

CLINICAL AND SYSTEMATIC REVIEWS

Clinical Genetic Testing in Gastroenterology

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Rapid advances in genetics have led to an increased understanding of the genetic determinants of human disease, including many gastrointestinal (GI) disorders. Coupled with a proliferation of genetic testing services, this has resulted in a clinical landscape where commercially available genetic tests for GI disorders are now widely available. In this review, we discuss the current status of clinical genetic testing for GI illnesses, review the available testing options, and briefly discuss indications for and practical aspects of such testing. Our goal is to familiarize the practicing gastroenterologist with this rapidly changing and important aspect of clinical care.

Clinical and Translational Gastroenterology (2016) 7, e167; doi:10.1038/ctg.2016.23; published online 28 April 2016

Subject Category: Clinical Review

INTRODUCTION

The landscape of genetic testing in clinical medicine is continuously evolving, and this has been driven primarily by rapid advances in sequencing technology that reduce costs and turnaround time,¹ the proliferation of genetic testing services offered by both private companies and academic institutions,² and ongoing investigations that identify new genetic contributions to human disease. Where genetic testing was once the sole purview of select research centers, it has become easy for clinicians in both academic and community practices to order genetic testing for a growing number of medical conditions, and the ease of ordering tests can approach that of routine blood work. However, the nuances involved in the interpretation of genetic test results and their clinical implications are more complex. As two of the primary barriers for ordering these tests continue to decrease, namely cost and turnaround time, genetic testing for specific conditions will likely become increasingly common as a part of routine clinical practice.

In the fields of cancer genetics, personalized cancer medicine,^{3,4} and prenatal risk assessment,^{5,6} the use of genetic testing has become well integrated into standard practice. However, the applications for genetic testing are steadily growing in other areas of medicine. In this review, we seek to review the current spectrum of gastrointestinal (GI) disorders for which germline genetic testing is clinically available, discuss the general indications for genetic testing, review the types of genetic tests available and their possible results, and discuss practical issues related to interpretation of these test results. Specific testing indications and medical management guidelines for each condition, which in many cases are complex and nuanced, are beyond the scope of this review.

CLINICALLY AVAILABLE GENETIC TESTS

Table 1 is a review of genetic tests for GI disorders that are clinically available as determined by a review of the Genetic Testing Registry through the National Center for Biotechnology Information,² and we briefly summarize the broad categories below. This list excludes those conditions that are confined to the neonatal and pediatric populations, such as neonatal acute liver failure syndromes. All testing discussed below refers to germline testing unless otherwise specified.

Hereditary colon cancer syndromes. The hereditary colon cancer syndromes can be broadly categorized into polyposis and nonpolyposis conditions, but this distinction is becoming blurred as many conditions appear to overlap with both categories. The polyposis syndromes comprise a group of genetically distinct conditions that are associated with a dramatically increased risk of both colonic polyps and cancer. The polyposis syndromes can be subdivided based upon the predominant histologic subtype observed: adenomatous, hamartomatous, or serrated polyps. The adenomatous polyposis syndromes include the classic and attenuated forms of Familial Adenomatous Polyposis, caused by germline mutations in the *APC* gene,⁷ an autosomal recessive form caused by mutations in the *MYH* base-excision repair gene,⁸ and a newly described syndrome due to a germline mutation in either the *POLE* or *POLD1* gene called Polymerase proofreading-associated polyposis (PPAP).⁹ Hamartomatous polyposis syndromes include Juvenile Polyposis, Cowden Disease, or Peutz–Jeghers syndrome.¹⁰ These are all transmitted in autosomal dominant patterns. The genetics of serrated polyposis remains incompletely defined. Collectively, these polyposis syndromes account for ~1% of all colorectal cancers.¹¹ Genetic testing for polyposis syndromes is generally considered in the context of suspicious endoscopic findings

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Received 3 September 2015; accepted 11 March 2016

Table 1 GI disorders for which clinical genetic testing is currently available

Class	Condition	Gene(s)	Inheritance
Colon cancer (polyposis syndromes)	Familial adenomatous polyposis (FAP)	<i>APC</i>	AD
	Gardner syndrome	<i>APC</i>	AD
	Attenuated FAP (AFAP)	<i>APC</i>	AD
	MYH-associated polyposis (MAP)	<i>MUTYH</i>	AR
	Polymerase proofreading-associated polyposis (PPAP)	<i>POLD1, POLE</i>	AD
	Peutz–Jeghers syndrome	<i>STK11</i>	AD
	Cowden syndrome	<i>PTEN</i>	AD
	Bannayan–Riley–Ruvalcaba	<i>PTEN</i>	AD
	Juvenile polyposis	<i>BMPR1A, SMAD4</i>	AD
	Lynch syndrome	<i>MLH1, MSH2, MSH6, PMS2, EPCAM</i>	AD
Colon cancer (nonpolyposis)	Hereditary diffuse gastric cancer	<i>CDH1</i>	AD
Gastric cancer	Familial pancreatic cancer	<i>BRCA1&2, ATM, CDKN2A, PALB2, STK11, Lynch syndrome genes</i>	AD
Pancreatic cancer	MEN-1 syndrome	<i>MEN1</i>	AD
Pancreatic endocrine tumors	Crohn's disease	Multiple, including <i>ATG16L1, NKX2.3, STAT3, IL-10, NOD2</i>	Complex
	Inflammatory bowel disease	Ulcerative colitis	Multiple, including <i>NKX2.3, STAT3, ECM1, IL-10</i>
Pancreatitis	Hereditary pancreatitis	<i>PRSS1, CFTR, SPINK1</i>	AR (SPINK1, CFTR) AD (PRSS1) Complex (CFTR)
			Complex (CFTR)
Celiac disease	Celiac disease	Haplotypes <i>HLA-DQ2, HLA-DQ8</i>	Complex
Metabolic liver disease	Wilson disease	<i>ATP7B</i>	AR
	Alpha-1-antitrypsin deficiency	<i>A1AT</i>	Autosomal codominant
Hyperbilirubinemias	Hereditary hemochromatosis	<i>HFE, TFR2, SLC40A1</i>	AR (HFE, TFR2) AD (SLC40A1)
	Crigler–Najjar syndrome, type II	<i>UGT1A1</i>	AR
	Gilbert's syndrome	<i>UGT1A1</i>	AR
	Dubin–Johnson syndrome	<i>ABCC2</i>	AR
Auto-inflammatory disorders	Rotor syndrome	<i>SLCO1B1, SLCO1B3</i>	AR
	Familial Mediterranean fever	<i>MEFV</i>	AR
	Hibernian fever (TRAPS)	<i>TNFRSF1A</i>	AD
	Hereditary GIST	<i>CKIT</i>	AD
GIST	Autosomal dominant polycystic liver disease	<i>LRP5, PRKCSH, SEC63</i>	AD
	Hirschsprung disease	Multiple	
	Acute porphyrias	<i>PBGD, ALAD, CPOX, PPOX</i>	AD (PBGD, CPOX, PPOX) AR (ALAD)

AD, autosomal dominant; AR, autosomal recessive; GIST, gastrointestinal stromal tumor.

(i.e., cumulative adenoma count >10) or a family history of established polyposis.

Most cases of hereditary colon cancer are not associated with polyposis and are due to Lynch syndrome, previously referred to as hereditary non-polyposis syndrome colorectal cancer (HNPCC). Inherited in an autosomal dominant fashion, Lynch syndrome is associated with a germline mutation in one of the five genes that regulate DNA mismatch repair (*MLH1, MSH2, MSH6, PMS2, or EPCAM*) and is associated with an increased risk of several extra-colonic cancers, most notably endometrial, urinary tract, and ovarian cancer.^{12,13} Genetic testing should be considered in the setting of a strong family history as defined by the Amsterdam criteria (three family members with colon cancer, two affected family members that are first degree relatives of the third, and one colon cancer diagnosed before age 50 years),^{14,15} or when colorectal cancer tissues demonstrate high levels of microsatellite instability or immunohistochemical evidence of mismatch repair protein deficiency. Some guidelines suggest that all

newly diagnosed colorectal cancers be evaluated for mismatch repair deficiency in order to maximize the detection rate of Lynch syndrome in the population.¹⁶

Familial pancreatic cancer. Families with at least two first degree relatives with pancreatic cancer or any three relatives with pancreatic cancer are considered to meet clinical criteria for the “Familial Pancreatic Cancer” syndrome.¹⁷ Familial clustering of pancreatic cancer most commonly occurs in the absence of an identified genetic cause,¹⁸ but an increased risk of pancreatic cancer may occasionally be seen in the context of other cancer susceptibility syndromes such as hereditary breast cancer (due to mutations most commonly in *BRCA2*, but also *BRCA1* or *PALB2*^{19–22}), familial atypical multiple-mole melanoma syndrome (due to mutations in *CDKN2A*²³), Peutz–Jeghers syndrome,²⁴ Lynch syndrome,²⁵ or Ataxia-Telangiectasia (*ATM*).²⁶ It should also be noted that many individuals with a germline mutation in a gene associated with an increased risk of pancreatic cancer will

often not exhibit a family history of pancreatic cancer, and this raises additional challenges in identifying these patients. There is also a dramatically increased risk of pancreatic cancer in those with hereditary pancreatitis (discussed below). Overall, though, the genetic basis of most cases of familial pancreatic cancer is currently unknown, but large-scale sequencing studies continue to expand the list of genes implicated in this condition.²⁷ Genetic testing can be considered for those who meet clinical criteria for Familial Pancreatic Cancer syndrome or when pancreatic cancer occurs in the context of one of the cancer syndromes defined above. It should be noted that identification of an underlying mutation in the *BRCA1*, *BRCA2*, or *PALB2* gene has important therapeutic implications, as tumors in these individuals exhibit greater responses to PARP inhibitors.^{28,29}

Hereditary diffuse gastric cancer. Hereditary diffuse gastric cancer is a rare cause of familial gastric cancer that is due to a germline E-cadherin (*CDH1*) mutation.³⁰ The tumors are exclusively of the “diffuse”-type and not the more common “intestinal”-type, making early detection of cancer very challenging. This autosomal dominant condition is also associated with a high risk of lobular breast cancer in women. Genetic testing should be considered if a family exhibits two cases of confirmed diffuse gastric cancer with one diagnosis before 50 years, three cases of diffuse gastric cancer at any age, one case of diffuse gastric cancer before age 40 years, or the combination of diffuse gastric cancer and lobular breast cancer.

Inflammatory bowel disease. Although the majority of patients with inflammatory bowel disease (IBD) have no family history of the condition, twin studies have suggested a hereditary component,³¹ and many genes that are associated with an increased risk of IBD have been identified as low penetrance alleles through GWAS studies.³² However, the genetics of IBD are complex and poorly understood. The inheritance patterns are not strictly Mendelian, and the penetrance is variable. With rare exceptions,³³ no single genetic defect underlies the disease. A gene alteration associated with very early onset IBD in childhood is a homozygous mutation in *IL-10* or *IL-10* receptor.³⁴

Diagnostic genetic testing is not routinely indicated at this time, and clinical testing currently involves analysis of selected single nucleotide polymorphisms (SNPs) in conjunction with serologic markers to predict the likelihood of IBD, and to assist with distinguishing between a diagnosis of Crohn’s vs. ulcerative colitis.

Pharmacogenetic testing can be used to guide medication management in certain scenarios, and one notable example in the practice of gastroenterology is the use of *TPMT* testing to guide therapy with thiopurines in IBD.

Hereditary pancreatitis. Hereditary pancreatitis syndromes can present as either recurrent episodes of acute pancreatitis or as idiopathic chronic pancreatitis. They can be inherited in either autosomal dominant or recessive patterns. Mutations in serine protease 1 gene (*PRSS1*) result in an autosomal dominant form,³⁵ whereas mutations in *SPINK1* or more commonly *CFTR* can result in an autosomal recessive form,

the latter with or without other clinical manifestations of cystic fibrosis depending on the particular *CFTR* mutations.³⁶ Heterozygous mutations in *SPINK1* appear to increase the risk of developing pancreatitis when combined with other environmental or genetic insults.³⁷ Polymorphisms in additional genes, including *CLDN2* and *CPA1*, have recently been associated with pancreatitis.³⁸

Celiac disease. Celiac disease is a disorder caused by an immune response to dietary gluten consumption that most frequently affects the small bowel mucosa but is associated with other systemic manifestations. Although the genetics of celiac disease are complex, nearly all patients are HLA-DQ2 or HLA-DQ8 positive.³⁹ Although the presence of these haplotypes is not sufficient for development of the disease, HLA-DQ2 or HLA-DQ8 testing can be useful in excluding the condition. One particular scenario would be to exclude the diagnosis in an individual already on a gluten-free diet.

Metabolic liver disease. Although the majority of genetic causes of metabolic liver disease present early in life, Wilson’s disease, alpha-1 antitrypsin deficiency and hereditary hemochromatosis are the three most common genetic causes of metabolic liver disease encountered by the gastroenterologist in adults. Hemochromatosis, a disorder of iron overload with multiorgan pathology, is caused in most cases by autosomal recessive mutations in *HFE*, with C282Y homozygosity being the most common genotype.⁴⁰ Although the carrier frequency of pathologic *HFE* mutations is relatively high, the penetrance of a clinical disease phenotype with homozygous pathologic mutations is low.⁴¹ Genetic testing is indicated when serum chemistries indicate a transferrin saturation level of >45%.

Wilson’s disease is an autosomal recessive defect in copper transport due to defects in *ATP7B*, resulting in hepatic and neuropsychiatric pathology. The condition most commonly presents in children or adolescents, but the disease is not uncommonly first recognized in adults.⁴² Genetic testing should be considered in the context of unexplained liver disease, Kayser–Fleischer rings, neuropsychiatric symptoms, elevated urinary copper excretion, or low serum ceruloplasmin levels. Alpha-1-anti-trypsin deficiency, inherited in an autosomal co-dominant fashion, results in both lung and liver disease related to deficiency in the protease inhibitor alpha-1-anti-trypsin. Multiple pathogenic alleles of the gene encoding alpha-1-anti-trypsin exist, the most common of which is the “Z” allele.⁴³

Hyperbilirubinemias. The hereditary hyperbilirubinemias are a group of disorders resulting from defects in the metabolism of bilirubin. Gilbert’s syndrome, a form of unconjugated hyperbilirubinemia, is the most common and generally of no clinical significance, and is due to mutations in *UGT1A1*. More rare conditions include Crigler–Najjar syndrome (also caused by mutations in *UGT1A1*), Dubin–Johnson syndrome (mutations in *ABCC2*), and Rotor syndrome (*SLCO1B1* and *SLCO1B3*).

Hereditary GIST. Familial gastrointestinal stromal tumor (GIST) syndrome is an extremely rare autosomal dominant

condition that results from activating germline mutations in the c-Kit proto-oncogene.⁴⁴ In contrast to the vast majority of hereditary cancer syndromes that are due to germline mutations in a tumor suppressor gene, this condition results from a germline mutation in a tumor oncogene. Multiplicity of GIST lesions in the GI tract is common, and individuals with two or more independent GISTs or multiple first-degree relatives with GISTs should be referred for genetic testing.

Autoinflammatory disorders. The autoinflammatory disorders comprise a group of diseases characterized by aberrant activation of the innate immune system, often resulting in periodic fevers and bouts of abdominal pain. These conditions are often considered in the setting of a patient with recurrent episodes of abdominal pain with an otherwise unrevealing workup. Two of the most common conditions are Familial Mediterranean fever, caused by a mutation in the *MEFV* gene, and TRAPS (TNF receptor-1-associated periodic syndrome), also known as Hibernian fever, due to mutations in the *TNFR1* gene.

INDICATIONS FOR GENETIC TESTING AND WHEN TO REFER

As the number of available tests has increased, so has the scope of indications for genetic testing. Currently accepted clinical indications for genetic testing include diagnostic testing, presymptomatic testing, newborn screening, carrier testing, prenatal testing, and pharmacogenetic testing.⁴⁵ Despite the increasingly broad indications for genetic testing in general, genetic testing ordered by the practicing gastroenterologist will typically be only for diagnostic purposes, occasionally for presymptomatic testing among family members once a genetic condition is identified, or pharmacogenetic testing to guide thiopurine use. Genetic testing in general is indicated when the diagnosis is likely to change to management, and establishing a genetic diagnosis can have important implications for medical management.

Diagnostic testing refers to a genetic test that is ordered to confirm a suspected diagnosis, or to establish a diagnosis considered in a differential diagnosis. For example, *APC* testing might be ordered in a patient who on routine screening colonoscopy is found to have multiple adenomatous polyps, or *HFE* testing for a patient with a high transferrin saturation and abnormal liver function tests. (see case 1—Appendix). Another scenario in which genetic testing might be considered is presymptomatic testing. Presymptomatic testing is targeted to an individual at risk for developing a syndrome when there is a known disease-causing mutation in a family member or a concerning family history. For example, testing the asymptomatic siblings of a patient with confirmed Wilson's disease can determine whether they are also at risk of developing the syndrome.

In general, pre-test genetic counseling is recommended before ordering a genetic test to ensure that patients are well informed about all the potential ramifications of the test results. An additional logistical consideration is determining which family member may be the ideal person in whom to initiate testing. In most cases, testing should ideally be initiated

in an "affected" family member who exhibits clinical features of the suspected condition. If a mutation is identified, testing for this specific mutation can then be offered to at-risk relatives.

Given the rapid proliferation of available genetic tests for GI disorders, the ease of ordering such tests, and an increasing familiarity with the genetic landscape of the subspecialty, many gastroenterologists have gained facility with ordering and interpreting genetic tests for specific disorders they have clinical experience with, such as hereditary hemochromatosis. However, for asymptomatic patients who are at increased risk for a hereditary condition in the context of a specific family history, for those with a potential diagnosis of a cancer predisposition syndrome such as Lynch syndrome, and when testing for children is being considered, it is more appropriate to refer to a genetic counselor or a center with expertise in genetic testing given the complex nature of the risks and benefits in these scenarios as well as the increasingly complex legal framework that governs the informed consent process for these indications in many states.

Clinics offering genetic counseling and diagnostic services can be found through the Genetic Testing Registry website.²

INFORMED CONSENT

An essential part of any genetic testing is obtaining informed consent from the patient. Although the specifics will vary somewhat based on the clinical circumstance and specific test (such as whole-genome sequencing⁴⁶ or cancer susceptibility testing⁴⁷), essential elements include a detailed explanation of the test being ordered, the possible test results including findings of unknown significance (see below), the risks associated with the test including potential genetic discrimination, how the test results will be communicated, the medical and emotional implications that positive testing will have for the patient and family members, and the implications that test results will have for disease management. We recommend referring a patient to a genetic counselor if the physician is unfamiliar or uncomfortable with any aspects of this process.

In the context of increasing concern over the privacy and health implications of genetic testing, many states such as Massachusetts⁴⁸ have specific regulations regarding the consent process for genetic testing, particularly in the setting of pre-symptomatic testing.

TYPES OF GENETIC TESTING AND RESULTS

Although genetic testing encompasses a wide range of techniques ranging from karyotype analysis to whole genome sequencing, the great majority of genetic tests in gastroenterology involve DNA sequencing, where the test is designed to detect the presence or absence of a specific mutation in the DNA sequence known to be causative of or associated with the specific condition. The scope of the DNA sequencing pursued for a specific test can vary greatly, often as a consequence of the known genetic architecture of the specific condition. It is important to have a basic familiarity with types of tests available, as tests that involve more extensive analysis can result in complex and unexpected results, and knowledge

of the possible types of results of a specific test is an essential prerequisite to providing informed consent.

Specific gene sequencing or targeted exon sequencing is the most common technique employed. These tests will sequence the entire coding region of a gene, or the areas of the gene in which the most common disease-causing mutations are found. These tests may fail to identify causative mutations for many reasons, including mutations present outside of the regions sequenced (such as introns or promoter regions), or inherited epimutations (such as promoter methylation).

Genetic testing that is not restricted to a single gene but instead includes a broad panel of disease-associated genes is becoming increasingly common.⁴ In this case, multiple genes associated with an increased risk of a specific condition (such as colon cancer) are all analyzed using next-generation sequencing techniques. Although this approach can be more efficient in that it eliminates the need for serial gene testing, these tests increase the risk of identifying variants of unknown significance (see below).

Finally, whole exome sequencing, in which the coding regions of the entire genome are sequenced, and whole genome sequencing, in which the entire genome is sequenced, are clinically available. They remain primarily research tools at this time, though successful clinical applications of these tests have been demonstrated.⁴⁹ Although powerful in their breadth, the analysis and interpretation of these test results is complex. Unexpected mutations in genes not associated with the disease under consideration, and identification of multiple variants of unknown significance are common. It can be challenging to explain the significance of such findings to patients, and some basic guidelines have been proposed.⁵⁰ The rapidly decreasing costs of these tests will only increase their use in the coming years, and will likely result in many incidental findings of unknown significance in otherwise asymptomatic persons. The decision to pursue such expansive and undirected testing must be considered carefully.

SEQUENCING RESULTS

DNA sequencing can identify variants that fall into different categories based upon their likelihood of causing disease. Although the specific categories reported may vary,^{51,52} they generally include benign variants or polymorphisms, pathologic or pathogenic variants, and variants of unknown significance.

Benign variants are alterations in the genetic code that are known or strongly predicted to have no influence on the affected gene and thus not associated with specific disease. An example would be a DNA base change within a codon that does not alter the final amino-acid sequence of the encoded protein. A pathologic mutation is a mutation that has been demonstrated to be disease causing or is strongly predicted to be disruptive in protein function, such as a missense mutation in a critical protein domain (e.g., C282Y in the *HFE* gene) or a mutation that leads to a premature truncation of the protein.

Variants of unknown significance are those mutations where the impact on protein function and thus disease risk is unknown. They are typically point mutations that result in a single amino-acid substitution in a region of the protein where it may not appear to affect protein function. The presence of a

variant of unknown significance in the absence of a clear disease-causing mutation can be a challenge to interpret. Such results are generally considered inconclusive, and clinical management decisions should not be based solely upon such results. The emotional and psychological impact upon patients when receiving such an uncertain result can be variable (see case 2—Appendix).

PRIVACY AND DISCRIMINATION

In the United States, the Genetic Information Nondiscrimination Act (GINA) was passed in 2008 and provides protection from discrimination in health insurance and employment based upon genetic test information on the federal level.⁵³ However, genetic information may still be used in determinations of life and disability insurance and GINA does not apply to certain groups such as the military, health benefits plans for federal employees, and veterans obtaining health care through the Department of Veterans Affairs.⁵⁴

PRACTICAL CONSIDERATIONS

Once a decision has been made to proceed with genetic testing, there are several practical issues to consider.

Turnaround time continues to be an important factor, with even highly targeted testing taking several weeks to return results from commercial laboratories.

Genetic testing remains costly, with billed costs reaching as high as several thousand dollars. Given great variation in insurance coverage for this type of testing, prior authorization is generally recommended.

Finally, choosing a specific company or academic laboratory for a genetic test can seem somewhat challenging given the multiple choices offered by different companies. As a starting point, we recommend consulting the Genetic Testing Registry through the NCBI² to identify CLIA-approved testing facilities.

CONCLUSION

The landscape of genetic testing continues to change dramatically with exponential advances in sequencing technology and a greater understanding of the genetic contributions to human disease. Tests that were once available only in research settings are increasingly available for clinical use, and as the barriers to genetic testing continue to shrink, clinical genetic testing for a variety of GI disorders will be increasingly adopted. As such, a working knowledge of basic genetics, the landscape of genetic disorders in gastroenterology and hepatology, the importance of informed consent, and practical issues surrounding genetic testing will be increasingly important and valuable for clinicians.

CONFLICT OF INTEREST

Guarantor of the article: Daniel C. Chung, MD.

Specific author contributions: Both authors were involved in the design, writing, editing, and final approval of the manuscript.

Financial support: None.

Potential competing interests: None.

Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ Genetic testing plays an important role in the diagnosis of many commonly encountered GI conditions including hereditary GI cancer syndromes, metabolic liver diseases, and inflammatory disorders.
- ✓ Genetic testing can be useful to screen pre-symptomatic relatives for disease.

WHAT IS NEW HERE

- ✓ There are some limitations to genetic testing, and pre-test counseling is recommended.
- ✓ The range of genetic test options will continue to increase in the future.

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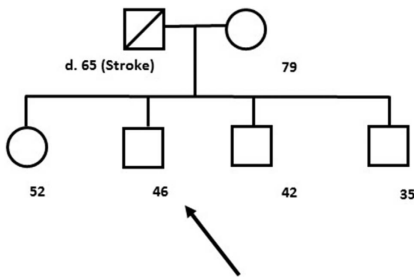
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APPENDIX

Case 1—screening family members

A 46-year-old Caucasian man is referred to your clinic for abnormal LFTs. He was discovered to have a mild elevation of his transaminases during routine blood work for an insurance physical, and has had an unremarkable workup for autoimmune and infectious causes of liver disease. His family history is notable for a father who died at the age of 65 due to a stroke, and he has two brothers and a sister who are healthy, as well as a young daughter. As part of your workup you obtain serum iron and transferrin levels that reveal a transferrin saturation of 60%. You have a clinical suspicion for hereditary hemochromatosis and decide to pursue diagnostic testing.



After obtaining appropriate informed consent, you order HFE testing through a national genetic testing company. This specific test looks for two specific point mutations, H63D and C282Y, which are thought to be associated with most cases of hemochromatosis. As full gene sequencing is not offered, variants of unknown significance are not a potential issue.

The testing results return positive for C282Y homozygosity, consistent with a diagnosis of hereditary hemochromatosis, and you initiate therapeutic phlebotomy. Given the diagnosis and the autosomal recessive inheritance pattern, the patient's siblings should be screened for hemochromatosis. Each would have a 25% chance of also carrying homozygous mutations. You feel comfortable with the management of the disease as well as the risks and benefits of genetic testing, and therefore arrange to screen the other family members in your clinic.

Case 2—variants of unknown significance

A 32-year-old woman is referred to you for a screening colonoscopy because her mother was diagnosed with multiple polyps and colon cancer at the age of 43. During the endoscopy you find and remove 11 polyps, 10 of which are adenomatous by pathology. Given the family history and endoscopic findings, you suspect attenuated familial adenomatous polyposis (AFAP) and decide to pursue diagnostic testing.

You order *APC* gene testing, and a variant of unknown significance is identified.

This testing result raises many challenging clinical questions, including whether this is a previously unrecognized pathologic mutation causing AFAP in this individual, what diagnosis you would offer to the patient, how to proceed with further testing and endoscopic surveillance in this individual, and whether to screen the patient's first-degree relatives including her young children. In general, this type of result would be considered inconclusive and clinical management decisions would be based primarily upon the clinical and family history features. Given the complexity of these issues, you refer the patient to an academic center with expertise in hereditary colon cancer and polyposis syndromes.

