

7.03 PROBLEM SET 7

BASED ON LECTURES 30-36
THIS PROBLEM SET WILL NOT BE GRADED

1) Cancer is a term used to describe a number of diseases characterized by unregulated cell growth. Cancers typically are associated with genetic changes, which can range from point mutations to large-scale chromosome abnormalities. The net effect of such mutations generally is the release of cells from their normal growth constraints.

The results from a sarcoma study involving ten individuals recently were reported. Wild-type and tumor cells were analyzed to determine the genotype of a gene involved in cell cycle regulation. In all cases, wild-type cells were heterozygous, carrying a wild-type allele and a previously uncharacterized allele. In contrast, all tumor cells were homozygous with two copies of the uncharacterized allele.

a) Based on the above information, is it more likely that this gene is an oncogene or tumor suppressor gene? Explain.

Given this information, it seems more likely to be a tumor suppressor gene. The unknown allele may contain a loss of function mutation. If this were the case, then the cancer cells would lack a functional allele resulting in unregulated growth.

The unknown allele identified in this study was sequenced. A mutation was detected in the promoter region of one study participant. In the other nine study participants, sequencing disclosed a mutation in the coding region of this allele.

b) Describe how these mutations could result in a cancer phenotype.

The promoter region mutation may prevent transcription, so the absence of gene product could lead to unregulated cell growth. In contrast, the coding region mutation may abolish the wild-type activity of the encoded polypeptide, preventing it from executing its role in cell cycle regulation.

c) Offer an explanation for the different frequencies observed between the two types of mutations detected in this study.

Nine of the individuals in the study are from the same family.

One allele is more common in the population than the other.

The coding region may contain a hotspot for mutation.

If the coding region were considerably longer than the promoter region, then the probability of a coding region mutation event would be comparatively greater.

The results from a carcinoma study recently were reported. Of the ten individuals examined, nine were homozygous recessive for a gene involved in the detection of altered DNA.

d) Is this gene more likely to be an oncogene or tumor suppressor gene? Explain.

It is more likely a tumor suppressor gene. If this were the case, then the homozygous recessive individuals would be without any functional gene product. The absence of functional gene product could lead to tumor formation.

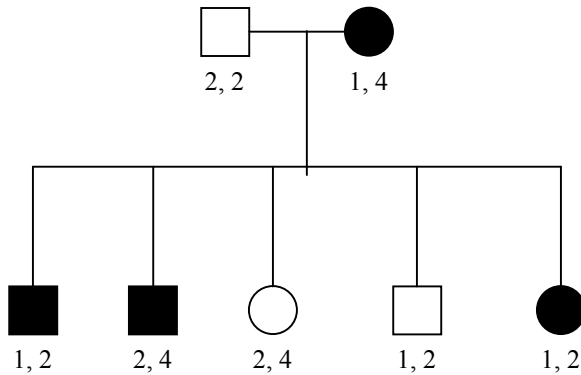
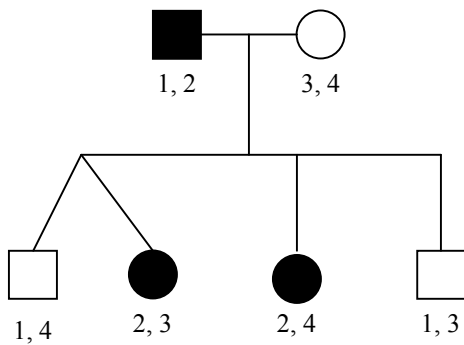
e) Propose a hypothesis to account for how the homozygous recessive condition could lead to carcinoma development.

The absence of gene product prevents the cell from detecting DNA damage due to mutation. This defect leads to the accumulation of deleterious mutations, potentially ranging from point mutations to chromosome abnormalities. These mutations eventually will lead to cell-cycle de-regulation.

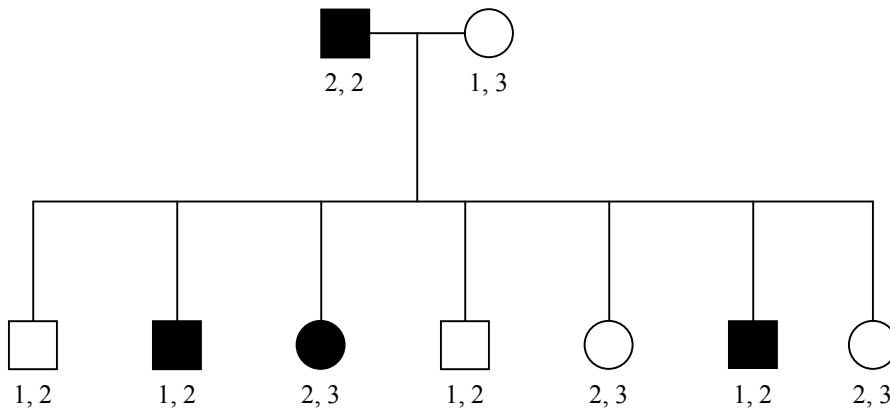
2) You have been interested in a rare genetic disorder for some time. After conducting an exhaustive search, you identified three families with this disorder in the US. Preliminary findings suggest that the gene causing this disorder may be linked to a specific SSR. This SSR locus has four alleles, each with a different number of repeats. To begin your analysis, you constructed a pedigree for each of the three families. The pedigrees are listed below (affected individuals are darkened and the SSR genotype is listed below each individual).

FAMILY #1

FAMILY #2



FAMILY #3



a) What is the most likely mode of inheritance for this disorder?

Based on its appearance in both generations, it would seem to be autosomal dominant.

We will use information in the pedigree to calculate a LOD score for each family.

b) Which of these families can be used to calculate a LOD score to test for linkage between the SSR marker and the gene of interest? Explain.

Families one and two can be used. The relevant parents in each of these families are heterozygous for the SSR and exhibit the disorder.

c) Calculate the LOD score at theta (θ) values of (.05, .1, .2, .3, and .4). Which θ value shows the highest odds of linkage for each family?

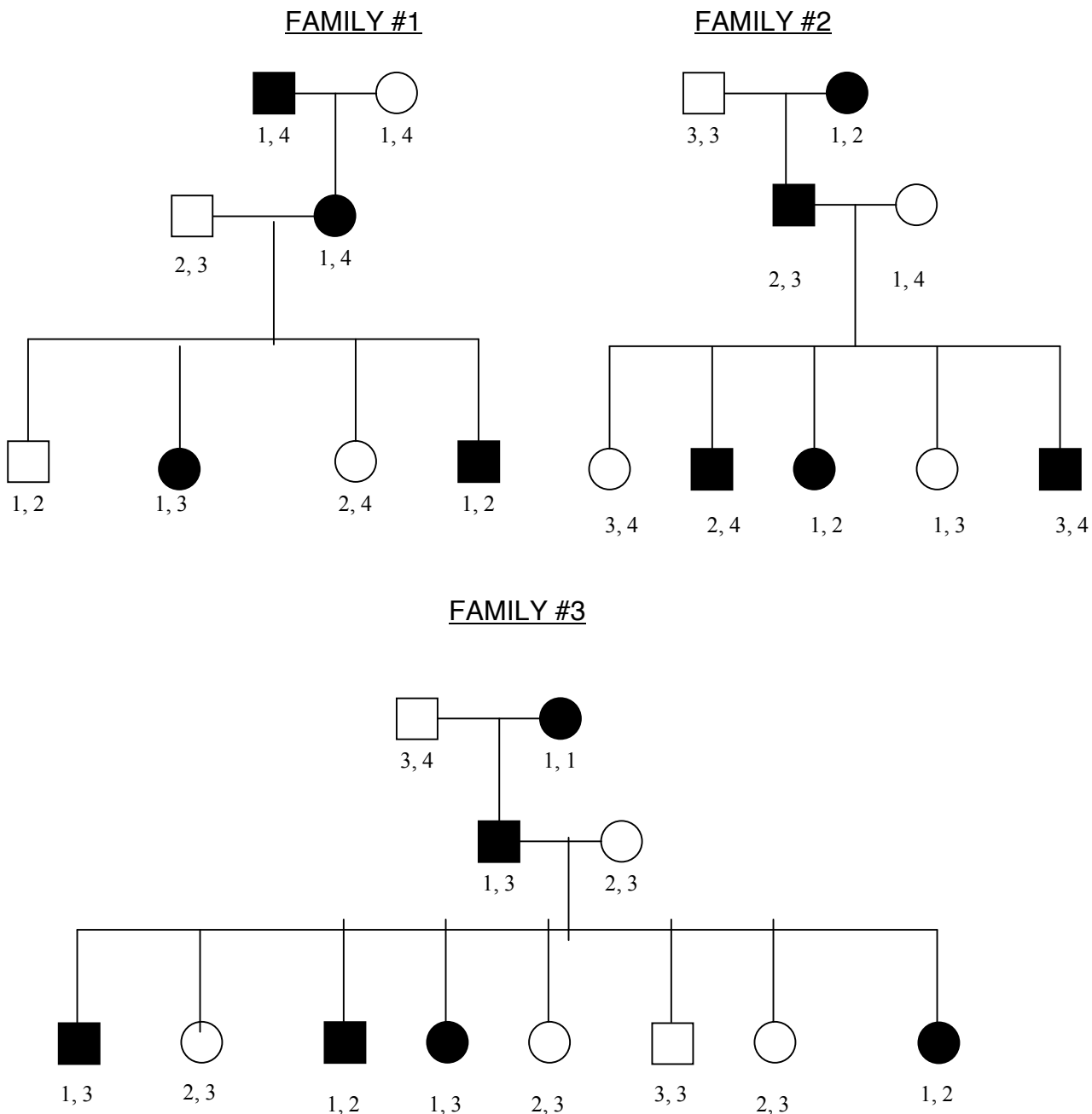
Calculating the LOD scores for the US families requires accounting for both possible phases in the relevant parent, as the phase is unknown.

US FAMILIES

<u>THETA</u>	<u>FAMILY1</u>	<u>FAMILY2</u>
0.05	0.8139	-1.4424
0.1	0.7201	-0.8873
0.2	0.5171	-0.3876
0.3	0.2978	-0.1514
0.4	0.0939	-0.0354

Among the theta values tested, a theta value of 0.05 showed the highest odds of linkage for family #1. Keep in mind, though, that this low LOD score does not suggest linkage. For family #2, a theta value of 0.4 showed the highest odds of linkage. Again, this low LOD score does not suggest linkage between the disorder-causing gene and the SSR.

Three families with this disorder also were identified in Argentina. In contrast to the US families, genetic information is available for three generations in these families. The pedigree for each family is listed below.



d) Which of these families can be used to calculate a LOD score? Which is the relevant parent in each pedigree?

All three families can be used to calculate LOD scores. In family 1, the mother in the second generation is the relevant parent. As her phase is unknown, both possible phases must be accounted for in the LOD score calculation. For family 2, the mother in the first generation is relevant to establish the phase of the father in the second generation, who is the relevant parent. For family 3, the mother in

the first generation is relevant to establish the phase of the father in the second generation, who is the relevant parent.

e) Calculate the LOD score at theta (θ) values of (.05, .1, .2, .3, and .4). Which θ value shows the highest odds of linkage for each family?

The phase of the relevant parent in family #1 is unknown and must be accounted for in the LOD score calculation.

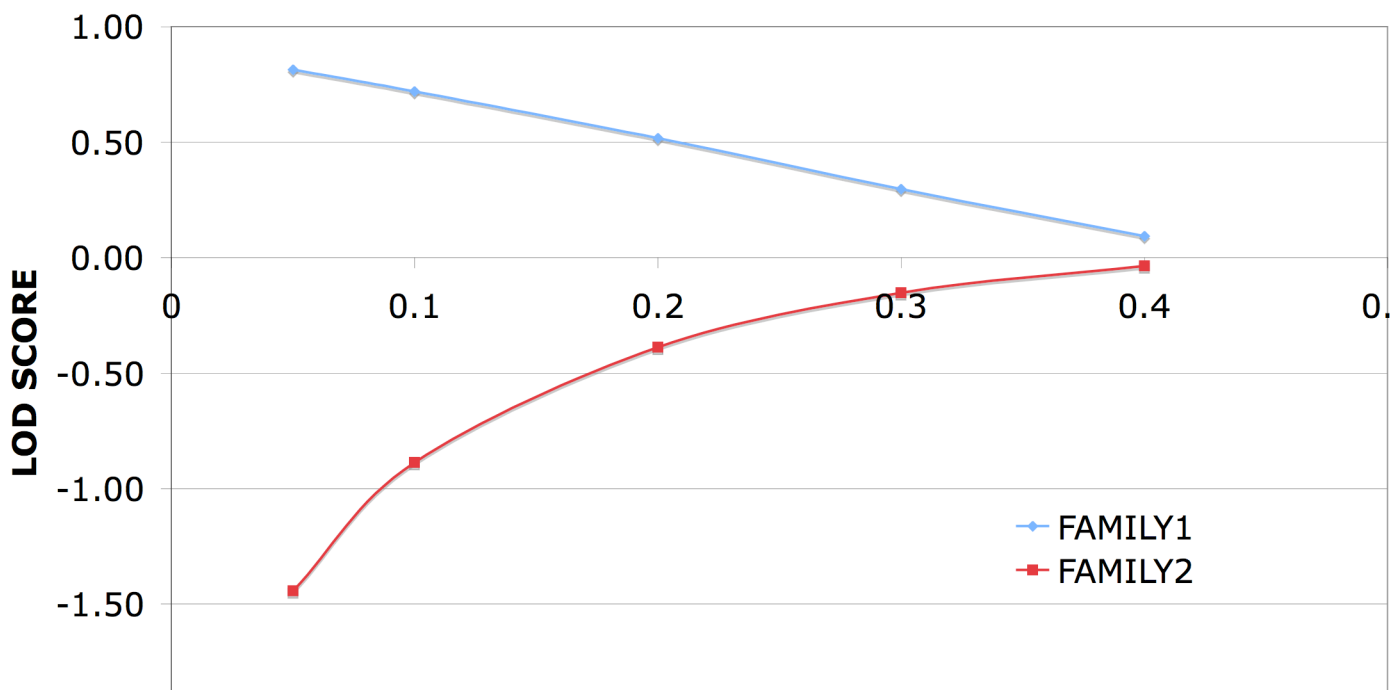
<u>LOD Score</u>			
<u>ARGENTINA</u>			
<u>THETA</u>	<u>FAMILY1</u>	<u>FAMILY2</u>	<u>FAMILY3</u>
0.05	-0.4635	0.115	2.23
0.1	-0.2288	0.3221	2.042
0.2	-0.0602	0.4185	1.6329
0.3	-0.0112	0.3627	1.169
0.4	-0.0007	0.2198	0.6334

For family 1, a recombination fraction of 0.4 showed the highest odds yet does not suggest linkage. A recombination fraction of 0.2 yielded the highest odds for family 2 and does not suggest linkage. In contrast, family 3 showed the highest odds of linkage at a recombination fraction of .05 without suggesting linkage. Based on this analysis, linkage between the two loci is unlikely for all three families. Due to the independence of the pedigrees, though, the LOD scores at each theta value can be pooled across families. The combined LOD scores still fall below the conventional standard (LOD score of ≥ 3) necessary to suggest linkage.

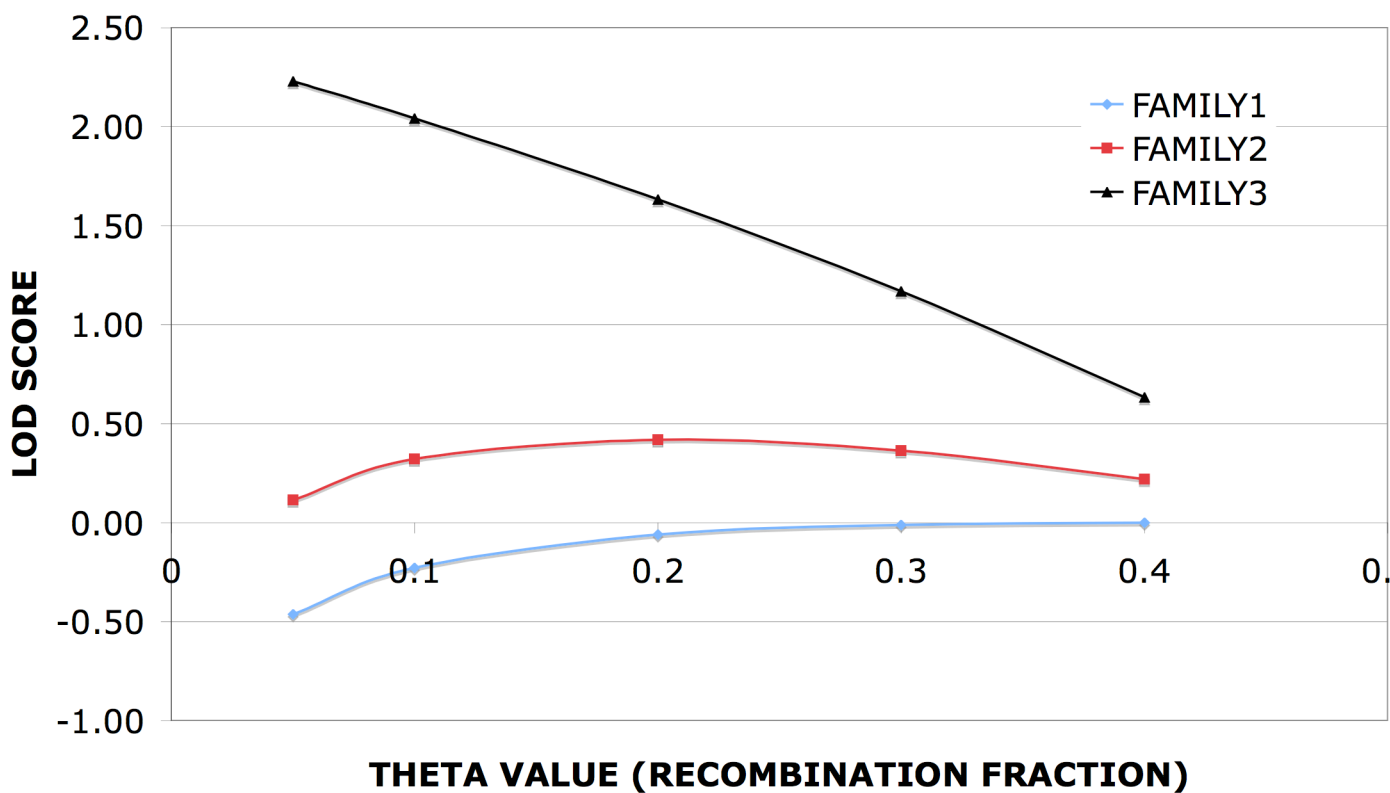
f) Plot the LOD score for each theta value for the US and Argentine families. Is the curve similar for the various families?

Except for the Argentine families #1 and #2, the curves differ substantially. The curves illustrate the low odds of linkage between the two loci under investigation.

ANALYSIS FOR THE US FAMILIES



ANALYSIS FOR THE ARGENTINE FAMILIES



g) Do the LOD scores for the US families suggest linkage? Do the LOD scores for the Argentine families suggest linkage? Explain.

For the US families, the LOD scores associated with each theta value do not suggest linkage. In addition, combining the LOD scores for families 1 and 2 at each theta value fails to suggest linkage.

For the Argentine families, none of the LOD scores for the theta values tested suggest linkage. Pooling the LOD scores likewise fails to suggest linkage at any of the tested theta values.

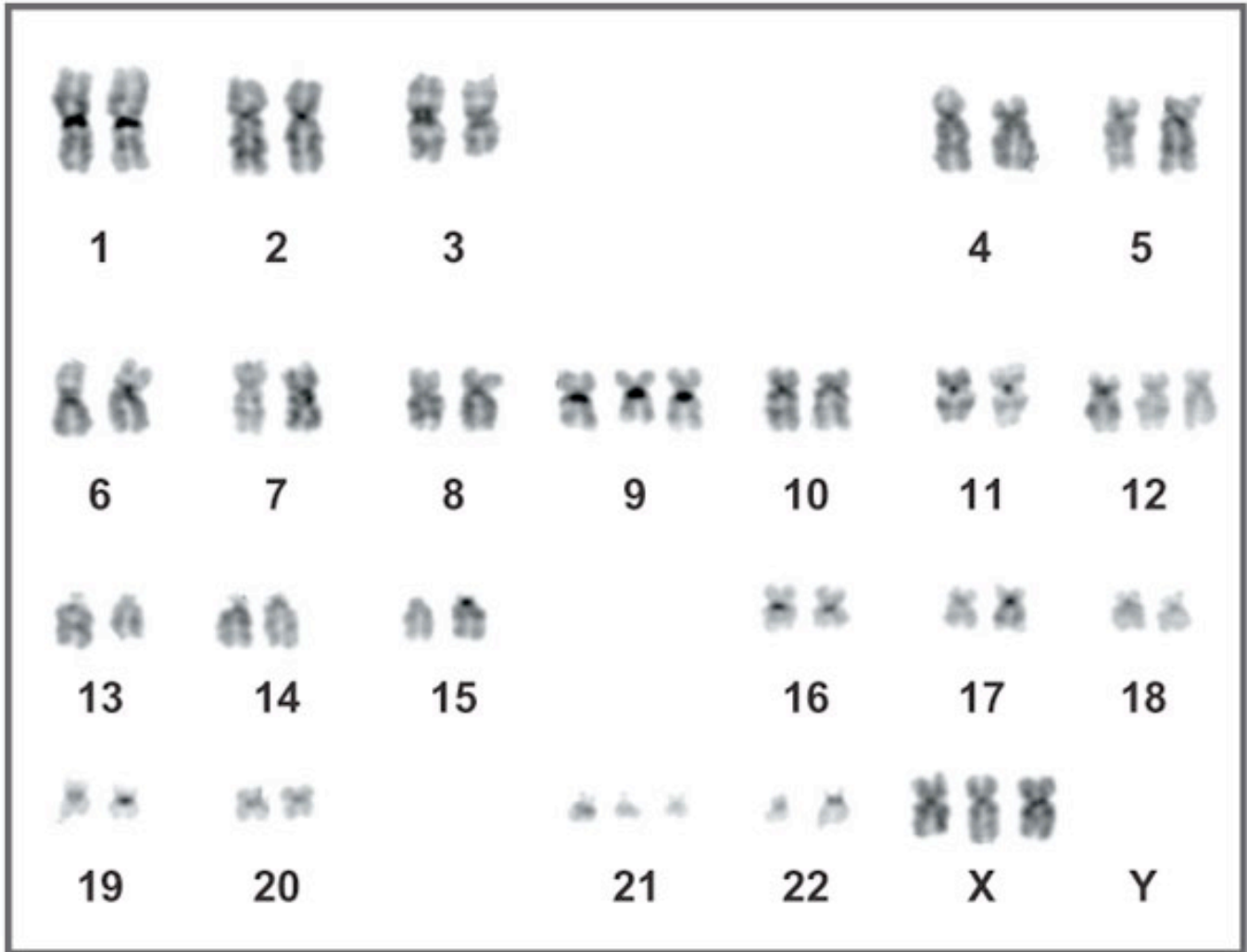
h) Can the results from the Argentine and US families be combined? If so, what is your final conclusion regarding linkage between the gene of interest and SSR marker?

Yes, the LOD scores can be combined. All five of the pedigrees are assumed to be independent since no one individual can be placed in more than one pedigree.

Combining the LOD scores between the US and Argentine families does not suggest linkage between the disorder-causing gene and the SSR.

3) Chromosome abnormalities are associated with a number of disorders including some cancers. Duplications, for example, have been detected in some carcinomas and inversions in some lymphomas. It is believed that the analysis of such abnormalities could provide important clues to key oncogenic events and perhaps suggest potential avenues for cancer treatment.

The karyotype for a specific type of carcinoma is displayed below.



a) Identify the chromosome abnormalities evident in this karyotype.

Chromosomes 9, 12, and 21 are trisomic as is the X-chromosome.

There appears to be either a duplication or deletion in chromosome 22 as well.

Sixteen individuals with a specific carcinoma were screened for chromosomal aberrations. In eleven of these individuals, the short arm of chromosome eight is longer in wild-type cells compared to cancer cells.

b) Which type of abnormality exists in the cancer cells? Explain.

The data suggest that the cancer cells are homozygous for a deletion in the short of chromosome eight.

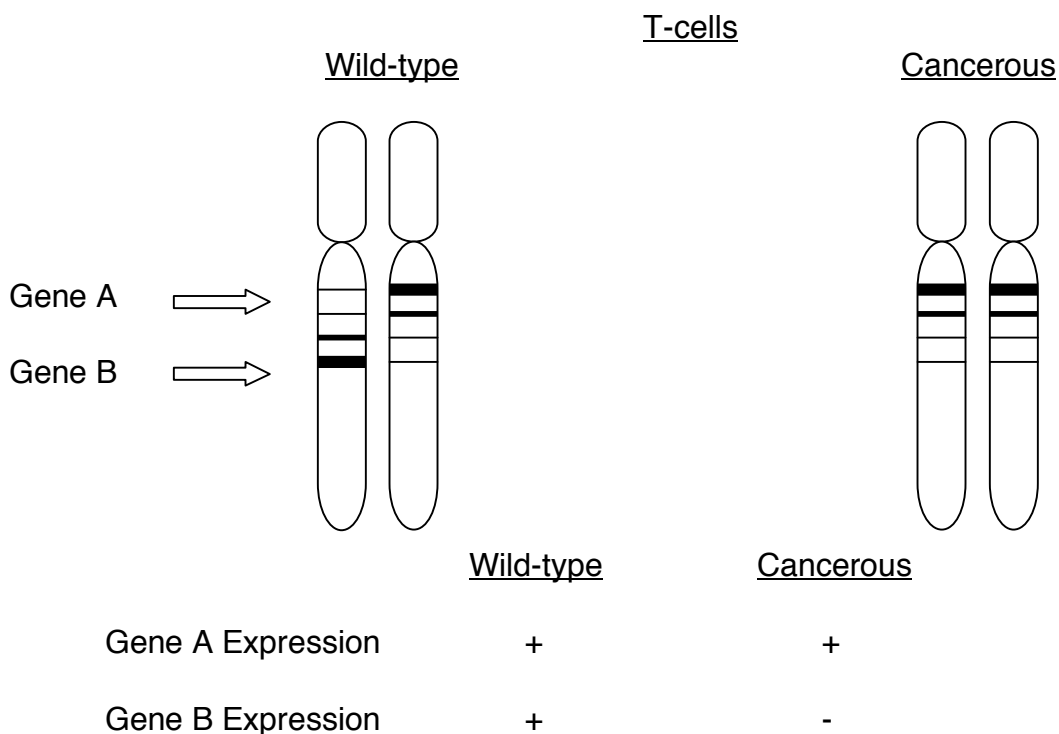
c) Describe how the chromosome abnormality listed in **part “b”** could lead to carcinoma development.

The deletion event may have eliminated a key tumor suppressor gene. As the cancer cells are homozygous for the deletion, there would be no functional gene product to participate in cell cycle regulation.

d) In each of the eleven individuals described above, a subset of cancer cells was aneuploid. Why wasn't aneuploidy observed in all cancer cells?

Tumors consist of clonal populations of cells that are continually mutating. Thus, not all cells of the tumor share the same genotype. In this case, some of the cancer cells have one or more mutations that result in aneuploidy.

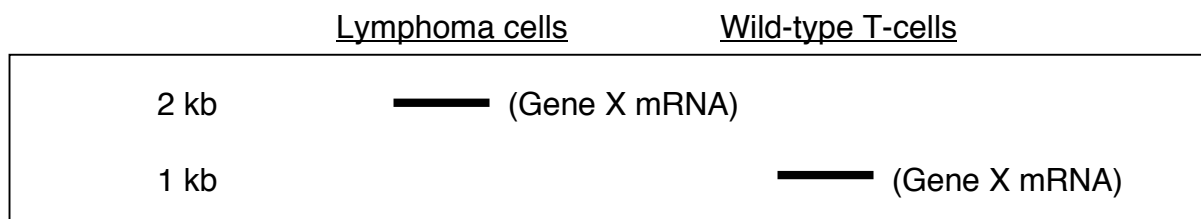
One goal of an on-going lymphoma study is to determine if a correlation exists between a chromosome #2 aberration and lymphoma development. Some early results from this study are listed below.



e) Is there a chromosome abnormality? Describe a mechanism to account for the development of the cancer phenotype.

The results show that an inversion has occurred. The cancer cells are homozygous for this inversion in chromosome two. In the cancer cells, the inversion is associated with the absence of Gene B expression. One hypothesis is that Gene B is a tumor suppressor gene, and the inversion silenced its expression. Without any Gene B product, the cell cycle no longer is regulated in a wild-type manner.

Wild-type T-cells express Gene X at high levels. A type of T-cell lymphoma is heterozygous for a specific translocation that is absent in wild-type cells. Despite the translocation, lymphoma cells continue to show high levels of Gene X expression. The results from Northern blot analysis of lymphoma and wild-type T-cells are shown below).



f) Propose a hypothesis to account for the results shown above.

The elongated transcript in lymphoma cells indicates that the translocation caused a gene fusion between Gene X and another gene.

The Gene X transcripts from lymphoma or wild-type T-cells were translated using an *in vitro* system. The resulting polypeptides were assayed for tyrosine kinase activity (see results below).

	<u>Transcripts from lymphoma cells</u>	<u>Wild-type transcripts</u>
<u>Activity Level</u>	+++	-

g) Based on the above results, propose a mechanism to account for development of the cancer phenotype.

The translocation event resulted in a gene fusion between the highly expressed Gene X and a tyrosine kinase gene. The high expression of the gene fusion resulted in constitutive, or at least more frequent, tyrosine kinase activity. As tyrosine kinases play key roles in cell cycle regulation, the cancer phenotype may be due to the over-stimulation of cell division.