Assessment: Colorectal, Endometrial, and Gastric (Version 3.2024) [Available from: https://www.nccn.org/professionals/physician_gls/pdf/genetics_ceg.pdf
3. Win AK, Jenkins MA, Dowty JG, Antoniou AC, Lee A, Giles GG, et al. Prevalence and penetrance of major genes and polygenes for colorectal Cancer. Cancer Epidemiol Biomarkers Prev. 2017;26(3):404–12.
4. Vasen HF, Watson P, Mecklin JP, Lynch HT. New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the international collaborative group on HNPCC. Gastroenterology. 1999;116(6):1453–6.
5. Hegde M, Ferber M, Mao R, Samowitz W, Ganguly A, Working Group of the American College of Medical G. ACMG technical standards and guidelines for genetic testing for inherited colorectal cancer (Lynch syndrome, Familial adenomatous polyposis, and MYH-associated polyposis). Genet Med. 2014;16(1):101–16.

6. Fulk K, Turner M, Eppolito A, Krukenberg R. RNA sequencing uncovers clinically actionable germline intronic MSH2 variants in previously unresolved Lynch syndrome families. BMJ Case Rep. 2022;15(4).
7. Tournier I, Vezain M, Martins A, Charbonnier F, Baert-Desurmont S, Olschwang S, et al. A large fraction of unclassified variants of the mismatch repair genes MLH1 and MSH2 is associated with splicing defects. Hum Mutat. 2008;29(12):1412–24.
8. Sijmons RH, Hofstra RMW, Review. Clinical aspects of hereditary DNA mismatch repair gene mutations. DNA Repair (Amst). 2016;38:155–62.
9. Le DT, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 Blockade. Science. 2017;357(6349):409–13.
10. Morak M, Pineda M, Martins A, Gaildrat P, Tubeuf H, Drouet A, et al. Splicing analyses for variants in MMR genes: best practice recommendations from the European mismatch repair working group. Eur J Hum Genet. 2022;30(9):1051–9.
11. Morak M, Schaefer K, Steinke-Lange V, Koehler U, Keinath S, Massdorf T, et al. Full-length transcript amplification and sequencing as universal method to test mRNA integrity and biallelic expression in mismatch repair genes. Eur J Hum Genet. 2019;27(12):1808–20.
12. Feliubadalo L, Tonda R, Gausachs M, Trotta JR, Castellanos E, Lopez-Doriga A, et al. Benchmarking of whole exome sequencing and ad hoc designed panels for genetic testing of hereditary Cancer. Sci Rep. 2017;7:37984.

Publisher's note