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HST.161 Molecular Biology and Genetics in Modern Medicine
Fall 2007

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Problem Set # 3 Hst 160 Fall 2007
Due November 27,2007

1. Shania and Elton Sinatra had four children, all boys. The four children were born spread over a fifteen year period. Shania was 23 when the first child was born, Elton was 36. The youngest, George, was noted to have trouble focusing his right eye at the age of eight months. A tumor was discovered in this eye which covered half the retina. George's right eye was removed at this time. Several months later a tumor was discovered in his left eye. Despite efforts to halt the growth of this tumor by radiation and drug treatment, this tumor grew to the point where George could no longer see. The tumor was then surgically removed. George has successfully survived to adulthood and now comes to you for counseling at the age of 35. George's three brothers did not have retinoblastoma as children and have not had any form of cancer as an adult. Neither Shania nor Elton or any members of their immediate families had retinoblastoma. Elton died at the age of 67 of a sudden heart attack.

- a. George is now 37 years old and wishes to start a family. Explain to George the risk to his children of getting retinoblastoma. (2 points)
- b. George and his wife, Dianna, wish to obtain as much information as possible prior to the birth of their first child on that child's risk of retinoblastoma. You suggest to George that DNA testing be carried out to identify the mutation responsible for his retinoblastoma. You suggest the performance of two types of DNA testing:

1. Southern blotting
2. PCR for each of the exons of the retinoblastoma (RB1) gene, followed by DNA sequencing.

Explain to George what types of mutations would be revealed by each type of testing and how you would recognize a relevant mutation using each technique.

Despite careful analysis of test results, no mutation could be identified in George's copies of the RB1 gene. Explain to George why the testing has failed to reveal the mutation responsible for his retinoblastoma, yet you remain confident that a mutation is present in one of his copies of his RB1 gene. Propose at least one type of mutation which could have escaped detection in the screen for mutation you have carried out and yet be responsible for George's retinoblastomas.

- c. George has read that in cases like his where the mutation in the RB1 gene which is responsible for his retinoblastoma can not be identified, it is possible to determine through testing based on genetic linkage whether he has transmitted the copy of the RB1 gene from his father, Elton, or his mother Shania, to his child. He has also read that determining this information would have been relevant to his child's risk of receiving a defective copy of the RB1 gene. Give an explanation to George as to why knowing whether he has transmitted Elton's or Shania's copy of the RB1 gene will affect the risk of retinoblastoma to his child.

- d. DNA samples are obtained from George, George's mother, Shania, his wife, Dianna and his brothers John, Paul and Richard. A DNA sample from Elton is unavailable. Four single nucleotide polymorphisms within the retinoblastoma gene are tested. The following results are obtained.

Individual tested	Snp 842	Snp657	Snp351	Snp899
George	2,2	1,1	2,2	1,1
Shania	2,2	1,1	1,2	1,1
Dianna	1,1	1,2	2,2	1,2
Richard	2,2	1,2	1,2	1,1
John	2,2	1,2	1,2	1,1
Paul	2,2	1,2	1,2	1,1

Based on this data what is Elton's genotype for SNP657. (1 point)

Do these data make it possible to determine which copy of the RB1 gene, the one received from Shania or the one received from Elton, has been transmitted to a child of George? Explain.

- e. Dianna becomes pregnant. During the last trimester of Dianna's pregnancy, a new VNTR DNA marker from the retinoblastoma gene becomes available and it is tested on the DNA samples from George's family. The following results are obtained.

Individual	VNTR 505
George	5,8
Shania	5,7
Dianna	4,5
Richard	5,7
John	5,7
Paul	5,7

Give an explanation to George of how these new data make it possible to give an assessment of the risk of the fetus for retinoblastoma which is different from the risk assessment possible before the VNTR 505 data was obtained.

- f. Two months later George and Dianna's daughter Alanis is born. The genotypic data obtained from Alanis's DNA is as follows:

	Snp 842	Snp657	Snp351	Snp899	VNTR 505
Alanis	1,2	1,1	2,2	1,2	5,5

What is your assessment of Alanis's risk based on these data.

- g. Alanis is examined for retinoblastoma tumors every three months for the next five years, but no tumors are found. Dianna and George decide to have another child. They in fact have fraternal twins, Jewel and Sid. When Sid's eyes are examined at birth he has no retinoblastoma tumors, but Jewel is found to have tumors in both his eyes. The genotypes of the twins are shown below. Explain to George and Dianna the risk for retinoblastoma to Sid based on these data.

	Snp 842	Snp657	Snp351	Snp899	VNTR 505
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Sid	1,2	1,2	2,2	1,1	4,5
Jewel	1,2	1,2	2,2	1,1	4,8

h. Jewel's tumors are removed and DNA is isolated for later genotyping. What genotype for these markers would you expect in a tumor from Jewel if the second hit in the tumor analyzed was a point mutation in exon 4 of the RB1 gene causing a CAG codon to be changed to a UAG codon?

i. What genotype for these markers would you expect to observe in a tumor from Jewel in which non-disjunction of chromosome 13 was the event responsible for the second hit in his tumor?

2.

The following questions relate to the paper by Cavenee et. al. Nature 305: 779 published in 1983 which is the first description of the somatic genetic events in retinoblastoma. This paper is available for download in pdf form on our class website.

a. All of the "tumor DNA" used in the studies in the Cavenee et. al. paper was derived from either tumor cell lines in culture or tumor cells passaged by growth in mice. It is often difficult to remove all the normal tissue from a tumor as it is surgically removed? What do you think the Southern blots in this paper would have looked like if the DNA for the studies had been obtained directly from tumors removed from the eyes of affected children?

b. In Figure 4 of Cavenee, the data for which marker is critical in concluding that the event which led to homozygous inactivation of the RB1 gene in this tumor was a mitotic crossover rather than a non-disjunction event?

c. What outcome would have been expected for this marker, if the event in this tumor had been a non-disjunction event not a mitotic crossover.