

Meeting Report

Testing for Germ Line *p53* Mutations in Cancer Families

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A workshop on germ line *p53* testing in members of families with the Li-Fraumeni cancer syndrome was held at the National Institutes of Health, Bethesda, Maryland, May 8-9, 1991.² The meeting was cosponsored by the National Cancer Institute and the National Center for Human Genome Research.

Virtually all forms of cancer in humans show a tendency to aggregate in families. These aggregates can be due to the inheritance of a cancer susceptibility gene, although chance association and shared exposures to environmental carcinogens are alternative explanations. Advances in molecular genetics have revealed the chromosomal locations of many of these cancer genes. A few have been cloned, including the hereditary retinoblastoma gene, *WT1* gene for Wilms' tumor, neurofibromatosis type I gene, and *p53* gene, which is altered in six reported families with Li-Fraumeni syndrome (1, 2).

The work of the Human Genome Project can be expected to identify many more genes for inherited diseases, including cancer (3). The proper use of the genetic data is an issue of growing concern. To address the relevance of these issues to cancer genes, a workshop was held on testing for germ line *p53* mutation in families with Li-Fraumeni syndrome. This autosomal dominant disorder predisposes to at least six forms of cancer. The syndrome might serve as a paradigm for future testing of clinically healthy individuals for a variety of site-specific cancer susceptibility genes. The participants in the workshop encompassed diverse fields of study including clinical medicine, laboratory sciences, epidemiology, biostatistics, medical ethics, law, psychology, and cancer control.

Joseph F. Fraumeni, Jr. (National Cancer Institute, Bethesda, MD) noted that this syndrome was initially recognized through clinical observations at the bedside, followed by descriptive and analytic epidemiology studies of defined patient populations. The syndrome is a clinical diagnosis based on the aggregation of elements of at least six forms of cancer in young family members (4). In affected families, the cancers show no distinguishing clinical and histopathological features. However, 39% of these cancers in one series occurred before 20 years of age (Table 1). The most common childhood cancers

have been soft-tissue sarcomas in the first 5 years of life and osteosarcomas in adolescence. Acute leukemia and brain tumors also occur throughout childhood and young adulthood, whereas the few adrenal cortical carcinomas occur primarily in infancy. In young adults, premenopausal breast cancer is, by far, the most common neoplasm. Cancer patients in these families who survive the first neoplasm are prone to develop second cancers, particularly within the field of radiation therapy. In addition, a few young adults who developed cancer of the lungs or larynx reported a history of cigarette smoking, suggesting a host-environment interaction. The possibility of cancer prevention in family members through avoidance of environmental carcinogens was raised. While evidence is still limited that families with Li-Fraumeni syndrome are unusually sensitive to environmental carcinogens, it was agreed that counseling regarding a healthy life style should be part of any intervention.

Stephen Friend (Harvard Medical School, Charlestown, MA) described the finding of germ line *p53* mutations in families with Li-Fraumeni syndrome. In general, cancers arise from normal cells through a series of genetic alterations. The *p53* mutations found in the germ line cells of cancer families represent only one change in the multistep process of carcinogenesis. Germ line mutations in *p53*, like somatic mutations in this gene, arise in several regions of the gene. The definitive method for the identification of germ line mutations is gene sequencing, but this is laborious and costly. Detection of germ line *p53* mutation by gene sequencing should not produce false positive results unless a polymerase chain reaction artifact or sample mix-up occurs. Because of practical difficulties in sequencing the *p53* gene in large numbers of specimens, several gel electrophoresis methods have been applied to screen blood specimens likely to contain *p53* mutations. Unfortunately, the false negative rate of these methods is unknown. The major advantage of screening methods, such as single-strand conformational polymorphism and constant denaturing gel electrophoresis, is the feasibility to screen dozens of specimens weekly in a moderate-sized laboratory. Several laboratories worldwide are currently screening tissues of sporadic and familial cancer patients for germ line *p53* mutations. Tsunematsu (Tokyo) and Birch (Manchester, England) each presented two families with features of Li-Fraumeni syndrome and a germ line *p53* mutation. Many more families with the syndrome are under study.

S., a patient who has had three primary cancers since infancy, gave a personal perspective of the impact of Li-Fraumeni syndrome in her family. Two of her siblings had died of cancer in early adulthood, one of a second primary cancer of bone. She has three healthy children under ten years of age and constantly worries about their health. She wants her family to be tested for the *p53* mutation. She said that finding a mutation in one

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Table 1 Distribution of 191 cancers, by tumor type and age at diagnosis, among 991 members of 31 families with Li-Fraumeni syndrome^a

Tumor type	Age at diagnosis (years)					All ages
	0-19	20-29	30-39	40-49	>50	
Soft tissue sarcoma	24	3	4	6	3	40
Osteosarcoma	22	5	0	2	1	30
Breast cancer	0	12	12	14	7	45
Brain tumor	9	5	2	1	0	17
Leukemia	8	1	0	0	2	11
Adrenal cortical carcinoma	5	0	0	0	0	5
Other cancer types	7	6	9	6	15	43
Total	75	22	27	29	28	191

^a Excludes second and subsequent primary cancers in affected persons.

or more of her children would not make her more worried or anxious than she is at present. S. has personally not experienced job discrimination because of her medical history and has always had health insurance. Despite three cancers, she pointed out that her use of medical services has been modest over her lifetime. She believes the information about *p53* would help her and her children to plan their lives, including the decision to have a family. The information would also help her deal with the minor illnesses and physical complaints of her children. Her husband and her surviving relatives also want the family to be tested. During the discussion, she indicated that her older children had not been told of a familial susceptibility to cancer, but they recognize that several of their close relatives have died of the disease. S. reiterated that she and her family would be better served with more information about the individual risk of cancer development.

Alice Whittemore (Stanford University School of Medicine, Stanford, CA) discussed the biostatistical issues in screening for germ line *p53* mutations in defined populations. The predictive power of a positive test for *p53* is determined by three factors: prevalence of *p53* mutations in the study population; the sensitivity (probability of detecting a true positive) of the test; and its specificity (probability of detecting a true negative). In the example shown in Table 2, sensitivity and specificity are set at 99% (highly sensitive and specific). In a screen of 1000 persons with a *p53* prevalence of 0.01, however, the predictive power of a positive test is only 50%. In other words, only one-half of those with a positive *p53* test actually are cancer-prone individuals. Dr. Whittemore considered the effect of altering prevalence, sensitivity, and specificity. Predictive power is increased substantially by applying a highly specific test to a study population with high prevalence, preferably greater than 10%. One such group is likely to be members of families with clinical features of Li-Fraumeni syndrome. In contrast, the prevalence of germ line *p53* mutations is likely to be very low in the general population. Intermediate groups might include cancer patients with a neoplasm used to define Li-Fraumeni syndrome, such as breast cancer of early onset, but not a striking family history of cancers.

Jerome J. DeCosse (New York Hospital, New York, NY) recounted the experience with prophylactic colectomy and chemoprevention in patients with polyposis coli. Carriers of the polyposis gene have a lifetime risk of colorectal cancer that approaches 90%. Many of these

Table 2 Predictive power^a of positive test of 1000 persons, assuming 1% population prevalence of germ line *p53* mutations, and 99% sensitivity^b and 99% specificity^c of the assay

Tumor type	Age at diagnosis (years)					All ages
	0-19	20-29	30-39	40-49	>50	
Positive	10		10			20
Negative	0		980			980
All persons	10		990			1000
Predictive power = 10/20, or 50% ^a						

^a Predictive power: among persons who test positive, the proportion who are truly susceptible.

^b Sensitivity: among susceptible persons, the proportion positive for the test.

^c Specificity: among nonsusceptibles, the proportion who test negative.

patients develop multiple primary cancers within the large intestine, starting at age 20 years. Screening proctoscopy can be performed every 2 to 3 years, in place of annual colonoscopy, to minimize the morbidity of screening children in affected families. Data show that cancer rarely occurs within the colon of polyposis patients without rectal polyps. An eye finding, congenital hypertrophy of retinal pigment epithelium, can be a useful diagnostic tool for identifying gene carriers at an early age. Management of polyposis coli requires a total colectomy, together with periodic surveillance of any residual rectal tissue. Additionally, these patients may be at an increased risk of mortality from desmoid tumors of the abdomen and cancers of the upper gastrointestinal tract, notably carcinoma of the ampulla of Vater. Evidence of dietary influences in colon cancer development suggests a role for education to avoid environmental carcinogens. The possibility of chemoprevention has been tested in polyposis patients. In a small study, wheat fiber appeared to inhibit the development of new adenomas. There may also be a benefit from using tamoxifen in the prevention of or treatment for invasive desmoid tumors.

Stephen E. Sallan (Dana-Farber Cancer Institute, Boston, MA) examined the risks and benefits, if any, of periodic medical surveillance of children with germ line *p53* mutations. Survival of brain tumors and sarcomas, which account for nearly one-half of all cancers in children with Li-Fraumeni syndrome, may be improved through early diagnosis. However, population screening of neuroblastoma by urinary catecholamine levels has not yet improved survival statistics. When testing children, issues of informed consent and explanation of findings to the children merit careful consideration. Magnetic resonance imaging appears to be the surveillance procedure of choice because no radiation is delivered and multiple anatomical sites can be examined; the main drawbacks are the cost and availability of the study. The possibility of chemoprevention was also raised, although the agent of choice is uncertain. Given the marked loss of human potential that results from the death of a child, pilot research protocols are needed to evaluate the possibility of early cancer detection in *p53* mutation carriers.

Jason Brandt (Johns Hopkins Medical Center, Baltimore, MD) reviewed the experience at his institution with presymptomatic testing for Huntington's disease. This autosomal dominant trait has 100% penetrance, variable age of onset in adulthood, and no available treatment. Carriers are identified by linkage analysis be-

cause the gene has not been isolated. Initial surveys indicated that 80% of individuals at risk said they wanted the test for purposes of planning for the future, relieving anxiety, and making childbearing decisions. The testing program has been restricted to adults with at least 25% disease risk, who are living near Baltimore and are mentally competent to give informed consent. The protocol for testing involves physical and neuropsychological examinations, which have revealed early signs of Huntington's disease in some individuals. Pretest counseling is given over 3 to 5 visits of approximately 90 min each. Thereafter blood samples are tested and results are reported to the patient, who is followed for at least 3 years. To date, the effect of disclosure on the well-being of patients has been modest. Psychological tests revealed no marked differences in depression among those found to have a high likelihood as compared to those with a low likelihood of carrying the gene. The results of testing generally produced temporary changes in the individual's perception of personal risk of disease. Among those who tested positive, there have been anecdotal losses of jobs as a consequence, and one patient required psychiatric hospitalization. The yearly cost of the program was approximately \$5000/patient (grant supported at present).

Philip R. Reilly (Shriver Institute, Waltham, MA) reviewed the legal and ethical aspects of screening and counseling of cancer families, particularly children. A historical overview of laws regarding testing was presented, including the 1962 law on testing for phenylketonuria in newborns, the Human Genome Privacy Act, and the Americans with Disabilities Act. With regard to children, the law assumes that parents have a right to act as proxy for their children. The decision to test children must be based on concern for the welfare of the child to be tested. Provisions are needed to protect the privacy of the individual tested. Furthermore, there should not be testing outside of the clinical context, e.g., as a requirement for insurance, employment, or admission to school. Candidates for testing should be advised to reexamine their insurance status before disclosure of the results. For testing of children, three alternatives were considered: testing at-risk children when appropriate safeguards and follow-up procedures are in place; monitoring (but not testing) all at-risk children as though they were carriers; or a moratorium on any testing of children until a pilot study has been made on adult carriers of *p53*.

Eric Juengst (National Cancer Institute, Bethesda, MD) described the Project's Ethical, Legal and Social Implications Program. The program supports scholarly research and facilitates policy making and public education on issues related to the Genome Project. The history of testing for cystic fibrosis was reviewed. A recent workshop on cystic fibrosis concluded that testing should be limited to families at risk and that population screening for the disease is not the standard of care. An advisory group was formed, and support was generated for studies of cystic fibrosis testing. A similar process might be used in the future to conduct studies of testing for *p53* and other mutations in cancer susceptibility genes.

Leroy Walters (Kennedy Institute for Ethics) described the process of review for human gene therapy protocol. Many of the issues relevant to gene therapy also apply to testing for disease susceptibility genes. Also, similar issues arise with the availability of new genetic

tests, and the possibility of establishing a National Advisory Group on Genetic Testing and Screening was presented. This group would provide a coordinated national approach to issues of genetic testing, which should be more effective and efficient than the *ad hoc* approach of the past.

Louise C. Strong (M. D. Anderson Cancer Center, Houston, TX) gave an overview of the presentations and discussions and listed the unknowns and uncertainties identified by the discussants. Clinically, the range of cancers in the syndrome remains to be defined. The new mutation rate of Li-Fraumeni syndrome is uncertain. There are technical problems in testing, which is labor intensive and can yield false positive as well as false negative results. A distinction was made between surveying selected populations for the frequency of *p53* mutation and presymptomatic testing among members of high-risk families. In both instances, statistical aspects need to be considered in the study design. The benefits of testing, as eloquently outlined by patient S., include relief of anxiety for those who test negative and opportunities for early cancer detection. Benefits are to be balanced against the risks, including harmful psychological, economic, and other effects. There is a need for education of both clinicians and at-risk patients with regard to opportunities for early detection and avoidance of environmental carcinogens. Although the experience of Huntington's disease testing indicates little change in the life style of patients after testing, the impact of testing might have been minimized by the extensive support provided for participants. The impact of testing in less supportive environments cannot be assumed to also have minimal adverse effects. The issue of testing children has received relatively little discussion in the genetics literature. Testing of presymptomatic children requires attention to developing appropriate forms and procedures for obtaining informed consent. The issue of cost of the early detection procedures requires further consideration, particularly since equal access to testing should be a fundamental principle in future programs. In the ensuing discussion, the need to distinguish between testing Li-Fraumeni syndrome families and research surveys of other populations was reemphasized. For research purposes, banking of DNA specimens should be encouraged, particularly in the context of ongoing cancer therapy and chemoprevention trials. Screening of the general population to determine the frequency of *p53* mutations seems impractical at present because of the high cost and low prevalence, which requires enormous sample sizes. However, surveys of subsets of cancer patients without the classical features of Li-Fraumeni syndrome were encouraged. There will be patients who have new germ line *p53* mutations and unimpressive family histories of cancer.

Day 2 of the workshop was devoted to the discussion of issues that required attention before the development of guidelines for presymptomatic testing of members of families with Li-Fraumeni syndrome. It was agreed that members of these families, who have a high prevalence of *p53* mutations, should be considered separately from cancer patients and members of the general population. The issues of testing family members can be separated into two categories. The first category is broad and applies to testing clinically healthy individuals for any genetic disease, including hereditary susceptibility

to cancer. The participants agreed that these broad issues have been previously addressed by other expert committees and should not be the main focus of the workshop (5, 6). The establishment of a National Advisory Group on Genetic Testing and Screening, as recommended by Dr. Walters, was endorsed as a reasonable mechanism for addressing these issues.

The second category encompasses questions that are specific to germ line *p53* mutations; the following issues were identified:

(a) Li-Fraumeni syndrome remains to be fully defined, primarily because cancers that occur as part of the syndrome are indistinguishable from cancers due to other factors. Li-Fraumeni syndrome may be genetically heterogeneous, and only a portion of affected families may have germ line *p53* mutations. Better characterization is required of the cancers that comprise the syndrome. Features of interest include the anatomical location of specific subtypes of cancer, presenting symptoms, clinical stage at diagnosis, and survival rates. The recently established International Working Group and the Germ Line *p53* Mutation Registry can help to resolve these questions.

(b) Evidence for germ line *p53* mutations as the underlying defect in some Li-Fraumeni syndrome families can be strengthened by additional work. These include linkage analysis and *p53* testing of additional families; distinguishing the spectrum of mutations associated with this syndrome from *p53* polymorphisms; and the use of the polymerase chain reaction to amplify paraffin-embedded tissue of deceased family members for studies of the *p53* genotype.

(c) The potential benefits and risks of *p53* testing in cancer-free children and young adults need to be examined. Possible benefits include relief from anxiety among those who test negative and surveillance of those who test positive for early detection of curable cancers. Risks to those who test positive include depression, isolation, economic losses, and discrimination by insurance companies.

(d) Protocols for counseling patients and families need to be developed and tested. Baseline information on the psychological health of family members and tools for psychological testing must be identified.

(e) Aside from families with Li-Fraumeni syndrome, surveys are needed on the frequency of germ line *p53* mutations in specific subgroups. Several dozen groups of investigators are currently examining tissue banks for *p53* mutations in patients with diverse cancers. Efforts must be made to collect this information in a uniform manner so that findings can be pooled.

In summary, the workshop identified issues in testing for germ line *p53* mutations in members of Li-Fraumeni syndrome families. Committees were organized to address a series of difficult questions that are specific to *p53* and Li-Fraumeni syndrome but which might also apply to other tumor suppressor genes. Participants agreed to reconvene in several months to review and act on committee reports. The second *p53* workshop will be expected to provide guidelines and recommendations for future research on predictive testing among members of families with Li-Fraumeni syndrome, which can serve as a model for testing for other cancer susceptibility genes.

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