

Appendix

Preparatory materials for students

Case 1:

A four-year-old Caucasian boy presents with slowly progressing loss of muscle tone. His mother reports that he had previously achieved all developmental milestones appropriately. She remarks that, although he used to have no problem, getting up off the floor has become difficult for her son and he now has to push himself up with his hands by walking them closer to and then up his legs. You note that his gait is somewhat abnormal and that his calves are large for a child his size. His mother reports the child's six-year-old sister does not have similar issues and that there is no family history of a muscle disorder. The mother is eight weeks pregnant.

You order serum creatine kinase levels, which come back markedly elevated. (This is a measure of muscle damage).

Learning issues:

You should be able to explain and justify your answer to each learning issue.

1. What diagnosis is the most likely and why?
2. What type of testing would you like to pursue and why: a blood test? a muscle biopsy? a genetic test? And what exactly will the test be evaluating? If you request a genetic test, you need to name a specific type of assay and the relevant type of mutation you want to test for (e.g., FISH to look for a specific large deletion).
3. If the result of this first assay is negative, what type of test would you order next?
4. If a genetic disorder is found in the boy, is it appropriate to proceed with testing in other members of the family? If so, what approach would you take?
5. If the mother wants prenatal testing for her current pregnancy, can it be done? What approach would you take?

Resources for preparation:

In addition to using the textbook (Thompson & Thompson *Genetics in Medicine*), below is a list of resources that will be useful in doing the research for the labs. These are invaluable tools that are used everyday by geneticists and other medical professionals.

GeneTests:

<http://www.genetests.org/>

This is the key resource for disease-specific genetic testing strategies and for the clinical labs that do the testing. The website includes GeneReviews, which are reviews of particular genetic conditions authored by experts in those conditions. The reviews include genetic testing and counseling strategies. A good glossary of medical genetics terms can be found in the educational resources section.

NCBI:

<http://www.ncbi.nlm.nih.gov/>

This is the website for the National Center for Biotechnology Information (NCBI), which is a series of public databases for molecular biology information. It is part of the National Library of Medicine.

Some of the databases at NCBI:

PubMed (scientific literature)

Entrez (genes)

RefSeq (reference genomes)

OMIM (Mendelian genetic diseases)

dbSNP (human single nucleotide polymorphisms, SNPs)

dbGaP (genome-wide association studies)

OMIM:

Online Mendelian Inheritance in Man

<http://www.ncbi.nlm.nih.gov/sites/entrez?db=OMIM>

This is an extensive database of Mendelian diseases in humans. It is searchable using several different parameters, including phenotypic features, disease name, or gene.

dbGaP is also a database that is searchable based on the same features, so you have to know which is the appropriate database for your question. dbGaP is a newer database that reports the results of genome-wide association studies for complex traits. At this point, dbGaP is much more relevant to genetic researchers than to clinicians; it's more of a data repository, whereas OMIM is a great information resource on genetic diseases, although the information is not synthesized.

Genetic Alliance

<http://www.geneticalliance.org/>

Genetic Alliance is a coalition of advocacy organizations for genetic disorders. On their website, you can search for information about particular diseases and you can sometimes get a wider range of reviews on particular disease topics, including those written for a lay audience. An important feature of the Genetic Alliance website is the ability to search for patient support groups, which can be a tremendous resource to provide to patients.

Another place with information for a patient audience is Genetics Home Reference at

<http://ghr.nlm.nih.gov/>

The American College of Medical Genetics has practical information on follow-up for newborn screening. This includes the ACT sheets, which outline the short-term actions a healthcare professional should take when confronting an abnormal newborn screening result.

http://www.acmg.net/AM/Template.cfm?Section=Act_Sheet&Template=/CM/HTMLDisplay.cfm&ContentID=1858

Emory Genetics Lab's clinical tests webpage

<http://genetics.emory.edu/egl/tests/> has helpful information on some of the tests that are offered locally. Test descriptions often include recommendations for the order in which tests should be performed (if there are options). Guidance on appropriate testing context is also provided.

Classroom materials for students: Case 1

Report 1

Patient Information	Final Report
PATIENT NAME: Affected Child	LABORATORY #: 08EG00000
DATE OF BIRTH: 2/4/2009	TYPE OF SPECIMEN: Whole blood, green & purple top
CROSS REFERENCE #: N/A	DATE COLLECTED: 3/7/2009
REFERRING DIAGNOSIS: 359.1 - hereditary progressive muscular dystrophy	DATE RECEIVED: 3/8/2009
PATIENT ID:	FINAL REPORT: 3/18/2009
Duchenne/Becker Muscular Dystrophy: EmArray DMD CGH Array	

Results: NO MUTATION DETECTED**Interpretation**

A sample from this individual was referred to our laboratory for molecular testing for Duchenne muscular dystrophy. Duchenne and Becker muscular dystrophies (DMD, BMD) are allelic X-linked muscular diseases that result from abnormalities of the dystrophin protein. Mutations in the *DMD* gene located on the X chromosome cause DMD and BMD. In males, a mutation of the single copy of the *DMD* gene causes disease. The mutation spectrum in the *DMD* gene is ~60% deletions, ~5% duplications, and ~35% point mutations.

No evidence of a deletion, duplication, or other structural abnormality was detected in the *DMD* gene. Analysis for *DMD* gene point mutations is recommended for individuals with a family history of DMD/BMD who have tested negative for deletion/duplication mutations. Genetic counseling is recommended.

METHODOLOGY: A DNA sample extracted from the blood sample was analyzed using a comparative genomic hybridization (CGH) array custom designed for analyzing the *DMD* gene.

NOTE: Direct analysis of *DMD* gene deletions is highly accurate. Possible diagnostic errors include sample mix-ups, genotyping errors and rare genetic variants that interfere with analysis, and other sources. Genomic coordinate numbering is based on GRCh36/hg18.

Pursuant to the requirements of CLIA '88, this test was developed and its performance validated by Emory Genetics Laboratory. It has not been cleared or approved by the U.S. Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary. This test is used for clinical purposes.

Jane Adams, MD, FAAP, FACMG
Medical Consultant

John Jones, PhD, FACMG
Laboratory Director

This case has been reviewed and electronically signed by a Laboratory Director.

Report 2

Patient Information	Final Report
PATIENT NAME: Affected Child	LABORATORY #: 08EE0000
DATE OF BIRTH: 2/4/2009	TYPE OF SPECIMEN: Whole blood, green & purple top
CROSS REFERENCE #: N/A	DATE COLLECTED: 3/7/2009
REFERRING DIAGNOSIS: 359.1 - hereditary progressive muscular dystrophy	DATE RECEIVED: 3/25/2009
PATIENT ID:	FINAL REPORT: 4/8/2009

DMD/BMD: DMD Gene Sequencing

Results: Mutation detected. One copy of a c.8443C>T (p.Q2815X) mutation was detected in the *DMD* gene of this individual.

Interpretation

A sample from this individual was referred to our laboratory for molecular testing for Duchenne muscular dystrophy. Duchenne and Becker muscular dystrophies (DMD, BMD) are allelic X-linked muscular diseases that result from abnormalities of the dystrophin protein. Mutations in the *DMD* gene located on the X chromosome cause DMD and BMD. In males, a mutation of the single copy of the *DMD* gene causes disease. The mutation spectrum in the *DMD* gene is ~60% deletions, ~5% duplications, and ~35% point mutations. Previous testing performed in our laboratory did not detect any deletions or duplications in the *DMD* gene of this individual (see report 08EG00000).

Sequence analysis of the entire coding region, flanking intronic sequence, eight promoters, and five previously described deep intronic mutations in the *DMD* gene identified one copy of a c.8443C>T mutation in exon 57. The c.8443C>T mutation is predicted to result in the replacement of the codon for the amino acid glutamine at position 2815 with a premature stop codon (p.Q2815X; CAG>TAG). The c.8443C>T (p.Q2815X) mutation has been reported in a patient with DMD.^{1,2} Genetic counseling is recommended.

Emory Genetics Laboratory offers targeted mutation analysis for family members at risk for carrying the mutations identified in this individual. For more information on targeted testing, please visit <http://www.geneticslab.emory.edu> or call (404) 778-8500 to speak with the laboratory genetic counselor.

Variants: Sequence analysis also detected 6 hemizygous (1 copy) sequence variants in the *DMD* gene of this individual: IVS17+13T>C (intron 17), c.2645A>G (p.D882G, exon 21), c.5234G>A (p.R1745H, exon 37), IVS54+11C>T (intron 54), c.8810G>A (p.R2937Q, exon 59) and IVS66+15T>C (intron 66). These variants are common in the general population and are not likely related to disease.³

Methodology: PCR was used to amplify the 79 coding exons, flanking intronic sequence, eight promoters, and five previously described deep intronic mutations of the *DMD* gene. The PCR products were sequenced in the forward and reverse directions. Nucleotide numbering is based on GenBank accession number NM_00406.1; nucleotide 1 corresponds to the A of the start codon ATG.

References:

1. <http://www.dmd.nl/>
2. Prior *et al.* 1995. *Am. J. Hum. Genet.* 57: 22-33.
3. www.ncbi.nlm.nih.gov/snp

NOTE: The interpretation of nucleotide changes is based on our current understanding of the *DMD* gene. These interpretations may change over time as more information about this gene becomes available. Possible diagnostic errors

include sample mix-ups, genetic variants that interfere with analysis, and other sources.

Pursuant to the requirements of CLIA '88, this test was developed and its performance validated by Emory Genetics Laboratory. It has not been cleared or approved by the U.S. Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary. This test is used for clinical purposes.

Jane Adams, MD, FAAP, FACMG
Medical Consultant

John Jones, PhD, FACMG
Laboratory Director

This case has been reviewed and electronically signed by a Laboratory Director.

Report 3

Patient Information		Final Report
PATIENT NAME:	Mom of Affected Child	LABORATORY #: 08KM00000
DATE OF BIRTH:	7/7/1971	TYPE OF SPECIMEN: Whole blood
CROSS REFERENCE #:	N/A	DATE COLLECTED: 4/13/2009
REFERRING DIAGNOSIS:	Carrier testing for DMD	DATE RECEIVED: 4/13/2009
PATIENT ID:		FINAL REPORT: 4/22/2009

Familial Mutation Testing: DMD/BMD (Targeted *DMD* Gene Sequencing)

Results: No mutation detected.

Interpretation

A sample from this individual was referred to our laboratory for carrier molecular testing for Duchenne muscular dystrophy (DMD). Previous molecular analysis performed in our laboratory detected one copy of a c.8443C>T (p.Q2815X) mutation in the *DMD* gene of this individual's affected son (see report 08EE0000).

Sequence analysis of exon 57 of the *DMD* gene, in which the familial mutation was located, did not detect the c.8443C>T (p.Q2815X) mutation. This result indicates that a new *DMD* mutation occurred in this individual's affected son; however, it is possible that she has gonadal mosaicism for this mutation. Therefore, we recommend that prenatal testing be considered in subsequent pregnancies. Genetic counseling is recommended.

Emory Genetics Laboratory offers prenatal testing and targeted mutation analysis for family members at risk for carrying the mutation identified in this family. For more information please visit <http://www.genetics.emory.edu> or call (404) 778-8500 to speak with the laboratory genetic counselor.

Methodology: Genomic DNA from this individual was used to amplify the coding region and the flanking intronic sequence of the *DMD* gene in which the familial mutation is located, and the corresponding PCR products was sequenced in the forward and reverse directions. Other regions of the gene were not analyzed. Nucleotide numbering is based on GenBank accession number NM_00406.1; nucleotide 1 corresponds to the A of the start codon ATG.

NOTE: The interpretation of nucleotide changes is based on our current understanding of the *DMD* gene. These interpretations may change over time as more information about these genes becomes available. Possible diagnostic errors include sample mix-ups, genetic variants that interfere with analysis, and other sources.

Pursuant to the requirements of CLIA '88, this test was developed and its performance validated by Emory Genetics Laboratory. It has not been cleared or approved by the U.S. Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary. This test is used for clinical purposes.

Jane Adams, MD, FAAP, FACMG
Medical Consultant

John Jones, PhD, FACMG
Senior Laboratory Director

This case has been reviewed and electronically signed by a Laboratory Director.

Report 4

Patient Information**Final Report**PATIENT NAME: **Mom of Affected, Fetus of**LABORATORY #: **08EQ00000**DATE OF BIRTH: **7/7/1971**TYPE OF SPECIMEN: **Cultured chorionic villi**

CROSS REFERENCE #:

DATE COLLECTED: **4/23/2009**REFERRING DIAGNOSIS: **Son has features of DMD**DATE RECEIVED: **5/14/2009**

PATIENT ID:

FINAL REPORT: **5/22/2009****Prenatal: DMD/BMD (Targeted *DMD* Gene Sequencing)**

Results: Mutation detected. One copy of the c.8443C>T (p.Q2815X) mutation was detected in the *DMD* gene of this individual.

Interpretation

A cultured chorionic villi sample (sample 08EQ0000) from Mom of Affected's current pregnancy (collected 04/23/09) was submitted for molecular testing for DMD/BMD. Previous molecular testing performed in our laboratory on Mom of Affected's affected son (see report 08EE00000) identified one copy of a c.8443C>T (p.Q2815X) mutation in the *DMD* gene. Subsequent targeted testing on Mom of Affected (see report 08KM00000) did not identify the c.8443C>T (p.Q2815X) mutation. The possibility of Mom of Affected being a gonadal mosaic cannot be ruled out. Information provided to us indicates that chromosome analysis performed at Genzyme Genetics of this cultured chorionic villi sample determined that the fetus is male.

Sequence analysis of exon 57 of the *DMD* gene, in which the familial mutation was located, detected one copy of the c.8443C>T (p.Q2815X) mutation. This result indicates that the fetus did inherit this familial *DMD* gene mutation. These results also indicate that Mom of Affected is a gonadal mosaic for the familial mutation and at risk for passing this mutation to future children. Genetic counseling is recommended.

Comparative analyses of the maternal and fetal DNA samples have been conducted using PCR of polymorphic sites in other regions of the genome. These data distinguish between the maternal and fetal samples and did not show any evidence of maternal cell contamination of the cultured chorionic villi sample.

Methodology: Genomic DNA from this individual was used to amplify the coding region and the flanking intronic sequence of the *DMD* gene in which the familial mutation is located, and the corresponding PCR products were sequenced in the forward and reverse directions. Other regions of the gene were not analyzed. Nucleotide numbering is based on GenBank accession number NM_00406.1; nucleotide 1 corresponds to the A of the start codon ATG.

NOTE: The interpretation of nucleotide changes is based on our current understanding of the *DMD* gene. These interpretations may change over time as more information about these genes becomes available. Possible diagnostic errors include sample mix-ups, genetic variants that interfere with analysis, and other sources.

Pursuant to the requirements of CLIA '88, this test was developed and its performance validated by Emory Genetics Laboratory. It has not been cleared or approved by the U.S. Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary. This test is used for clinical purposes.

Jane Adams, MD, FAAP, FACMG
Medical Consultant

John Jones, PhD, FACMG
Senior Laboratory Director

This case has been reviewed and electronically signed by a Laboratory Director.

Summary material for students: Case 1

Case 1: Duchenne muscular dystrophy

There are a few aspects of this case that suggest Duchenne muscular dystrophy (DMD) as a possible diagnosis. This is the most common progressive muscle disorder in young boys, the boy in our case is the right age for presentation, he has the Gower sign, and he has enlarged calves, as would be expected. Also, DMD is an X-linked disorder, which means that, typically, boys are affected.

DMD is caused by mutations in the gene that encodes the protein dystrophin. This gene is named *DMD*, and it is one of the largest genes in the human genome.

Comparison of testing approaches:

There are two main testing strategies for DMD: protein-based testing via muscle biopsy or DNA-based testing via blood sample. After muscle biopsy, you would use fluorescently labeled antibodies to detect the dystrophin protein. Boys with Duchenne muscular dystrophy do not produce this protein, no matter what mutation they carry, so this is a definitive test for DMD. This technique avoids issues with identification of the mutation in this large gene. However, muscle biopsy is an invasive test, and you can't do further risk analysis in the family if you don't know what mutation the boy has. On the other hand, if the boy does have DMD, the immunological assay on the muscle biopsy will be positive (meaning you won't see any dystrophin) no matter what the underlying mutation is.

For DNA-based testing, the most common types of mutation in the *DMD* gene are large deletions and duplications, so you would first do some sort of testing to detect this type of mutation. Southern blots are one way to do this, but a quicker and less labor-intensive approach that we have discussed in class is array-based comparative genomic hybridization (CGH). The resolution of a whole-genome CGH array is not sufficient for this application; however, a CGH array specific to the *DMD* gene allows a complete analysis of the gene for deletions and duplications. If no deletion or duplication is found by this method, you would next look for a point mutation, because this is the next most common type of mutation. A very recent development in *DMD* testing is the capability of complete sequencing of this enormous gene. Few testing labs do this. Other labs might sequence particular parts of the gene. This means that to interpret a sequence result for *DMD*, you need to know exactly how much of the gene was sequenced and the sensitivity of the test, which should be provided by the testing lab. If DNA sequencing does not yield a mutation in a boy who has signs of DMD, it could be that a mutation is present in *DMD* but it wasn't found because the gene was only partially sequenced. This caveat holds for other genetic diseases: you must know the limitations of a genetic test in order to interpret test results, particularly if they are negative. A negative DNA-based test result does not negate a clinical diagnosis of DMD; even with the most thorough testing strategies, approximately 1-2% of mutations in *DMD* will be missed. If no mutation is found in the patient in our scenario, following *DMD* array CGH and *DMD* sequencing, a muscle biopsy could be ordered to check for dystrophin in the muscle, if that hasn't been done already.

Test results and risk analysis for this family:

In our case, the patient did have a point mutation in the *DMD* gene. His mother was concerned with other risks in the family. Because this is an X-linked disorder, if the patient inherited the mutation, it would have come from his mother. Without knowing anything else about the family, the risk that the mother carries the mutation is ~67%, based on the new mutation rate for *DMD* of ~33%. To better estimate the family risks, mutation-specific testing was performed for the mother. The complete gene analysis is not performed in the mother because we know the mutation that is carried in the family. This approach is less expensive and more easily interpreted in the context of the family history. In a blood sample, the mother in our case did not carry the mutation that was found in her affected son. Because the new mutation rate for Duchenne MD is ~33%, this is not unexpected; the child could be affected due to a random new mutation in one oocyte. However, the mother already has another child and is currently pregnant and is concerned with the risks to her other children. Does the lack of the mutation in a blood sample from the mother mean the siblings aren't at risk? The answer is no; we know from experience that approximately 15% of mothers who have a child with DMD have germline mosaicism for the relevant mutation. This means that the mutation is not detectable in the mother's blood, but it will be present in some of her oocytes and could be passed to other children. This is a fairly unusual situation for a Mendelian disorder. It's always possible for any mutation, but the mutability of the *DMD* gene makes it more common with this disorder. We can't look for germline mosaicism without harvesting eggs, so it's not something we test for; we can simply quote the empirical risk that has already been observed.

Because the mom in this case is already pregnant, we can do prenatal diagnosis in the child if the parents are concerned. To do this, we would perform a chorionic villus sampling or amniocentesis and do a karyotype to determine whether or not the fetus is male and then, since we know what mutation the affected brother has, we would again do mutation-specific testing for that particular mutation in the fetus. If the specific mutation in the family is not known, prenatal testing would not be offered. Also, if the fetus is a girl, prenatal diagnosis is not advised, due to the inability to predict the severity of the phenotype in a female with a *DMD* mutation¹.

The affected child in this case also has a six-year old sister. We could do carrier testing on this girl to determine whether or not she inherited the mutation. In many medical situations, parental autonomy to make medical decisions for a child is an unquestioned right. However, according to statements by professional organizations such as the American Society of Human Genetics, genetic carrier testing is an exception and should not be performed on children unless there is a timely medical benefit to the child. This principle preserves the child's future autonomy to elect genetic testing. Therefore, some might argue that we shouldn't do this testing until the girl is an adult and seeks this information herself, because it is not until that point that she can provide full informed consent to the testing. However, female carriers of *DMD* mutations can have medical issues, so this carrier testing is arguable for medical reasons. If she does carry the mutation, she will need to be more closely watched for features related to the mutation, including cardiomyopathy, muscle weakness and cramping, and left ventricular dilation. Students may wish to discuss the pros and cons to testing this girl and their thoughts on the age at which this testing should occur.

Other implications for test results

Mutation-specific molecular therapies for treatment of Duchenne muscular dystrophy (and other genetic disorders) are in clinical trials. These include therapies that cause mutated *DMD* exons to be skipped² and others that cause translating ribosomes to read-through nonsense mutations in *DMD*³. The goal of these approaches is to induce production of dystrophin in muscle, albeit at less than normal levels and, in the case of the exon skipping approaches, of a shorter than normal length. Because these strategies are specific to mutation type and/or mutation location within *DMD*, the *DMD* gene mutation must be identified before patients can be enrolled in clinical trials. Further, genetic testing is recommended by the DMD Care Considerations Working Group, which was established by the Centers for Disease Control and Prevention to establish guidelines for the treatment and management of DMD. This working group argues that genetic testing for DMD is always required, even if the diagnosis has been established by the absence of dystrophin expression in a muscle biopsy, because it is required for the most accurate genetic counseling and for consideration of these mutation-specific therapies⁴.

Preparatory materials for students

Case 2:

A 35-year-old African American female comes to your office for her annual physical. She is very concerned because her paternal uncle died of colon cancer two months previously at the age of 53. Her father died of colon cancer at the age of 49. She also knows of other relatives who have had “various cancers.” The patient demands screening for “all kinds of cancer.” Physical exam reveals no malignancies and a fecal occult blood test is negative. How would you proceed?

Learning issues:

You should be able to explain and justify your answer to each learning issue.

1. What are the most common hereditary forms of colon cancer? How common are they?
2. How will you determine:
 - a. whether the family history fits the expected pattern for a hereditary cancer?
 - b. whether the family history suggests the type of hereditary cancer in the family?
 - c. whether your patient is at risk of this hereditary cancer?
3. What are the appropriate genetic testing strategies for the common hereditary forms of colon cancer? This includes:
 - a. methodologies
 - b. determining who is a valid candidate for each type of testing
 - c. outlining a strategy for testing at-risk individuals in an affected family
4. What is the appropriate follow-up:
 - a. for somebody who tests positive for a mutation associated with a hereditary colon cancer?
 - b. for somebody who tests negative for a mutation?
5. How likely is somebody who carries a hereditary colon cancer-associated mutation to get cancer?

Additional information provided to students during the class discussion:

The patient’s paternal aunt had endometrial cancer at age 40

This aunt’s daughter had ovarian cancer at age 36

The patient’s father had a single, poorly differentiated lesion of the right colon. There is an archived sample from the father’s surgery that contains tumor tissue, as well as some surrounding normal tissue.

The results of microsatellite instability testing are positive. (Representative data from this test are shown to the students during class).

Classroom materials: Case 2 Report 1

Patient Information

Final Report

PATIENT NAME: Father of Ms. Gerard

LABORATORY #: **07HN00000**

DATE OF BIRTH:

TYPE OF SPECIMEN: **Paraffin imbedded tissue**

CROSS REFERENCE #:

DATE COLLECTED: **1/7/1997**

REFERRING DIAGNOSIS: **colon cancer**

DATE RECEIVED: **1/8/2007**

PATIENT ID:

FINAL REPORT: **1/15/2007**

Lynch Syndrome Panel: *MLH1*, *MSH2*, and *MSH6* Gene Sequencing

Results: Mutation detected. One copy of a c.643delCA deletion mutation was detected in the *MSH2* gene of this patient.

Interpretation

It is our understanding that this patient/individual had a possible diagnosis of Lynch syndrome (hereditary nonpolyposis colon cancer, or HNPCC). Sequencing of the coding region of three genes, *MLH1*, *MSH2*, and *MSH6*, was requested for this individual.

Sequence analysis identified one copy of a c.643delCA deletion mutation in the *MSH2* gene of this individual (GenBank mRNA accession no. NM_000152.3; note nucleotide number 1 corresponds to the A residue of the start codon ATG). The c.643delCA deletion mutation is predicted to result in the introduction of a premature translation stop downstream of the mutation. This mutation has not been previously observed in patients with Lynch syndrome, but is of a type that is predicted to be disease causing. No mutations were observed in the *MLH1* or *MSH6* genes.

This result is consistent with a diagnosis of Lynch syndrome for this individual. This result must be interpreted in the context of this individual's clinical profile and family history. Genetic counseling is recommended.

Sequence analysis detected several homozygous and heterozygous sequence variants in *MSH2* and *MSH6* genes, currently classified as polymorphisms according to the database of the International Collaborative Group on HNPCC. It is important to note that this assay cannot differentiate the presence of the variant in both copies of a gene from the presence of the variant in one gene combined with a deletion of/in the second gene.

MLH1: None

MSH2: Heterozygous sequence variants- IVS5+27delAA, IVS10+12G>A and IVS12-6C>T

MSH6: Homozygous sequence variants- IVS2-52T>G, intron2, c.540T>C (p.D180), IVS3+26G>T
Heterozygous sequence variant- IVS9-13delT

Emory Genetics Laboratory offers targeted mutation analysis for family members at risk for carrying the mutation identified in this individual. For more information on custom diagnostic testing, please visit <http://www.geneticslab.emory.edu> or call (404) 778-8500 to speak with the laboratory genetic counselor.

METHODOLOGY: A PCR-based assay was used to amplify the coding exons and immediate flanking regions of the *MLH1*, *MSH2*, and *MSH6* genes. The PCR products were sequenced in both the forward and reverse directions.

NOTE: The interpretation of nucleotide changes is based on our current understanding of the *MLH1*, *MSH2*, and *MSH6* genes. These interpretations may change over time as more information about these genes becomes available. This analysis will not detect large deletions or mutations in the promoter or other regulatory regions. Some intronic mutations will not be detected by this assay. Possible diagnostic errors include sample mix-ups, genetic variants that interfere with analysis, and other sources.

This test was developed and its performance determined by Emory Genetics Laboratory. It has not been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary. This test is used for clinical purposes.

Jane Adams, MD
Medical Director

John Jones, PhD, FACMG
Laboratory Co-Director

This case has been reviewed and electronically signed by a Laboratory Director.

Genetic counseling letter

Dear Ms. Gerard:

It was a pleasure meeting with you again, along with your husband on 2/15/2007 at Emory Genetics. This letter will summarize our conversation regarding your genetic test results.

The result of your DNA mismatch repair gene mutation analysis (*MSH2*) was positive. The c.643delCA *MSH2* gene mutation was found in you. Finding this mutation confirms the diagnosis of hereditary nonpolyposis colorectal cancer (HNPCC), also known as Lynch syndrome, in your family. You received a copy of your test results and the brochure *Understanding Your Genetic Test Result*.

Cancer Risks

A recent literature review proposed the following predicted lifetime cancer risks associated with Lynch syndrome:

- | | |
|---------------------------------|--------|
| • Colorectal cancer in men | 28-75% |
| • Colorectal cancer in women | 24-52% |
| • Endometrial cancer | 27-71% |
| • Ovarian cancer | 3-13% |
| • Gastric cancer | 2-13% |
| • Urinary tract cancer | 1-12% |
| • Brain tumor | 1-4% |
| • Bile duct/gall bladder cancer | 2% |
| • Small bowel cancer | 4-7% |

Vasen et al. Journal of Medical Genetics, 2007; 44:353-362

Screening Recommendations from NCCN® Practice Guidelines in Oncology – v.1.2007

Colorectal cancer – colonoscopy beginning at age 20-25 or 10 years younger than the youngest age at diagnosis in the family; repeated every 1-2 years

Renal cancer – consider annual urinalysis with cytology and imaging of the renal collecting system (CT urogram)

Endometrial and ovarian cancer – annual transvaginal ultrasound and endometrial sampling starting by age 30-35 or 5 to 10 years earlier than the youngest diagnosis of these cancers in the family, CA-125 blood test every 6 months, and consideration of prophylactic hysterectomy and bilateral salpingo-oophorectomy when child bearing is completed

Other abdominal cancer – consider periodic evaluation (typically done with endoscopy)

Next Steps

You have not yet had a colonoscopy or other cancer screening. Given your family history of colon and gynecologic cancers we would recommend that you establish care with both a

gastroenterology (GI) and a gynecology group. At your request, we provided you with the names and contact numbers for doctors in the Emory HealthCare System.

It would be helpful for the gynecologist to have more specific information about the cancers that were diagnosed in the family in order to provide you with more accurate information about available screening methods and detection rates.

Family Implications

We reviewed the implications of these test results for your family. The cancer risk associated with *MSH2* gene mutations is inherited in a dominant pattern. This means that a mutation in only one *MSH2* gene in the pair is enough to cause an increased risk for cancer. Since parents share half of their genes with their children, there is a 50% chance to pass the *MSH2* mutation to any future children. Your sister also has a 50% chance to have the gene mutation. You think that she has decided to pursue genetic testing.

Some individuals might decide to take measures to prevent passing along a specific gene mutation. There are reproductive options available, including pre-implantation genetic diagnosis, sperm donation, and adoption. Prenatal testing with CVS or amniocentesis procedures is also available. Genetic testing for an HNPCC-related gene mutation would not be recommended for minor age children, since it is very rare for colon cancer to present in the teens. Without a family history of very early-onset cancer, it is recommended to wait until age 18 or older to pursue presymptomatic testing on at-risk children. Genetic counseling and testing would be appropriate prior to initiating any invasive screening protocol.

Again, it was a pleasure to meet with you for genetic counseling. You are planning to schedule appointments with a gastroenterologist and gynecologist to initiate screening. We also encouraged you to enroll in a colorectal cancer registry. You may wish to talk with your relatives to see if any of them are already part of a registry. Being part of a registry helps to further knowledge about Lynch syndrome and may enable you to participate in research projects if you desire. Many registries also provide periodic updates about advances in cancer screening or treatment to their members.

As new advances are being made in cancer genetics, additional information may become available in the future that would benefit your family. We suggest that you to contact our office annually to see if there is new information that relates to your situation. Please do not hesitate to contact us at any time if you have any questions or we can be of further assistance.

Sincerely,

Carrie Smith, MS, CGC
Cancer Genetics Program

John Jones, MD, FAAP, FACMG
Associate Professor of Human Genetics

Summary material for students: Case 2

The two major hereditary forms of colon cancer are: familial adenomatous polyposis (FAP) and Lynch syndrome (also known as hereditary nonpolyposis colon cancer, HNPCC). In either case, you expect an autosomal dominant pattern of transmission. In contrast to sporadic colon cancers, with FAP and Lynch syndrome you typically see multiple affected family members, an earlier age of onset than you usually see for the cancers, and possibly multiple types of cancer in the same person. You could also see other non-colon cancers in the family, with the type of cancer depending on the gene that is mutated in the family. Although genomic analysis of tumors is increasingly being used in a research setting to understand the evolution of cancers, it is not the appropriate analysis in this situation. The central question we want to answer is: is there a germline (inherited) mutation in this family that is associated with a cancer predisposition?

Some of the questions that you would want to ask to distinguish the two hereditary cancer syndromes in a family are:

- Are there other cancers in the family and, if so, what kind?
- Do we know anything about the colon cancer seen in the affecteds?
- At what age were the affecteds diagnosed?

FAP overview:

The additional cancers associated with FAP are:
osteomas and soft tissue tumors (Gardner syndrome)
CNS tumors, usually medulloblastoma (Turcot syndrome)
cancer of the small bowel

FAP can also be associated with extra teeth and other dental anomalies, gastric polyps, duodenal polyps, desmoid tumors, and congenital hypertrophy of the retinal pigment epithelium.

Untreated, individuals with FAP will almost inevitably get colon cancer (near complete penetrance). Hundreds to thousands of precancerous colonic polyps develop beginning at a mean age of 16 years. The mean age of diagnosis without treatment is 39 years.

Lynch syndrome overview:

In addition to colon cancer, Lynch syndrome is associated with:
endometrial cancer
ovarian cancer
brain tumors, particularly glioblastoma
gastric cancer
cancers of the hepatobiliary tract
cancers of the upper urinary tract
pancreatic cancer

Individuals with a Lynch syndrome mutation have an approximately 80% lifetime risk of developing colon cancer, with the average age of diagnosis being age 60 (previous data had suggested the mean age of onset around 44 years).

One set of criteria for the clinical diagnosis of Lynch syndrome in a family are the Amsterdam II criteria (there are others, including the Bethesda guidelines). The Amsterdam II criteria are:

- 3 or more family members (1 of whom is a first-degree relative of the other two) with colorectal or another Lynch-related cancer
- 2 successive generations affected
- 1 or more of the cancers diagnosed before age 50
- FAP has been excluded

Genetic testing

If you want to perform genetic testing for either FAP or Lynch syndrome in a family, it is highly preferable to look for mutations in an affected individual first (as we have already discussed for hereditary breast and ovarian cancer syndrome). Remember, genetic tests for colon cancer don't have 100% sensitivity, so a negative result (i.e. no mutation was found) in an unaffected person cannot distinguish between the following situations:

No FAP (or Lynch) mutation in the family

The tested individual didn't inherit the mutation that is in the family

You just didn't find the mutation that this person inherited

Keep in mind that if you don't find a mutation in an affected individual, you cannot rule out the presence of a mutation altogether, because the sensitivity of the test is not 100%. In general, only the most commonly mutated genes for Lynch syndrome are sequenced when genetic testing for Lynch syndrome is performed.

Mutations in *APC* cause FAP. These mutations tend to be point mutations, and there are many different mutations that have been found across the gene. Because of this, the first genetic test to order for FAP testing is sequence analysis of *APC* looking for germline mutations (so you need to do this in a blood or normal tissue sample) in an affected individual. You can do deletion/duplication analysis if no point mutation is found.

There are multiple genes that cause Lynch syndrome when they are mutated, and this makes genetic testing somewhat more complicated than it is for FAP. All of these genes encode proteins involved in DNA mismatch repair, and we can use this shared function in our approach to testing. The Evaluation of Genomic Applications in Practice and Prevention (EGAPP) working group is a group established by the Centers for Disease Control and Prevention to use an evidence-based system to evaluate genetic tests. EGAPP found sufficient evidence to recommend that genetic testing for Lynch syndrome be offered to individuals with newly diagnosed colorectal cancer⁵. Their analysis suggested improved health outcomes in family members as a result of this testing. They found several genetic testing strategies for Lynch syndrome that were effective, but did not identify a single most effective strategy. Some of the options for the testing strategy were compared in our discussion, as described below.

A flowchart for genetic testing for Lynch syndrome is provided (Supplementary figure). If Lynch syndrome is suspected in a family, a set of clinical criteria, such as the Amsterdam criteria, can be used to determine whether a Lynch syndrome diagnosis is likely. If a family meets these criteria, one can look for evidence of a mismatch repair defect in a tumor from an affected person in the family. This functional analysis is used to increase suspicion of Lynch

syndrome but it is not a diagnostic test, nor does it tell you which gene is mutated in the germline. We assess mismatch repair through an analysis of microsatellite instability (MSI); microsatellites are prone to mutation when the mismatch repair systems aren't working well. You will only see this instability in the tumor, because in the rest of the cells in the body, one copy of the relevant mismatch repair gene is intact, so cells can correct mismatches that occur (i.e., only cells in the tumor have the second "hit" mutation). Colon tumors due to Lynch syndrome will very often exhibit microsatellite instability. However, MSI is not diagnostic of Lynch syndrome, because some sporadic colon cancers also exhibit this property. Once MSI has been documented, you can look for the inherited Lynch syndrome mutation in one of the mismatch repair genes. This is done through sequence analysis in a blood (or other normal tissue) sample of the affected individual. This test can be informed by an immunohistochemistry analysis on the tumor that is used to detect reduced or absent staining for any of the mismatch repair proteins, indicating there might be a mutation in the gene for that protein. If the immunohistochemistry doesn't lead you to an obvious culprit to sequence, you can sequence the major mismatch repair genes for Lynch syndrome all at once. The most commonly mutated genes in Lynch syndrome are *MLH1*, *MSH2*, *MSH6*, and some labs also sequence *PMS2*.

Case results and risk analysis:

In our scenario, the family history is most suggestive of Lynch syndrome. An archived sample is available from our patient's deceased father, and testing of the tumor indicates MSI. Sequence analysis of normal tissue from the sample indicates there is a likely cancer-predisposing mutation in *MSH2*. This means our patient (and her siblings) is at 50% risk of inheriting the mutation. Mutation-specific testing in our patient indicates that she did inherit the mutation.

The use of a deceased person's tissue sample brings up ethical discussions related to its use. Who decides whether this testing is appropriate? How does one access the sample? Genetic counselors can coordinate this testing and work with physicians to argue the medical benefit of this testing for at-risk relatives.

Follow-up:

If somebody is found to carry an FAP mutation prior to getting colon cancer, the recommendation would be for them to prophylactically have their colon removed, because mutations are 100% penetrant. With Lynch syndrome, they would be advised to have increased surveillance. If a cancer develops, they should be advised to have a complete colectomy, which you would usually try to avoid with a sporadic colon cancer. They should also be advised on a cancer surveillance strategy for the other types of cancers associated with Lynch syndrome. Please refer to the genetic counseling letter for further details.

If no mutation is found in a family that fits the diagnostic criteria for Lynch syndrome, asymptomatic individuals in the family would still need to be under increased cancer surveillance, because they are still at increased risk of cancer due to their family history—we just didn't find the relevant mutation. This could be because there is a mutation in one of the genes we sequenced, but we didn't find it (maybe it fell outside the sequenced region or it was a big deletion), or because it was in a gene that we didn't sequence. The cancer could also be due to a more complex contribution of genetic and/or environmental risk factors.

Suppose our patient has siblings, who as a result of this testing would be at 50% risk of carrying the cancer-predisposing mutation. Our patient does not wish to inform them of her genetic test results and the related cancer risks. What is the physician's duty to warn the siblings of these health risks? What is the physician's duty to maintain patient confidentiality?

Respect for patient confidentiality is a central tenet of ethical medicine, but it is permissible to breach this confidentiality when failure to do so may expose others to harm. Guidelines from the American Medical Association and the American Society of Clinical Oncology state that a physician is obligated to warn the *patient* about family members' risks but does not have the duty to warn the family members directly. There has been disagreement in United States courts as to this duty to warn with some cases deciding that this duty is satisfied by warning the patient only; others have found that the physician does have a duty to warn the family members.

1. Abbs S, Tuffery-Giraud S, Bakker E, Ferlini A, Sejersen T, Mueller CR. Best practice guidelines on molecular diagnostics in Duchenne/Becker muscular dystrophies. *Neuromuscul Disord*. 2010;20(6):422-427.
2. Goemans NM, Tulinius M, van den Akker JT, et al. Systemic administration of PRO051 in Duchenne's muscular dystrophy. *N Engl J Med*. 2011;364(16):1513-1522.
3. Pichavant C, Aartsma-Rus A, Clemens PR, et al. Current Status of Pharmaceutical and Genetic Therapeutic Approaches to Treat DMD. *Mol Ther*. 2011;19(5):830-840.
4. Bushby K, Finkel R, Birnkrant DJ, et al. Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and pharmacological and psychosocial management. *Lancet Neurol*. 2009;9(1):77-93.
5. Evaluation of Genomic Applications in Practice and Prevention Working Group. Recommendations from the EGAPP Working Group: genetic testing strategies in newly diagnosed individuals with colorectal cancer aimed at reducing morbidity and mortality from Lynch syndrome in relatives. *Genet Med*. 2009;11(1):35-41.