



Gene Expression Profile in B-cell Chronic Lymphocytic Leukemia

Background:

Chronic Lymphocytic Leukemia (CLL) is an adult leukemia associated with problems in the apoptotic pathways of B-cells. There is a great deal of variation in the prognostic outlook of different patients. An accurate prognostic indicator for in CLL is the chromosomal aberrations detected in each patient. Some of the abnormalities include a 13q deletion, an 11q deletion, trisomy 12, and a 17p deletion. The 17p and 11q deletions usually have shortened survival compared to other CLL patients. 11q patients are generally associated with extensive lymphadenopathy.

Objective:

In this study, the gene expression in the 11q group of patients was looked at compared to the non 11q patients.

Methods:

Fluorescence in situ hybridization (FISH) was used to detect the chromosomal abnormalities in each patient. A blood separation was done to separate the mononuclear cells, and the B-cell percentage was confirmed by flow cytometry. Then, total RNA was isolated from the cells using a Trizol-phenol-chloroform method. The gene expression was looked at using Clontech Hematology/Immunology arrays containing 415 genes. The RNA was used in a reverse transcription reaction to produce DNA probes labeled with P³². The probes were hybridized to the Clontech nylon membranes and then allowed to expose for a week in a phosphorimaging cassette. The images were then scanned to detect the radiation levels on each gene spot. The images were analysed using Pathways Universal Array Analysis software which normalized the intensities of each spot against the background.

Results:

Ten genes were found to be differentially expressed between the 11q and the non 11q CLL patients. Nine genes were overexpressed in the 11q group, and one was underexpressed. Also, we found that abdominal/mediastinal lymphadenopathy was significantly associated with the 11q deletion. Blood counts seemed to be lower in the 11q deletion group, but the results did not prove to be statistically significant.

Conclusion:

The results suggest that there is differential gene expression between the 11q and the non-11q groups. However, further studies need to be done in order to obtain conclusive results.

Chronic Lymphocytic Leukemia (CLL)

- CLL is the most common leukemia, accounting for 25% of all leukemias.
- The average age for the diagnosis of CLL is about 70 years.
- This disease is mainly associated with apoptotic problems in the leukemic cells.
- Common symptoms of CLL patients are lymphocytosis, lymphadenopathy, splenomegaly, hepatomegaly, anemia (low red cell count) and thrombocytopenia (low platelet count).



Dohner, Hartmut. 11q Deletions Identify a New Subset of B-Cell Chronic Lymphocytic Leukemia Characterized by Extensive Nodal Involvement and Inferior Prognosis. Blood. 1997 Apr 1;89(7):2516-22.

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Chromosomal Aberrations

In CLL, there are certain chromosomal aberrations detected in 82% of the cases that can give an idea of the prognosis for each patient

Aberration	Frequency	Prognosis
13q14 deletion	55%	-most common aberration -longest survival time of all CLL patients
11q22-23 deletion	18%	-poor prognosis -often associated with extensive, bulky lymphadenopathy -some studies claim that the ataxia-telangiectasia protein (ATM) is mutated in these cases
Trisomy 12	16%	-associated with atypical cell morphology -better prognosis than 11q or 17p
17p13 deletion	7%	-worst CLL prognosis -affects tumor suppressor gene p53 -resistant to chemotherapy

Dohner, Hartmut. Genomic Aberrations and Survival in Chronic Lymphocytic Leukemia. The New England Journal of Medicine. 2000 Dec 28; 343(26): 1910-6

Dierlamm, J. Genetic abnormalities in chronic lymphocytic leukemia and their clinical and prognostic implications. Cancer Genet Cytogenet. 1997 Mar;94(1):27-35.

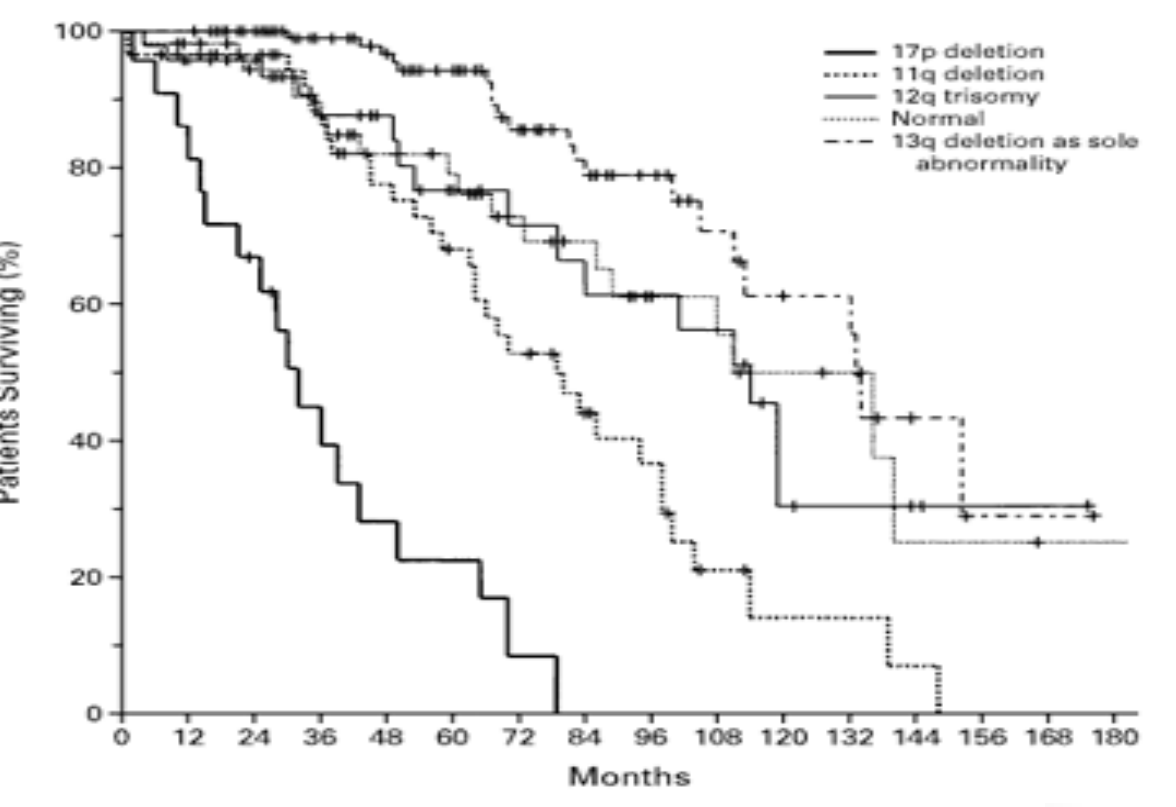
Objective

The objective of this study was to see if the varying symptoms and clinical prognosis of the 11q versus non-11q patients correlates to a differential gene expression pattern in the two groups.

Rational

The 11q abnormality in CLL patients has been shown to predict a worse prognostic outlook then patients with the 13q deletion or trisomy 12. Patients with the 11q deletion also have a great amount of lymphadenopathy. We wanted to look at any genes that could possibly be involved with these patients

Chromosome Abnormalities Influence Over-all Survival in Chronic Lymphocytic Leukemia



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Materials and Methods

- Peripheral blood samples from 13 different CLL patients were obtained after informed consent.
- The mononuclear cells were separated out from the samples by density gradient centrifugation using lymphocyte separation medium.
- The percentage of B-cells was confirmed by flow cytometry.
- A Trizol-phenol-chloroform method was then used to isolate Total RNA from the samples.

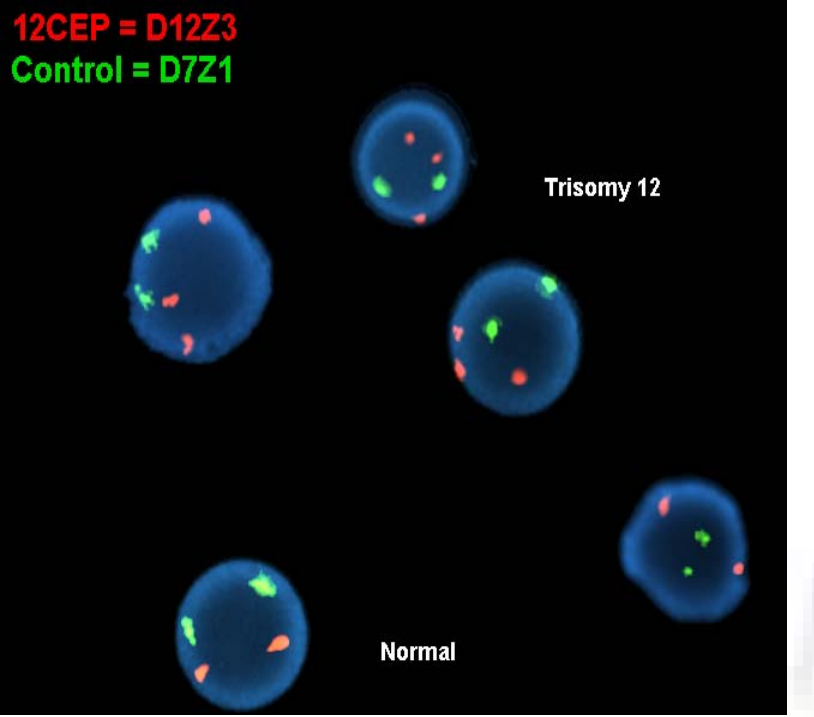


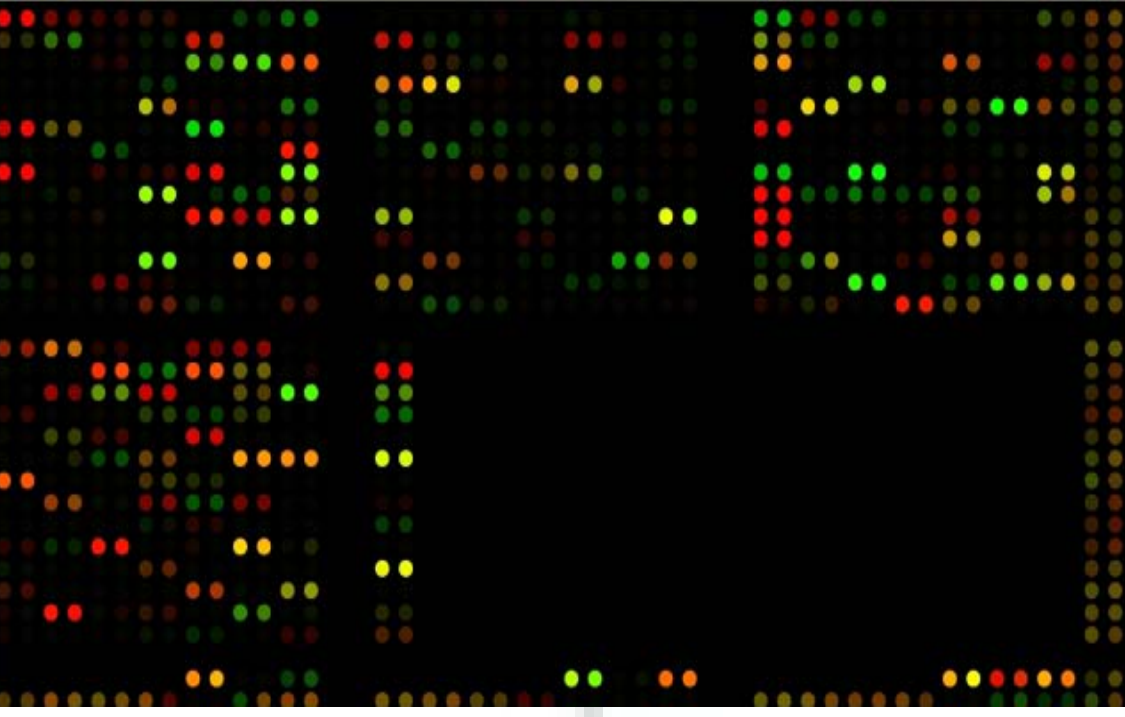
Image courtesy of Dr. Warren Sanger, UNMC Department of Human Genetics.

Fluorescence in situ hybridization (FISH)

- In FISH, the nuclei are first stripped from the cells.
- Then, fluorescent probes are designed to hybridize to the chromosomes along the region of the aberration.
- The results can be seen by the fluorescence given off by the cell nuclei in situ.

Gene Expression Array Hybridization

- Probes were prepared using either CLL patient RNA or Universal Reference RNA (Stratogene).
- The RNA was then reverse transcribed with dATPs labeled with P³².
- The radioactively labeled cDNA was then hybridized to a Clontech nylon membrane with 415 hematology related genes.



Array Scanning

- After exposing the membrane for a week on a phosphorimaging screen, the membranes were then scanned into the computer.
- The images were analyzed using the Pathways Universal Array Analysis software (Invitrogen) to measure the gene expression ratios.

Gene Expression Ratios

Ratios were obtained comparing the intensity of CLL genes over the intensity of the genes from the Universal Stratogene Reference RNA. Expression ratios were normalized by the global intensity of each array.

T-test

The t-test was used to compare the mean of the 11q expression ratios with the means of the non-11q expression ratios on the hematology/immunology arrays. The t-test was also used to compare the mean values of the 11q vs non-11q white counts and lymphocyte counts.

Fisher Exact Test

The Fisher Exact Test was used to determine if abdominal /mediastinal lymphadenopathy was statistically associated with the presence of chromosome abnormalities.

Results

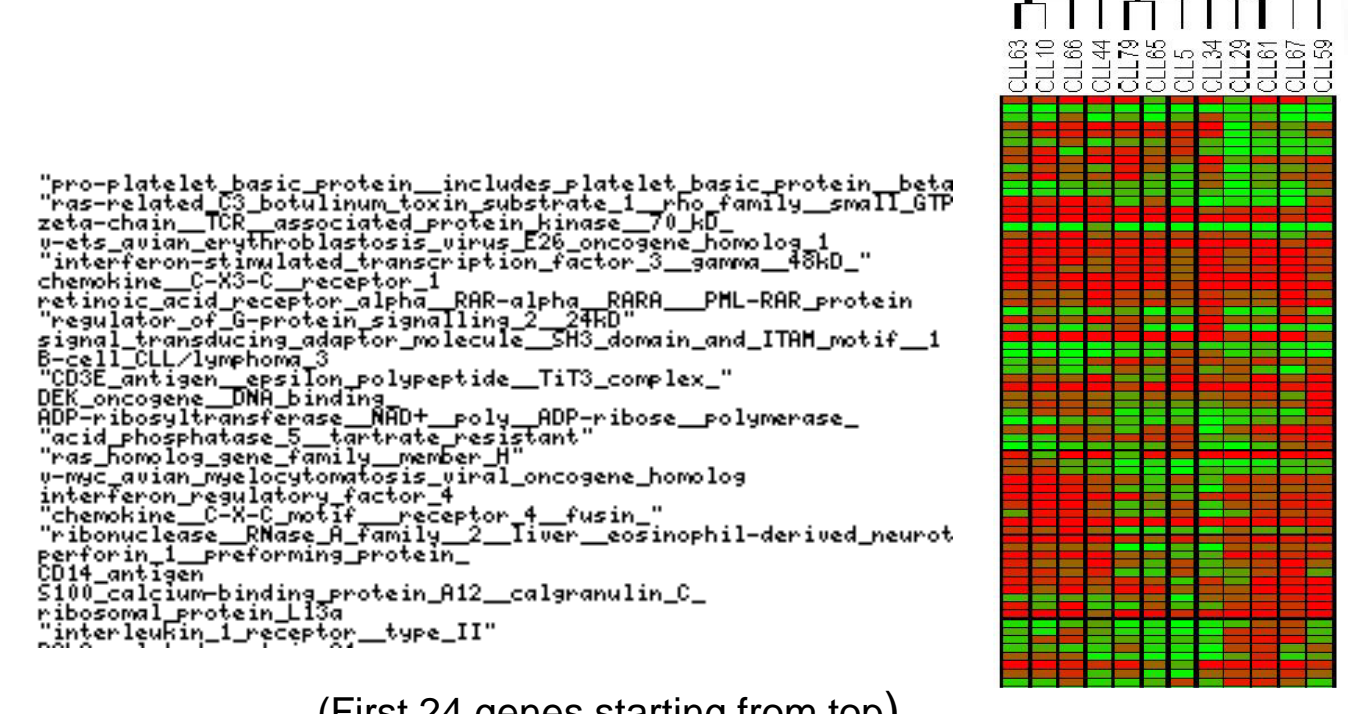
Ten genes were found to be differentially expressed in the 11q vs non-11q samples.

11q mean ratio	Non-11q mean ratio	p-value	gene name
1214.4	46.0	0.00018	small inducible cytokine subfamily A 17
.654	.146	0.022762	acute myeloid leukemia 1 oncogene
.245	.065	0.014554	annexin A2
192.5	292.1	0.016782	nuclear factor of activated T-cells, cytoplasmic 1
.103	.0017	0.017319	protein tyrosine phosphatase, receptor type, F
23.5	2.98	0.023145	lymphocyte-activation gene 3
122.7	77.0	0.0273	tumor necrosis factor (TNF alpha)
14.9	2.76	0.008673	interleukin 13
2.05	1.21	0.033848	RNA binding motif, single stranded interacting protein 1
.787	.300	0.047071	interleukin 1, beta

RED – overexpressed in 11q WHITE – underexpressed in 11q

Cluster Analysis

11qs did not cluster together. This suggests that a different group of genes may contribute more to the differences in 11q vs non-11q



Association of CLL 11q deletion with clinical data

The 11q patients were found to have a statistically greater amount of lymphadenopathy.

	No Lymphadenopathy	Abdominal Lymphadenopathy	Total
Non-11q	5	1	6
11q	1	6	7
Total	6	7	13
Fisher's Exact Test	p=0.029		

The average white count and lymphocyte count are much higher in the non-11q group. However, we cannot say that this is statistically significant since we did not obtain a p-value < .05. More samples are needed for a more accurate result.

	Mean White Count	Mean Absolute Lymphocyte Count
11q deletion	33100.00	27385.71
Non-11q deletion	60816.67	51216.67
p value	0.106842512	0.124536054
Student T-test		

Discussion

-According to this study, ten genes were found to be statistically differentially expressed. These results suggest that there is differential gene expression between the 11q and non-11q groups.
-Also, lymphadenopathy was found to be significantly associated with the 11q deletion. More samples should be tested before any conclusions are drawn.

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