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

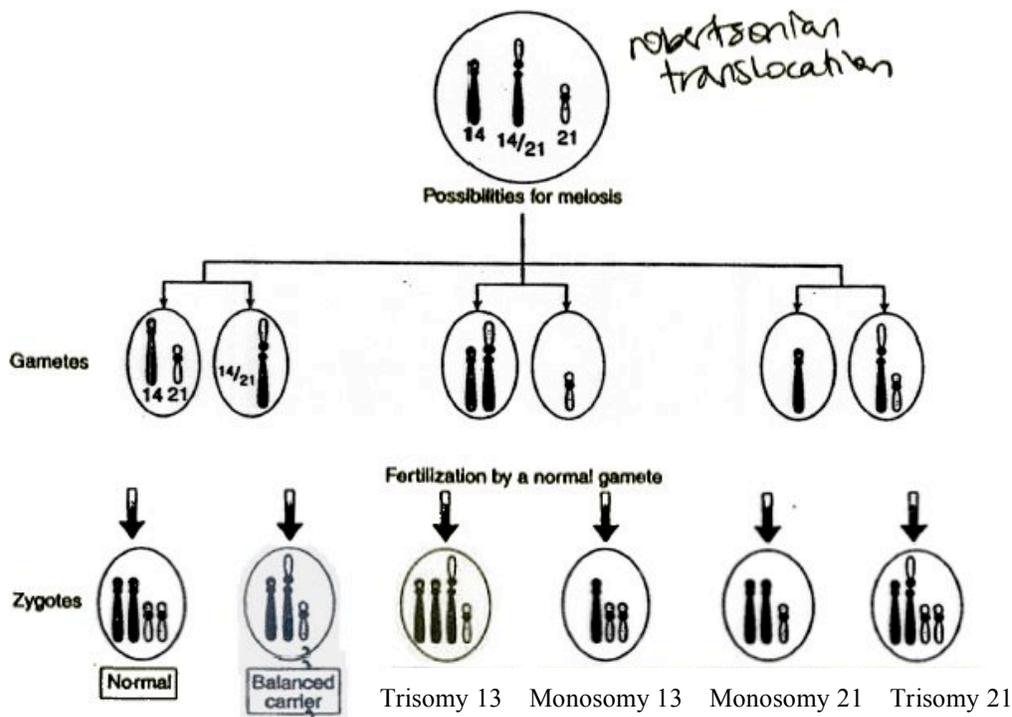
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HST 161 – Fall 2004
Molecular Biology & Genetics in Modern Medicine
September 30, 2004
Quiz #1

This quiz should take you less than an hour to complete, but you may use the full hour if necessary. Please print your name on each page. Please write concise, to-the-point answers rather than long explanations. Good luck! ☺

1. (32 points) *A couple trying to begin a family has three miscarriages. They then have a child born with clinical features characteristic of trisomy 13. The child lives only a short time and karyotype studies are not performed. The couple is then karyotyped and the father is shown to have a 13:21 Robertsonian translocation.*

a. *Draw the possible [karyotypes for chromosomes 13 and 21 of the] gametes that the father could produce following meiosis (12 points). Indicate below each gamete whether you expect a normal, balanced carrier, monosomy, or trisomy if that gamete were combined with a normal gamete (12 points). Indicate the two major risks the couple has if a pregnancy is carried to term (8 points).*



2 points for each of the six gametes. 2 points each for identifying normal, balanced carrier, trisomy 13, monosomy 13, monosomy 21, and trisomy 21.

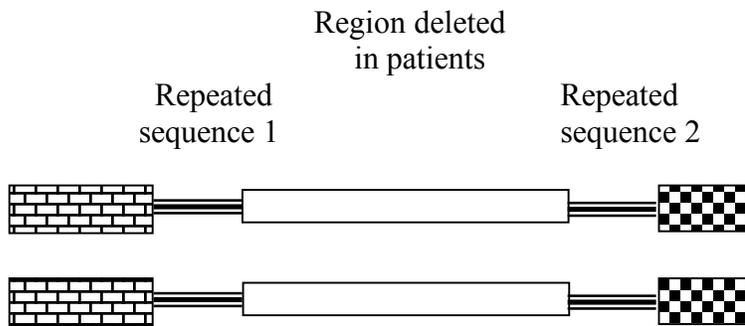
The major risks for a viable, term pregnancy are trisomy 13/Patau's syndrome (one-quarter of term pregnancies) and trisomy 21/Down's syndrome (one-quarter of term pregnancies) (4 points each). Partial credit (2 points) for mentioning risk of balanced carrier. The fetuses with monosomy 13 and monosomy 21 will not be viable.

2. (20 points) Prader-Willi syndrome (PWS) and Angelman syndrome can both be caused by a deletion of DNA sequences on chromosome 15. Ten cases of each disorder, which arose independently, were shown to be due to deletion and the deletion endpoints were determined. Note that a DNA sequence was found to be duplicated on chromosome 15 in normal individuals. The two copies of the duplicated DNA sequence on chromosome 15 normally flank the region which was deleted in each disorder.

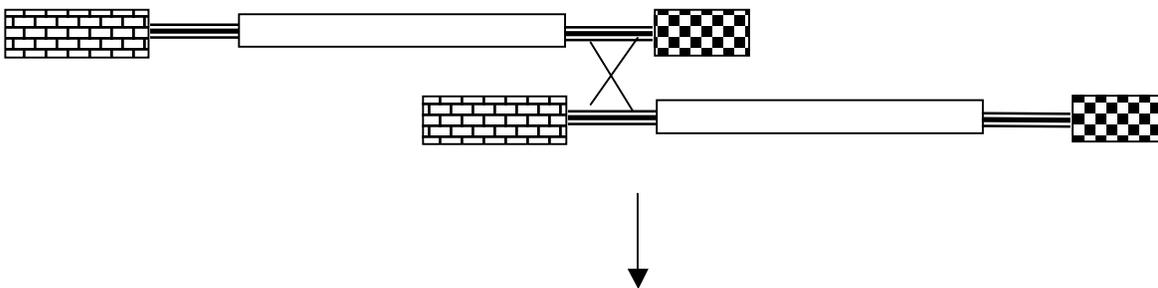
a. Explain using a diagram how the presence of the duplicated sequence has led to the deletions in these cases. (10 points)

There are 2 acceptable mechanisms: unequal crossing over (shown below) and also recombination of the chromosome with itself (with looping out of deleted DNA sequence).

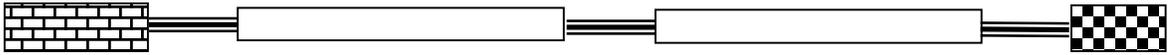
The original sequence would look like this (3 points for drawing repeat sequences around the DNA sequence that is deleted):



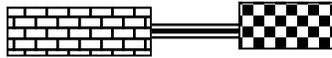
Unequal crossing over during DNA replication would cause the following (4 points for drawing mechanism, either unequal crossing over or recombination with itself):



Resulting in duplication of the region between the repeat elements



OR



Deletion of the region between the repeat elements: this would lead to either Prader-Willi or Angelman syndrome (3 points for showing products of unequal recombination OR products of recombination with itself with deleted sequence in a loop and deletion of sequence between repeat elements)

b. Explain why some children get PWS and others get Angelmann syndrome when all of them carry the same deletion. (10 points)

The Prader-Willi/Angelman region is imprinted, causing differential expression of genes from the chromosome of maternal origin and the chromosome of paternal origin (4 points). If there is no paternal copy of 15q11.2-q12 (because the deletion happened during generation of the sperm), the child will have only the maternal copy for that region and be likely to get Prader-Willi syndrome (3 points). In contrast, if there is no maternal copy of 15q11.2-q12 (because the deletion happened during generation of the egg), the child will have only the paternal copy for that region and be likely to get Angelman syndrome (3 points).

3. (18 points) The factor VIII protein is approximately 300 kilodaltons and has a repetitive structure. Give a molecular explanation for the following findings:

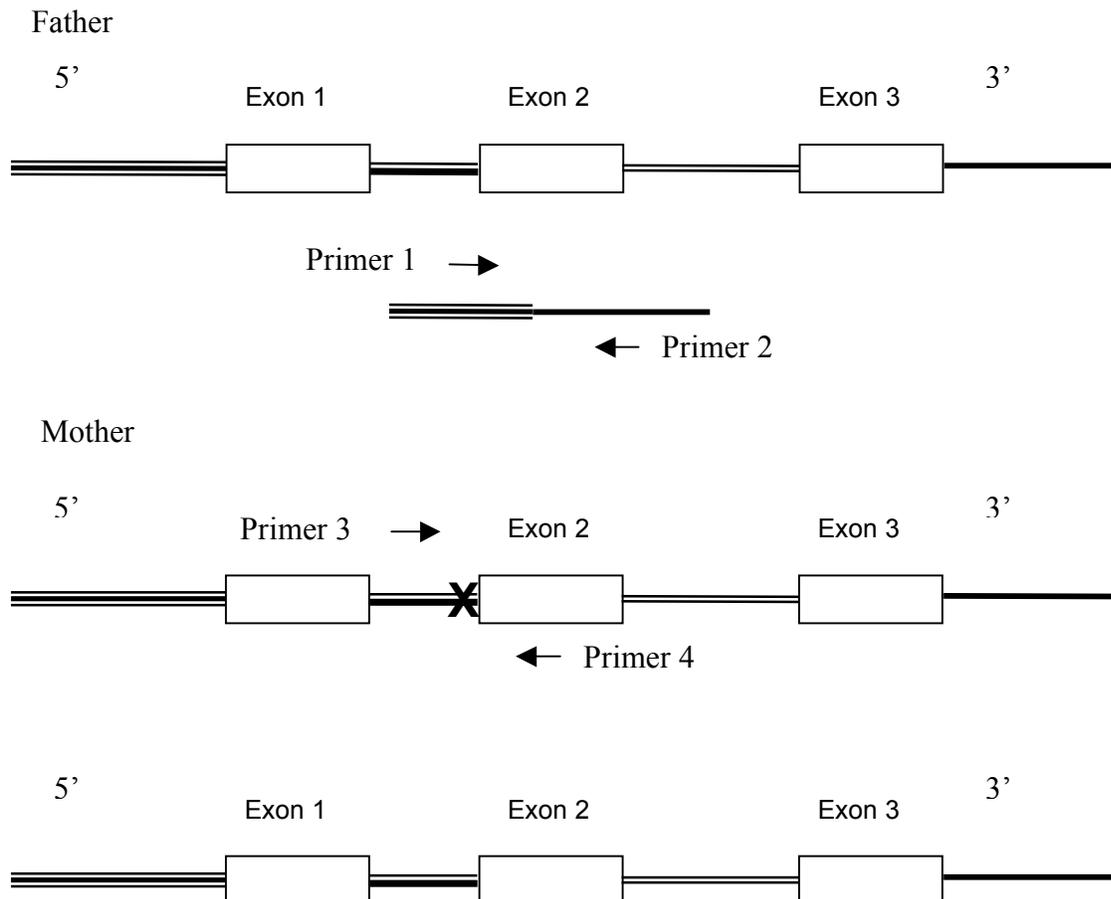
a. Deletions which remove some of the exons of the factor VIII gene can cause hemophilia, but the size of the deletion does not necessarily correlate with the severity of the hemophilia. Some larger deletions give a mild hemophilia, with quite significant circulating factor VIII levels, while other smaller deletions give a severe hemophilia with no detectable circulating factor VIII detectable. It has been shown that factor VIII molecules with a number of the repeats removed still retain factor VIII function.

Only a fraction of the repeats are necessary for factor VIII protein function, as the protein can still function without the repeats. This means that deletions that merely remove a set of repeats will give rise to mild hemophilia, even when large. Note that these deletions delete multiple exons. In one set of the factor VIII gene deletions leading to mild hemophilia, RNA splicing maintains the open reading frame of the protein; if the exon before the deletion ends at the 3rd base of a codon, the exon following the deletion begins at the 1st base of a codon, and so forth. In contrast, in the set of deletions leading to the more severe forms of hemophilia, RNA splicing does not maintain the open reading frame, causing a “frameshift” mutation. For example, the exon prior to the deletion might end at the 3rd base of a

codon while the exon following the deletion might start with the 2nd base of a codon (10 points for partial explanation, 15 points for discussing importance of maintaining reading frame, 18 points-full credit- for role of splicing in maintaining or disrupting reading frame).

4. (30 points) A couple from Italy are each carriers of beta-thalassemia. The husband's family is from the Ferrara region and he is a carrier of a deletion of the entire beta-globin gene. The father's deletion starts at the AUG cocon and ends in exon 3 of the beta globin gene. The wife's family is from the area of Naples and she is a carrier of a point mutation in intron 1 of the beta-globin gene which causes an abnormal splicing pattern. The couple wishes to have prenatal diagnosis for their pregnancies. They come to you.

Explain how PCR could be used to devise tests for prenatal diagnosis. First draw the two beta-globin genes of each parent; recall that there are 3 exons and 2 introns in the beta globin gene. (4 points for mother, 4 points for father) Clearly indicate where you would design PCR primers. (5 points for mother, 5 points for father) Explain what the PCR products for an affected child, and any unaffected children, will show for each PCR reaction (12 points).



PCR of an affected child would indicate a small PCR product from the paternal allele upon amplification with primers 1 and 2 (2 points: ALTERNATIVE small and very large products, full credit at 2 points) and a normal-sized allele upon amplification with primers 3 and 4 (2 points).

The mutation in intron 1 would be seen upon DNA sequencing of the product amplified with primers 3 and 4. The mutation would be homozygous because the paternal allele is deleted.

For a carrier of the paternal mutation with a normal maternal beta globin gene, amplification with primers 1 and 2 would give rise to a small product (2 points) while amplification with primers 3 and 4 would give rise to a normal sized allele without mutation (1 point).

For a carrier of the maternal mutation with a normal paternal beta globin gene, amplification with primers 1 and 2 would give rise to no product (2 points: ALTERNATIVE very large product, full credit at 2 points) and amplification with primers 3 and 4 would give rise to a normal sized allele with mutation (1 point).

For a completely normal child (not a carrier of either mutation), PCR amplification with primers 1 and 2 would give rise to no product (2 points: ALTERNATIVE very large product, full credit at 2 points) and amplification with primers 3 and 4 would give rise to a normal sized allele without mutation (1 point).

Multiple other experimental designs are possible- including designing a primer that only annealed to mutated site on maternal allele, so that an intronic band was present only if the point mutation on the maternal allele was inherited. Also full credit for use of RT-PCR from mRNA to cDNA that would detect differences in size of product due to splicing defect in maternal allele. You are not able to detect the father's deletion by using PCR of exon 1, 2, 3. You would still get all three bands from the other allele.