CNV and RNA analysis reveal a germline pathogenic duplication of MSH2 exon 15 in a family with Lynch syndrome: A case report

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Received: Jan 23, 2023
Accepted: Feb 09, 2023
Published: Feb 16, 2023
Archived: www.jcimcr.org
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DOI: www.doi.org/10.52768/2766-7820/2289

Abstract

Background: MSH2 germ line pathogenic variants are a well-recognised cause of Lynch syndrome, predisposing individuals to a variety of malignancies, most usually colorectal and endometrial cancer. Partial duplications of MSH2 gene, due to their position in the genome and frequently unclear mechanisms of pathogenicity, are often classified as Variants of Uncertain Significance (VUS).

Case presentation: CNV (Copy Number Variation) analysis revealed a duplication of MSH2 exon 15 in a young male patient with colorectal cancer as well as in his affected family members. RNA analysis elucidated the impact of this duplication on RNA, revealing that it leads to an abnormal transcript, thus providing experimental evidence for a pathogenic effect.

Conclusions: We show that the combination of CNV and RNA analysis provides critical information for the identification and proper classification of pathogenic/likely pathogenic variants which, in turn, is of great importance for the patients as well as for their family members with an actionable impact in clinical practice.

Keywords: Colon cancer; RNA study; Copy number variation; MSH2 duplication; Variant classification.

Abbreviations: VUS: Variants OF Uncertain Significance; CNV: Copy Number Variation; MMR: Mismatch Repair; MSI: Microsatellite Instability; CMMRDS: Constitutional Mismatch Repair Deficiency Syndrome; FFPE: Formalin-Fixed and Paraffin-Embedded; IHC: Immunohistochemistry; NGS: Next Generation Sequencing.
MAD4
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BMPR1A
APC (NM_000038), 36 genes (Roche Nimble Gen Seq Cap EZ Choice) consisting of:

Generation Sequencing (NGS) was performed with a panel of
targeted Next genes involved in hereditary cancer predisposition was per
generated using a solution-based capture approach. Targeted Next
sequencing from this panel was carried out using the “MSICall”
ples as indicated by the manufacturer. Analysis of the sequenc

Patients with LS have up to an 80% lifetime risk of developing
colon cancer and, in women, a 60% lifetime risk of developing
endometrial carcinoma [4]. Apart from colorectal and endome
tumors, LS related cancers include gastric, ovarian, pancre
as, urothelial (kidney, renal pelvis, ureter, bladder and prostate),
brain, biliary tract, small intestinal cancers, as well as sebaceous
adenomas, sebaceous carcinomas and keratoacanthomas [5].

Approximately 40% of the pathogenic germline variants causative for LS are in MSH2 gene [4]. Structural variants such as
inversions and Copy Number Variations (CNVs) are more com
mon in MSH2 gene than in other MMR genes [6]. Although in
versions and deletions are, in majority, classified as pathogenic,
exonic duplications are, due to their position in the genome and
frequently unclear mechanisms of pathogenicity, often classi
fied as Variants of Uncertain Significance (VUS) [6].

Here, we present a family with Lynch Syndrome and different
types of cancer with a pathogenic duplication of MSH2 exon 15.

Case presentation

Patient

A 23-year-old male of Turkish origin diagnosed with colorec
tal cancer was referred to our private diagnostic laboratory for genetic testing with a hereditary cancer panel. Peripheral
blood samples from the proband and his family members, when
available, were drawn for diagnostic purposes after obtaining
a signed informed consent and permission for the anonymous
use of their data for research purposes and/or scientific publica
tions.

Gene testing

Genomic DNA was extracted from peripheral blood using Mag Core® Genomic DNA Whole Blood Kit (RBC Bioscience)
according to the manufacturer’s instructions. The analysis of genes involved in hereditary cancer predisposition was per
formed using a solution-based capture approach. Targeted Next Generation Sequencing (NGS) was performed with a panel of
36 genes (Roche Nimble Gen Seq Cap EZ Choice) consisting of:

For the MSI analysis, genomic DNA was isolated from pro
band’s FFPE tumor biopsies using the Mag Max Total Nucleic
Acid Isolation Kit (Thermo Fischer Scientific, Waltham, MA,
USA) according to the manufacturer’s instructions in the Seq Cap EZ
Choice Library User’s Guide (Roche Nimble Gen, Pleasanton,
CA, USA). Sequencing was carried out using the Miseq illumina
NGS (Illumina, San Diego, CA, USA) technology and sequence
crashes were identified and interpreted in the context of a
single clinically relevant transcript using the commercially avail
able software suite Seq Nextversion 4.4.0 (JSI Medical Systems
GmbH, Ettenheim, Germany). The presence of CNVs was inves
igated using the commercial computational algorithm Seq Pilot
(JSI medical systems GmbH, Germany) and verified by the use
of MLPA method (Multiplex Ligation-dependent Probe Amplifi
cation, MRC Holland). Sample preparation of the proband, his
family members and normal references was performed accord
ing to the manufacturer’s instructions. Briefly, specific MLPA
probes were hybridized to each denatured DNA sample, fol
lowed by ligation of the hybridized probes, PCR amplification of
ligated probes and fragment separation by capillary electropho
resis. Results were analysed with Coffalyser. Net.

Immunohistochemistry MMR and MSI analysis

Immunohistochemical (IHC) examination and evaluation of Mismatch Repair proteins (MMR) in the paraffin-embedded tis
sue was performed using the following antibodies: clone M1,
ROCHE (MLH1 Ab), clone G219-1129, ROCHE (MSH2 Ab), clone
tSP93, ROCHE (MSH6 Ab), clone A16-4, ROCHE (PMS2 Ab) ac

RNA analysis

In order to investigate the impact of this variant on RNA lev
el, total RNA was extracted from peripheral blood lymphocytes
using Trizol (Invitrogen, Paisley, UK) following a standard pro-

Background

MSH2 belongs to the Mismatch Repair (MMR) proteins, which are responsible for the repair of DNA replication errors. These errors preferentially accumulate in regions of repetitive DNA sequences called microsatellites, causing Micro Satellite Instability (MSI) [1]. MSI is a main characteristic of Lynch Syn
drome (LS), one of the most common hereditary cancer syndromes which are inherited in an autosomal dominant manner. LS, previously known as Hereditary Nonpolyposis Colorectal Cancer (HNPPC), is caused by germline mutations of the MMR genes MLH1, MSH2, MSH6, and PMS2 and, rarely, deletions of the 3’ UTR region of the non-MMR gene EPICAM, which lead to hypermethylmation of the MSH2 promoter and loss of MSH2 expression [2]. Biallelic inherited MMR pathogenic variants in MLH1, MSH2, MSH6, and PMS2 are associated with a rare condition called constitutional mismatch repair deficiency syn
drome (CMMRDS) [3].

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tocol. C DNA was synthesized using the Super Script™ VILO™ c DNA Synthesis Kit (Thermo Fisher Scientific) as described by the manufacturer. The resulting c DNA was amplified using specific primers designed on MSH2 cDNA (NM_000251.1) so that the forward primer was downstream of the reverse primer within the duplicated region (MSH2EX15dup Forward primer: 5’–GCT AAA CAG AAA GCC CTG GAA C–3’, MSH2EX15dup Reverse primer: 5’–TAG CAA GCT CTG CAA CAT GAA–3’). The PCR product was purified using the Nucleo Fast® 96 PCRCl eanupkit (Macherey-Nagel GmbH and Co., Düren, Germany). The purified PCR product was used for each sequencing reaction performed using the BigDye® Terminator v1.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA). Sequencing reaction products were purified prior to electrophoresis using the Montage™SEQ96 Sequencing Reaction kit (EMD Millipore Corp., Billerica, MA, USA) and sequenced using a Seq Studio Genetic Analyzer (Applied Biosystems).

**Results**

The proband, a 23-year-old male of Turkish origin diagnosed with colorectal cancer at the age of 23 had a strong family history of endometrial, bladder and brain cancer from his mother’s side (Figure 1).

CNV analysis revealed that the proband carried a heterozygous duplication of exon 15 in MSH2 gene [NG_007110 (NM_000251): c.(2458+1_2459-1)_dup(2634+1_2635-1)] confirmed by MLPA microdeletion/microduplication analysis (MRC Holland, Amsterdam, Netherlands, SALSA KIT P003-D1) (Figure 2). No other clinically relevant variants were found. MLPA analysis of family members (affected and unaffected) showed that in this family the variant segregates with the MSH2-associated cancer (the patient’s mother (II: 5) and her two affected siblings (II: 6, II:7) carried the variant, whereas the unaffected father (II:4), sister (III:2) and maternal aunt (II:8) of the patient were normal for the above-mentioned variant) (Figure 1).

Immunohistochemical examination of MMR proteins in the proband’s Formalin-Fixed And Paraffin-Embedded (FFPE) tumor tissue indicated significantly reduced expression of the MSH2 protein (<10%), while the expression of MLH1, MSH6, and PMS2 proteins was 60%, 70%, and 100%, respectively (Figure 3). During MSI analysis of the patient’s tumor tissue MSI-high was observed (MSI score=111,19). A sample is considered positive if the MSI score is >30. The sample therefore was classified as Microsatellite-High (MSI-H).

RNA analysis revealed that MSH2 exon 15 duplication was in tandem leading to a frame shift and a premature stop codon a few amino acid residues downstream the end of exon 15 [MSH2 EX15dup, p.(Q879Vfs*21)] (Figure 4).

**Discussion and conclusions**

Duplication of MSH2 exon 15 has been described before in families affected with Lynch syndrome (HNPCC) [10,11] but, to our knowledge, no experimental studies were performed concerning this variant. In addition, the mutation database Clin Var
expression of MSH2 protein but the expression of MSH6 was
the proband’s tumor tissue indicated significantly reduced ex-

tions in MSH2 gene provided evidence for the reclassification of
be disrupted leading to NMD, in which case PVS1 can be ap-

crion, if a duplication of a portion of the gene of a defined length
was classified as pathogenic with a total score of 1.2 and the
following evidence used: 1A, 2I (PVS1, assigned points: 0.9), 3A,
4F, 5D. RNA analysis, which proved that the duplication was in
was found to segregate with cancer in the proband’s family, further

c evidence was needed to classify this variant as pathogenic.

CVN analysis alone, although very important to reveal the
structural variant, could not elucidate the impact of this MSH2
partial duplication on functional level, so RNA analysis was

It revealed that this variant leads to a frame shift and the cre-
ation of a novel translational termination codon 21 residues
later, thus resulting in a truncated and non-functional protein
product. Several variants resulting in premature stop codon in
the neighbouring regions of MSH2 gene have been reported as
pathogenic (Cln Var Variation IDs: 428483, 993962, 1392097).
According to the ACMG/Clin Gen recommended guidelines for
the classification of CVN variants [13], the MSH2 exon 15 duplication
found in the proband and his affected family members
was classified as pathogenic with a total score of 1.2 and the
following evidence used: 1A, 2I (PVS1, assigned points: 0.9), 3A,
4F, 5D. RNA analysis, which proved that the duplication was in

in a young-onset, node-positive stage III left colon cancer patient
underwent left hemicolectomy: pT3N1 (2/38). Since MSH2-high
status did not predict unresponsiveness to chemotherapy in
stage III colon cancer, the patient was administered 12 courses
of adjuvant FOLFOX [19,20]. Adjuvant treatment was complet-
ed in six months and clinical follow-up was initiated. In addition,
since MSH2-high was observed during MSI analysis of the pa-
tient’s tumor tissue, the patient is eligible for immunotherapy
administration [21].

According to the surveillance/prevention strategies of the
NCCN guidelines for carriers of pathogenic/likely pathogenic
variants colonoscopy every 2 years for colon cancer screening
and Esophago Gastro Duodenoscopy (EGD) every 3-5 years for
gastric cancer screening were recommended for the proband
and his MSH2 positive family members, as well as annual gyn-
aecological examination for female individuals [5]. In addition,
it was recommended that they continue with other cancer
 screenings in accordance with their age. Last but not least,
knowledge of the genetic background allows the carriers of
pathogenic/likely pathogenic variants in Lynch syndrome genes
to perform prenatal or preimplantation genetic diagnosis [22].

Conclusion

In conclusion, in this study we have identified and character-
ized as pathogenic aMSH2 exon 15 duplication in a family with
Lynch syndrome. CVN analysis in Lynch syndrome patients is of
great importance since it can identify pathogenic/likely patho-
genic variants with actionable clinical significance. Currently,
exonic duplications of MSH2 gene are often classified as VUS
because their effect on gene expression is unknown. As in this

RNA analysis can contribute to the classification of cer-
tain exonic duplications in MSH2, as well as in other genes, as
pathogenic. As with sequence variants, proper classification of
partial MSH2 gene duplications, by performing RNA analysis, is
crucial for the management of the patients, since it can lead
to targeted therapeutic and risk-reducing interventions, and of
their family members, allowing cascade family screening.

Declarations

Ethics approval and consent to participate: The present
study was approved by the Ethics Committee of Özel Memorial
Antalya Hospital (Antalya, Turkey). All tested individuals pro-
vided signed informed consent form before molecular genetic

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testing and permission for the anonymous use of their data for research purposes and/or scientific publications.

Consent for publication: The proband provided a signed informed consent form prior to molecular genetic testing for the permission of the anonymous use of her data for research purposes and/or scientific publications.

Availability of data and material: All data generated or analyzed during this study are included in this published article. The clinical interpretation of the genomic variant along with the experimental information has been submitted to Clin Var (SUB12060189).

Competing interests: The authors declare that they have no competing interests.

Funding: Not applicable.

Authors’ contributions: DA drafted the manuscript. DA, KA, NT and EP designed the study. DA, KA, GP, and KP carried out the DNA extraction, sequencing and contributed to the analysis and interpretation of the variant data. GNT performed the bio-informatics analysis. MO, OK and MOC provided the materials, demographic data and family history. EP performed the Mismatch Repair (MMR) analysis by immunohistochemistry. GN conceived of the study and participated in its design and coordination.

Acknowledgements: Not applicable.

References


