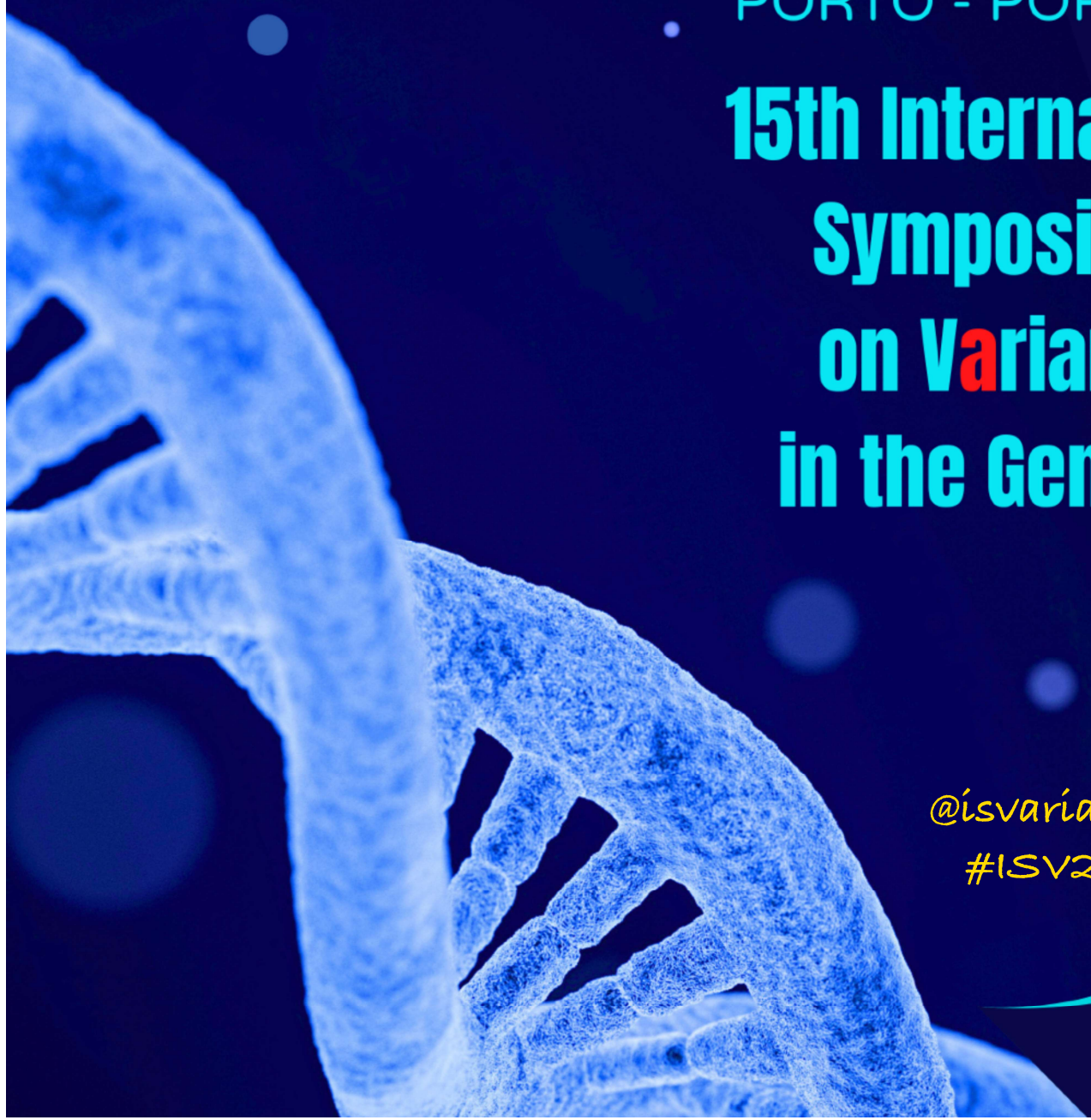
HUMAN GENOME ORGANISATION
HUGO

ISV24

12 - 15 MAY

PORTO - PORTUGAL

15th International Symposium on Variants in the Genome



@isvariants

#ISV24

15th International Symposium on Variants in the Genome

12 - 15 May 2024

Axis Vermar Conference & Beach Hotel
Póvoa de Varzim, Porto, Portugal

Scientific Programme Committee

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INSTITUTO DE INVESTIGAÇÃO E INOVAÇÃO EM SAÚDE
UNIVERSIDADE DO PORTO

Previous Meetings

1991 Oxford, UK
1993 Lago D'Orta, Italy
1995 Visby, Sweden
1997 Brno, Czech Republic
1999 Vicoforte, Italy
2001 Bled, Slovenia
2003 Palm Cove, Australia
2005 Santorini, Greece
2007 Xiamen, China
2009 Paphos, Cyprus
2011 Santorini, Greece
2013 Lake Louise, Canada
2015 Leiden, Netherlands
2017 Santiago de Compostela, Spain
2020 Piran, Slovenia (cancelled)

with skipping of exons 15-16 causing in-frame deletion of 120 residues.The *de novo* duplication of 5q31.1 (chr5:133839470-134410436, hg19) also affects several genes important for developmental pathways, and a case of a patient with a similar developmental delay phenotype and a similar duplication has been published previously.Biochemical testing showed that homocysteine, methylmalonate and methionine levels were within the normal range, suggesting no defect in cobalamin metabolism. This is consistent with the localisation of the *HCFC1* variant outside the Kelch domain and with the fact that several missense variants in the region of ZBTB17 and GABP2 binding sites have been reported in patients with developmental delay without cobalamin deficiency.The case illustrates difficulties in the interpretation of results in patients carrying multiple variants. It is not clear if both the 5q31.1 duplication and the *HCFC1* variant participate in the patient's disorder, and, especially when the phenotypic features are rather unspecific, if combination of both *de novo* events plays a role. Regarding the *HCFC1* splicing variant itself, the exact ratio of transcripts resulting from abnormal splicing of the gene must be considered. The transcript with a premature stop codon can exert a loss-of-function effect, while the transcript resulting in the shorter in-frame deletion of the basic region of HCFC1 may possibly mimic the effect of missense mutations observed in this region.Supported by AZV NU22-07-00165.

POS 39

Identification of two novel germline *MLH1* mutations in colorectal carcinoma patients: Variant interpretation in practice

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Germline mutations in mismatch repair (MMR) genes, *MLH1, MSH2, MSH6* and *PMS2*, are associated with Lynch syndrome, the most common hereditary syndrome of colorectal cancer. Detecting pathogenic or likely pathogenic mutations in these genes is vital to identify high-risk individuals, enabling early diagnosis of colorectal cancer and other Lynch-associated cancers in affected families.
In our routine screening for Lynch syndrome mutations in eligible subjects, we detected two novel *MLH1* germline mutations, NM_000249.4:c.496_497insCACTT and NM_000249.4:c.1759_1767delATGCTTGCC, in two young female patients with colorectal carcinoma using next generation sequencing. During the process of variant annotation and classification we used American College of Medical Genetics and Genomics (ACMG) and Association for Molecular Pathology guidelines. To the best of our knowledge, these two mutations have not been described in the literature or reported in genetic databases such as NCBI ClinVar[1], LOVD - human mismatch repair genes[2], Canadian Open Genetics Repository (COGR)[3], or the International Society for Gastrointestinal Hereditary Tumours (InSiGHT)[4] database. Furthermore, they are absent in population databases GnomAD and ExAC among healthy people which contributes to their pathogenicity[5]. *In silico* prediction tool MutationTaster have assessed these mutations as frameshift insertion and in-frame deletion, and as disease causing. Therefore, the moderate pathogenicity (PM2) and supportive pathogenicity (PP3) evidences could be applied to the both of these variants. According to the guidelines we used, the frameshift insertion NM_000249.4:c.496_497insCACTT in exon 6 of the *MLH1* gene is considered a variant of very

strong pathogenicity, meeting the criteria for PVS1.

Annotating the second mutation in the *MLH1* gene was more challenging because of the in-frame nature of the mutation. The NM_000249.4:c.1759_1767delATGCTTGCC mutation is an in-frame deletion of 9 nucleotides in exon 16 of the *MLH1* gene, resulting in the loss of 3 amino acids in the MLH1 synthesized protein. These 3 amino acids are located in the C-terminal functional domain of the MLH1 protein, which is responsible for interaction with human exonuclease 1 (hEXO1). Since there is a change in protein length due to the in-frame deletion in a non-repeat region, which is actually a functional domain of the protein, we applied the moderate pathogenicity evidences PM4 and PM1.
The combined evidences of PVS1, PM2, and PP3 strongly suggest that the detected frameshift mutation should be classified as pathogenic (class 5). For the second in-frame mutation, evidences PM1, PM2, PM4, and PP3 support its classification as likely pathogenic (class 4).
Moving forward, it is important to monitor the status of these mutations and conduct further segregation and functional analysis in order to better understand their impact.
The clinical interpretation of genetic testing results remains a significant challenge in successfully integrating genomics into modern medicine. Mistakes in variant interpretation can result in inappropriate medical interventions or missed therapeutic options. Moreover, scientific resources frequently present conflicting data and interpretations for a particular variant, necessitating thorough scrutiny of both content and study validity. This can be a challenging task, even for experienced variant scientists.

Acknowledgements: This study was funded by the Horizon Europe Twinning Project STEPUPIORS (Agreement No. 101079217) and the Ministry of Education and Science of the Republic of Serbia (Agreement No. 451-03-47/2023-01/200043).

References:
[1]. https://www.ncbi.nlm.nih.gov/clinvar/
2. https://databases.lovd.nl/shared/genes/MSH2
3. https://opengenetics.ca/
4. http://www.insight-database.org/genes
5. https://gnomad.broadinstitute.org/

POS 40

The importance of complementary metabolic studies in accessing the pathogenicity of variants of uncertain significance

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2 - Unidade Multidisciplinar de Investigação Biomédica, Instituto de Ciências Biomédicas Abel Salazar (UMIB/ICBAS), Universidade do Porto and Laboratory for Integrative and Translational Research in Population Health (ITR), Porto, Portugal;
3 - Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal;
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The classification of genetic variants is of utmost importance in today's medical practice. Several organizations have published systems for classifying genetic variants, with the American College of Human Genetics (ACGM) recommendations (1) being the most frequently used in medical genetics practice. These guidelines use criteria such as population data, bioinformatic tools, and patients' phenotypes to classify rare variants, and often, the result is a Variant of Uncertain Significance (VUS). Despite initiatives such as GnomAD (https://gnomad.broadinstitute.org/), ClinGen (https://clinicalgenome.org/), and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), there is still much unaccounted variation in our genome. With the increase of indications for genetic testing, the interpretation of VUS has become a crucial element of the daily practice of clinical geneticists. We reflect on the field of Inborn Errors of Metabolism (IEM) as an area where various metabolic studies are invaluable tools to aid the interpretation of the functional impact of genetic variations and thus exclude or confirm diagnoses. Currently, there are 1904 inborn metabolic

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Institute for Oncology and Radiology of Serbia

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In our routine screening for Lynch syndrome mutations in eligible subjects, we detected two novel *MLH1* germline mutations, NM_000249.4:c.496_497insCACTT and NM_000249.4:c.1759_1767delATGCTTGCC, in two young female patients with colorectal carcinoma using next generation sequencing. Both of our patients had positive family history for Lynch syndrome-associated diseases and increased probability of beeing a MMR mutation carrier calculated by MMRpro tool (Figure 1 and Figure 2). During the process of variant annotation and classification we used American College of Medical Genetics and Genomics (ACMG) and Association for Molecular Pathology guidelines.

To the best of our knowledge, these two mutations have not been described in the literature or reported in genetic databases such as NCBI ClinVar, LOVD - human mismatch repair genes, Canadian Open Genetics Repository (COGR), or the International Society for Gastrointestinal Hereditary Tumours (InSiGHT) database. Furthermore, they are absent in population databases GnomAD and ExAC among healthy people which contributes to their pathogenicity.

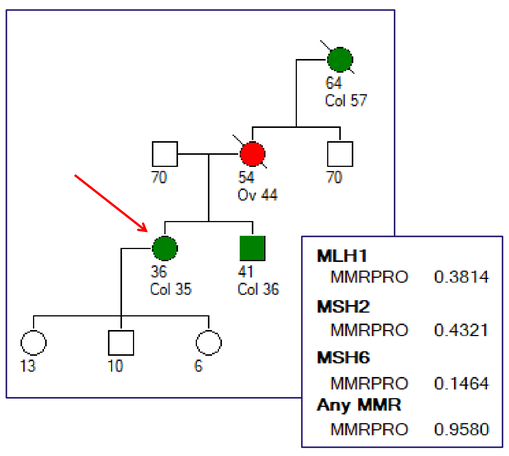


Figure 1. Pedigree of Family 1

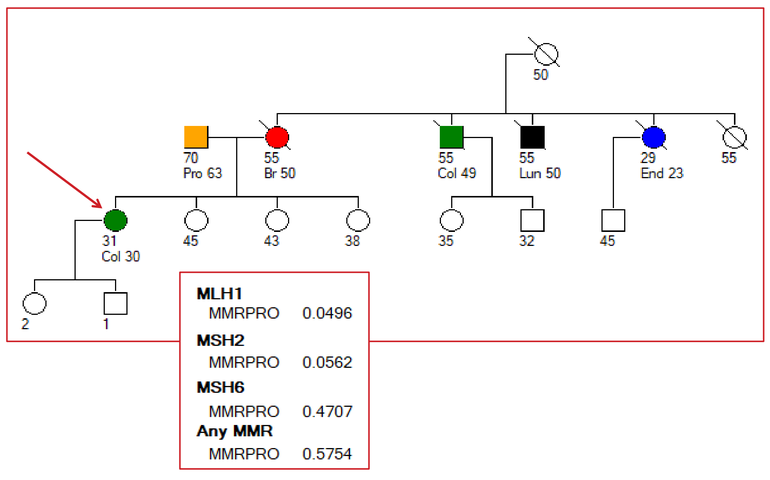
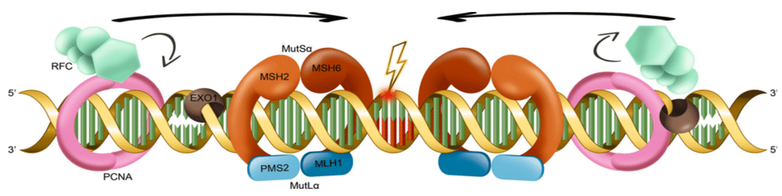


Figure 2. Pedigree of family 2

In silico prediction tool MutationTaster have assessed these mutations as frameshift insertion and in-frame deletion, and as disease causing. Therefore, the moderate pathogenicity (PM2) and supportive pathogenicity (PP3) evidences could be applied to the both of these variants.



Sajjadi E, Venetis K, Piciotti R, Invernizzi M, Rocco EG, Haricharan S, et al. Mismatch repair - deficient hormone receptor - positive breast cancers : Biology and pathological characterization. Cancer Cell Int 2021;1–11.

According to the guidelines we used, the frameshift insertion NM_000249.4:c.496_497insCACTT in exon 6 of the *MLH1* gene is considered a variant of very strong pathogenicity, meeting the criteria for PVS1 (Figure 3).

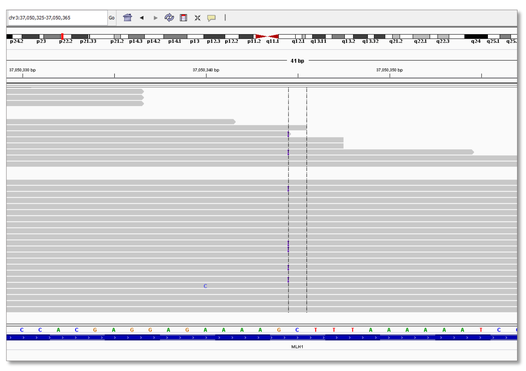


Figure 3. The frameshift insertion in exon 6 of the MLH1 gene

Annotating the second mutation in the *MLH1* gene was more challenging because of the in-frame nature of the mutation.
The NM_000249.4:c.1759_1767delATGCTTGCC mutation is an in-frame deletion of 9 nucleotides in exon 16 of the MLH1 gene, resulting in the loss of 3 amino acids in the MLH1 synthesized protein (Figure 4).

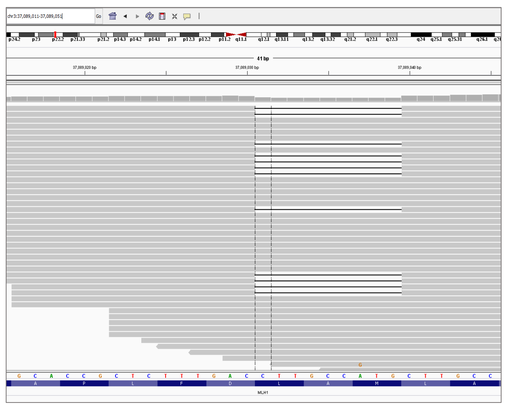


Figure 4. The in-frame deletion in exon 16 of the MLH1 gene

	Benign		Pathogenic			
	Strong	Supporting	Supporting	Moderate	Strong	Very strong
Population data	MAF is too high for disorder BA1/BS1 **OR** observation in controls inconsistent with disease penetrance BS2			Absent in population databases PM2	Prevalence in affecteds statistically increased over controls PS4	
Computational and predictive data		Multiple lines of computational evidence suggest no impact on gene /gene product BP4 Missense in gene where only truncating cause disease BP1 Silent variant with non predicted splice impact BP7 In-frame indels in repeat w/out known function BP3	Multiple lines of computational evidence support a deleterious effect on the gene /gene product PP3	Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before PM5 Protein length changing variant PM4	Same amino acid change as an established pathogenic variant PS1	Predicted null variant in a gene where LOF is a known mechanism of disease PVS1
Functional data	Well-established functional studies show no deleterious effect BS3		Missense in gene with low rate of benign missense variants and path. missenses common PP2	Mutational hot spot or well-studied functional domain without benign variation PM1	Well-established functional studies show a deleterious effect PS3	
Segregation data	Nonsegregation with disease BS4		Cosegregation with disease in multiple affected family members PP1	Increased segregation data →		
De novo data				De novo (without paternity & maternity confirmed) PM6	De novo (paternity and maternity confirmed) PS2	
Allelic data		Observed in *trans* with a dominant variant BP2 Observed in *cis* with a pathogenic variant BP2		For recessive disorders, detected in trans with a pathogenic variant PM3		
Other database		Reputable source w/out shared data = benign BP6	Reputable source = pathogenic PP5			
Other data		Found in case with an alternate cause BP5	Patient's phenotype or FH highly specific for gene PP4			

Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405–24.

These 3 amino acids are located in the C-terminal functional domain of the MLH1 protein, which is responsible for interaction with human exonuclease 1 (hEXO1). Since there is a change in protein length due to the in-frame deletion in a non-repeat region, which is actually a functional domain of the protein, we applied the moderate pathogenicity evidences PM4 and PM1.

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gnomAD genome aggregation database · InSiGHT · ClinVar

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