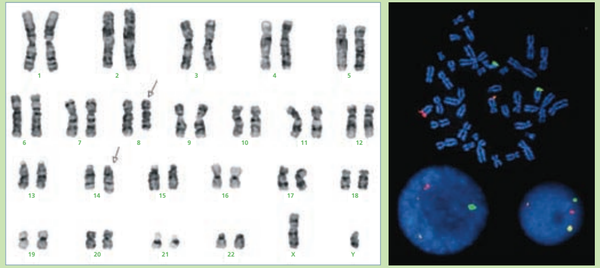
unlocking the genetic code:

THE INFLUENCE IN CLL

Cancer can be a genetically linked disease; chronic lymphocytic leukemia (CLL) is no exception. Researchers study the genetics of cancer by evaluating chromosomes. Chromosomes are made up of DNA which contains genes that carry genetic codes of information. This information directs the development and function of the body. Individuals normally have 23 pairs of chromosomes, all of which code for different traits and functions. By evaluating genes and chromosomes, researchers are able to figure out the root cause of cancer and other diseases. However, CLL cells seldom divide, making the traditional evaluation of chromosomes for genetic purposes difficult.



Left: Cytogenetic analysis of CLL patient chromosomes with abrerrations on chromosomes 8 and 14. Right: Florescent In Suto Hybridization (FISH) detection of chromosomes 8 and 14. The top portion of the photograph shows chromosomes after they have divided. The bottom of the photograph shows cells with non-dividing chromosomes.

Until the last decade limited genetic research was available on CLL. Development of new technology such as florescent in situ hybridization (FISH) now allows researchers to evaluate the frequency of some commonly described genetic abnormalities that have been found in patients with CLL. These abnormalities most frequently involve chromosomes 11, 12, 13, and 17.

Historically, chromosome 17 abnormalities, and to a lesser degree chromosome 11 abnormalities, were associated with a poor response to treatment and short survival. Patients with a chromosome 12 abnormality had a similar prognosis to those with no abnormality. Abnormality to chromosome 13 alone was considered to be somewhat better than other abnormalities associated with CLL. Ongoing research is better defining chromosomal characteristics, and identifying new chromosomes that add to the understanding of CLL characteristics.

CHROMOSOME 11

Chromosome 11 contains genes that play a role in the DNA repair process and that regulate abnormal cell growth. In 10-15% of patients with CLL, important DNA repair genes present on the long arm of chromosome 11 are lost. This loss is generally associated with very large lymph nodes and extensive disease. Combination regimens, fludarabine and cyclophosphamide (FC) and FC+ rituximab (FCR), have significantly reduced the negative impact of this chromosomal abnormality.

CHROMOSOME 12

Some CLL patients have three number 12 chromosomes instead of two, an abnormality known as trisomy 12. This abnormality makes the cells look somewhat unusual for CLL. Patients with trisomy 12 do not generally exhibit the chromosomal changes commonly associated with CLL.

The overall outcome of patients with trisomy 12 is similar to those who have no abnormalities on FISH testing. Trisomy 12 is generally associated with an increased likelihood of eventual disease progression and need for treatment. These patients have a very high expression of CD20, which is the target for rituximab and other emerging antibodies such as ofatumumab and GA-101. These drugs may prove to be very beneficial to trisomy 12 patients.

Patients with trisomy 12 often have abnormalities involving chromosomes 6, 8, 14 and 19. Individuals with trisomy 12 alone tend to exhibit a better clinical response than those with the less common abnormalities. In the next two to three years it is likely that there will be treatments specifically targeting these abnormalities.

CHROMOSOME 13

A normal chromosome 13 suppresses the development of tumors. At the time of initial presentation, more than two thirds of CLL patients have loss of genetic material on chromosome 13. Unsuccessful efforts have been made to explain the loss of genetic material in CLL patients.

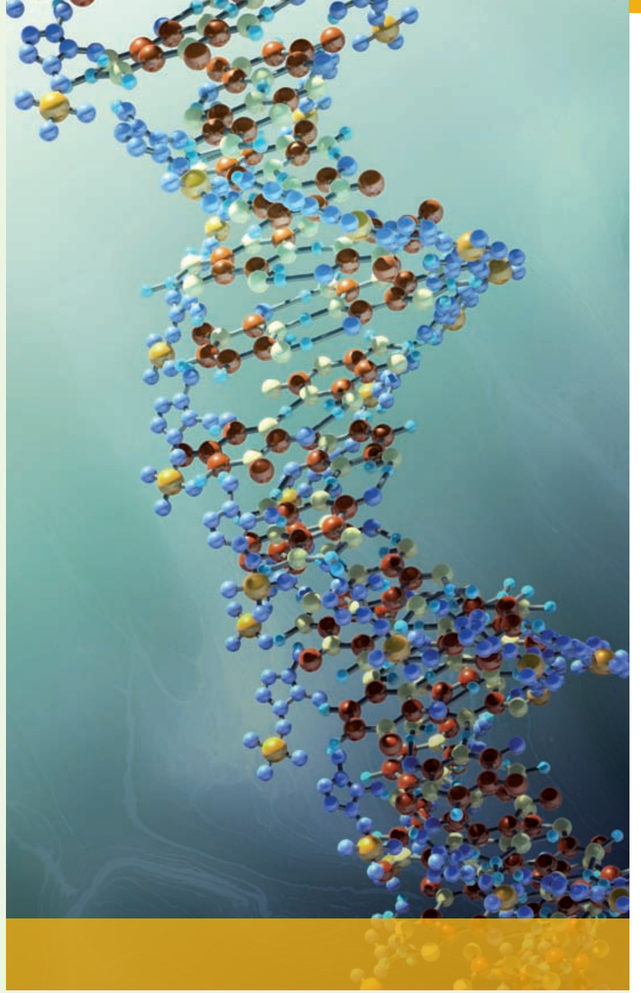
A collaboration among investigators in the Clinical Research Consortium (CRC), including Dr. Carlo Croce of Ohio State University, Dr. Kanti Rai at Long Island Jewish Medical Center, and Dr. George Calin (previously at OSU, now at M. D. Anderson Cancer Center) identified a region on chromosome 13 that is associated with the loss of genetic material. It was suggested that the missing genetic material might be linked to microRNA genes.

MicroRNA genes were first discovered in worms in 1993, and later found in other species. When discovered in humans, microRNA was considered to be "junk DNA" (DNA with no function) because these genes were so small and did not code for anything. Almost 10 years later, the investigators mentioned above began looking at microRNA in CLL. They found that two microRNAs, 15 and 16, were lost from chromosome 13. CLL is considered to be a genetically silent disease. Therefore, it was an unexpected breakthrough that CLL would lead to the conceptual understanding of microRNA's role in regulating cancer.

CHROMOSOME 17

The loss of information on the long arm of chromosome 17 (17q deletion) is often associated with a mutation in a very important regulatory gene, p53. A properly functioning p53 gene is needed for a beneficial response to chemotherapy and radiation. Previously, chromosome 17 abnormalities did not indicate a positive prognosis. Now, many early stage patients with a 17q deletion show no evidence of progression for long periods of time. The true impact of the loss of 17q continues to be defined.

The path to understanding the role of genetics in CLL will not be straight or short. However, exciting breakthroughs will come at a steady pace. Eventually researchers will understand what causes CLL to occur and to progress. In the meantime, the role of clinicians is to develop treatments that will cure the disease without necessarily understanding all the genetic ramifications of the disorder. : :



what does this all mean to me?

Are treatment decisions determined by my genetic factors?

Genetic factors can help predict rapid disease progression and the requirement of early treatment intervention; however, these decisions are always made in conjunction with a patient's clinical features including symptoms, blood counts and evolution of disease. Recently it has been found that genetic factors may also help in determining the type of therapy to use for a greater likelihood of response.

Am I born with the genetic abnormalities for CLL or do they develop? What causes the development?

Although this is not completely known, most people likely acquire genetic abnormalities throughout their life. The causes for these changes can be varied. During the DNA replication process (which occurs many times a day in blood cells), genes sometimes make mistakes when copying the genetic material. Problems can also evolve in the structure of the genes. Some changes may occur randomly while others may be caused by external or environmental factors. Although environmental factors seem like an attractive explanation, no specific environmental factor has yet to be convincingly identified.

Is there a classification of subgroups in CLL based on genetics?

At the moment, clinical features are generally used for CLL subgroup classification. A standardized form of genetic classification is needed to better understand which patients should be treated earlier, who can be observed, and what treatments would most benefit patients. This form of classification is in developmental stages as newer technologies are improving the understanding of CLL genetics.

Can my prognostic markers change?

It is possible that a patient's prognostic markers may change over time. Some people will develop new genetic abnormalities if they have progressive disease. However, the majority of CLL patients with a good prognosis will have slowly progressive or non-progressive disease and their prognostic markers are unlikely to change significantly.

What is cytogenetics?

Genetic material is packaged into cells in the form of chromosomes. Cytogenetic tests examine all 23 pairs of chromosomes to look for genetic and molecular abnormalities that may be associated with a malignancy. This test provides limited information relative to CLL because it has to be performed on cells preparing to divide, and CLL cells generally do not divide. Cytogenetics does provide useful information in a subset of CLL patients with complex abnormalities that are not detected by FISH tests.

Why are fluorescence in situ hybridization (FISH) tests useful in CLL?

FISH tests look at specific chromosomes commonly linked to a malignancy. Fluorescent light is used to determine if specific genetic abnormalities are present on these chromosomes. The features identified help in deciding the best course of treatment. Unlike cytogenetics, FISH tests can be applied to chromosomes during any phase of cell division. FISH is also more sensitive than cytogenetics, and therefore more likely to pick up an abnormality if present.

How is flow cytometry used?

Cells have tags on them called surface markers which are identifiable to other cells and molecules in the body. Flow cytometry analyzes these surface markers. CLL cells have a very characteristic set of markers on their surface, and flow cytometry helps distinguish CLL from similar lymphocyte disorders, making it an important test for diagnosis. Flow cytometry can also offer useful prognostic information (measurement of CD38 and ZAP-70) and is useful in determining if patients have any residual disease in their bone marrow after chemotherapy.

What is IgVH?

Immunoglobulin gene variable heavy (IgVH) chain is a gene in lymphocytes. IgVH rearrangement occurs as lymphocytes mature. Maturing lymphocytes undertake a high number of random mutations to allow the immune system to recognize a wide variety of antigens. The cell stores a library of these mutations so antigens can be easily recognized and destroyed. The IgVH mutation process is part of the normal function of the immune system to recognize foreign organisms.

Why is IgVH mutation a good prognostic factor?

Patients with a mutated IgVH are capable of recognizing a larger number of antigens and are genetically stable, resulting in a lower likelihood of disease progression. CLL cells with unmutated IgVH are likely to be more immature and genetically unstable, thus associated with a higher chance of progressive disease.

Why does a mutation to chromosome 11 cause enlarged lymph nodes?

A number of genes on chromosome 11 are involved in the regulation of abnormal cells. When these genes become mutated or deleted in CLL cells, the abnormal cells (in this case lymphocytes) are allowed to grow uncontrollably. This uncontrolled growth forces the lymph nodes to enlarge.

What causes a patient to be refractory to chemotherapy?

Most chemotherapy drugs target essential functions of a cell that help them divide or grow. CLL cells seldom divide and can also develop tricks to bypass these functions. They may repair the damage caused by chemotherapy drugs, use alternative mechanisms to grow or they may develop proteins to pump the chemotherapy out of the cell. Some of the genetic changes that underlie the malignancy can also help the cell to fight the effects of chemotherapy.

Why does CLL generally not show up until age 50 or older?

Most malignant disorders increase with age. The immune system is constantly growing and dividing, and is generally kept in check by regulatory genes and self-regulation. Unfortunately, mistakes are made and occasionally these mistakes take place in genes that can predispose to cancer. Since it is believed that more than one event must occur to produce diseases like leukemia, it takes time for CLL mutations to accumulate. Also, it appears that the immune system may be less able to control the malignant cells as people get older. : :