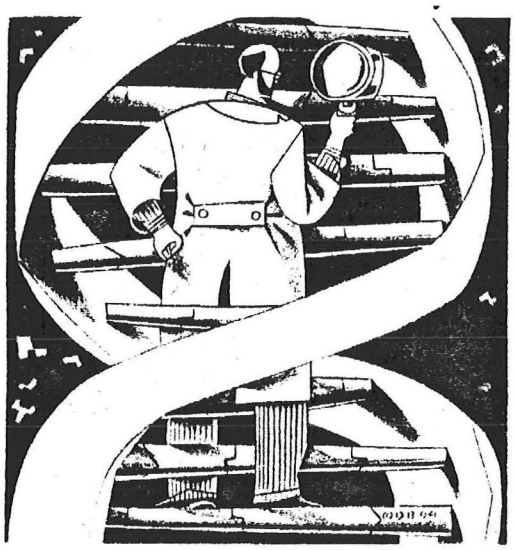
Predictive Genetic Testing for Cancer

Andrew R. Zinn, M.D., Ph.D.

Internal Medicine Grand Rounds
UT Southwestern Medical School
August 14, 1997



"Genetic testing should be considered in the same way as a new drug. It can have efficacy, and it can have toxicity."
–Dr. Francis Collins, Director, National Center for Human Genome Research, 1994

OncorMed Announces Breast Cancer Gene Analysis Now Available To High Risk Patients
Gaithersburg, MD–Jan. 11, 1996. OncorMed, Inc. (AMEX:ONM) announced today that its BRCA1 genetic testing service is now available to any person in the United States who is considered to be at high risk for inherited breast-ovarian cancer, under specific guidelines and with the recommendation of their physician.

"We will fight any sale of this test before there is consensus on how it should be used."
–Mary Jo Ellis Kahn, National Breast Cancer Coalition, March 1996

OncorMed Introduces BRCA2 Mutation Analysis To Hereditary Breast Cancer Testing Service
Gaithersburg, MD--July 23, 1996. OncorMed, Inc. (AMEX:ONM) announced today that on August 1, 1996 it will begin offering a genetic testing service for mutations found in the recently discovered BRCA2 gene that lead to inherited breast, ovarian and other cancers.

Andrew R. Zinn, M.D., Ph.D.
Assistant Professor of Internal Medicine
Division of Endocrinology
Eugene McDermott Center for Human Growth and Development

Research interests: genetic disorders of human growth, development, and reproduction; sex chromosome disorders; infertility; obesity
http://mcdermott.swmed.edu/mcd_center/zinn/

BRCA1 Testing Vignette

DP is a 33 year old American woman of Ashkenazi Jewish descent living in southern Italy. Her paternal grandmother and aunt died at relatively early ages of ovarian cancer. The aunt's only daughter was in her late 20's, unmarried, and very concerned about finding a husband and having children as soon as possible so that she could have a prophylactic oophorectomy. The aunt lived in Jerusalem, and she participated in the studies that led to identification of the 185delAG *BRCA1* mutation, present in about 1% of Ashkenazim. She was found posthumously to have this mutation. Her daughter was counseled that there was a 50% chance that she inherited the mutation, and she agreed to undergo genetic testing as part of ongoing Israeli studies. She did not have the mutation.

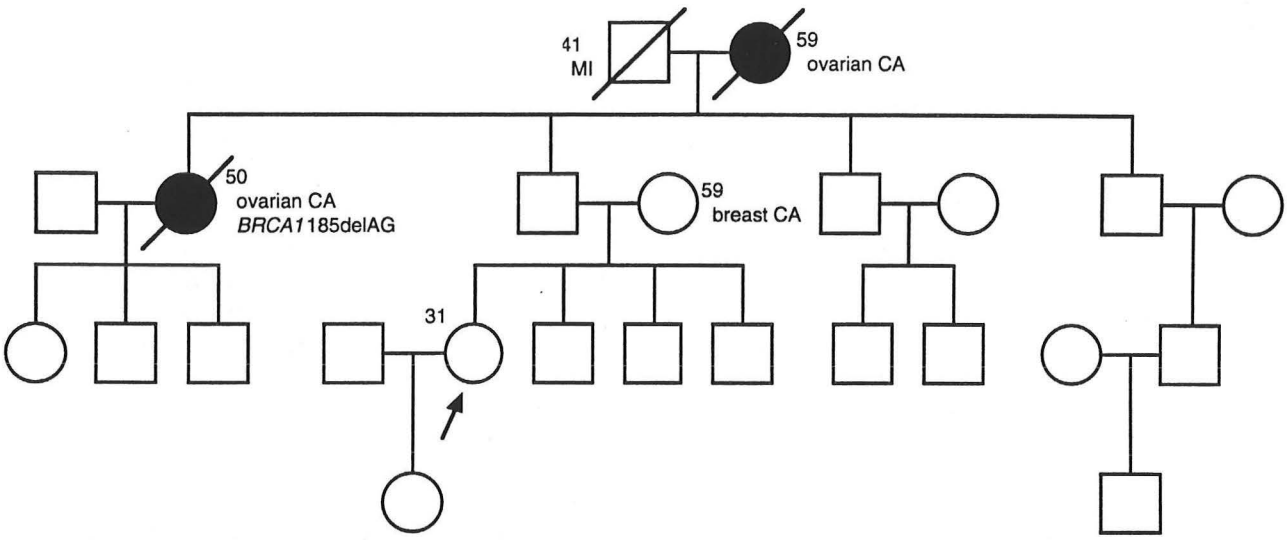


Fig 1. Pedigree of 31 year old Ashkenazi Jewish woman seeking *BRCA1* mutation testing.

DP's American family learned that they are a *BRCA1* kindred from their Israeli relatives. They had not previously thought women in the family to be at any increased risk of breast cancer. Even the Israeli members who received formal counseling were concerned principally with the risk of ovarian cancer, which was of more immediate emotional significance to them. DP's father and two of her three brothers are physicians, and she holds a doctoral degree in the social sciences. The family contacted me to request genetic testing. Like her cousin, DP intended to have prophylactic oophorectomy if the test was positive. My experience with predictive genetic testing for DP prompted me to review the subject for today's grand rounds.

Their request seemed reasonable. DP's risk of carrying the 185delAG mutation was 25%, and her father's risk was 50%. The best estimates at that time for Ashkenazi Jewish women in high-risk families were lifetime risks of ~85% for breast cancer and ~50% for ovarian cancer. This information had been widely reported by the media. Most experts think that increased surveillance and/or prophylactic surgery will lessen these risks, although the benefit is not proven. I discussed the risk of breast cancer with DP if carried the mutation, but she did not want to consider prophylactic mastectomy. Like the rest of her family, she was much more concerned about ovarian cancer (even though her mother had a modified radical mastectomy 14 years earlier for a low-grade malignancy). As an aside, she reported being told by the Gilda Radner Foundation in the past that ovarian cancer susceptibility is inherited only through the maternal lineage, a misconception that is still common.

I contacted several researchers who were conducting *BRCA1* testing, including Dr. Gail Tomlinson in the Department of Pediatrics here at UT Southwestern, Dr. Lawrence Brody at the NIH, Dr. Sue Richards at Baylor College of Medicine, and Dr. Dvorah Abeliovich at Hadassah Hospital in Jerusalem, who found the aunt's mutation. They all considered *BRCA1* testing to be appropriate for DP, but none could provide the test without seeing her in person as part of their study protocol. I also contacted several Italian researchers, but they were performing only linkage studies, not direct mutation analysis.

Linkage studies were impractical and unnecessary since the aunt's mutation was known. One researcher offered to do the test "off the record" but would not guarantee the accuracy of the results. Dr. Brody suggested that I contact OncorMed, Inc., who performed some of their testing on a contract basis.

At that time the company was just starting to market *BRCA1* and *BRCA2* mutation analysis under an in-house IRB-approved protocol. I spoke with the laboratory director, Dr. Patricia Murphy. She first asked that I provide her with the patient's pedigree. DP met their criteria for testing. I inquired about confidentiality. Even though DP is now an Italian citizen and enjoys nationalized health care, I was concerned about the risk that she could suffer insurance discrimination should she ever wish to return to America. She also wanted her test results to be confidential, telling me to my astonishment that the Italian government was considering adopting a U.S.-style health insurance system to contain costs. OncorMed agreed to protect confidentiality.

Per their protocol, I next had to provide them with the names of an oncologist, a cancer surgeon, a geneticist (me), and a mental health professional who had agreed to provide pre- and post-test counseling and interpretation of the results. They required DP to sign a pre-test counseling checklist and a consent form. I told her to sign illegibly. Evidently the only critical signature was my name on the credit card slip; payment in advance of $150 was required. I next encountered a technical hurdle. I had asked DP's father to draw a purple top tube and freeze it during a brief visit by her to America. OncorMed will not accept frozen blood, since they have difficulty extracting DNA from it. Fortunately my own laboratory routinely extracts DNA from frozen blood samples, and Dr. Murphy agreed to test purified DNA that I provided. They tested specifically for the 185delAG mutation using allele-specific oligonucleotide hybridization (see below). I also gave an aliquot to the researcher who offered to test it "off the record" by DNA sequencing. I received the results from this researcher within a week and from OncorMed approximately 6 weeks later. Both tests were negative for the mutation.

Post-test counseling was easy in this fortunate circumstance. Absent any other unknown predisposing mutations, DP's estimated lifetime risk of breast and ovarian cancer is the same as that of the general population, approximately 12.6% and 1%, respectively (American Cancer Society, 1996). She was relieved to learn of the results, although much of the anxiety had been provoked in the first place by learning that she was at risk of carrying a cancer susceptibility gene. I informed her father that his chance of carrying the mutation was now 1/3 (Bayes theorem), and if he is a carrier, his risk of prostate cancer might be increased several-fold over the general population. He declined genetic testing. He was 66 years old, in good health, and planned to continue routine prostate cancer surveillance by annual rectal examination and serum PSA. He did feel that members of his extended family on the maternal side should be tested for the mutation, and spoke with a cousin who is a practicing oncologist in New York. The cousin had not previously thought about*BRCA1* and *BRCA2* testing in his family with their history of ovarian cancer. To my knowledge, other family members have not been tested.

What has changed in the past 18 months with regard to genetic testing for *BRCA1* and *BRCA2* mutations? A second *BRCA1* mutation and one *BRCA2* mutation have been collectively found in about 1% of Ashkenazi Jewish women (see Taurog, 1997). DP could carry one of these other mutations. Present estimates of the lifetime risks of breast or ovarian cancer in unselected Ashkenazi women presenting for cancer risk assessment are now ~50% and 15-20%, respectively (Struewing et al., 1997), significantly lower than the penetrance estimates from high-risk families. Commercial labs now offer testing for all three common Ashkenazi mutations, ranging in price from $300-400. At least one lab, Genetics and IVF Institute, provides the test to physicians on demand. OncorMed's current price for testing a relative for a known mutation is now $300, up from $150, and the turnaround time for the 'Ashkenazi panel' is now two weeks. Most New York oncologists are by now probably very familiar with *BRCA1* and *BRCA2* testing in Ashkenazi women with a family history of either breast or ovarian cancer. Many states now have laws protecting genetic privacy, and federal legislation prohibiting health insurance discrimination on the basis of genetic information appears imminent. And I now agree with others that *BRCA1* and *BRCA2* testing is best done in an academic research setting, even for high-risk patients. I hope to convince you in today's grand rounds that this is the case for most predictive genetic tests for cancer.

Introduction

The Human Genome Project aims to decipher the complete nucleotide sequence of human DNA by the year 2005. The results promise to have a dramatic impact on the practice of medicine in the next century. High resolution genetic maps are already being used to study the heredity of common diseases such as atherosclerosis, diabetes, Alzheimer's disease, and other multifactorial disorders, whose genetic components were until recently too complex to dissect. The results of these studies will empower physicians to test patients for disease susceptibility even in the absence of signs, symptoms, or biochemical abnormalities (Caskey, 1993), with the goal of optimizing the prevention and treatment of illness.

The role of genes in disease is exemplified by the discovery over the past several decades of oncogenes and tumor suppressor genes. Indeed, understanding cancer was a raison d'être for the Human Genome Project. Even after the importance of specific genes in cancer was recognized, it was thought that most cancer-causing mutations were sporadic. Studies of familial cancer syndromes have led to an appreciation of the extent to which inherited, or germ-line, mutations in oncogenes and tumor suppressor genes predispose individuals to a variety of tumors, including cancers of the kidney, thyroid, breast, ovaries, colon, uterus, skin, prostate, and others. One reason for studying hereditary cancer syndromes is the possibility that the same genes will play a role in much more common sporadic tumors, and this is proving to be true in some cases. Another motive is the potential profit to be realized from screening tests, and there are ever-increasing numbers of biotechnology companies engaged in genetic research, with significant pressures to market new screening tests soon after genes are discovered.

Most of the projected applications of predictive genetic testing for common diseases are still well in the future. For this reason, I have limited the discussion to genetic testing for cancer, for which several tests are already being marketed, although the same principles will apply to genetic tests for other conditions. I will begin with a brief discussion of the methods used for genetic testing. I will then discuss specific cancer syndromes, the relevant genes, and the status of predictive genetic testing in each syndrome, emphasizing disorders that are relevant to internists. I will conclude by considering some of the ethical, legal, and social implications of genetic testing in the United States today.

Genetic Testing Methods

Linkage. The location of a cancer-predisposing gene that shows Mendelian inheritance may be deduced by genetic linkage analysis. In fact, the demonstration of significant linkage (LOD score >3, or $p<0.05$) is considered proof of the existence of a gene, e.g. the *BRCA1* gene was designated in 1991, three years before it was cloned (Solomon and Ledbetter, 1991). Linkage analysis involves studying the segregation in pedigrees of polymorphic genetic markers, e.g. restriction fragment length polymorphisms, variable number tandem repeats, microsatellites, etc. A cancer-predisposing gene and a marker that is near by will tend to be co-inherited, or show linkage, while unlinked markers will show independent assortment.

Once linkage of a phenotype like cancer susceptibility is established, the linked polymorphic markers can be used to determine whether an individual has inherited the susceptibility gene before the gene has been cloned. Some women who participated in *BRCA1* studies were provided with genetic testing on this basis. In order to use linkage analysis for genetic testing, the family in question must be shown to have linkage. This requires analysis of multiple family members. Furthermore, unless a marker is very close to or within the disease gene, there is a possibility that genetic recombination will result in false results. For these reasons, and because of technical demands, linkage analysis is usually performed only by research laboratories. The time lag from finding linkage to cloning a gene and identifying mutations has become much shorter recently, and linkage analysis is increasingly less common as a means of predictive genetic testing except for families participating in studies.

Mutation detection. Routine genetic testing for cancer susceptibility relies upon detecting mutations. In some cases the mutation in question is known, e.g. the 185delAG *BRCA1* mutation, while in other cases the mutation has not been previously identified. Specific detection strategies can be tailored to known mutations. One simple approach suitable for a subset of mutations is to look for the

gain or loss of a restriction enzyme recognition site by Southern blotting of genomic DNA, usually after polymerase chain reaction (PCR) amplification. Restriction sites can sometimes be intentionally introduced during the PCR with specially designed primers. Another very common method involves hybridizing allele-specific oligonucleotide (ASO) primers to PCR-amplified genomic sequences. Conditions are chosen so that each ASO anneals specifically to its complementary target. This method is easily automated and is used by several labs to detect Ashkenazi *BRCA1* and *BRCA2* mutations. Other methods using ASO primers include allele-specific amplification, ligation, or primer extension (Cotton, 1993).

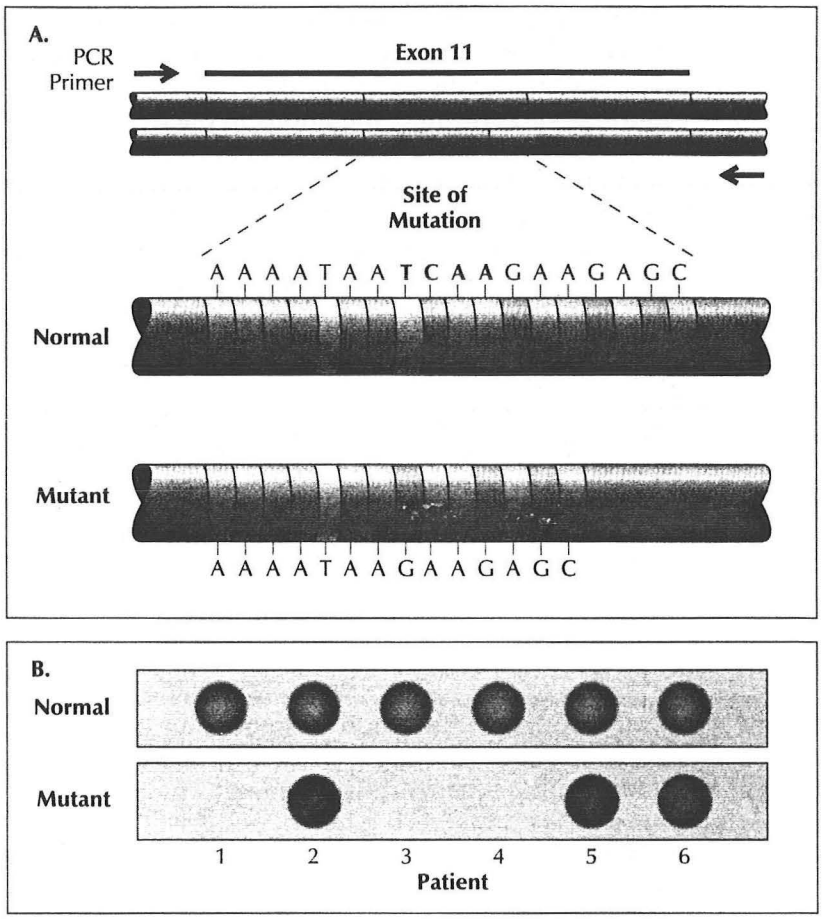


Fig. 2. Allele-specific oligonucleotide (ASO) hybridization analysis (Weitzel, 1996)

The other class of mutations are those whose sequences are not known a priori. Detecting new or unknown mutations is one of the most challenging technical problems in genetic research. A number of rapid techniques for scanning genes have been developed, including single strand conformation polymorphism (SSCP) analysis, heteroduplex analysis, denaturing gradient gel electrophoresis (DGGE), and chemical or enzymatic mismatch detection (Cotton, 1993). Of these, SSCP and heteroduplex analysis are the simplest and most widely used. All of these techniques are rapid and non-labor intensive, but none has adequate sensitivity for diagnostic purposes.

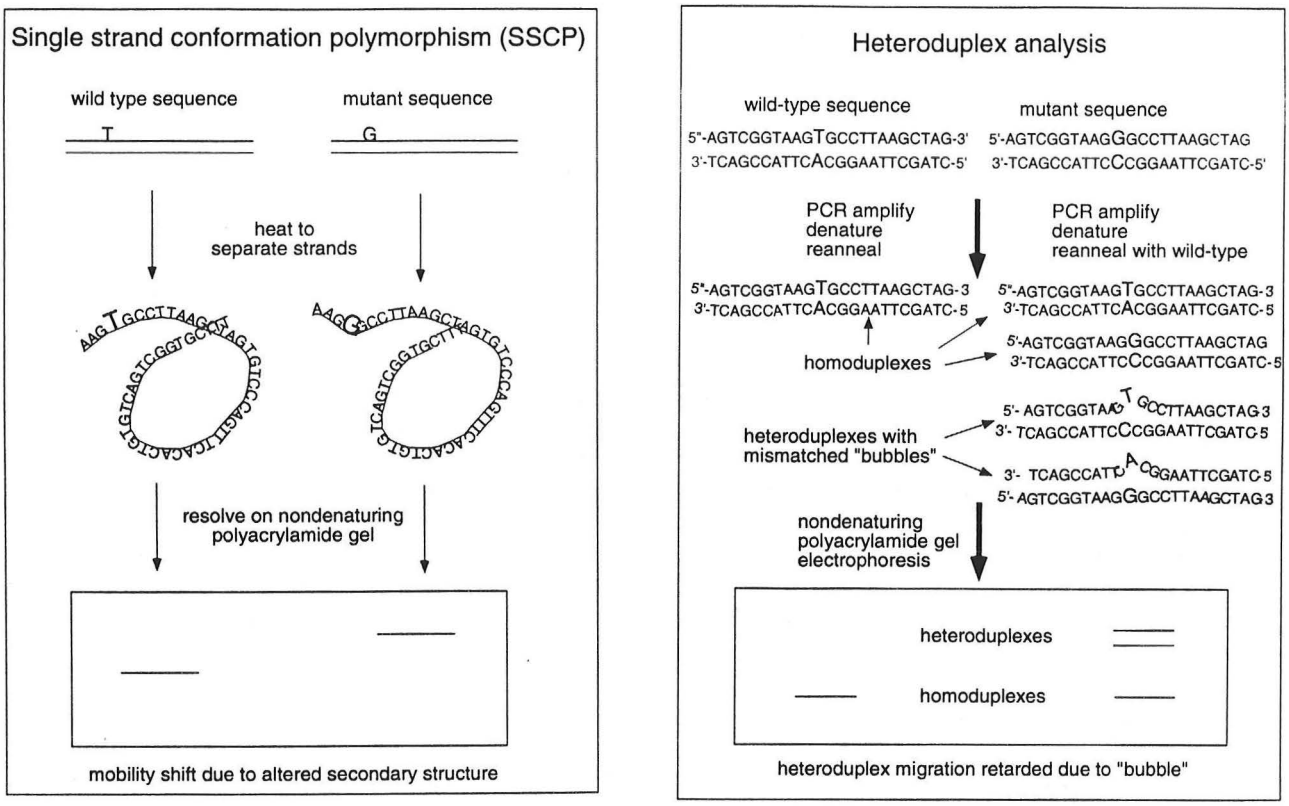


Fig. 3. Two popular methods for detecting unknown mutations.

Another technique growing in popularity is the protein truncation test (PTT) or *in vitro* synthesized protein (IVSP) assay. Stretches of coding sequence are amplified by RT-PCR from RNA (or in some cases by PCR from genomic DNA), along with a promoter sequence for T7 RNA polymerase and a consensus sequence for initiation of translation. Coupled in vitro transcription/translation reactions are then performed using the PCR products as templates, and the protein products are resolved by SDS-polyacrylamide gel electrophoresis. The presence of a stop codon or a frameshift mutation that results in premature termination of translation will result in a truncated protein product.

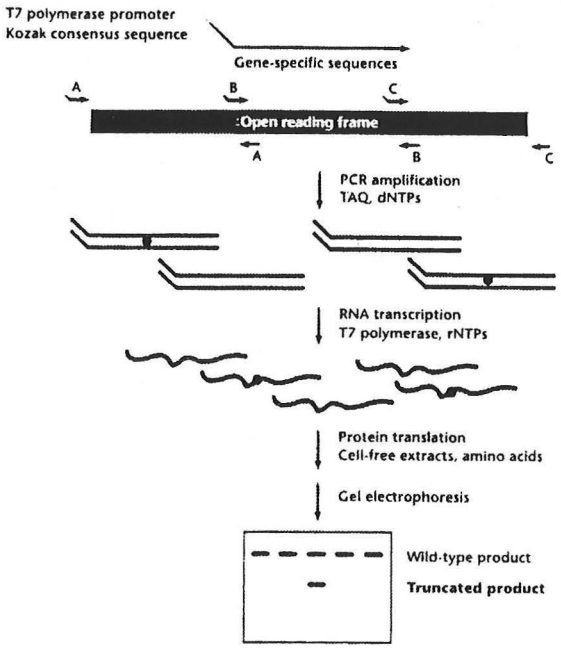


Fig. 4. Protein truncation test (Plummer and Casey, 1996).

The gold standard for detecting new mutations is DNA sequencing, usually of individual exons and flanking splice sites that have been PCR-amplified from genomic DNA. Even for sequencing the sensitivity of detecting new mutations is not 100%. There are regions that are difficult to sequence, such as DNA with high G+C content or repetitive sequences that interfere with the chemistry. Mutations outside of coding regions that affect transcription or RNA splicing can be missed. It is often difficult to know whether newly identified missense mutations that result in amino acid substitutions are deleterious to the protein's function. Most laboratories employ a hierarchical approach to look for new mutations in large genes. One of the simpler scanning techniques is employed first. Any mutations detected are verified and characterized by targeted sequencing. If no mutation is found, then the entire coding region is systematically sequenced.

Because of the laborious nature of DNA sequencing, there is great interest in developing alternative technologies for genetic testing. These include immunoassays to detect protein products and biochemical assays for their functions. However, the technology on the horizon that appears to be the most promising for genetic testing involves hybridizing PCR products from genomic DNA to an array of immobilized oligonucleotides, so-called biochips. As yet no commercial chip-based genetic tests are available, but their feasibility for *BRCA1* mutation detection has been demonstrated (Hacia et al., 1996), and experts predict the debut of biochips for clinical genetic testing applications within the next few years (Eng and Vijg, 1997). Drs. Glen Evans and Harold Garner in the Genome Science and Technology Center at UT Southwestern are helping to develop this technology.

Specific inherited cancer syndromes

The hallmarks of a familial cancer syndrome are as follows (Weitzel, 1996):

- Onset of cancer 15-20 years earlier than average
- Occurrence of cancer in several close relatives
- More than one type of cancer in the same close relative
- Bilateral tumors in paired organs (e.g. retinas, kidneys, breasts)
- Occurrence of cancer in the sex that is less often affected (e.g. male breast cancer)
- Multiple cancers in several generations
- Clustering of rare cancers

Table 1 lists cloned genes for dominantly inherited familial cancer syndromes in chronological order of discovery, along with their chromosomal locations, associated hereditary syndromes, major cancers, and probable functions. For some of these disorders, such as neurofibromatosis or tuberous sclerosis, cancer predisposition is one part of a characteristic phenotype. Such disorders can usually be diagnosed clinically, and the utility of predictive genetic testing for identifying mutation carriers is limited. I will focus my discussion on those disorders where cancer is the major or sole phenotype, for which genetic testing assumes greater importance. These include retinoblastoma, Li-Faumeni syndrome, familial adenomatous polyposis, multiple endocrine neoplasia 2, hereditary melanoma, hereditary nonpolyposis colon cancer, and familial breast and ovarian cancer. I will briefly review each syndrome, the gene(s) implicated, and the status of genetic testing for that syndrome.

Retinoblastoma. This rare childhood cancer usually develops before age 4. Mutations in the *RB1* tumor suppressor gene on human chromosome 13 are an early event in tumorigenesis. Cytogenetic abnormalities involving band 13q14 are observed in some cases. *RB1* mutations are also implicated in osteosarcoma, the risk of which is increased 500-fold in hereditary retinoblastoma patients. Approximately 5-10% of retinoblastomas are due to familial mutations, 20-30% to new germline mutations, and 60-70% to sporadic somatic mutations. Familial cases are often bilateral. The epidemiology of retinoblastoma led Knudson to formulate his seminal two-hit hypothesis for tumor suppressor genes, of which *RB1* is the prototype (Knudson Jr., 1971). The molecular correlate of this hypothesis is that both alleles of *RB1* must be inactivated for tumor formation. In familial cases, the first hit is inherited; only one additional hit is then required for tumorigenesis. The RB1 protein is appears to negatively regulate progression through the cell cycle by sequestering a variety of nuclear proteins involved in cellular growth (Weinberg, 1995).

With prompt recognition and treatment, many children survive retinoblastoma, making it important to monitor individuals who may carry an *RB1* mutation. Conventional screening for tumor formation includes a complete retinal examination under general anesthesia eight times during the first three years of life in order to detect tumors early enough to preserve vision (Noorani et al., 1996). Predictive genetic testing for retinoblastoma by linkage analysis was suggested as long ago as 1979 (Sparkes et al., 1979). The *RB1* gene has been completely sequenced; it contains 27 exons spanning 180 kilobases. With enough effort an *RB1* mutation can be detected in up to 70-80% of hereditary retinoblastoma patients (Eng and Vijg, 1997), but implementation of mutation detection has been hampered by the gene's large size and the scattering of mutations across the entire coding region. Due to the technical difficulties and limited demand, genetic testing for retinoblastoma by linkage or mutation analysis is presently limited to research laboratories. A cost-benefit analysis recently found that genetic testing compares favorably to conventional screening ($8,674 versus $31,430 in Canadian dollars) for relatives of individuals affected with retinoblastoma (Noorani et al., 1996). The testing strategy involved identifying the mutation in the proband and then testing family members at risk, using a hierarchical approach to mutation detection as outlined above. Conventional clinical screening for tumors is then continued in relatives found to carry the mutation.

Li-Fraumeni Syndrome (LFS). Germline mutations in the *TP53* tumor suppressor gene are associated with a familial cancer syndrome of diverse tumors, including breast cancer, soft tissue sarcomas, brain tumors, osteosarcoma, leukemia, lymphoma, adrenocortical carcinoma, and others. Tumors characteristically develop unusually early, and there are often multiple primary tumors. The risk of developing an invasive cancer has been estimated to be as high as 50% by age 30 (Strong et al., 1987), and 90% by age 70 (Malkin et al., 1990).

The *TP53* gene maps to human chromosome 17 and consists of 11 exons. Wild-type p53 protein acts as a negative regulator of cell growth (Levine, 1997). The protein also serves important functions in arresting cell division after DNA damage and in directing damaged cells toward apoptosis. Mutations in *TP53*, particularly gain-of-function mutations that promote cell division, may be the most common genetic change in human cancers.

Much effort has been devoted toward developing laboratory tests for *TP53* mutations because of their potential utility as tumor markers in sporadic cancers. Immunohistochemical tests for tumor samples are available, using monoclonal antibodies specific for altered forms of the protein. There has been less interest in developing predictive genetic testing for germline mutations, because LFS families are rare, germline mutations do not appear to be involved in a significant proportion of common familial cancers, and the benefits of identifying *TP53* mutations carriers are unclear given the difficulty of monitoring for early tumors in numerous organs. Early experience with testing found that many members of LFS kindreds decline genetic screening (Schneider et al., 1995). Nevertheless, the potential market for testing sporadic tumors for*TP53* mutations is driving the development of tests which can also be applied to germline mutations. OncorMed, Inc. offers sequencing of the entire coding region and a functional assay for DNA binding activity under an investigational protocol. The functional assay may be helpful if a mutation is discovered whose significance is unclear. A biochip for detecting *TP53* mutations is available for research purposes from Affymetrix (H. Garner, UT Southwestern, personal communication).

Gene	Locus	Hereditary Syndrome	Chief cancers	Year	Function
RB1	13q14	Retinoblastoma	retinoblastoma osteosarcoma	1986	cell cycle regulation
WT1	11p13	Wilms' tumor	Wilms' tumor (kidney)	1990	zinc-finger transcription factor
TP53	17p13	Li-Fraumeni	breast sarcoma brain tumors	"	transcription factor/regulator of apoptosis in response to stress
NF1	17q11	Neurofibromatosis 1	sarcoma brain tumor	"	GTPase activator (RAS signaling)
APC	5q21	Familial adenomatous polyposis	colorectal	1991	binds to beta-catenin (WNT signaling)
NF2	22q12	Neurofibromatosis 2	acoustic neuroma brain	1993	band 4.1 family member; links cytoskeleton to plasma membrane
VHL	3p25	Von Hippel-Lindau	renal pheochromocytoma brain	"	elongin (transcriptional elongation)
RET	10q11	Multiple endocrine neoplasia 2	medullary thyroid pheochromocytoma	"	receptor tyrosine kinase
TSC2	16p13	Tuberous sclerosis 2	renal brain	"	tumor suppressor
MSH2	2p16	Nonpolyposis colorectal cancer 1	colorectal endometrial stomach	"	DNA mismatch repair
MLH1	3p21	Nonpolyposis colorectal cancer 2	colorectal endometrial stomach	1994	DNA mismatch repair
PMS1	2q32	Nonpolyposis colorectal cancer 3	colorectal endometrial stomach	"	DNA mismatch repair
PMS2	7p22	Nonpolyposis colorectal cancer 4	colorectal endometrial stomach	"	DNA mismatch repair
CDKN2A	9p21	Melanoma	melanoma	"	p16-cyclin-dependent kinase inhibitor
BRCA1	17q21	Breast/ovarian cancer	breast ovary	"	tumor suppressor gene
BRCA2	13q12-13	Breast cancer	breast	1995	tumor suppressor gene
EXT1	8q24.1	Multiple exostoses 1	chondrosarcoma	"	tumor suppressor gene
EXT2	11p12-11	Multiple exostoses 2	chondrosarcoma	1996	tumor suppressor gene
CDK4	12q13	Melanoma	melanoma	"	cyclin-dependent kinase
PTC	9q22.3	Gorlin (basal cell nevus) syndrome	basal cell	"	Hedgehog protein signaling

Table 1. Dominant familial cancer genes (Murphy and Bray, 1997)

Familial Medullary Thyroid Carcinoma (FMTC). FMTC and the associated multiple endocrine neoplasia type II (MEN2) syndromes are a paradigm for how predictive genetic testing can be used to prevent cancer. MTC, a malignancy of the parafollicular C cells, accounts for approximately 5-10% of all thyroid cancer. MEN2A is characterized by MTC plus increased risk of pheochromocytoma and parathyroid hyperplasia. MEN2B is characterized by aggressive MTC plus pheochromocytoma and ganglioneuromas of the GI mucosa; parathyroid hyperplasia is not seen. These disorders were reviewed in these grand rounds recently (Petty, 1995). Mutations in the *RET* proto-oncogene can be detected in about 90-95% of FMTC and MEN2 families. *RET* encodes a protein tyrosine kinase receptor for a circulating glial cell line-derived neurotrophic factor (GDNF) and appears to function in the migration, proliferation, and differentiation of kidney and neural crest cells (Robertson and Mason, 1997).

Genetic testing for *RET* mutations was introduced soon after the gene was implicated in FMTC and MEN2 in 1993 and is rapidly becoming the standard of care for families with these conditions (Utiger, 1994). In his editorial, Dr. Robert Utiger also advocated testing any individual diagnosed with MTC for a germline *RET* mutation. The goal is to determine whether the cancer is inherited or sporadic. If the cancer is inherited, family members may be tested for the same mutation, and cancer averted in individuals who carry the mutation by prophylactic thyroidectomy. Relatives without a mutation are relieved of the need for repeated burdensome clinical screening using biochemical markers of MTC (pentagastrin calcitonin stimulation test) or pheochromocytoma (urinary catecholamines).

The *RET* gene has 21 exons. Mutations in exons 10 and 11 are associated with MEN2A, in exon 16 in MEN2B, and in exons 10, 11, 13, and 14 in FMTC. The test presently offered in the Laboratory for Molecular Diagnostics at UT Southwestern uses SSCP analysis to screen for mutations in exons 10, 11, and 16; putative mutations are verified by DNA sequencing. OncorMed, Inc. tests commercially for *RET* mutations by PCR amplification and sequencing of exons 10, 11, 13, 14, and 16.

Several factors make FMTC/MEN2 ideal for predictive genetic testing. First, nearly all familial cases involve mutations in one gene, *RET*, and mutations can be identified in upwards of 95% of families. Second, individuals who carry a mutation are highly likely to develop the characteristic tumors, and individuals without a mutation are not, since the incidence of sporadic tumors is low. In other words, genetic testing is **predictive** of clinical outcome. Third, effective prophylaxis with acceptable morbidity is available for MTC (thyroidectomy). Fourth, genetic testing is more cost-effective than clinical screening, which is already established as the standard of care. Thus insurers and managed care providers are willing to pay for it.

Familial melanoma. Linkage studies of kindreds with multiple cases of malignant melanoma have implicated susceptibility genes on human chromosomes 1 and 9. In 1994, the tumor suppressor gene *CDKN2A* (also known as *p16*) was shown to be mutated in most families in whom the disease is linked to chromosome 9, about half of all melanoma kindreds (Hussussian et al., 1994). The role of *p16* was supported by the finding of homozygous deletions or mutations of the gene in about 75% of melanoma cell lines (Kamb et al., 1994). The p16 protein appears to antagonize the interaction between cyclin dependent kinase 4 (CDK4) and cyclin D by binding to CDK4, inhibiting phosphorylation of Rb and subsequent progression of the cell cycle through the G1 phase (Serrano et al., 1993). A germline mutation in the p16 binding domain of CDK4 was recently reported in two unrelated melanoma families who did not show linkage to chromosome 9 (Zuo et al., 1996).. OncorMed already offers genetic testing for germline *p16* mutations in familial melanoma patients on an investigational basis using a sequencing assay. Tests for *CDK4* mutations will no doubt appear soon. The American Society of Clinical Oncology (ASCO) has recommended that these tests not be offered in a clinical setting, since the clinical implications of finding a mutation are uncertain (ASCO, 1996).

Familial adenomatous polyposis (FAP). FAP is characterized by the development of hundreds of colonic polyps early in life, with inevitable progression to colon carcinoma unless prophylactic colectomy is performed. The disease has an incidence of about 1 in 5000 and accounts for fewer than 1% of all colon cancers. Extracolonic manifestations may include polyps of the upper GI tract, which can become malignant, osteomas of the jaw, congenital hypertrophy of the retinal pigment epithelium, sebaceous cysts, and a variety of non-GI tumors. The constellation of FAP and extracolonic features is also known as Gardner syndrome, which is no longer considered a distinct disorder. Standard therapy

consists of prophylactic subtotal colectomy or intensive colonoscopic surveillance beginning before age 25, with colectomy once polyps are detected. Nonsteroidal anti-inflammatory drugs are also used to suppress upper GI polyps.

FAP is due to mutations in the *APC* gene on chromosome 5q21. Although the gene was cloned in 1991, its precise function is still unclear. The protein binds to the E-cadherin associated protein ß-catenin and may be involved in WNT (Wingless) signaling (Rubinfeld et al., 1993). The *APC* gene is large. The reading frame encompasses more than 8,500 base pairs encoded by 15 exons, with disease-associated mutations scattered among these exons. For this reason, genetic testing for *APC* mutations is difficult unless a mutation has already been identified in a relative . Powell et al. (1993) reported finding *APC* mutations in 54 of 62 FAP patients (87%), using a protein truncation test to detect frameshifts, nonsense mutations, and splicing mutations (82%), and an allele-specific expression assay to detect mutations that cause reduced levels of normal transcripts (5%), e.g. mutations that affect promoter or splicing efficiency. Commercial testing for *APC* mutations using the protein truncation test was introduced within a year of this publication.

The test has now been available for several years and its use in clinical practice has been examined. Giardiello et al. (1997) looked at 182 tests performed by one commercial lab in 1995. Information was available on 177 patients from 125 families. These investigators considered two indications for *APC* testing to be appropriate: diagnostic confirmation of FAP in patients with typical colorectal polyps or multiple adenomas, and presymptomatic diagnosis in first degree relatives of affected patients. They estimated that the protein truncation test used has a sensitivity of about 80% to detect *APC* mutations. A positive result indicated that a mutation was identified. A negative result meant that no mutation was found **in a member of a family in whom a mutation was previously detected**. A third result, "no mutation detected," was reported when no mutation was detected and a mutation had not yet been identified in the family. In this case, the result does not rule out FAP, because the test fails to detect about 20% of *APC* mutations. The authors looked at whether the tests that were ordered were indicated, whether informed consent was obtained and documented in writing, whether formal genetic counseling was provided prior to testing, and whether the results would have been interpreted correctly by the ordering physicians without additional discussions with a gastroenterologist and/or genetic counselor familiar with the test.

Gastroenterologists were the most frequent specialists who ordered the test (47%). Medical geneticists or genetic counselors ordered 18% of tests, and another 13% were ordered by surgeons. The indication was considered appropriate for 83% of tests ordered. Inappropriate indications included a family history of colorectal cancer (9.6%), the presence of other cancers (1.7%), and a family history of brain tumor representing possible Turcot's syndrome (concurrent primary brain tumor and multiple colorectal adenomas) (one patient). Forty five percent of appropriate tests and 2.3% of inappropriate tests (one patient) were positive for an *APC* mutation. The one positive inappropriate test was from a woman with metastatic colon cancer at age 38 and no family history of colorectal cancer or polyps.

Pretest counseling and informed consent have been deemed essential for genetic susceptibility testing (NACHGR, 1994; ASCO, 1996). Genetic counseling was provided prior to testing only 18.6% of the time, and written consent obtained in only 16.9% of cases. Equally concerning, the ordering physicians' interpretations of test results prior to additional discussions were incorrect in 31.6% of cases. In particular, physicians did not understand that "no mutation detected" could represent a false negative result if a mutation had not been previously identified in the family. The consequences of this type of misinterpretation could be catastrophic: patients might be falsely assured they are not at risk, and endoscopic surveillance might not be instituted. There were no statistically significant differences among the various specialists (gastroenterology, surgery, medical genetics, or other) with regard to use or interpretation of the test.

Hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome). Colorectal cancer affects approximately 6% of the U.S. population. In certain families the risk is much greater; these have been termed hereditary nonpolyposis colorectal cancer (HNPCC) kindreds . The term "nonpolyposis" serves to distinguish the disorder from FAP but is a bit of a misnomer, since HNPCC tumors also arise

from premalignant adenomas according to the well-established sequence for sporadic colorectal cancer (Lynch et al., 1996). HNPCC involves an increased probability of malignant transformation.

A kindred with HNPCC, or Lynch syndrome, is defined by The International Collaborative Group on HNPCC by the following criteria: 1. At least 3 family members with colorectal cancer, two of whom are first degree relatives. 2. At least two generations are affected. 3. At least one individual diagnosed with colorectal cancer prior to age 50 (Vasen et al., 1991). Estimates of the prevalence of HNPCC in the general population vary widely, ranging anywhere from 0.05% to 15% of all colorectal cancers, or about 1/3000 to 1/100 individuals (Ponz de Leon, 1994). HNPCC gene carriers have a lifetime risk of 65-90% for developing colorectal cancer. HNPCC-associated tumors develop earlier than sporadic colorectal cancer, with a median age of onset of 45 versus 70 years, and are more often right sided (proximal to the splenic flexure) (70%) (Lynch et al., 1996). There is also a greater tendency to form multiple tumors, both synchronous and metachronous; the risk of a second colorectal cancer is 40-45% over 10 years if the first cancer is not treated with subtotal colectomy. Characteristic extracolonic carcinomas are also seen in some HNPCC kindreds (sometimes called Lynch II syndrome), including cancers of the endometrium, ovary, small intestine, biliary tree, ureter, renal pelvis, stomach, and pancreas.

The first HNPCC gene was identified in 1993 by groups led by Bert Vogelstein and Kenneth Kinzler at Johns Hopkins University (Leach et al., 1993) and Richard Kolodner at Harvard's Dana-Farber Cancer Institute (Fishel et al., 1993), after genetic linkage studies had mapped the locus to human chromosome 2. The gene, *hMSH2*, turned out to encode a protein involved in DNA mismatch repair. It accounts for about 60% of HNPCC. Less than 4 months later, the same two groups found that another mismatch repair gene linked to chromosome 3, *hMLH1*, accounted for another 30% of HNPCC (Bronner et al., 1994; Papadopoulos et al., 1994). Subsequently, mutations in two more DNA repair genes, *PMS1* and *PMS2*, have been found to cause some of the remaining 10% of HNPCC, but these mutations appear to be rare (Liu et al., 1996).

Progression to malignancy in HNPCC appears to involve inactivation of both alleles of a DNA repair gene via the familiar Knudson two-hit model. The tumors are characterized by a replication error phenotype (RER+), manifest as instability of microsatellite repeat sequences (Aaltonen et al., 1993). The RER+ phenotype is found in ~90% of HNPCC tumors , many sporadic right-sided sporadic colorectal cancers, and most colorectal cancers from patients younger than age 35 (Liu et al., 1995; Liu et al., 1996). In other common cancers such as lung or testis the RER+ phenotype is rare or absent. It also appears that a significant fraction of the young colorectal cancer patients with RER+ tumors have inherited germline mutations, even in the absence of a striking family history suggestive of HNPCC (Liu et al., 1995). As with most hereditary cancer genes, the penetrance, or likelihood of cancer, of HNPCC-associated mutations has undoubtedly been overestimated because the genes were isolated by studying highly cancer-prone families.

There is general agreement on the utility of genetic testing for management of HNPCC. The following strategy for kindreds meeting the ICG-HNPCC criteria has been proposed (Liu et al., 1996): The youngest affected family member should be first tested for germline mutations in these two genes. If an alteration is identified, other family members are then tested for the same mutation. These authors also suggest that the RER+ phenotype be used as a screening test for individuals not meeting the stringent HNPCC criteria but with colorectal cancer prior to age 35 or with a strong family history. An accompanying editorial pointed out some of the problems with HNPCC genetic testing (Plummer and Casey, 1996). First, not all mutations are detected. Some kindreds have *PMS1* or *PMS2* mutations, for example. Liu et al (1996)were able to detect *hMSH2* or *hMLH1* mutations in only 34 of 48 (71%) HNPCC families using a protein truncation test and, if that failed, complete analysis of coding sequences. But from their previous linkage studies, they estimated that *hMSH2* or *hMLH1* mutations should explain 90% of HNPCC kindreds (Liu et al., 1994). Second, the initial step requires obtaining a blood sample from a living affected relative; none may exist or be willing to participate. Third, the RER+ phenotype is not precisely defined --- how many microsatellite markers should be tested for instability, and what percentage should be unstable for the tumor to be considered positive? Last, it is not known whether tumors in persons who carry a mutation in a mismatch repair gene are always RER+, so this phenotype may not be a reliable guide for further testing of families not meeting the ICG-HNPCC criteria.

The cost effectiveness of genetic testing for HNPCC has also been examined (Brown and Kessler, 1996). A fundamental determinant is the population prevalence of HNPCC, estimates of which vary widely. The estimated cost per life-year saved ranged from $333,000 to $11,000, depending on whether the percentage of colorectal cancer due to HNPCC-associated genes is 0.5% or 15%. By comparison, the cost-effectiveness for breast cancer screening has been estimated in the range of $20,000 to $50,000 per life-year saved, similar to that for screening the general population for colorectal cancer by conventional modalities. Genetic screening for HNPCC would be economically dubious by conventional standards if the population prevalence is less than 1 in 500, or about 3% of all colorectal cancers. The other major factor is the unit cost of the gene test, which the authors assumed to be $1000 (a favorable estimate in my and their opinion). Until the unit cost of HNPCC gene testing is markedly lower, or better estimates of HNPCC prevalence are available, it seems prudent to limit testing to members of families who are at high risk clinically for carrying HNPCC-associated mutations. The ASCO recommends that there is colorectal carcinoma in at least three individuals, one of whom is a first-degree relative of the other two, that two generations are affected, and that one of the cases is diagnosed before age 50 (ASCO, 1996).

Despite the controversy over who should be tested for HNPCC-associated mutations, testing is already occurring in both academic and commercial research settings, and guidelines for follow-up care for individuals who test positive for HNPCC-associated mutations recently appeared in JAMA (Burke et al., 1997). The guidelines were developed over a 14 month period by a task force organized by the National Human Genome Research Institute. For known mutation carriers with colon cancer, subtotal colectomy with ileorectal anastamosis rather than limited resection is recommended to prevent metachronous tumors. Subtotal colectomy should also be considered as an alternative to polypectomy for mutation carriers with adenomas. The recommendations for mutation carriers without recognized mucosal pathology are summarized as follows:

Intervention	Provisional recommendation	Evidence	Cautions
Colonoscopy	q 1-3 yrs. beginning at age 20-25	multiple time series with and without the intervention	optimal screening interval unknown
Transvaginal ultrasound or endometrial aspirate	Annually, beginning at age 25-35	expert opinion only	benefit not proven, limited sensitivity
Prophylactic hysterectomy and oophorectomy	none	expert opinion only	efficacy uncertain; risk not fully eliminated
Subtotal colectomy	none	expert opinion only	efficacy uncertain; risk not fully eliminated

To quote from the conclusions, "options... are imperfect and controversial," "current methods of surveillance are of uncertain efficacy, expensive, and variably covered by health insurance," and "early screening or the documentation of high-risk status may jeopardize access to health insurance," but "information about options for prevention and surveillance should be included as an integral part of cancer-related genetic counseling," "full disclosure of the lack of proven benefit... should be incorporated into the informed consent process... and reviewed when genetic test results are provided to individuals found to be mutation carriers," and "much weight should be given to individual preferences." The task force also recommended "that care for individuals with cancer-predisposing mutations be provided whenever possible within the context of research protocols designed to evaluate clinical outcomes."

Tests for *MSH2* and *MLH1* germline mutations using unspecified mutation analysis and DNA sequencing is available under an investigational protocol from OncorMed. The *MSH2* gene is tested first ($1175); if no mutation is detected, then *MLH1* is tested ($1175). A test for tumor RER+ phenotype is also offered for research purposes. Criteria for HNPCC testing are as follows:

• A person with colorectal cancer (CRC) and either:

- three relatives (at least one being first degree to the other two) with CRC or any of the following cancers: endometrial carcinoma, transitional cell carcinoma of the ureter and renal pelvis, adenocarcinoma of the stomach, small intestine, ovary, or biliary tract
- two or more first or second degree relatives with CRC
- onset of disease before age 30
- onset between ages 30 and 50 and at least one other first or second degree relative with CRC
- multiple colon cancers, synchronous or metachronous
- a primary tumor of the endometrium, ovary, stomach, small intestine, ureter, gallbladder, or liver

or

- A first or second degree relative of an individual with a documented MSH2 or MLH1 mutation.

Hereditary breast and ovarian cancer. The much-heralded discovery of the *BRCA1* and *BRCA2* genes that predispose to cancer of the breast and ovary led to an explosive growth in the interest in genetic testing on the part of physicians, patients, the media, the public, and politicians. Our present knowledge regarding the biology and genetic epidemiology of *BRCA1* and *BRCA2* were comprehensively reviewed by Dr. Joel Taurog in these Grand Rounds very recently (Taurog, 1997), and I will not go into detail on the topics that he covered.

BRCA1 and *BRCA2* epitomize many of the conundrums of genetic testing. Both genes are quite large, and mutations are dispersed throughout the coding sequences (a few are even outside), making mutation detection technically challenging and expensive. Germline *BRCA1* and *BRCA2* mutations account for only a small fraction of breast cancer in the general population. Both genes have incomplete penetrance: the risk that a mutation carrier will develop cancer is substantially less than 100%, even in high-risk families. No interventions have been proved to improve the outcome of women who carry a mutation. Nevertheless, genetic testing for *BRCA1* and *BRCA2* is already commercially available and is being aggressively marketed.

The situation with susceptibility testing for breast cancer is similar to HNPCC. There is general agreement that testing may benefit members of high-risk families with multiple affected members. In these select patients, there is a significant likelihood of finding a mutation, and the probability that a woman who carries a mutation will develop cancer over her lifetime is high. The controversy, of course, is over what constitutes high risk, and whether women at moderate risk would also benefit from testing. The following table shows the prior probability that a woman carries a *BRCA1* mutation under various scenarios (Shattuck-Eidens et al., 1995). Comparable data are not yet available for *BRCA2*. For a discussion of the prevalence of *BRCA1* and *BRCA2* mutations specifically in Ashkenazi Jewish women please refer to Dr. Taurog's grand rounds.

Single affected	
breast ‹30 years	12%
breast ‹40 years	6%
breast 40-49 years	3%
ovarian ‹50 years	7%
Sister pairs	
breast ‹40 years, breast ‹40 years	37%
breast 40-49 years, breast 40-49 years	20%
breast ‹50 years, ovarian ‹50 years	46%
ovarian ‹50 years, ovarian ‹50 years	61%
Families	
breast only, ›3 affecteds ‹50 years	40%
›2 breast with ›1 ovarian	82%
›2 breast with ›2 ovarian	91%

Couch et al. (1997) reexamined the probability of a *BRCA1* mutation in a study of 263 unrelated women with breast cancer who were referred to clinics for evaluation of genetic risk. Of these women,

169 were referred specifically because of a familial risk factor, and 94 because breast cancer was diagnosed before age 40. Among the 169 women with a positive family history there was an average of four breast cancers per family. Each exon of *BRCA1* was screened by heteroduplex analysis, and any abnormally migrating exons were sequenced.

Risk factor	% with *BRCA1* mutation detected
Positive family history	16
Diagnosis before age 40	13
Family member with bilateral breast cancer	18
No family member with ovarian cancer	7
At least one family member with ovarian cancer	40
Breast and ovarian cancer in a single family member	67
Ashkenazi Jewish descent	26

The median age at diagnosis was 41.0 years for families with BRCA1 mutations and 50.7 years for families without *BRCA1* mutations. The risk of a *BRCA1* mutation of 7% in women from families with breast cancer alone contrasts sharply with previous estimates of up to 45% from linkage studies of high risk families. The presence of bilateral breast cancer did not significantly increase the probability of finding a mutation. Surprisingly, the number of breast cancer cases in the family was not by itself predictive of the presence of a *BRCA1* mutation; this number may simply reflect family size. The authors suggest that many carriers could be missed if women with few affected relatives are not offered testing.

A whole host of population genetic studies of *BRCA1* and *BRCA2* have appeared within the past few months. The data from these studies were integrated by Szabo and King (Szabo and King, 1997), who drew several conclusions. First, the proportion of high-risk families with breast or ovarian cancer varies widely among populations. High risk here is defined as at least three affected females with breast cancer or at least two affected relatives if one has ovarian cancer or male breast cancer. In Russia, 79% of these families have a *BRCA1* mutation, 47% in Israel, 29% in Italy, and 20-25% in Britain, France, Scandinavia, and Hungary. Second, *BRCA1* and *BRCA2* mutation carriers collectively account for 6-10% of breast and ovarian cancer unselected for family history in most populations (15% in Israel). Third, almost a third of high risk families have no detected *BRCA1* or *BRCA2* mutations. Fourth, *BRCA2* mutations are more common than *BRCA1* mutations in families with male breast cancer. Finally, *BRCA1* mutations are 1.5-2.0-fold more frequent than *BRCA2* mutations in high risk families everywhere but Iceland, which shows a strong founder effect with *BRCA2* 999del5 explaining virtually all hereditary breast and ovarian cancer. Clearly, the population from which a patient derives will have a major influence on the prior probability of finding a *BRCA1* or *BRCA2* mutation, and this information will have to be taken into account for optimal use of genetic testing.

The ASCO recommends the following criteria for testing, thought to give a prior probability of ›10% of finding a *BRCA1* mutation:

- Family with more than 2 breast cancer cases and one or more cases of ovarian cancer diagnosed at any age.
- Family with more than 3 breast cancer cases diagnosed before age 50
- Sister pairs with two of the following cancers diagnosed before age 50: two breast cancers, two ovarian cancers, or a breast and ovarian cancer.

OncorMed offers *BRCA1/BRCA2* testing under an investigational protocol. Testing is done in stages, beginning with mutation analysis to detect the more common mutations, and continuing until a mutation has been found or the genes have been completely sequenced. The first stage ($500) identifies approximately 45% of BRCA1 and 30% of BRCA2 mutations, including the three mutations common in Ashkenazi women. The second stage ($800) detects an additional 37% of BRCA1 and 32% of BRCA2 mutations. The last stage ($800) detects another 16% of BRCA1 and 36% of BRCA2 mutations, for a cumulative sensitivity of about 98% for both genes at a final cost of $500-$2100. Testing for just the

three common Ashkenazi mutations using allele-specific oligonucleotide hybridization is also offered ($350). Testing a relative for a known mutation is $300 (Stage I mutation) or $375 (stage II or III mutations). Testing is offered for the following persons with informed consent:

- Patients with breast and/or ovarian cancer who
  - have two or more relatives* through a single lineage with either breast or ovarian cancer
  - have one relative* with breast or ovarian cancer at ≤ 50 years
  - developed cancer at ≤ 50 years
  - have breast and ovarian cancer or bilateral disease
- Males with breast cancer diagnosed at any age
- Relatives* of persons with a documented *BRCA1* or *BRCA2* mutation
- Persons who have a family history of
  - one relative* with breast cancer at ≤ 40 years
  - one relative* with ovarian cancer at ≤ 50 years
  - one relative* with both breast and ovarian cancer
  - two relatives* with breast cancer at ≤ 50 years
  - one relative* with breast cancer at ≤ 50 years and one relative with ovarian cancer
  - two relatives* with ovarian cancer

*first or second degree relatives related through a single lineage

Myriad Genetics Laboratories, Inc. offers a simpler version of *BRCA1/BRCA2* testing. Informed consent is required. Comprehensive sequencing of the coding regions of both genes ("BRACAnalysis™") is $2400, testing for the Ashkenazi mutations, ("*Multisite 3* BRACAnalysis™") is $450, and testing a relative for a known mutation is $395. The company offers assistance in preauthorizing coverage from health plans or insurance carriers. Myriad states that it supports the ASCO guidelines, but literature they supply suggests that any of the following persons may benefit from *BRCA1/BRCA2* testing:

- those with a diagnosis of breast and ovarian cancer, especially premenopausal breast cancer
- those with a family history of breast or ovarian cancer
- those with a blood relative who is known to have a mutation in *BRCA1* or *BRCA2*
- Ashkenazi Jewish women who have breast or ovarian cancer or a family history of one or both diseases

Myriad also suggests that testing may also be appropriate for women who develop breast or ovarian cancer at an early age or have bilateral breast cancer, even in the absence of a family history.

The ASCO recommendations fail to address the unique population genetics of *BRCA1* and *BRCA2* mutations in Ashkenazi Jewish women, which makes the considerations for testing this population somewhat different. At least 2% of these women are thought to harbor one of three specific mutations: *BRCA1* 185delAG, 5382insC, or *BRCA2* 6174delT. One company, Genetics & IVF Institute, markets testing specifically for Ashkenazi Jews and tests only for these three mutations. The high prevalence of these mutations in Ashkenazi Jews make population-based screening feasible, and it should be possible to obtain relatively unbiased estimates of the frequency of mutations and their associated penetrance (i.e. the likelihood of developing cancer) and expressivity (i.e. which types of cancers are increased). Pilot studies addressing these issues were reviewed by Dr. Taurog.

Just as with HNPCC, the NHGRI has issued recommendations for cancer surveillance and risk reduction for individuals carrying *BRCA1* or *BRCA2* mutations. These recommendations implicitly acknowledge the fact that testing is already occurring both within and outside of research protocols. The recommendations, developed over a 14 month period, are as follows:

Intervention	Provisional recommendation	Evidence	Cautions
Breast self-examination	educate regarding monthly self-exams	expert opinion only	benefit not proven
Clinical breast exam	q 6-12 mo., starting at age 25-35 yr.	expert opinion only	benefit not proven

mammography	annually starting at age 25-25 yr.	expert opinion only (randomized trial of average-risk women aged 50-69 yr.)	risks and benefits not established for women under age 50 yr.
transvaginal ultrasound with color Doppler and CA-125 level	q 6-12 mo., starting at age 25-35 yr.	expert opinion only	benefit not proven; risk of ovarian cancer probably lower for BRCA2 mutation carriers
prostate cancer surveillance (BRCA1 mutation carriers only)	inform about options for screening with annual rectal examination and PSA, beginning at age 50 yr.	expert opinion only	benefit not proven; many agencies do not recommend screening due to uncertain benefit of early detection
colon cancer	fecal occult blood test annually and flexible sigmoidoscopy every 3-5 yr. beginning at age 50 (same as general population).	randomized trial (fecal occult blood test) and case control study (sigmoidoscopy) of average-risk population	relevance of population-based data uncertain
prophylactic bilateral mastectomy	no recommendation	expert opinion only	efficacy uncertain; risk not fully eliminated
prophylactic bilateral oophorectomy	no recommendation	multiple time series ± intervention; no significant risk reduction seen in one study	efficacy uncertain; risk not fully eliminated

As with HNPCC, the recommendations (or non-recommendations, in the case of prophylactic surgery) are a departure from the practice of evidence-based medicine. Recent reports that *BRCA1* and *BRCA2* may interact with RAD51 and play a role in DNA repair (reviewed by Dr. Taurog) raise further concern about the risks of increased radiation exposure in mutation carriers and highlight the need for studies of the risk/benefit ratio of early mammography in this population.

A few studies have looked at the psychological consequences of testing for hereditary breast/ovarian cancer mutations. So far the published studies have been of selected high-risk families who participated previously in genetic studies of breast cancer, so the results may not be applicable to the larger population who will inevitably be tested. Lynch et al. (1997) looked at 388 members of 14 *BRCA1* families from various U.S. locations who underwent linkage analysis. Of these, 181 elected to receive the results: 78 were positive (i.e. carried a mutation), 100 were negative, and 3 were inconclusive. Three fourths were women. The chief reason given for seeking risk assessment was concern about children and/or family (56%), followed by surveillance (30%), curiosity (17%), consideration of prophylactic surgery (7%), anxiety (5%), and research purposes (5%). More than a third of those who tested positive expressed sadness, anger, or guilt, while 80% of those who tested negative expressed relief or happiness. About a fourth of the patients declined to release their test results to their physicians because of fear of insurance and employment discrimination. Prophylactic mastectomy was considered by 35% of those who tested positive, and prophylactic oophorectomy by 76%.

An earlier study by these authors found that carriers of *BRCA1* mutations in high risk families showed no increase in depressive symptoms or functional impairment by one month after notification of test results, whereas noncarriers showed statistically significant improvement in psychosocial functioning (Lerman et al., 1996). As was found to be the case with genetic testing for Huntington's disease (Wiggins et al., 1992), knowing one's *BRCA1* status may enhance quality of life for members of high-risk families, even for mutation carriers, by relieving the anxiety associated with prolonged uncertainty.

Croyle et al. (1997) reported initial results of a study designed to assess the impact of *BRCA1* testing in a large Mormon hereditary breast cancer family. They interviewed 60 women who had received test results in the prior 1-2 weeks: 25 carried a mutation, and 35 did not. In contrast to the data of Lynch and colleagues, mutation carriers in this study had significantly higher levels of general and test-related psychological distress than noncarriers. Distress was greatest in women who had never experienced cancer or cancer-related surgery but who were found to carry the mutation. The results of reassessment at later time intervals are pending.

A decision analysis of prophylactic surgery for *BRCA1* or *BRCA2* mutation carriers was recently published . Because estimates of penetrance vary widely depending on the population studied, the authors modeled a range of values for the risks of breast cancer (40-85% by age 70) and ovarian cancer (5-40%). They assumed prophylactic mastectomy and oophorectomy reduce the cumulative risks of breast and ovarian cancer 85% and 50%, respectively, based upon expert opinion and limited experience from women in linkage studies. The risk of breast cancer after mastectomy reflects the presence of residual breast tissue (Goodnight et al., 1984). The reason oophorectomy fails is less clear. There may be residual ovarian epithelial cells or embryologically equivalent cells in the peritoneum. There were also assumptions regarding stage of tumors and they assumed that prognosis for breast cancer was the same for mutation carriers as for women in the general population. They used figures from one study suggesting that the prognosis of ovarian cancer is more favorable for mutation carriers than for women in general (Rubin et al., 1996). The major results were as follows:

Age at time of surgery	30	40	50	60
Low penetrance				
Life expectancy without surgery	45.9	37.3	29.6	22.4
Gain from mastectomy	2.9	2.0	1.0	0.2
Gain from oophorectomy	0.3	0.3	0.1	0
Gain from both	3.2	2.3	1.1	0.2
Medium penetrance				
Life expectancy without surgery	42.3	34.2	27.9	22.0
Gain from mastectomy	4.1	2.9	1.6	0.3
Gain from oophorectomy	1.0	1.0	0.4	0.1
Gain from both	5.3	4.0	2.0	0.4
High penetrance				
Life expectancy without surgery	37.5	30.1	25.4	21.2
Gain from mastectomy	5.3	3.7	2.3	0.5
Gain from oophorectomy	1.7	1.7	0.8	0.3
Gain from both	7.6	5.9	3.3	0.9

At 60 years of age, gain in life expectancy was less than one year regardless of assumed penetrance. At each penetrance level, gain in life expectancy was much greater for mastectomy than for oophorectomy. This difference is probably due to the relatively low estimate of 50% efficacy for prophylactic oophorectomy, based largely on one small study (Struewing et al., 1995). The failure rate was only 2% in larger series of women with familial ovarian cancer, but this study was conducted prior to *BRCA1* mutation testing (Piver et al., 1993).

The authors examined the effect of delaying surgery until age 40, by which time most women will have completed childbearing and lactation. The effect of delaying oophorectomy was minimal, while delaying mastectomy reduced the expected gain for a 30 year old woman by 1.1 to 2.9 years. They noted that the expected gain from prophylactic mastectomy and oophorectomy for a 35 year old member of a high-penetrance family (6.5 years) is comparable to that of reducing cholesterol from >300 mg/dl to 200 mg/dl (6.3 years). Even assuming low penetrance, the expected gain (2.6 yr.) is comparable to that of smoking cessation (2.8 yr.) and is greater than the expected gain from eliminating moderate obesity or lowering moderately elevated diastolic blood pressure. The gain in life expectancy from prophylactic surgery also compares favorable to that of adjuvant chemotherapy for breast cancer (0.9 yr. for node negative, 1.4 yr. for node positive). The authors acknowledge that their model is highly sensitive to the

initial assumptions, and at present serves only as a rough guide. They also caution that actuarial risks reflect the average benefit to a cohort , not the outcomes for individual patients. Some women will benefit not at all, others considerably. Ironically, deciding just who will benefit from intervention is often cited by proponents as a reason for offering genetic testing.

Ethical, Legal and Social Issues

Predictive genetic testing for susceptibility to common cancers raises a host of ethical, legal, and social concerns. These concerns include but are not limited to issues of insurability, employment discrimination, and the privacy of family members. In a survey of families with genetic disorders, 22% of persons felt that they or a family member had been denied health insurance on the basis of genetic test results (Lapham et al., 1996). In one study, fewer than half the well-educated, insured members of high risk families with known *BRCA1* mutations chose to learn their status. Fear of insurance and/or loss of privacy was cited as a major reason by the nearly one third of persons at high risk for familial breast cancer who decline genetic testing at two large referral centers (Collins, 1997). There is widespread agreement that the risks of insurance or employment discrimination must be disclosed for a patient to give informed consent to genetic testing. Some protection from employment discrimination may obtain from the American with Disabilities Act of 1990. The Equal Employment Opportunity Commission issued a ruling in 1995 that the definition of disability covers individuals at risk for future illness on the basis of genetic abnormalities (EEOC order 915.002, section 902, March 14, 1995), but this interpretation has not been tested in the courts.

The subject of informed consent for cancer susceptibility testing was recently reviewed by a task force of the Cancer Genetics Study Consortium (Geller et al., 1997). They argued that genetic testing deserves special consideration because it affects entire families rather than just individuals, our culture tends to consider genes as deterministic of future health and behavior, tests are probablilistic, and the primary risks and benefits of testing at present are psychological and social rather than physical. They point out that the consent process needs to take into account cultural, ethnic, and gender differences among patients. For example, Ashkenazi Jewish women may react differently than African-American women regarding the availability of breast cancer susceptibility testing and their obligation to inform family members if the test is positive (Geller et al., 1995).

The issue of health insurance risk from genetic testing has been in the news recently. Bills have been introduced before both houses of the 105th Congress to prohibit discrimination on the basis of genetic information, and these legislative efforts have been publicly endorsed by President Clinton (Collins, 1997). At least 19 states have already enacted laws to restrict the use of genetic information in health insurance, and over 75 similar bills are under consideration in more than 30 states, including Texas (TX 75RSB 98). Not surprisingly, these efforts are opposed by the Health Insurance Association of America. Much of this state legislation is overshadowed by provisions of the Health Insurance Portability and Accountability Act of 1996 (HIPAA), which takes effect this year. The new law forbids group health insurers from denying coverage on the basis of "preexisting conditions" that predate the 6-month period prior to enrollment. It also forbids group health insurance plans from applying the preexisting conditions rule to genetic information unless the person has been diagnosed with the illness predicted by the genetic test. However, this law has some gaps in its coverage. First, the law does not apply to individual health insurance policies. While these policies are only ~5% of the group policy market, many persons need individual coverage at some point in their lives, e.g. while between jobs. Second, HIPAA prohibits individuals within a group from being singled out, but it leaves open the possibility that every individual within the group may be charged higher premiums because of genetic information about one or more members. Finally, the law does not limit an insurer's access to or release of genetic information. This last point reflects the public's concern about privacy.

Of the bills presently before Congress, The Genetic Information Nondiscrimination in Health Insurance Act of 1997 (H.R. 306 and S. 89) most closely meets the 1995 recommendations of the National Action Plan on Breast Cancer (NAPBC) and the NIH-DOE Working Group on Ethical, Legal, and Social Implications of Human Genome Research (ELSI-Working Group). This bill closes all of the loopholes in HIPAA mentioned above. The Genetic Confidentiality and Nondiscrimination Act of 1997 (S. 422) introduced by Senator Domenici (R-NM) goes even further, setting up a system for regulating

the conduct of genomic research. Under this bill, persons who provide DNA samples would exercise ongoing control over the use of data obtained from the sample. IRB review would be extended to all genetic research regardless of funding source. The bill also creates a duty to disclose clinically relevant research findings to families of deceased study subjects. A number of research organizations have opposed this bill as being too burdensome.

The genetic privacy issue is part of a larger concern about privacy of health information in general. As directed by HIPAA, the Department of Health and Human Services is developing recommendations on the protection of privacy of all individually identifiable health information. The case has been made that genetic information deserves special protection because of its predictive nature, its fundamental link to personal identity and kinship, and the speed of development of genetic technologies (Collins, 1997). Others have argued that as genetic testing becomes more and more a part of medical care, it will be difficult in practice to separate genetic from other clinical information. For instance, the family history we teach every medical student and resident to take could be construed as genetic information, defined as "information about genes, gene products, or inherited characteristics that may derive from the individual or family member" by the NAPBC and ELSI Working Group (Collins, 1997).

Who Should Be Tested?

Because of the uncertain benefits of genetic testing and the potential harm if the information is misused, the American Society of Human Genetics, the National Breast Cancer Coalition, and the National Advisory Council for Human Genome Research, and previous NIH director Dr. Bernadine Healy have all come out in opposition to the use of genetic testing outside of carefully controlled research settings (NACHGR, 1994; Visco, 1996; Healy, 1997). The American Society of Clinical Oncology has outlined three criteria for when cancer predisposition testing should be offered: 1. The person to be tested has a strongly positive family history of cancer or a very early age of onset of disease. 2. The test can be adequately interpreted. 3. The results will influence the medical management of the patient or family member (ASCO, 1996). The ASCO also distinguishes three categories of disorders for consideration of cancer predisposition testing. The first category includes well-defined hereditary syndromes where either a positive or negative result will alter medical care, and for which genetic testing may be considered part of standard management. MEN2, familial adenomatous polyposis, and retinoblastoma are in this category. The second category is hereditary syndromes for which the medical benefits of carrier detection are presumed but not established. Diseases in this category include HNPCC, Li-Fraumeni syndrome, and familial breast and ovarian cancer. The third category is hereditary syndromes for which the significance of a germline mutation is unclear, germline mutations have been identified in only a few families, or the benefit of mutation detection is unclear. Familial melanoma falls into this category. The ASCO recommends that oncologists consider offering genetic testing only for syndromes in the first two categories. Genetic testing for diseases in the last category is considered "research with unknown clinical implications that should not be offered in clinical practice."

Conclusions

Genetic testing is already having an impact on the prevention and treatment of cancer. As more cancer susceptibility genes are cloned, many new tests will be rapidly introduced in the future. Tests for susceptibility to hereditary cancers are being aggressively marketed by biotechnology companies. Each new test should be viewed critically, keeping in mind that "commercial availability of a new genetic test does not ensure that the test is indicated for clinical application" (ASCO, 1996). For some diseases such as MEN2 testing is enormously helpful. For more common diseases such as breast cancer the benefit of testing is less clear, and risks of genetic discrimination are real. Most predictive genetic tests for cancer susceptibility at present violate a dictum of medical practice: "Don't order a test unless you know what to do with the information." Whenever possible, predictive genetic testing should be offered as part of research protocols to protect patients' privacy, obtain accurate interpretation of the results, ensure adequate pre- and post-test counseling, and facilitate the long-term outcome studies that are desperately needed. Referring patients for research protocols should become easier with the launch this year of the Cancer Genetic Network by the National Cancer Institute, a plan for six to eight research and testing sites and one data management center to coordinate genetic testing studies (Nelson, 1997).

"We did not find a mutation. This could be due to the fact that (1) you truly do not have a mutation in either of these two genes, or any other gene, *and therefore may have the general population risk for colorectal cancer;* or (2) you have a mutation in one of the two tested genes that the test was unable to pick up, *and therefore you may be at very high risk for colorectal cancer;* or (3) you have a mutation in one of the other two genes associated with HNPCC, *and therefore may be at very high risk for colorectal cancer;* or (4) you may have a mutation in another, yet to be identified gene, *and therefore may be at very high risk for colorectal cancer.* All in all, the prudent conclusion is that your test is inconclusive."

—Hypothetical post-test counseling for a negative result from a commercially available HNPCC protein truncation test for a person whose family has no previously identified mutation (Codori, 1997).

Aaltonen L, Peltomaki P, Leach F, Sistonen P, Pylkkanen L, et al. (1993). Clues to the pathogenesis of familial colorectal cancer. Science 260: 812-6.

American Cancer Society (1996). Cancer facts and figures.

ASCO (1996). Statement of the American Society of Clinical Oncology: genetic testing for cancer susceptibility, Adopted on February 20, 1996. J Clin Oncol 14: 1730-6.

Bronner CE, Baker SM, Morrison PT, Warren G, Smith LG, et al. (1994). Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary non-polyposis colon cancer. Nature 368: 258-61.

Brown ML and Kessler LG (1996). Use of gene tests to detect hereditary predisposition to cancer: what do we know about cost effectiveness? Int J Cancer 69: 55-7.

Burke W, Daly M, Garber J, Botkin J, Kahn MJ, et al. (1997). Recommendations for follow-up care of individuals with an inherited predisposition to cancer. II. BRCA1 and BRCA2. Cancer Genetics Studies Consortium. JAMA 277: 997-1003.

Caskey C (1993). Presymptomatic diagnosis: a first step toward genetic health care. Science 262: 48-49.

Codori A-M (1997). Psychological opportunities and hazards in predictive genetic testing for cancer risk. Gastroenterol Clin North Am 26: 19-39.

Collins F (1997). Health insurance in the age of genetics. NHGRI Statement, July 1997

Cotton R (1993). Current methods of mutation detection. Mutation Research 285: 125-144.

Couch FJ, DeShano ML, Blackwood MA, Calzone K, Stopfer J, et al. (1997). BRCA1 mutations in women attending clinics that evaluate the risk of breast cancer. N Engl J Med 336: 1409-15.

Croyle RT, Smith KR, Botkin JR, Baty B and Nash J (1997). Psychological responses to BRCA1 mutation testing: preliminary findings. Health Psychol 16: 63-72.

Eng C and Vijg J (1997). Genetic testing: the problems and the promise. Nat Biotechnol 15: 422-6.

Fishel R, Lescoe M, Rao M, Copeland N, Jenkins N, et al. (1993). The human mutator gene homolog *MSH2* and its association with hereditary nonpolyposis colon cancer. Cell 75: 1027-1038.

Geller G, Bernhardt B, Helzlsouer K, Holtzman N, Stefanek M, et al. (1995). Informed consent and *BRCA1* testing. Nat Genet 11: 364.

Geller G, Botkin JR, Green MJ, Press N, Biesecker BB, et al. (1997). Genetic testing for susceptibility to adult-onset cancer. The process and content of informed consent. JAMA 277: 1467-74.

Giardiello FM, Brensinger JD, Petersen GM, Luce MC, Hylind LM, et al. (1997). The use and interpretation of commercial APC gene testing for familial adenomatous polyposis. N Engl J Med 336: 823-7.

Goodnight J, Quagliana J and Morton D (1984). Failure of subcutaneous mastectomy to prevent the development of breast cancer. J Surg Oncol 26:

Hacia J, Brody L, Chee M, Fodor S and Collins F (1996). Detection of heterozygous mutations in BRCA1 using high density oligonucleotide arrays and two-colour fluorescence analysis. Nat Genet 14: 441-447.

Healy B (1997). BRCA genes--bookmaking, fortunetelling, and medical care. N Engl J Med 336: 1448-9.

Hussussian CJ, Struewing JP, Goldstein AM, Higgins PA, Ally DS, et al. (1994). Germline p16 mutations in familial melanoma. Nat Genet 8: 15-21.

Kamb A, Gruis N, Weaver-Feldhaus J, Liu Q, Harshman K, et al. (1994). A cell cycle regulator potentially involved in genesis of many tumor types. Science 264: 436-440.

Knudson Jr. A (1971). Mutation and cancer: statistical study of retinoblastoma. Proc Nat Acad Sci USA 68: 820-823.

Lapham EV, Kozma C and Weiss JO (1996). Genetic discrimination: perspectives of consumers. Science 274: 621-4.

Leach F, Nicolaides N, Papadopoulos N, Liu B, Jen J, et al. (1993). Mutations of a MutS homolog in hereditary non-polyposis colorectal cancer. Cell 75: 1215-1225.

Lerman C, Narod S, Schulman K, Hughes C, Gomez-Caminero A, et al. (1996). BRCA1 testing in families with hereditary breast-ovarian cancer. A prospective study of patient decision making and outcomes. JAMA 275: 1885-92.

Levine AJ (1997). p53, the cellular gatekeeper for growth and division. Cell 88: 323-31.

Liu B, Farrington SM, Petersen GM, Hamilton SR, Parsons R, et al. (1995). Genetic instability occurs in the majority of young patients with colorectal cancer. Nat Med 1: 348-52.

Liu B, Parsons R, Papadopoulos N, Nicolaides NC, Lynch HT, et al. (1996). Analysis of mismatch repair genes in hereditary non-polyposis colorectal cancer patients. Nat Med 2: 169-74.

Liu B, Parsons RE, Hamilton SR, Petersen GM, Lynch HT, et al. (1994). hMSH2 mutations in hereditary nonpolyposis colorectal cancer kindreds. Cancer Res 54: 4590-4.

Lynch HT, Lemon SJ, Durham C, Tinley ST, Connolly C, et al. (1997). A descriptive study of BRCA1 testing and reactions to disclosure of test results. Cancer 79: 2219-2228.

Lynch HT, Smyrk T and Lynch JF (1996). Overview of natural history, pathology, molecular genetics and management of HNPCC (Lynch Syndrome). Int J Cancer 69: 38-43.

Malkin D, Li F, Strong L, Fraumeni Jr J, Nelson C, et al. (1990). Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science 250: 1233-1238.

Murphy P and Bray W (1997). Hereditary cancer (poster). Trends Genet, May 1997.

NACHGR (1994). Statement on use of DNA testing for presymptomatic identification of cancer risk. National Advisory Council for Human Genome Research. JAMA 271: 785.

Nelson N (1997). Cancer Genetics Network gets under way with 5 years of funding. J Natl Cancer Inst 89: 10-1.

Noorani HZ, Khan HN, Gallie BL and Detsky AS (1996). Cost comparison of molecular versus conventional screening of relatives at risk for retinoblastoma. Am J Hum Genet 59: 301-7.

Papadopoulos N, Nicolaides NC, Wei YF, Ruben SM, Carter KC, et al. (1994). Mutation of a mutL homolog in hereditary colon cancer. Science 263: 1625-9.

Petty K (1995). Multiple endocrine neoplasia. UT Southwestern Internal Medicine Grand Rounds, December 14, 1995

Piver M, Jishi M, Tsukada Y and Nava G (1993). Primary peritoneal carcinoma after prophylactic oophorectomy inwomenwith a family history of ovarian cancer: a report of the Gilda Radner Familial Ovarian Cancer Registry 1981-1991. Cancer 71: 2751-2755.

Plummer SJ and Casey G (1996). Are we any closer to genetic testing for common malignancies? Nat Med 2: 156-8.

Ponz de Leon M (1994). Prevalence of hereditary nonpolyposis colorectal carcinoma. Ann Med 26: 209-214.

Powell SM, Petersen GM, Krush AJ, Booker S, Jen J, et al. (1993). Molecular diagnosis of familial adenomatous polyposis. N Engl J Med 329: 1982-7.

Robertson K and Mason I (1997). The GDNF-RET signalling partnership. Trends Genetics 13: 1-3.

Rubin S, Benjamin I, Behbakht K, et al. (1996). Clinical and pathological features of ovarian cancer in women with germ-line mutations of *BRCA1*. N Engl J Med 335: 1413-1416.

Rubinfeld B, Souza B, Albert I, Muller O, Chamberlain S, et al. (1993). Association of the APC gene product with beta-catenin. Science 262: 1731-1734.

Schneider KA, Patenaude AF and Garber JE (1995). Testing for cancer genes: decisions, decisions. Nat Med 1: 302-3.

Serrano M, Hannon G and Beach D (1993). A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. Nature 366: 704-707.

Shattuck-Eidens D, McClure M, Simard J, Labrie F, Narod S, et al. (1995). A collaborative survey of 80 mutations in the BRCA1 breast and ovarian cancer susceptibility gene. Implications for presymptomatic testing and screening. Jama 273: 535-41.

Solomon E and Ledbetter D (1991). Report of the committee on the genetic constitution of chromosome 17. Cytogenet Cell Genet 58: 686-738.

Sparkes R, Sparkes M, Wilson M, Towner J, Benedict W, et al. (1979). Regional assignment of genes for human esterase D and retinoblastoma to chromosome band 13q14 (Abstract). Cytogenet Cell Genetic 25: 209.

Strong L, Stine M and Norsted T (1987). Cancer in survivors of childhood soft tissue sarcoma and their relatives. J Nat Cancer Inst 79: 1213-1220.

Struewing J, Watson R, Easton D, Ponder B, Lynch H, et al. (1995). Prophylactic oophorectomy in inherited breast/ovarian cancer families. Hereditary breast cancer, ovarian and colon cancer 17: 33-35.

Struewing JP, Hartge P, Wacholder S, Baker SM, Berlin M, et al. (1997). The risk of cancer associated with specific mutations of BRCA1 and BRCA2 among Ashkenazi Jews. N Engl J Med 336: 1401-8.

Szabo C and King M-C (1997). Population genetics of BRCA1 and BRCA2. Am J Hum Genet 60: 1013-1020.

Taurog J (1997). BRCA1 and BRCA2: genes with frequent mutations that predispose to breast and ovarian cancer. UT Southwestern Internal Medicine Grand Rounds, July 3, 1997

Utiger RD (1994). Medullary thyroid carcinoma, genes, and the prevention of cancer. N Engl J Med 331: 870-1.

Vasen H, Mecklin J, Meera Khan P and Lynch H (1991). The international collaborative group on hereditary nonpolyposis colorectal cancer (ICG-HNPCC). Dis Colon Rectum 34: 424-425.

Visco F (1996). Commentary on the ASCO statement on genetic testing for cancer susceptibility. J Clin Oncology 14: 1737.

Weinberg R (1995). The retinoblastoma protein and cell cycle control. Cell 81: 323-330.

Weitzel JN (1996). Genetic counseling for familial cancer risk. Hosp Pract 31: 57-69.

Wiggins S, Whyte P, Huggins M, et al. (1992). The psychological consequences of predictive testing for Huntigton's disease. N Engl J Med 327: 1401-1405.

Zuo L, Weger J, Yang Q, Goldstein AM, Tucker MA, et al. (1996). Germline mutations in the p16INK4a binding domain of CDK4 in familial melanoma. Nat Genet 12: 97-9.

Appendix: Selected Laboratories Offering Predictive Genetic Tests for Cancer

Laboratory	Contact Information	Test(s) offered	Cost (commercial labs)
OncorMed, Inc. Gaithersburg, MD	info@oncormed.com Phone: (800) 662-6763 Fax: (301) 926-6125	*p53* sequencing (IRB-approved protocol)	$650*
		p53 functional assay (IRB-approved protocol)	$650*
		RET (sequencing)	$230 (exon 16 only) $430* (exons 10, 11, 13, 14, 16)
		CDKN1A (p16) sequencing (IRB-approved protocol)	$550*
		MSH2, *MLH1* mutational analysis & sequencing (IRB-approved protocol)	$1175 each*
		RER phenotype (IRB-approved protocol)	$570*
		BRCA1/BRCA2 mutational analysis & sequencing (IRB-approved protocol)	$500-$2100* (testing is done in stages until a mutation is found)
			*$375 if testing a relative for a known mutation
		BRCA1/BRCA2 "Ashkenazi" mutations only (IRB-approved protocol)	$350 ($300 if testing a relative for a known mutation
Myriad Genetic Laboratories, Inc. Salt Lake City, UT	BRACA@myriad.com Phone: (800) 469-7423 Fax: (801) 584-3615	*BRCA1/BRCA2* comprehensive sequence analysis	$2400
		BRCA1/BRCA2 known mutation	$395
		BRCA1/BRCA2 "Ashkenazi" mutations only	$450
Genetics and IVF Institute	Phone: (800) 552-4363 Fax:	*BRCA1/BRCA2* "Ashkenazi" mutations only	$295
Laboratory Corp. of America Ctr. of Mol. Biol. & Pathology Research Triangle Park, NC	Genetic Services Phone: (800) 345-4363 Fax: (919) 361-7798	*APC*	
Mayo Clinic Molecular Genetics Laboratory Rochester, MN	Stephen N. Thibodeau, PhD Phone: (507) 284-9185 Fax: (507) 284-0043	*RET* (initial testing of an affected family member recommended)	

Appendix: Selected Laboratories Offering Predictive Genetic Tests for Cancer

Rush-Presbyterian-St. Luke's Medical Center Genetic Laboratory Chicago, IL	Katarina Szego/Nancy Becker Phone: (312) 942-6298 Fax: (312) 942-2857	*p53*	
U. Alabama Immunogenetics/DNA Diagnostic Laboratory Birmingham, AL	Leigh Harman, PhD acton@acton.dom.uab.edu Phone: (205) 934-7107 Fax: (205) 934-4062	*APC*	
Univ. Minnesota Hospital/Clinic Molecular Diagnostics Laboratory Minneapolis, MN	David Olson olson074@maroon.tc.umn.edu Phone: (612) 624-8445 Fax: (612) 625-6994	*p53* (research testing only)	
Univ. Vermont Molecular Diagnostics Burlington, VT	Sandy Thompson jnicklas@zoo.uvm.edu Phone: (802) 656-4553 Fax: (802) 656-8892	*Rb1*	
Wash. U. School of Medicine Molecular Diagnostic Laboratory St. Louis, MO	Barbara Zehnbauer, PhD bzehnbau@imgate.wustl.edu Phone: (314) 454-6093 Fax: (314) 454-2075	*APC* (direct molecular analysis; protein truncation)	
Wash. U. School of Medicine Moley Laboratory (surgical research lab) St. Louis, MO	J.F. Moley Phone: (314) 362-5210 Fax: (314) 747-1407	*RET* (research testing only; direct and biochemical analysis)	Consider if mutation of unclear functional significance is found