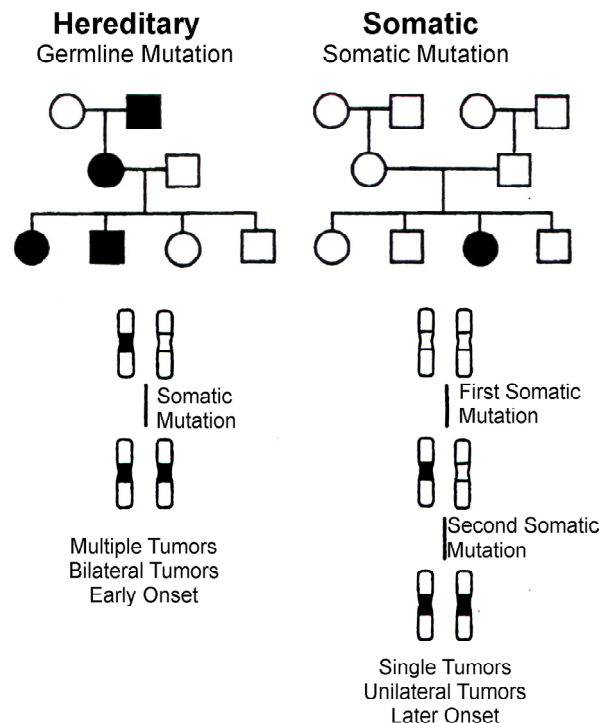


## Detection of Cancer Genes

No one knows why any one person gets cancer. A substance that causes a change in DNA that can lead to cancer is a **carcinogen** and exposure to a carcinogen is the first step in cancer. Some cancers are related to the environment, especially the smoker's environment. Radiation and the combustion products from tobacco are two of the most common carcinogens. Asbestos and some heavy metals in particulate form are also carcinogens. Many steroids in higher than normal concentrations are carcinogenic. For others, substances in foods may be cancer causing, cancer promoting or, conversely cancer preventing. A high fat, low fiber diet is suspected as being cancer promoting. A diet with abundant cruciferous vegetables may be anti-cancer promoting. Some viruses promote cancer formation.

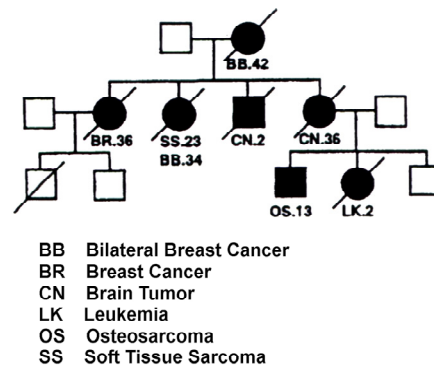
Current estimates are that 1/3 of the children born today will get some form of cancer in their lifetime. Most believe that the onset of cancer is an accumulation of mutations that affect the controls over cell division. This correlates with the increase in many cancers with aging. A gene that has the potential to induce cancer is called an **oncogene**. Some cancers are familial, and probably genetic. Familial clustering of cancers appears to be linked to the inheritance of mutated tumor suppressor genes, such as p53. Although familial cancers collectively constitute a very small fraction of the total reported cancers, they occur in dominant inherited patterns. Mutations that are directly inherited are referred to as **germline mutations**. Such mutations can be detected in family pedigrees. **Somatic mutations** do not have direct genetic links and are acquired during the life of the individual. Germline and Somatic mutation patterns of cancer formation in families is shown below.



In a germline inherited mutation, a single mutation within the suppressor gene will result in the inactivation of both alleles in the gametes that inherit the mutated homologue. (The non-mutated homologue will produce "normal" gametes.) In somatic tissues, normal, mutation-free tumor suppressor genes require two sequential somatic mutations to initiate tumors in a given cell line. This model for somatic mutation cancer induction is referred to as the **"two-hit"** hypothesis.

Cancer genes identified include the retinoblastoma (RB) gene, Wilm's' tumor (WTI), neurofibromatosis type II gene and Li-Fraumeni syndrome.

In Li-Fraumeni syndrome, a notable feature in family pedigrees is one individual with a sarcoma, at least two immediate relatives with other cancers before the age of 45, and multiple cancers in other family members. A typical family pedigree for Li-Fraumeni syndrome is shown.



Gene and chromosome maps are increasingly available as tools for the identifying predisposition for various diseases, including cancers. The procedures used to obtain such information include DNA isolation and the analysis of point mutations in cancer-related genes, such as p53. DNA sequencing is one method for detecting gene point mutations.

### Some Information about p53

The study of inherited cancers has given molecular biologists the opportunity to search for genes that are critical in normal cell development and carcinogenesis. At the molecular level, cancer formation is characterized by alterations in both dominant oncogenes and tumor suppressor genes, such as p53. Suppressors are normal cellular proteins that are involved in limiting cell growth; oncogenes are involved in promoting uncontrolled growth of cells.

Comparing how the p53 tumor suppressor protein functions in normal cells and cancer cells is a focus of many cancer biology studies. The gene for the p53 protein is located on the short arm of chromosome 17. It encodes a 53,000 molecular nuclear phosphoprotein. Normal (unmutated) p53 is a sequence-specific DNA-binding protein that is a transcription regulator. Mutated p53 loses its ability to bind to DNA. p53 genes that have mutations in specific DNA sequences, called "hot-spots" (sites where mutations are detected in high frequency) promote uncontrolled cell growth and function as oncogenes. For a tumor suppressor gene such as p53 to play a role in transformation in cancer, both alleles need to be altered.

The p53 protein can be divided into three domains. The first is the amino terminus region that contains the transcription activation region. The second domain, the central region within the protein, contains the majority of critical hot-spot mutations. 95% of the mutations are located within exons five through eight. Human cancer point mutations have been detected within five subregions of these exons. The third region of the p53 protein is the carboxyl section that contains the oligomerization and nuclear localization sequences.

Several known mutations result in an altered p53 protein conformation. These changes can result in increased stability of the mutant protein, increasing its ability to bind to the normal p53 protein and inactivate it.

Some mutations are differentially encountered within the body. In some cases, differences in frequencies of mutations at a specific site may reflect an enhanced growth advantage for a particular tumor tissue; in others, mutation depends on the organ in which it occurs. Some mutagens act differentially within certain organs. For example, the mutation at amino acid 175 of p53 is common in colon carcinoma but is rarely observed in lung carcinoma.

### Case Study of Li-Fraumeni Syndrome and p53

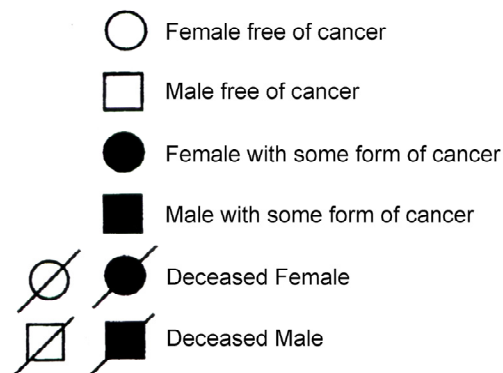
Li-Fraumeni syndrome is rare but when it occurs Li-Fraumeni affects young family members and has high mortality rates. Two physicians, Li and Fraumeni first described the syndrome after examining death certificates of 648 childhood sarcomas. Four families were studied in which siblings and cousins had childhood sarcomas. Further analysis showed more than 50% of the affected families had extended phenotypes that included brain cancers, breast cancers and leukemias. Cells of individuals with Li-Fraumeni syndrome have one normal p53 allele.

In this exercise you will construct a pedigree for a family that is suspected of having Li-Fraumeni syndrome. You will also observe the results of DNA samples that have been enzymatically digested and separated by gel electrophoresis.

### Using Family History to Construct the Pedigree

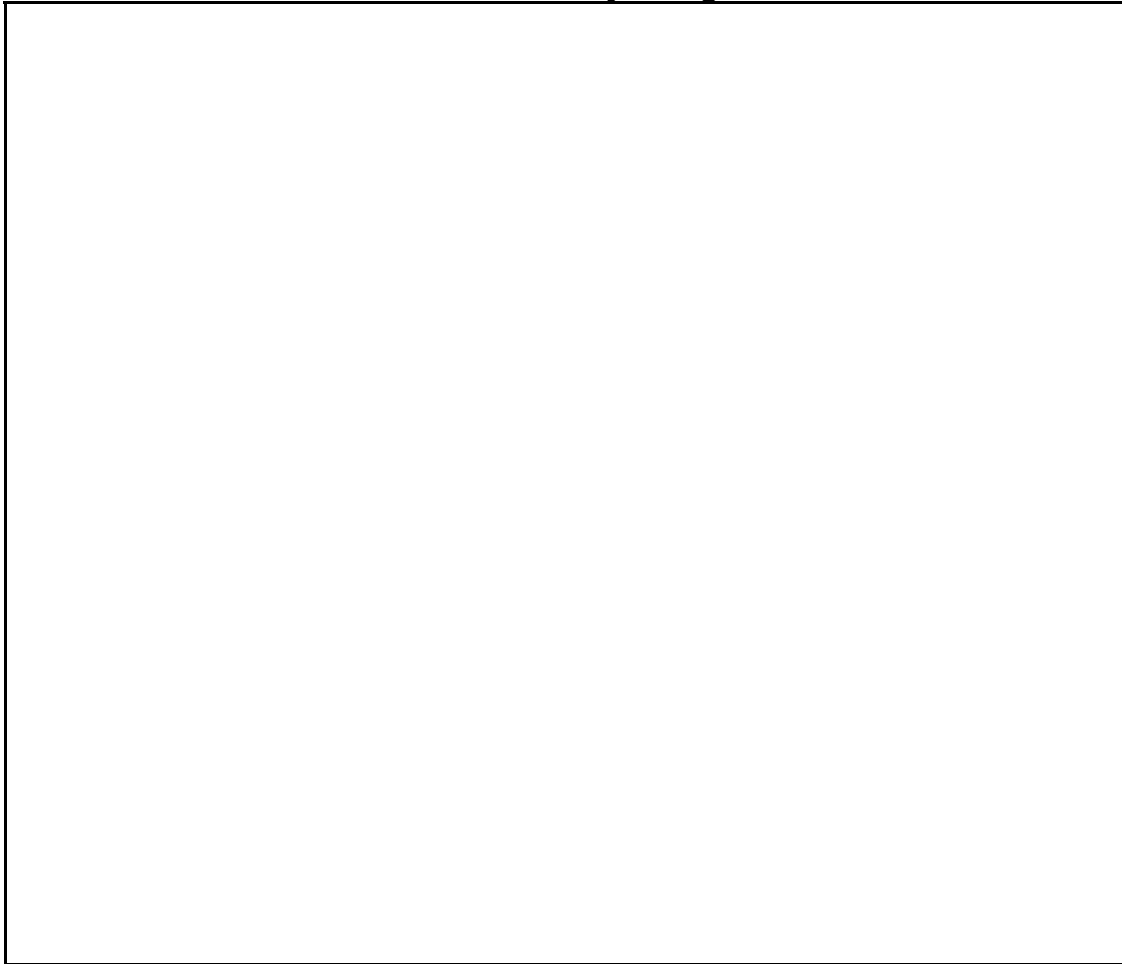
Valerie Brown, age 36, was diagnosed with breast cancer. Valerie's mother also had breast cancer when she was in her thirties. As part of the medical work-up, the oncologist inquired about Valerie's family history of cancer. Her father and his family appear to be free of cancer. However, in Valerie's mother's family, several cases of cancer have occurred.

Construct Valerie's family pedigree using the information given below. The following symbols are standard for constructing a pedigree.



- Valerie, age 36, has been diagnosed with breast cancer.
- Valerie's mother, Diane, was diagnosed and treated for breast cancer at the age of 39
- Diane had a sister, Mabel, who died at age 2 of a brain tumor.
- Diane's brother, James underwent surgery for colon cancer, followed by chemotherapy.
- Valerie's maternal grandmother, Elsie, died at age 42 from bilateral breast cancer.
- Valerie's maternal grandfather, Elmer, is 88 years old, and free of cancer.
- Valerie's maternal cousin, Patrick (son of James), died of brain cancer at 14.
- Patrick's sister, Jane, was diagnosed with childhood leukemia and died at age 2.
- Patrick's two brothers, Robert, 28 and Curtis, 30, are in good health and free of cancer.
- Valerie's younger sister, Nancy, is free of cancer.
- Nancy's son, Michael, now 19, was diagnosed at age of 3 with sarcoma. At 18, he was diagnosed with osteocarcinoma.
- Nancy's son, Justin, and daughter, Jessica, are free of cancer.
- Valerie has 5 children, 3 girls, Zoe, Maria and Eve, and 2 boys, Eli and Zachary, all of whom show no signs of cancer at this time.
- There is no indication of cancer in Valerie's husband, George, George's family, or in Valerie's paternal grandparents.

### Valerie's Family Pedigree



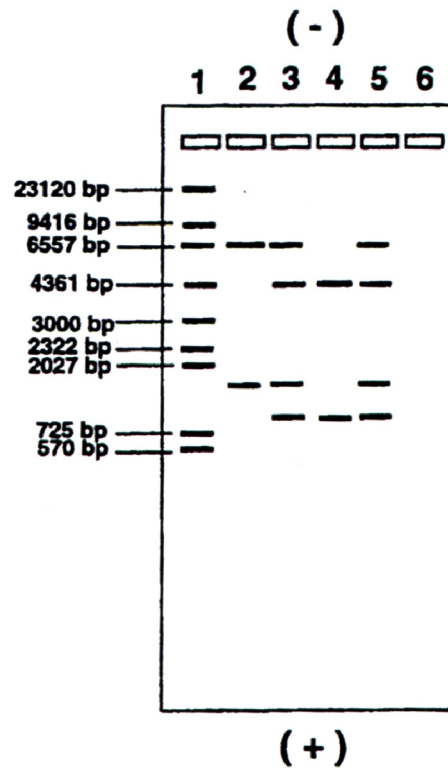
The pattern of cancer in Valerie's family pedigree strongly suggests Li-Fraumeni syndrome. In such a case, a secondary diagnostic test is normally conducted. Valerie was most interested in having the p53 diagnostic test to determine if she inherited mutations.

To do so, Valerie provides a sample of blood, normal breast tissue and breast tumor tissue to conduct DNA analysis for the p53 gene. PCR is used to obtain sufficient DNA followed by one of several methods to detect the presence of a point mutation at the p53 hot spots.

Valerie's amplified DNA samples were digested with a restriction enzyme that recognizes and cuts the mutant sequence (the palindrome CAGCTG ) at the hot spot site at nucleotide 165. A normal p53 control and a set of standard DNA marker fragments were also used. Valerie's predigested samples, the control type and DNA Markers were separated by agarose gel electrophoresis and stained. The stained gel pattern is shown below.

Digestion of the normal amplified DNA produced a characteristic DNA fragment banding pattern. DNA obtained from Valerie's blood lymphocyte digest produced an altered band pattern representing one normal allele and the second, mutant, allele. The DNA analysis from the tumor tissue showed only the pattern for the tumor allele.

- Well 1 Standard DNA fragments
- Well 2 Control DNA
- Well 3 Valerie's Peripheral Blood DNA
- Well 4 Valerie's Breast Tumor DNA
- Well 5 Valerie's Breast Normal DNA



**Discussion Questions**

1. Distinguish between a tumor suppressor gene and an oncogene.
  
2. How do hot spots affect p53 protein structure?
  
3. Why does Valerie's breast tumor DNA sample have fewer bands than her peripheral blood sample?
  
4. Why does the physician do a control DNA and a set of standard markers?
  
5. Does Valerie's family pedigree and DNA analysis indicate Li-Fraumeni syndrome is carried in her family? Why or why not?

Materials for this exercise were provided by BCC Life Science Instructors, Carol Burton and Melodye Gold.