Lynch Syndrome: Form, Function, Proteins, and Basketball

We are all different, every one of us. Just look around. Some are bigger, some are smaller, some can run faster, and others can jump higher. We come in all sizes, shapes, and colors. We are heterogeneous, in large part, because our genes are subtly different. In fact, there is variation in DNA sequence among individuals approximately every 1000 nucleotides, called single nucleotide polymorphisms, or SNPs (“snips”). This translates into more than 2 million variable sites per person.1 Most of this sequence heterogeneity is thought to be functionally meaningless because exons (ie, the DNA encoding functional proteins) make up only 1.1% of our genome.2 Suffice it to say, humans are even more diverse at the DNA level than we are in size and shape. Despite all the SNPs, we are still 99.9% identical to one another at the DNA level and closer yet at the protein level. Functionally, humans are almost interchangeable parts; we are members of a single species, and most of the variation is relatively minor biologically.

Interpreting Genetic Variation

In this context, how does one know when sequence variations in genes represent functional differences in proteins? Often, one cannot. Some sequence variations are easier to interpret than are others. The most obvious loss-of-function variation is when a gene has been deleted from the genome. The interpretation of a deleted gene is easy. However, curiously, it can be difficult to detect the loss of a non–sex-linked gene through DNA sequencing because it is not possible (yet) to tell whether there are 1 or 2 copies of the DNA sequence, and we are generally born with 2 copies of everything. Another relatively unambiguous mutation is when it creates a premature stop-codon within a gene. If the premature stop-codon occurs closer to the amino-terminus (ie, the beginning) of the protein, there will usually be a loss of function. However, the closer the premature truncation is to the carboxy-terminus (ie, the end or tail) of the protein, the more difficult it is to interpret. Sequence variations may also alter the splice sites of the noncoding introns within the transcribed messenger RNA (mRNA). Appropriate mRNA splicing is required to process the initial mRNA into a translatable gene. If an intron is left in the mRNA, the translated sequence will contain extra amino acids or, more likely, shift out of the appropriate translation-reading frame, adding a string of completely novel amino acids to a shortened protein.

Deletions, premature stop-codons, and splice site alterations are relatively easy to interpret compared with variations of a gene sequence that alter a single amino acid within the protein (missense mutations). These subtle alterations may change the shape of a protein or its ability to interact with others proteins. The loss of 3 consecutive coding nucleotides will lead to the deletion of a single amino acid from the peptide sequence—again, a subtle alteration of the protein. The interpretation of every sequence variation becomes critical when we perform genetic testing for familial colorectal cancer because every sequence variation will be reported. However, not every sequence variation has clinical consequences. How do we deal with this in a real clinical setting?

Lynch Syndrome and DNA Mismatch Repair (MMR)

Lynch syndrome (hereditary nonpolyposis colorectal cancer) is an autosomal dominant disease, which confers a very high risk of developing cancer. Lynch syndrome is caused by mutations in the human MMR genes. Lifetime cancer risk rates are as high as 90% for men and 70% for women.3 One does not assign a diagnosis of this gravity without being certain of the conclusion. The problem is particularly complex because there are multiple MMR genes, each contributing to the form and function of the MMR system. Genetic testing provides a great deal of information. Some sequence variations in these genes will lead to an inactivation of that gene, whereas others are simply part of the natural differences between human beings.

In this month’s issue of GASTROENTEROLOGY, Raevaara et al4 have tried to interpret the clinical significance of nontruncating mutations (27 missense and 4 deletions of single amino acids) in the \textit{hMLH1} gene. This article combined 4 functional tests of the \textit{hMLH1} proteins with different amino acid sequences. These data were compared with family history and the standard assays pathologists use to assess these tumors for Lynch syndrome.5

DNA MMR and Basketball

Perhaps this article is better understood by thinking of the MMR system like a basketball team. Each March, the National Collegiate Athletic Association
(NCAA) sends 64 basketball teams to a tournament to find out which college has the best team. We could save ourselves the trouble of all the games, perhaps, by measuring the heights of the players, their ability to jump, their shooting percentages, or their speed. However, to select the best team, we make them play the game. The winners may not necessarily be the tallest, the fastest, or the most accurate shooters. Individually, the skills are only useful if they function well as a team. The MMR system works the same way.

**The Team Concept of MMR**

MMR fixes misincorporation errors that arise during new DNA synthesis and requires a handful of proteins to work cooperatively. Like basketball, there are 5 core players: hMHS2, hMSH6, hMLH1, hPMS2, and hEXOI. To win the MMR game, these 5 players must recognize the error and zip down the court of newly synthesized DNA, excising all the intervening nucleotides between the site of the error and the DNA polymerase that made the mistake. This scoring drive will allow the polymerase to go back and try the copying process again—usually getting it right the second time!

hMSH2 and hMLH1 are on the court of MMR for the entire game. Without them, there is no MMR team. These proteins double-team the opposition by forming a heterodimer with a related (homologous) protein through specific interactive domains (Figure 1).6–13 For example, hMSH2 and hMSH6 proteins tightly associate with each other through specific interactive domain(s),6 and this complex recognizes the most common errors (mismatches) created by the DNA polymerase. A mismatch in the usually smooth contour of DNA appears to make the helix more flexible and easily bent—or more easily captured in a bent configuration—by the hMSH2-hMSH6 heterodimer. This initial contact provokes ADP release by the human MutS homolog (MSH) proteins, triggering an ATP-dependent conformational change of the pincer-like hMSH2-hMSH6 heterodimer, which ultimately creates a clamp that is capable of sliding down the double helix like a Disney monorail down the track. Once an MSH heterodimer slides off the mismatch, the process is reiterated until the region on either side of the mismatch contains multiple sliding clamps.14 The MSH heterodimer essentially marks the location and direction of the error. This is more easily visualized in an animated figure at the Web site http://mmr.med.ohio-state.edu/mmrmovie.html.

The next step is less well understood, but involves the recruitment of a second protein heterodimer of hMLH1+hPMS2 to the ATP-bound MSH sliding clamps.13 The hMLH1-hPMS2 heterodimer is required to target the fifth member of the MMR team, hEXOI (exonuclease I), to the region to complete the excision of the mismatch error, ultimately permitting resynthesis of the new strand. hMSH2 and hMLH1 require their partners to function as a team, but may remain on the court even in their absence. On the other hand, hMSH6 and hPMS2 require hMSH2 and hMLH1, respectively, to stay in the game. If either of the 2 major MMR proteins is missing, or if their ability to interact as a heterodimer is disrupted, the minor partners are rapidly degraded.15 Thus, these interaction regions are particularly important for predicting pathogenicity of mutations in the DNA MMR genes.6

**A Strong Bench Improves MMR**

The human MMR system has a “bench.” In the absence of hMSH6, another protein, MSH3, can heterodimerize with hMSH2 and partially compensate during MMR (likely accounting for the ability of hMSH2 to remain in the MMR game even in the absence of MSH6). Actually, the hMSH2-hMSH3 heterodimer has a higher affinity for repairing insertion/deletion loop-type mutations that may occur during the replication of large repetitive sequences such as those found in centromeres and telomeres.16 To some degree, this could be likened to having specialists on the floor, each with their own particular strengths. Unfortunately, the hMSH2-hMSH3 heterodimer does not play the regular MMR game very well. The story with MLH1 is even more complicated, and not completely understood. hPMS2 requires dimerization with hMLH1 for full MMR activity and for stability. However, 2 other proteins, hPMS1 and hMLH3, also heterodimerize with hMLH1 (Figure 1). These heterodimers have yet-unknown contributions to the MMR bench.

Germline mutations in *hMSH2* or *hMLH1* give rise to the majority of classic Lynch syndrome.3,17 These mutations are like losing your key players, and when this happens, you lose the game. Losing hMSH6 or hPMS2 appears relatively less important functionally, and Lynch syndrome families of the *hMSH6* or *hPMS2* types have a milder form of the disease.18,19 In the case of loss of hMSH6, it is possible that hMSH3 is a good substitute on the floor, although an *hMSH3*-mutated Lynch syndrome family has never been identified. It is not known whether the other minor partner protein(s) can compensate for the loss of PMS2, but there are relatively fewer *hPMS2*-type Lynch syndrome families than there are for the major 2 genes.19
Assessing Functionality of MMR Proteins

To assess hMLH1 functionality, Raevaara et al.4 devised 4 plays that put variant MLH1 proteins into the MMR game—1 virtual and 3 real. Similar analyses have been performed on the MSH gene family.20 For the first play, they performed a comparative phylogenetic sequence analysis to determine whether the variant was in a functionally conserved location of the protein (the virtual game). This is much like how the coaches use height, speed, and shooting accuracy to predict the most recruitable players. To assess the real MMR game, Raevaara et al.4 artificially created missense variants of hMLH1 found in putative Lynch syndrome families. For the second play, they asked whether the modified players knew how to move into the right court. To test this, they determined whether the variant hMLH1 localized to the nucleus or cytoplasm. A center will not score many points in the backcourt! For the third play, they asked whether the hMLH1 variants could pair up with their hPMS2 partner. This was assessed by immunoprecipitation and by examining whether hPMS2 becomes stable in the presence of the hMLH1 variant. Fourth, they asked the most important question: once on the court and

Figure 1. Protein interaction regions for the human MMR System. The interaction regions between the human MutS homologs (MSH) are shown in blue.6 Interactions appear solely between hMSH2 with either hMSH3 or hMSH6 as a heterodimer. The interaction regions between the human MutL homologs (MLH/PMS) are shown in green.7,8 Interactions appear solely between hMLH1 with hPMS2, hPMS1, or hMLH3. The interaction regions between the human excision exonuclease hEXOI and the human MSH and MLH/PMS proteins are shown in grey.9 The discovery that the human MSH2-MSH6 heterodimer was a primary MSH in MMR engendered the nomenclature of MutSα for this complex.10 This was soon followed by MutSβ for the human MSH2-MSH3 complex and MutLα for the human MLH1-PMS2 complex,11,12 which may also be found as descriptions in the article by Raevaara et al.4 Although this contraction clearly saves letter space, it does not appear to be completely descriptive or entirely accurate and has not been used in this review. Biochemical studies have shown that the MLH/PMS heterodimer will only form an active repair complex with the ATP-bound MSH heterodimer sliding clamps13 (see http://mmr.med.ohio-state.edu/mmrmovie.html). The binding of ATP by the MLH/PMS heterodimer then appears to stabilize an interaction between hEXOI with the active MSH-MLH/PMS complex to perform the DNA excision step of MMR. The ATP binding domains of both MSH (Walker-like) and MLH/PMS (GHKL-like) proteins are shown in red. Mutations in the ATP binding domains and interaction regions are candidates for loss of function. However, predictions are not always accurate, and the team must be put on the court to assess their ability to play the game together.
paired, would the variant hMLH1-hPMS2 complex actually play the MMR game and score?

**Who Can Play the MMR Game?**

These plays substantially contribute to our ability to understand the role of sequence variation in altering the function of the MMR system. In many cases, the functional tests were substantially better than the predictive approaches to mutational analysis (the virtual game). Fundamentally, each of these sequence variations had to be considered individually. In several instances, the sequence variations were not associated with the expected clinical-pathologic manifestations in Lynch syndrome tumors such as microsatellite instability, loss of protein expression, and interaction with hPMS2. In these instances, the sequence variation was judged not to cause Lynch syndrome. In other instances, sequence variations resulted in the loss of hPMS2 stabilization, failure of the hMLH1 variant to move into the nucleus, and loss of MMR activity in vitro. These are clearly causative of Lynch syndrome. A few of the variants remained ambiguous, despite these functional tests.

There are several important caveats to this remarkable, Herculean analysis. Nearly all of the cellular tests were conducted in 293T cells because they have lost hMLH1 expression. We should exercise caution here because 293T cells are virally transformed human embryonic kidney cells with an unusually aneuploid karyotype, and substantial overexpression of other human DNA repair proteins (such as RAD51). Such an unusual cell line could contain other defects, which might influence the functional tests. In addition, many of the MLH1/ PMS2 interaction tests in this article differ substantially with a previous report by Guerrette et al, whose biochemical studies introduced only the MLH1 and PMS2 partners. In the present article, Raevaara et al used a tagged-precipitation approach, which can aggregate ancillary protein partners that might influence the analysis. Finally, the in vitro MMR studies examined the impact of a single protein concentration. A detailed quantitative analysis and titration study would have been more appealing and might have revealed the grey areas of biochemical efficiency versus a singular black-and-white appraisal.

**The Role of the Competition**

Just to add to the challenge of this type of work, it will ultimately be necessary to put the players up against variable competition. Some sequence variations may function fine in laboratory conditions but may falter when facing the stresses of chronic inflammation or specific types of infection. Recently, Martinez et al discovered a sequence variation in the promoter of the ornithine decarboxylase gene, which provided a remarkable degree of protection against adenoma recurrence but only in aspirin users. Some teams can beat nonconference opposition all winter long, and even win the conference title in February. But the big games in March determine the NCAA champion.

**Winning the Tournament**

The real issue here is that “winning the tournament” for a MMR gene sequence variation would require the identification of every individual in a putative Lynch syndrome family with these sequence variations, and following them prospectively for a period of 50 years to look for tumors. These functional analyses serve as scrimmages that substitute for the real game, and certainly improve our ability to interpret the results of germline mutation reports. Raevaara et al have begun to provide insights into form and function for the MMR genes, and offer a foundation that will help assess the functional significance of sequence variations in hMLH1 in the future. This group scored some 3 pointers with their work. Back to basketball one last time, University of North Carolina Tar Heels had a pretty good year, and won the 2005 NCAA tournament. Four of the key players have just decided to turn professional next year. Just like with the variant MMR proteins, it will be interesting to see how each player performs in the context of his new team!

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