ABSTRACT
There are multiple laboratories that offer germline genetic testing, and it can be difficult to discern which one to use for testing. Some laboratories have more comprehensive analysis techniques and capability, which increases the accuracy of testing. The ordering provider has a responsibility to select the appropriate laboratory with technologic capability for the needed testing, inform the laboratory of prior testing results in the patient and family so known familial variants have targeted testing, and use appropriate terminology and nomenclature when communicating information to other healthcare professionals, patients, and families. This report presents a case illustrating the potential errors that can occur when a provider selects a laboratory that lacks the capacity to detect certain pathogenic variants, such as large deletions and duplications. False-negative germline testing results lead to missed opportunities in prevention and early detection for not only the patient but often multiple family members, which may lead to psychosocial distress and late-detected cancers. This case highlights the complexities of genetic care and why management by a genetics professional can facilitate more fiscally responsible care, appropriate genetic testing, and comprehensive care for all family members at risk.

Case Report
A 41-year-old female underwent germline genetic testing ordered by her medical oncologist. Results showed that the patient tested negative for pathogenic variants in the following genes: APC, AXIN, BMPR1A, CDH1, CHECK2, EPCAM, MLH1, MSH2, MSH3, MSH6, MUTYH, NTHL1, PMS2, POLD1, POLE, and PTEN.

The patient sought genetic counseling because she was concerned about risk from her maternal side. Her mother had been recently diagnosed with papillary thyroid cancer and a maternal great grandmother had breast cancer at approximately 70 years of age. The genetics professional constructed a pedigree (Figure 1). The maternal family structure was relatively small. The patient had been diagnosed with colorectal cancer at age 40 years and was treated with surgical resection. Her paternal first cousin was diagnosed with colon cancer at age 35 years and had a known pathogenic MLH1 variant. The same oncologist ordered identical testing in the same laboratory for the patient’s 23-year-old daughter because, according to the daughter, there was concern the pathogenic variant might “skipped” a generation.

After much discussion about laboratory science, what constitutes a duplication, and potential risk from the maternal side, the patient decided she wanted to be retested. Because her insurance had already paid for testing, the patient was forced to pay for the testing out-of-pocket.

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The genetics professional offered testing for the known pathogenic MLH1 variant or consideration of a more comprehensive panel given the maternal history. There was no additional cost difference to the patient. The patient opted for a panel of 84 genes with RNA analysis. The large panel was selected because of the maternal/paternal family history; RNA analysis was included to potentially reduce the number of variants of unknown significance and detect deep intronic pathogenic variants.² The genetics professional conferred with the laboratory to determine whether they could detect the gross duplication prior to submitting the order. When the test order was placed, the genetics professional specifically requested that the laboratory look for the known duplication in the MLH1 gene, and supplied a copy of the known positive test result in the cousin as well as the patient’s previous negative test results. The results of the expanded testing proved that the patient did carry the known MLH1 pathogenic variant, which was consistent with her diagnosis of colorectal cancer. No other pathogenic variants or variants of unknown clinical significance were detected.

The genetics professional advised her that each of her biological children had a 50% chance of having the MLH1 pathogenic variant, and that testing is typically offered at approximately age 20 to 25 years.³ The genetics professional recommended that her daughter be retested, because the testing in the previous laboratory had been unable to detect the pathogenic variant. The daughter was retested for the known pathogenic variant in the laboratory with the capability of detection, and unfortunately tested positive as well. The genetics professional provided recommendations for prevention and early detection for both the patient and her daughter.³ Because this was known to be a paternal transmission, other relatives were identified who should be offered testing for the MLH1 pathogenic variant and instructed the family on how they could access genetic testing.

Implications for Oncology Care

Laboratory Science Matters

The choice and number of laboratories that perform germline testing is ever expanding and has undergone considerable change in the past decade due to technologic advances, regulatory requirements, and the 2013 United States Supreme Court ruling invalidating patents on genes.⁴ Laboratories that perform germline genetic testing began in academic institutions as research laboratories, and in 1980 the first commercial laboratories became available. Since 1980, there have been significant advances in technology, leading to a substantial

Figure 1. Family pedigree.
Abbreviation: ALS, amyotrophic lateral sclerosis.
reduction in the costs of sequencing, allowing for multiple genes on a single panel test. Because cancer is a common disease with known hereditary predisposition, it has been considered a lucrative opportunity for commercial genetic testing companies to focus efforts and marketing.5 There are many laboratories that offer testing, making selection of an accurate and appropriate genetic test difficult.6

Federal regulation of genetic testing is complicated and fragmented; oversight is provided by the Centers for Medicare & Medicaid Services (CMS), the FDA, and the Federal Trade Commission (FTC).4,5 CMS regulates laboratories through the CLIA program. The CLIA regulation categorizes laboratory tests by their complexity. Molecular genetic testing for hereditary diseases is considered high-complexity testing, requiring that laboratories performing these tests must meet CLIA standards for quality, accuracy, personnel, and reliability of testing. There is no specific category for genetic testing, which means laboratories must meet basic criteria for high-complexity tests but no additional requirements. The FDA considers genetic tests a medical device and therefore approves tests. Less commonly, a test may be marketed as a commercial test kit of collection devices and reagents that are packaged together and sold to multiple laboratories. A large number of laboratory-developed tests (LDTs) enter the market without FDA review, because they are created and used in the same facility. The laboratories themselves are principally regulated by CMS under CLIA. The FTC prohibits unfair or deceptive trade and advertising practices by assuring that descriptions of the product or test are accurate and not misleading.

When ordering genetic testing there are 3 broad considerations for genetic test accuracy: analytical validity, clinical validity, and clinical utility.7 Analytic validity refers to how well the test can detect the presence or absence of a particular gene or genetic variant. Clinical validity refers to how well the genetic variants being analyzed are related to the presence, absence, or risk of developing a specific disease. Clinical utility refers to whether the test can provide information about diagnosis, treatment, management, or prevention of a disease that will be helpful to patients and their providers. In this case, the concern is with analytic validity. The laboratory the oncologist used did not have the technology to detect the presence of the known pathogenic variant. An altered MLH1 gene is part of Lynch syndrome, rendering clinical validity, for which there are known management recommendations to reduce the morbidity and mortality associated with Lynch syndrome, rendering clinical utility.8

A combination of Sanger sequencing and/or next-generation sequencing (NGS) can detect >80% of pathogenic variants in MLH1, including insertions, missense variants, nonsense variants, and small deletions.9 The difference between Sanger sequencing and NGS is sequencing volume. Although the Sanger method only sequences a single DNA fragment at a time, NGS is massively parallel, sequencing millions of fragments simultaneously per run, and hundreds to thousands of genes at one time. Many laboratories rely on Sanger sequencing to validate gene variants identified first through NGS or to confirm a specific familial variant.8

An important aspect of genomic evaluation is the detection of relatively large deletions and duplications of an exon or more, which account for 5% to 10% of pathogenic variants in the MLH1 gene.9 Single-exon and multiexon deletions and duplications may require other methodologies for detection; the American College of Medical Genetics and Genomics recommends multiplex ligation-dependent probe amplification (MLPA).9

In this case, the laboratory where the specimen was initially sent did not use technology to detect a large duplication, such as MLPA technology. Large deletions and duplications occur in at least 10% of cases in Lynch syndrome, and the clinician needs to ascertain whether the laboratory has the capability to detect these pathogenic variants.9 The clinician can confer with the laboratory about this capability; a credentialed genetics professional can also assist with identifying the best laboratory and/or panel of tests to order.10

In this case, the family was known to have a large duplication in the MLH1 gene. Prior to ordering testing, it is necessary to obtain a copy of the testing to confirm the known pathogenic variant, because families often lack accurate information about the specific change; this step also assures the correct specific variant is ordered.11 Ideally, the testing is ordered in the same laboratory where the pathogenic variant was identified, but if that is not possible, the laboratory where the testing is being performed should be provided with a legible copy of the test results. When the report is issued, it should contain language that states whether the requested variant was or was not detected. Patients want assurance they tested negative for the known pathogenic variant in the family; in this case it was a false-negative.

Constructing a 3-Generation Pedigree Matters
Individuals can inherit germline risk from either or both sides of their family. An estimated 3.1% of individuals who test positive on a multigene hereditary cancer test have more than one pathogenic variant.12

Assessment of potential risk germline risk for developing malignancy begins with construction of a 3-generation pedigree. Although this is the best way to visualize risk, it is seldom done in clinical practice, and there are system barriers that limit pedigree construction.13 Including lack of time, the fact that pedigree construction capability is
not readily available in many electronic health records, and lack of reimbursement for the effort.

The genetics professional did a careful review of the patient’s medical record. No pedigree had been constructed. The patient’s medical chart stated simply that the “family has Lynch syndrome.” Furthermore, no medical history was recorded about either side of the family or prior testing.

Without a careful assessment of both sides of the family history and prior testing, it is nearly impossible to select the appropriate gene/panel of genes to order. The rationale for the selection of the genetic test ordered by the oncologist was not clear, and it is not clear whether there was consideration of the maternal history of breast and thyroid cancer because the history was not documented.

**Terminology Matters**
The chart stated that the “family has Lynch syndrome,” but this is an inappropriate use of the term without qualifying that the family has a known pathogenic variant. Current guidelines from the National Society of Genetic Counselors and the Collaborative Group of the Americas on Inherited Gastrointestinal Cancer state that the term Lynch syndrome should only be used for individuals identified as having germline heterozygous pathogenic variants in MLH1, MSH2, MSH6, PMS2, and EPCAM to prevent confusion. Although this family actually did have Lynch syndrome, at the time the note was written there was no review or proof that the family had a pathogenic variant. When the term is not correctly used, assumptions about risk and prior testing are made that may or may not be correct. When a pathogenic variant is identified in the family, the genetics professional will update the pedigree with the gene and specific variant, so it is clear as to who has and has not been tested.

A second term was used incorrectly in this case. Research demonstrates that there is a need for foundational knowledge among oncology professionals, and unfortunately this case illustrates this. It is incorrect to tell a family member a pathogenic variant can “skip” a generation. The term “skip” should never be used. Theoretically, if the mother tested negative for pathogenic variants, there was no need to test the daughter, because if it is not detectable in the mother, maternal risk will not be detectable in the offspring, resulting in a waste of financial resources. The oncologist was referring to penetrance, which is the likelihood that a clinical condition will occur when a particular genotype is present. Not everyone with a specific pathogenic variant will develop malignancy depending on the penetrance of the pathogenic variant.

**Implications for Providers**
Ordering germline genetic testing is far more complicated than it appears. There were multiple errors in this case that could have resulted in adverse care for both the patient and multiple family members. Although it was probably easier for the oncologist to order the testing, the oncologist may not have had enough knowledge of genomic science to order the correct test, in the best laboratory, and to interpret the results.

Constructing a 3-generation pedigree can take 10 to 30 minutes; obtaining and reviewing prior genetic testing records takes additional time. A busy clinical practice may not have the resources or time to complete these critical steps due to demands on providers to increase patient volumes and decrease appointment time. The availability of telehealth for genetic counseling has removed a significant barrier in access to professionals trained to assess and order testing.

The patient and her daughter incurred financial expenses as a result of the inadequate initial testing. Testing was ordered in a laboratory that did not have the capability of detecting the known MLH1 pathogenic variant, rendering the results useless. Both the patient and her daughter had out-of-pocket costs for the initial testing, and both incurred additional costs for the testing ordered by the genetics professional because their insurance would not pay for the additional testing. Ever-increasing healthcare costs demand that healthcare providers be good stewards and take steps to order the best genetic test. Involving a genetics professional whose sole focus is on test selection, laboratory selection, and interpretation is a potential cost-effective strategy.

Recommendations for patients with a pathogenic MLH1 variant are extensive and typically implemented at approximately age 20 to 25 years. When this patient tested negative, the guidelines were not implemented, putting this patient at risk for other primary cancers that could potentially be prevented or detected at an earlier stage. Based on the results after retesting, the daughter was devastated because she thought she had not inherited the risk; however, learning that she had provided her with the opportunity to reduce the risk with surgery and intensive surveillance. The potential liability associated with ordering the testing incorrectly should not be overlooked. Currently there is no consensus or formal policy in either genetics or oncology to guide the choice of panel size so that the scope of testing is appropriate in every clinical situation. Rather, the responsibility of this decision often falls on the medical oncologist, who may not have sufficient background to order the best test. If a provider orders a test, they accept the liability if they order the wrong test; including a genetics professional may decrease the possibility of error. Some institutions require all specimens sent for testing to have systematic review for appropriateness by a molecular pathologist or genetics professional. Another strategy to reduce error is including a genetics professional on a tumor board for consultation.
Coordinating care for the rest of the family is time-consuming but critical to reap the full benefits of genetic testing.\(^\text{23}\) If a clinician accepts responsibility for ordering genetic testing, they also accept responsibility for coordinating this care. Failure to communicate potential risk to other family members and coordinate this care is also a potential liability, and there is case law as far back as the early 1980s finding physicians negligent for failing to warn other family members of potential risk.\(^\text{24}\)

**Conclusions**

Germline genetic testing is a powerful tool for cancer prevention and early detection in not only the patient but also the family members, but only if assessment is complete, testing is correctly ordered in a reputable laboratory, and the results are interpreted correctly by a provider with the knowledge and expertise to do so.

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**References**


