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REVIEW ARTICLE

Genetics and Genomics

Lynch syndrome, molecular mechanisms and variant classification

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Patients with the heritable cancer disease, Lynch syndrome, carry germline variants in the *MLH1*, *MSH2*, *MSH6* and *PMS2* genes, encoding the central components of the DNA mismatch repair system. Loss-of-function variants disrupt the DNA mismatch repair system and give rise to a detrimental increase in the cellular mutational burden and cancer development. The treatment prospects for Lynch syndrome rely heavily on early diagnosis; however, accurate diagnosis is inextricably linked to correct clinical interpretation of individual variants. Protein variant classification traditionally relies on cumulative information from occurrence in patients, as well as experimental testing of the individual variants. The complexity of variant classification is due to (1) that variants of unknown significance are rare in the population and phenotypic information on the specific variants is missing, and (2) that individual variant testing is challenging, costly and slow. Here, we summarise recent developments in high-throughput technologies and computational prediction tools for the assessment of variants of unknown significance in Lynch syndrome. These approaches may vastly increase the number of interpretable variants and could also provide important mechanistic insights into the disease. These insights may in turn pave the road towards developing personalised treatment approaches for Lynch syndrome.

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INTRODUCTION

Lynch syndrome (LS) is a hereditary cancer predisposition disease, caused by germline variants that impair the DNA mismatch repair (MMR) system and lead to the accumulation of spontaneously acquired somatic mutations. Thus, patients with LS suffer from a high cumulative lifetime cancer risk compared to the general population. As a result, LS underlies 3% of all colorectal cancer (CRC) cases, and 8% of cases in young CRC patients (<50 years) [1, 2]. This makes LS the most common cause of genetically predisposed CRC, giving rise to its previous term hereditary non-polyposis colorectal cancer (HNPCC). Accordingly, LS was the first familial cancer disorder to be described and was later found to cause 2% of all endometrial cancers and predispose patients to a range of other cancers, including stomach, brain and ovarian cancer [3–5].

The main therapeutic approach for LS-derived CRC is the partial or complete surgical removal of the colon or colon-rectum [6, 7], while prophylactic surgery has been suggested as an approach to treat LS-derived gynaecological cancers [8–10]. Although chemotherapeutic treatments are given as supplementary treatment in the later stages of LS-derived CRC, its effectiveness remains unclear [6], and probably new treatments such as immunotherapy will in time be implemented in combination with personalised medicine [11]. Thus, the strongest clinical tool at hand is an early diagnosis, together with frequent surveillance and surgical removal of early adenomas [5]. Importantly, early diagnosis relies on a thorough understanding of the underlying germline variant as well as the disease mechanism that relates to the specific variant, which is the focus of this review.

DNA MISMATCH REPAIR

The MMR system repairs spontaneously arising somatic mutations. During replication, the DNA polymerase may incorporate mismatched nucleotides. The polymerase is a highly "faithful" enzyme and performs proofreading, which reduces the error rate [12]. However, the proofreading works in strong cooperation with the MMR system, which is recruited to sites of replication to correct mismatched base pairs [13] and is necessary to bring down the overall mutation rate [12, 14]. Thus, loss of MMR function is detrimental to genome integrity and sets the stage for cancer development.

LS patients carry germline loss of function (LoF) variants in one of the four key genes involved in MMR: *MutL homolog 1* (*MLH1*), *MutS homolog 2* (*MSH2*), *postmeiotic segregation increased 2* (*PMS2*) and *MutS homolog 6* (*MSH6*). In more rare cases, LS is caused by

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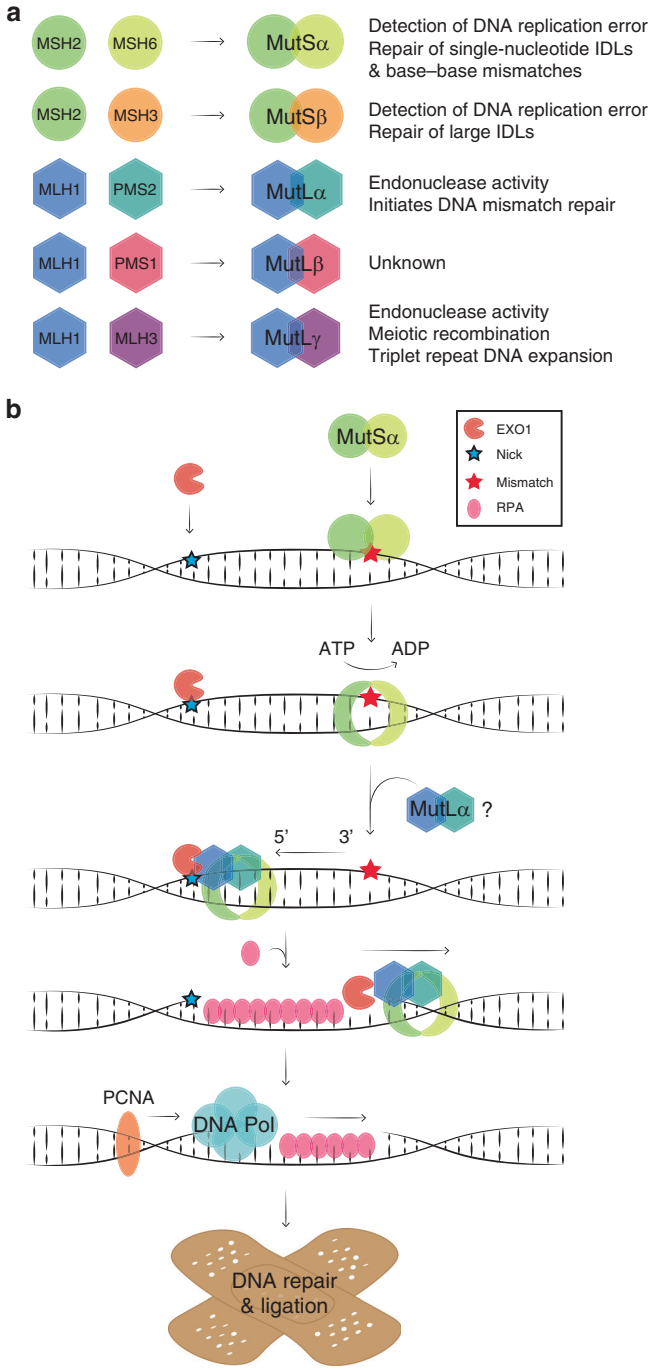

Fig. 1 The human DNA mismatch repair (MMR) system. a The MSH2 protein forms dimers with MSH6 (MutSα) and MSH3 (MutSβ). The MLH1 protein forms dimers with PMS2 (MutLα), PMS1 (MutLβ) and MLH3 (MutLγ). Heterodimer functions are listed. IDL, insertion/deletion loop. **b** A schematic illustration of the 5′ to 3′ MMR pathway. EXO1 binds a nick in the newly synthesised DNA strand 5′ to the mismatch. MutSα recognises the mismatch and undergoes an ATP-dependent conformational change, which locks the complex around the DNA to form a sliding clamp. MutSα moves along the DNA strand and interacts with MutLα, which further binds the DNA. MutSα/MutLα binds EXO1 and moves in the 5′ to 3′ direction allowing for the excision of the mismatch by EXO1. RPA protects the unpaired strand until the DNA polymerase bound to PCNA repairs the strand, after which the DNA ligase seals off any remaining nicks (not shown).

constitutional epimutations [15] that lead to the silencing of the *MLH1* gene [16, 17] or the *MSH2* gene [18]. Moreover, germline variants of *MutS homolog 3* (*MSH3*) have been implicated in LS when occurring together with other low-risk alleles [19].

The MMR proteins form heterodimers, MutSα (MSH2-MSH6) and MutLα (MLH1-PMS2) (Fig. 1a), which play key and distinct roles in the MMR pathway (Fig. 1b) [20]. Through the MSH6 protein, MutSα binds the DNA at the site of the mismatch, thus detecting base-base mismatches and small insertion/deletion loops (IDLs) [21]. An alternative complex (MutSβ) consisting of MSH2 and MSH3 corrects larger IDLs and exists in a 1:10 ratio to MutSα. Although the binding mechanism of MSH3 to DNA is unlike that of MSH6, redundancy between MutSα and MutSβ has been suggested [22].

The MMR pathway is bidirectional. The 5′ to 3′ repair proceeds as follows (Fig. 1b): first, a 5′ nick in the newly synthesised strand, which occurs randomly during replication and serves as strand discrimination to the MMR, allows exonuclease 1 (EXO1) to bind [23]. ATP exchange converts the mismatch-bound MutSα into a sliding clamp locked around the DNA [24]. The conformational change releases MutSα from the site of the mismatch and allows it to move along the DNA strand. Moreover, the change permits interaction with MutLα, which subsequently binds the DNA [24]. The MutSα/MutLα complex binds EXO1, after which 5′ to 3′ movement of the MutSα/MutLα/EXO1 complex allows for excision by EXO1 assisted by the single-stranded DNA binding protein complex replication protein A (RPA), which protects the exposed single-stranded DNA. Then, the DNA polymerase in complex with the proliferating cell nuclear antigen (PCNA) sliding clamp as well as the DNA ligase finish the repair process. The 3′ and 5′ directed repair processes seem to proceed differently, although some details remain to be resolved. For example, an in vitro study of the yeast MMR system suggests that 3′ to 5′ repair merely requires action from MutSα, EXO1 and RPA, while 5′ to 3′ repair requires additional action from MutLα and PCNA [25, 26]. Because EXO1 only excises DNA in a 5′ to 3′ direction, the main role of MutLα might be to travel with PCNA in the 5′ direction and nick the newly synthesised strand 5′ to the mismatch. This action is stimulated by PCNA and performed by PMS2 in an MLH1-dependent manner [26–28]. Moreover, whether EXO1 is essential for MMR also remains unclear [20, 29].

MLH1 forms alternative dimers with MLH3 and PMS1 (Fig. 1a). The MLH1-MLH3 dimer (MutLγ) plays a role in meiotic recombination [30] and triplet repeat DNA expansion [31], while the function of the MLH1-PMS1 dimer (MutLβ) is unknown, but may play a minor role in MMR [32]. Lastly, besides DNA repair, the MMR pathway promotes the DNA damage response, including cell cycle arrest and cell death, which further explains the tumorigenic load in MMR-deficient cells [33] and why LS tumours are often associated with resistance to a range of chemotherapies, including temozolomide, 5-fluorouracil and cisplatin [34].

DIAGNOSIS OF LYNCH SYNDROME

LS-derived tumours are recognised by loss of one of the four key MMR proteins visualised by immunohistochemical staining of the tumour cells, and by the acquisition of a microsatellite instability (MSI) phenotype. Microsatellites are spans of short tandem repeats within the DNA, which are especially prone to acquiring frameshift mutations, due to DNA polymerase slippage. Loss of MMR results in a hypermutagenic MSI phenotype with altered microsatellite patterns, which is considered a hallmark of LS [35–37]. However, MSI is not exclusive to LS, and is seen in as much as 15% of CRCs, most of which are caused by spontaneous hypermethylation of the *MLH1* promoter, leading to MMR loss [37, 38]. Thus, next to the molecular analysis of the tumour, the diagnosis must also rely on the family cancer history, individual cancer history and age of

cancer onset. A variety of risk assessment tools have been used over the years: the Amsterdam criteria II [39], the revised Bethesda guidelines [40], simple CRC risk assessment tools [41], and more recently, computational prediction models [42]. Probably, these will be gradually complemented by sequence-based gene panel analyses of the germline. This development is likely to be accelerated by the need for individualised treatment depending on in which gene a pathogenic variant is detected.

Moreover, the diagnosis should involve evaluation of the specific germline variant, and accordingly, the disease course differs dramatically. It is estimated that there is a high general risk in the population (1:279) of carrying a LS-linked MMR variant [43]. Most LS variants are detected in the *PMS2* (1:714) and *MSH6* (1:758) genes, whereas *MLH1* and *MSH2* variants are less common (1:1946 and 1:2148, respectively). However, there is a difference in disease penetrance, which seems to be correlated inversely with the population frequencies, since most LS-linked cancers arise from variants in the *MLH1* or *MSH2* genes [6, 44, 45]. The difference in penetrance has been recognised in several studies [3, 5, 6, 46], but is not well understood. Importantly, penetrance is affected by environmental factors and the individual genetic makeup of patients, like co-segregating germline variants and modifier genes that may cause stronger or milder clinical effects. Thus, diverging penetrance patterns between individual patients is a major caveat for the classification of disease-linked protein variants. Lastly, while LS is dominantly inherited, disease development follows Knudson's two-hit hypothesis [47], and thus relies on a "second hit" of the wild-type allele to ablate the MMR function and significantly increase the risk for further mutations [48], meaning the expressivity of the disease ultimately also relies on the timing of the second hit.

Notably, the MMR proteins are not equally dependent on each other, which may partially explain this difference in penetrance. For instance, the MSH2 protein is stabilised by MSH6, causing MSH2 levels to drop upon loss of MSH6 [49]. Moreover, interaction with MLH1 rescues PMS2 from degradation [50, 51], thus leaving PMS2 protein levels to depend on the presence of MLH1 [52], while MLH1 levels do not rely on PMS2. This means that the cell effectively can lose both MLH1 and PMS2 if MLH1 is destabilised [51], which is why the loss of both MLH1 and PMS2 visualised by immunohistochemical staining may indicate a germline *MLH1* variant. Indeed, this depends on the specific variant, as some MLH1 variants induce solitary loss of PMS2 [53]. Likely, these variants alter the PMS2-interaction interface of the MLH1 protein, thus preventing the stabilisation of PMS2.

The specific variants also define how the disease is expressed. For example, some studies find *MSH6*-linked cancers to induce a high risk of gynaecological cancers [3, 5, 54, 55], which indicates that the genes play different roles in tumorigenesis. Conclusively, a thorough description of individual disease-causative LS variants is crucial for correct diagnosis and treatment of patients.

VARIANTS AND DISEASE MECHANISM

To improve diagnosis, known LS-linked MMR variants have been collected in the InSiGHT database (http://insight-database.org/) and assigned to one of five classes of pathogenicity based on the data published on each variant [56]. The types of germline variants distribute differently between the classifications. For example, synonymous substitutions or intron variants would likely have no effect on the given MMR protein structure or function and predominate in class 1 (benign) and 2 (likely benign) [56]. In contrast, large IDLs, non-sense (truncating) and splice site variants would, in most cases, cause significant alterations to the protein sequence, expression level and structure. These will obviously be disruptive to the protein function and are highly represented in class 4 (likely pathogenic) and class 5 (pathogenic) [56]. However, missense variants are represented in all five different InSiGHT categories and make up the majority of variants in class 3 (variants of uncertain significance, VUS) and also constitute a significant part of the classified pathogenic MLH1 (40%), MSH2 (30%), MSH6 (50%) and PMS2 (60%) variants [45].

Missense variants are abundant in the VUS pool because their effects on a protein may range from undetectable to detrimental and greatly depend on the type and location of the amino acid substitution. Even a conservative amino acid substitution within or near an active site or binding interface may affect protein function, however, many non-conservative substitutions occur outside the active site, and thus mainly affect the stability of the protein.

Most protein structures are only marginally stable [57], and exist in an equilibrium between a folded and unfolded state. However, the folded and functional state of the protein—the native structure—is under normal conditions more stable than the unfolded state, which drives the equilibrium towards this native state. A germline variant that changes the protein sequence may destabilise the protein structure and shift the equilibrium towards the unfolded state. In the unfolded state, the protein may expose degradation signals (degrons) that are buried in the native structure and therefore becomes vulnerable to degradation. The exposed degrons recruit the cellular protein quality control (PQC) system, which directs the protein for proteasomal degradation (Fig. 2a). The PQC and degradation of misfolded proteins have recently been reviewed [58–62]. Briefly, most misfolded proteins are degraded through the ubiquitin–proteasome system (UPS) and thus rely on ubiquitin conjugation achieved by the sequential actions of three enzymes: a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2) and a ubiquitin-protein ligase

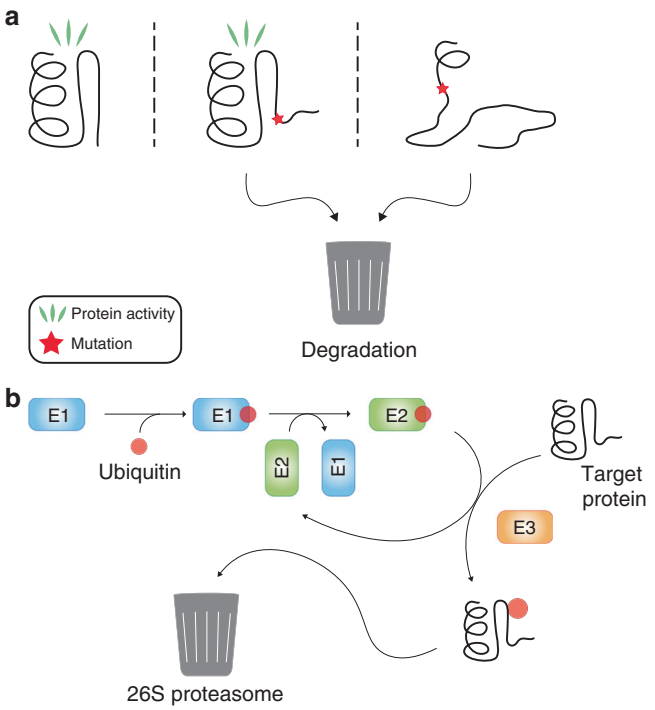

Fig. 2 Proteasomal degradation of misfolded proteins. **a** Overview of the ubiquitin–proteasome system (UPS). A ubiquitin moiety is activated by an E1 enzyme and transferred to an E2 enzyme. From here, the ubiquitin is transferred to the target protein by the means of an E3 ubiquitin-protein ligase. Ubiquitination promotes binding at the proteasome and subsequent degradation of the target protein. **b** A wild-type protein (left) mainly exists in the functional native structure that is not degraded. Mutations affect the protein structure to mild (centre) or more severe (right) degrees and may obstruct the protein function. Both mildly and severely misfolded proteins risk undergoing proteasomal degradation.

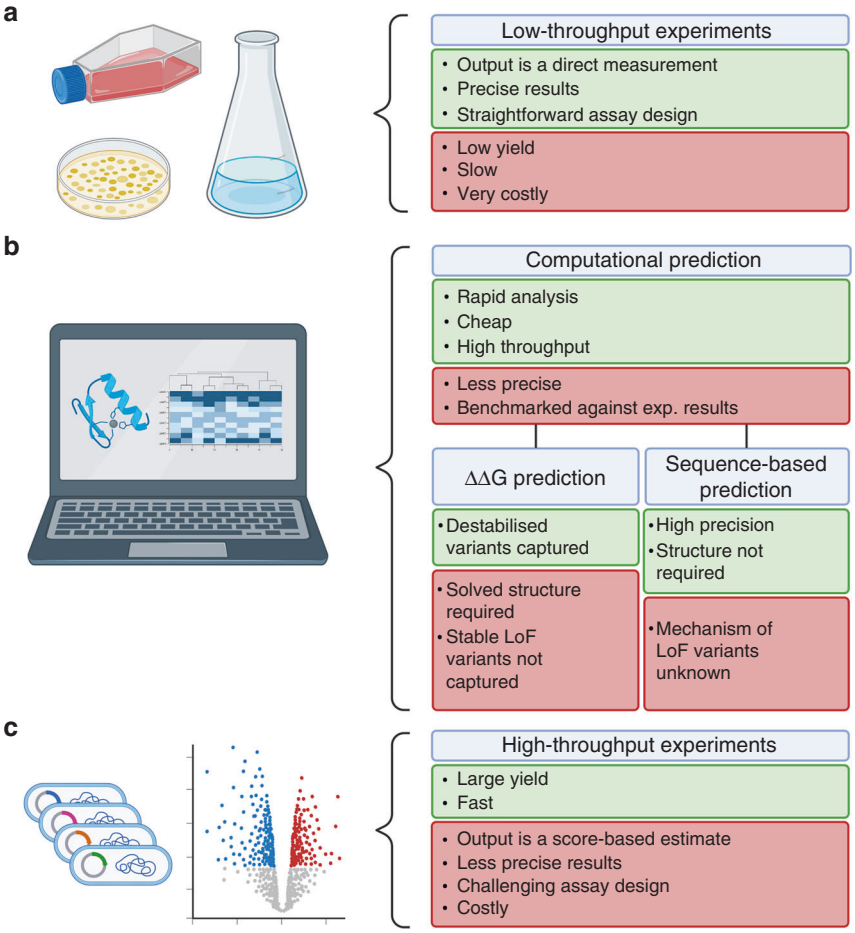

Fig. 3 Overview of tools used for testing individual variant effects. a Low-throughput lab-based experimental tools, i.e., individual variant testing. **b** Computational prediction tools, i.e., FoldX, GEMME, etc. **c** High-throughput lab-based experimental tools, i.e., MAVEs. Created with BioRender.com.

(E3) (Fig. 2a). Following activation by an E1 and transfer to an E2, the substrate-specific E3 enzyme covalently links the ubiquitin molecule to the target protein. This mediates the binding of the substrate to the 26S proteasome, which in turn catalyzes the degradation of the target protein.

In theory, even mild alterations to or destabilizations of the protein structure can cause protein degradation (Fig. 2b). Accordingly, the UPS also regulates the cellular abundance of wild-type proteins, including the MSH2 protein [49, 63–65]. However, the degradation of misfolded protein variants occurs more rapidly and is a well-established disease mechanism that appears to be the underlying cause for many cases of LS, causing low cellular levels of MSH2 and MLH1 variants [66–69], which is diagnosed by immunohistochemical staining of LS tumours. In some cases of LS, the unstable variant may still be functional. In the case of MSH2, inhibition of the UPS was shown to restore MMR function in cells with structurally unstable but functional MSH2 variants [70]. In these specific cases, inhibiting degradation could serve as a prophylactic treatment for patients carrying these variants [71]. Ideally, the rescue of a functional variant from degradation would restore the MMR function sufficiently and relieve the patient from the increased cancer risk—a strong incitement for obtaining a mechanistic understanding of individual LS variant effects at the molecular level [72].

THE EFFECTS OF VARIANTS OF UNCERTAIN SIGNIFICANCE

Interpretation of genetic variants is inherently difficult. Close to 90% of LS-linked missense variants are classified as VUS [66], and correct classification is a large and difficult task, which needs to be solved before clinicians can accurately diagnose and treat many suspected LS patients. The majority of variants identified by sequencing have never been observed in the human population before, hence the simple lack of preceding events in the population causes most variants to be classified as VUS [73]. Of course, the vast number of VUS is not specific for MMR proteins, and the majority (99%) of missense variants, including LS-linked VUSs, are rare [74] and have only been observed once or a few times in patients, which makes it difficult to determine potential disease causality. In contrast, variants that occur frequently in the population, such as founder mutations [75], are well-described, e.g., the highly penetrant MSH2 A636P variant, which underlies a third of all LS cancers in the Ashkenazi Jewish population [76]. A variant can also be categorised as a VUS if there exists conflicting evidence for its pathogenicity. Reasons for this could include co-segregation with a pathogenic variant in another MMR gene, inconclusive IHC or MSI analysis, or patients with a low prevalence of LS cancers among relatives. In such cases, functional assays can help elucidate the variant effect on MMR and thus reclassify the variant as likely pathogenic/benign [77–79].

Ideally, clinicians should have access to a complete map of the effects of every potential missense variant within a given protein. Once they detect a rare variant, a complete map of variant effects could be an excellent resource for classifying such rare variants. Figure 3 presents an overview of the methods used for variant interpretation. Traditionally, low-throughput studies of function

and abundance are applied retrospectively to analyse individual variants and ensure correct classification (Fig. 3a). Functional testing of individual variants in the MMR genes will possibly be made more readily available in the clinic with flow cytometry-based MMR assays that can be performed directly in blood cells from individuals that meet the Amsterdam II criteria [80]. However, individual verification is a slow, costly and labour-intensive process and applying such analysis to all possible missense variants in a protein is a daunting task.

Computational methods for variant interpretation

The implementation of computational prediction tools for missense variant effects has been an important step towards shedding light on the VUS pool. These tools were developed using both evolutionary conservation and structural data as input, and most are trained on clinically labelled data of benign and pathogenic variants (Fig. 3b) [73]. As evolution selects for protein function, residues that are critical for catalysis, protein or ligand binding, or structural stability can all be identified in this manner. However, methods based exclusively on sequence conservation will therefore on their own not provide any mechanistic information as to why a particular variant might be damaging. Conversely, structure-based methods may pinpoint variants that are likely to cause protein destabilization or loss of key interactions, but these methods will be blind to variants that may destroy enzymatic function without affecting the global protein stability. Combining evolution-based methods with structural stability calculations can provide an understanding of the various loss of function mechanisms inflicted by protein variants [81].

A detailed discussion of the many different approaches to predict variant effects and pathogenicity is beyond the scope of this review. These include, however, specific models for MMR genes and LS [82, 83]. Many of the models for variant effect prediction, such as PolyPhen-2 [84], PROVEAN [85], SIFT [86], EVMutation [87] and GEMME [88], generally use multiple sequence alignments of homologous proteins as input to construct a statistical model that estimates the likelihood of a given variant, and outputs an evolutionary sequence score. The algorithms consider both the conservation at individual positions as well as the co-evolution of amino acid pairs, the co-evolution term being the major difference between the algorithms. Evolutionary sequence analysis is also the basis of the recently published mutational effect predictor DeepSequence [89] and pathogenicity predictor EVE [90], and these types of prediction methods have been shown to work well for both MSH2 [67, 68, 90] and MLH1 [50].

Computational modelling of amino acid substitutions directly on the 3D structure of the given protein allows the prediction of the change in folding energy between the protein variant and the wild-type. The tools Rosetta [91] and FoldX [92] predict the change in stability with an accuracy of about 1 kcal mol^{-1}. The FoldX algorithm was developed to predict the structural destabilization afflicted by a missense substitution within a protein [93]. In simplified terms, these algorithms utilise information about the protein structure to calculate the difference in stability of a missense protein variant and the wild-type protein. Thus, one can predict the individual effects of all possible variants at any position within a structure. Several previous studies of the MMR proteins have found a correlation between LS and destabilised missense MMR variants [70, 94, 95]. Thus, in previous research, the FoldX and Rosetta algorithms were used to produce stability predictions of all possible missense variants of MSH2 [68], MLH1 [50] and a range of other proteins [96–98]. Here, it was shown that the effects of the majority of previously described missense variants with known clinical consequences could be accurately predicted, and that the predictions matched with cellular measurements of the individual variant abundances and protein function. Thus, an advantage of structure-based disease predictors is that they help provide the molecular mechanism underlying the damaging effect of a variant.

Multiplexed assays of variant effects

In another attempt to truly overcome the issues of variant interpretation, new laboratory-based high-throughput approaches have been developed, known as deep mutational scanning or multiplexed assays of variant effect (MAVE) technologies (Fig. 3c) [99]. These assays score the effects of all possible missense variants of a protein, and have been reported to outperform several computational prediction tools [66, 74, 100, 101]. However, in some cases, computational predictions have also been observed to outperform MAVEs in variant classification [90, 102].

MAVE technologies can be used to measure function, abundance, or interaction of libraries consisting of all possible single amino acid variants in a protein of interest. For example, the Variant Abundance by Massively Parallel sequencing (VAMP-seq) method measures abundance of fluorescently tagged protein variants, and thus scores their individual difference in pathogenicity in a high-throughput manner, when low abundance is the cause of loss of function [103]. Moreover, one can use the MAVE methods to test for variant function, by applying a selective pressure to the variant library, which was recently done for MSH2.

Multiplexed assays of MSH2 function

Recently, the function of missense MSH2 variants has been assayed in a high-throughput manner by Ollodart et al. [69] and Jia et al. [66]. In yeast, Ollodart et al. selected for LoF MSH2 variants by measuring the cellular mutation rate in a library of 185 different MSH2 variants. They selected cells with high mutation rates by measuring their resistance to canavanine, which is only tolerated by cells with a mutated *CAN1* gene. With this method, they successfully distinguished pathogenic and benign MSH2 variants. At an even larger scale, Jia et al. utilise a human *MSH2* knockout cell line in which they introduce a saturated library of MSH2 variants, including nearly all possible single amino acid missense variants. Treatment with 6-thioguanine (6TG) is toxic to MMR-proficient cells and allowed the authors to select for MMR-deficient MSH2 variants and identify these by sequencing. From this study, they estimate that 10–11% of missense variants are deleterious to MSH2 function [66] and that 7-8% of MSH2 VUS show a pathogenic phenotype, meaning the vast majority of MSH2 VUS are likely benign. This fits with predictions based on analyses of protein stability and sequence conservation that show that MSH2 is relatively tolerant to missense variants, and that most variants are predicted to have only minor effects [67, 68].

Current challenges in high-throughput variant classification

However, despite the promising progress, both functional MAVE analyses and computational prediction tools still wrongly classify some variants. In case of MAVE studies, misclassifications can occur if the employed assay is not sufficiently sensitive or does not capture all functional aspects of the protein. Further, it is important to establish guidelines for how to use MAVE functional data in clinical variant classification, as in addition to the guidelines that already exist for low-throughput functional assays [104]. Including functional data has been demonstrated to significantly aid in the reclassification of VUS [77].

Regarding computational pathogenicity predictions, the precision of the current prediction tools is promising, and it could certainly be a strong supportive tool in the clinic. Although some tools are challenged by reports of low sensitivity and a high number of false positives [105, 106], the advantages of computational prediction tools might in some situations outweigh their weaknesses, as the applicability, cost and efficiency is remarkable compared to individual variant testing. Indeed, new tools are being developed and perfected to overcome some of the inherent problems with the prediction algorithms [107, 108].

MAVE data can serve as useful benchmarks for computational variant effect predictors [73, 81, 87, 90, 102, 109], and may also be used to train variant effect predictors. This will likely lead to refined predictors in the near future.

POTENTIAL FOR RESTABILIZATION OF DISEASE-CAUSING MSH2 VARIANTS

MAVE technologies may also help to uncover particular disease mechanisms on the molecular level, for instance, by helping to determine which gene variants disrupt catalytic activity, interactions, or cause destabilization. For those variants that are degraded, the methods may shed light on the specificity of the PQC system, i.e., how does the PQC engage with proteins and what characterises the PQC-bound elements within the target proteins. For example, a recent study in *E. coli* shows how expression of PQC proteins, specifically the Lon protease, alters the mutational landscape of a model protein, and thus constrains which variants are allowed [110]. In a similar manner, the cellular PQC apparatus will also ultimately decide the threshold for which destabilised missense variants in MMR components will be pathogenic. Combining MAVE experiments with perturbations of the PQC apparatus will potentially allow the identification of variants that will regain function upon increased protein levels (Fig. 2b). This pool of variants is interesting, as they would be targetable from a clinical perspective. In principle, stabilisation of the protein would prevent disease development in the patient, and so far, this approach is used for the rescue of the cystic fibrosis CFTR F508Δ variant [111–113]. Proof-of-concept studies in yeast have demonstrated that restabilization of some pathogenic MSH2 variants can restore MMR function [70]. Thus, it may be possible to prevent cancer development for LS patients that carry "rescuable" MSH2 variants through the development of pharmaceuticals that act to increase the cellular amount of natively folded MSH2.

CONCLUDING REMARKS

MAVE-based experimental approaches combined with computational biology have already brought us key insights for VUS in Lynch Syndrome. Importantly, these tools have provided us with new approaches to study basic scientific questions focused on how individual variants operate through effects on catalysis, interactions or protein stability, and this knowledge can in turn improve the diagnosis and potential treatment of not only Lynch syndrome but also other genetic diseases.

At the moment, use of computational evidence is restricted to "supporting evidence only" according to clinical guidelines such as those provided by the ACMG-AMP [114]. These guidelines specify that multiple computational predictions may not be considered independent due to concerns over overlap in the underlying algorithms and the data that they are trained on. While it is true that some programmes use similar approaches and are thus not independent, others can be considered complementary. Moreover, rapid growth of sequence databases can lead to improved variant consequence predictions, such that the guidelines with respect to computational results should perhaps be revised.

The value of including MAVE data in clinical variant classification was demonstrated in a recent study on the TP53, PTEN and BRCA1 proteins [115], where MAVE data was integrated with available clinical data to correctly classify VUS. The same approach may be applied to MSH2, for which both MAVE and clinical data exist. Similar prospective studies for other MMR proteins could help provide broader knowledge on MMR variant effects and enable personalised treatment for patients suffering from Lynch Syndrome. Indeed, the importance of Lynch Syndrome and the promise of patient benefits from personalised approaches is also recognised in its listing among genes to be prioritised for systematic assessment of variant consequences [116, 117].

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Not applicable.

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ADDITIONAL INFORMATION

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