MCB C142 / IB C163 MIDTERM 2
Fall 2008

NAME (Please print): ____

STUDENT ID #:____

<u>*IMPORTANT REMINDERS***</u>**

<u>Print your name and ID# on every page of the exam.</u>
<u>You will lose 0.5 point/page if you forget to do this.</u>

Check that you have 17 pages total, including this cover page. The last five pages are work space only (no problems), that you may remove from the exam to use. <u>Work space pages will not be graded, but must be turned in, whether you use them or not</u>.

<u>Only the front of each page will be graded</u>. If you use the back of a page or a work space page, you must transcribe your answer to the space provided on the front of the page.

This is a closed book, closed note exam. No calculators allowed.

Look through the entire exam before starting. You do <u>not</u> have to start with Question 1.
Read each question entirely before beginning.
Write legibly. Show your calculations.

Written regrade requests must be presented to your GSI within 7 days after exams are returned. To be eligible for a regrade, your answers must be in pen. Answers in pencil, eraseable ink, or corrected with white-out will not be accepted for regrades.

(Do not write below this line)

PROBLEM 1	_____/6 PROBLEM 2_______/6	_____ / 12
PROBLEM 3	a____ b____ total____/7 PROBLEM 4_____/5	_____ / 12
PROBLEM 5	a____ b____ c____ d_____ e____ f____g____	_____ / 42
PROBLEM 6	a____ b____ c____ d_____ e____ f____	_____ / 30
PROBLEM 7	a____ b____	_____ / 24
PROBLEM 8	a____ b____ c_____	_____ / 15
PROBLEM 9	a____ b____ c_____	_____ / 15

TOTAL _____ / 150

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1. A deletion of the complete coding sequence of a particular gene could easily give rise to which of the following? (Circle all that apply) (6 pts)

a) a dominant phenotype due to haploinsufficiency

b) a dominant negative phenotype

c) a recessive loss-of-function phenotype

d) an informational suppressor of nonsense mutations in other genes

e) an interaction suppressor of missense mutations in other genes

f) a dominant enhancer of mutations in other genes

Highlighted answers (a,c, and f) should be circled

1 point per lettered answer (they get the point if appropriately circled or not circled)

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2. Protooncogenes can mutate such that they induce cancerous growth of a cell in a dominant fashion. Which types of mutation listed below could potentially cause a dominant oncogenic mutation? (Circle all that apply.) (6 pts)

a) a complete gene deletion

b) a missense mutation

c) a frameshift mutation

d) a silent mutation

e) a gene amplification

f) a chromosome translocation

Highlighted answers (b,c,e,f) should be circled

1 point per lettered answer (they get the point if appropriately circled or not circled)

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3. Homozygous mutations in the *Xeroderma pigmentosum* (*XP*) gene lead to a high risk of developing skin cancer. The *XP* gene encodes an enzyme involved in the DNA nucleotide excision repair pathway, which can remove and replace cross-linked thymine dimers or other chemical aberrations in DNA bases.

a) Is *XP* a tumor suppressor gene or a protoconcogene? Explain your reasoning. (3 pts)

***XP* is a tumor suppressor gene.**
Tumor suppressor genes are genes that play roles in maintaining genome integrity (by repairing DNA, or replicating or segregating chromosomes), in which loss-of-function mutations make cells more likely to become cancerous.
Full credit answers should include the correct "tumor suppressor" answer and some reasonable explanation – either the loss-of-function or the genome integrity idea, or both.

b) Skin cancer in patients with XP mutations is much more frequently seen among Japanese people living in Hawaii than those living in Japan. How would you explain this observation? (4 pts)

Exposure to ultraviolet (*UV*) radiation is higher in Hawaii than in Japan. *UV* is a mutagen that damages DNA (including creating thymine dimers). UV exposure would be expected to have a synergistic effect (or to "enhance") the defect associated with *XP* mutations. The combined effects of mutagen exposure and a defect in DNA repair would make the skin cells more likely to undergo additional mutations that would lead to cancer progression.

for full credit, the answer should include the idea that sunlight/UV radiation damages DNA (or is a "mutagen") and the idea that multiple mutations are necessary to cause cancer.

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4. Estrogen stimulates the growth of many human tissues, including breast tissue. Tamoxifen is one of the most successful drugs used to treat breast cancer. This compound is an estrogen antagonist – that is, a chemical that binds to the estrogen receptor (ER) on the surface of cells and *inhibits* its activity.

Breast cancer cells from some patients have abnormally high expression of Estrogen Receptors on their surfaces, while other breast cancers actually show low or undetectable ER expression. Which of these two classes of breast cancers (high ER and low ER expression) would you expect to be better candidates for tamoxifen therapy, and why? (5 points)

Tamoxifen is a more effective treatment for breast cancer cells expressing <u>high</u> levels of ER. ER upregulation is likely to contribute to the hyperproliferation of such cells, so downregulating the activity of the ER would be likely to suppress this effect. On the other hand, low ER breast cancer cells would *not* be likely to respond to tamoxifen – they are likely to have gain-of-function mutations downstream of the estrogen receptor, so even if tamoxifen downregulates ER activity, the cells will continue to proliferate.

For full credit, the answer (high ER cells) should be correct, and there should be some statement that low ER cells would probably not be affected by downregulation of ER activity.

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5. (42 points total) All eukaryotic cells have a "Spindle Assembly Checkpoint" that prevents cells from entering anaphase until all the chromosomes have attached to the mitotic spindle. In the late 1990s, researchers identified mutations that disrupted this checkpoint in budding yeast (*S. cerevisiae*). They mutagenized haploid yeast cells and plated them on normal rich media plates. They replica plated these colonies onto plates containing benomyl, a drug that interferes with the mitotic spindle. Most of the colonies did not grow on the drug-containing plates, and the cells arrested at metaphase, because the benomyl prevented some of the chromosomes from attaching to the spindle. However, some mutant cells did continue to divide on the benomyl plates, although they usually divided for only a few generations before the cells died. The lab that found such mutations called them "Mad" mutants, for "Mitotic arrest deficient."

a). Why do you think the Mad mutant cells were able to survive and grow for many generations on the plates lacking benomyl but not on the benomyl-containing plates? (6 points)

The Mad mutants are defective in a cell cycle checkpoint. Such checkpoints are usually important for the long-term fidelity of cell division, but are dispensable for normal cell cycle progression. On the benomyl-containing plates, the mitotic spindle is partially disrupted, so cells lacking the Spindle Assembly Checkpoint continue to divide but they eventually die to due aneuploidy (from chromosome loss or nondisjunction).

b). You repeat this screen to find new Mad mutants. You find 7 colonies of yeast cells that are able to continue dividing on the benomyl plates, and you pick the yeast from the corresponding colonies on the drug-free plates to analyze.

You first cross each of your 7 Mad mutants to a wild-type yeast strain to generate mutant cells of both mating types. In the course of these crosses, you realize that one of your mutant strains, Mutant #7, probably has a gain-of-function mutation. What observation might lead you to this conclusion? (5 points)

When you cross Mutant #7 to wild-type cells, you generate heterozygous diploid cells (*m*/+). If you observed that these cells continued to divide in the presence of benomyl, then the mutation is dominant and therefore likely to be gain-of-function.

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5. (continued)

c). Describe how you would carry out complementation tests to determine the total number of genes affected by the other 6 Mad mutations you have found. Specifically, how would you generate cells carrying combinations of the 6 different mutations, how would you test these cells for complementation? Also, why would you be unable to carry out complementation tests with Mutant #7? (5 points)

You would mate haploid yeast cells carrying each of the 6 mutations with haploid cells of the opposite mating type carrying the other 5 mutations. You would test the resulting diploid cells by putting them onto plates containing benomyl to see if they continue to divide. If they don't divide, then the mutations complement each other, and if they do divide, then the mutations fail to complement (they don't have to say this explicitly). You would be unable to carry out complementation tests with Mutant #7 because it is dominant, so in the diploid cells you would always see the Mad phenotype.

d). You do the complementation tests and get the following results (- means failure to complement, + means complementation):

mutant	1	2	3	4	5	6
1	-	-	+	+	+	-
2		-	+	+	+	-
3			-	+	-	+
4				-	+	+
5					-	+
6						-

How many genes are affected by these 6 Mad mutations, and which mutations are alleles of the same gene? (8 points)

The 6 Mad mutants are in 3 different genes. Mutants # 1, 2, and 6 are allelic to each other (or in the same gene), Mutants # 3 and 5 are in the same gene, and Mutant #4 is in a different gene than any of the others.

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5. (continued)

e). You clone the affected gene from your Mad Mutant #7. Based on the sequence, you realize that the gene encodes a kinase enzyme, so you name the gene *CHK1* (for checkpoint kinase). From the sequence of the gene in the mutant strain you learn that your mutant has a missense mutation. Is this consistent with the idea that it's a gain-of-function mutation? Why or why not? How might the missense mutation alter the function of the kinase? (6 points)

Yes, a missense mutation is consistent with Mutant #7 being gain-of-function. The mutant kinase could be constitutively active (rather than induced upon activation the Spindle Assembly Checkpoint) (this is the best answer). Another totally acceptable answer is that the missense mutation makes the protein more stable (e.g., by deleting a destruction signal), but I'd be very surprised if anyone came up with this one since we didn't really talk about destruction signals. It's a remote possibility that a missense mutation could lead to the protein being it more highly expressed, and I would give partial credit (4/6) for this answer.

f). You suspect that the kinase affected by Mutant #7 might regulate the activity of the Anaphase Promoting Complex (APC). Would you guess that the normal function of the kinase is to activate or inhibit the APC? Explain your reasoning. (6 points)

The kinase is more likely to <u>activate</u> the activity of the APC, since a gain-of-function mutation in the kinase makes cells continue to divide (or proceed to anaphase) rather than arresting at metaphase.

g). What phenotype(s) might you expect to see in haploid yeast cells carrying a temperature-sensitive loss-of-function mutation in *CHK1*? (6 points)

If the kinase is necessary to activate the APC, then loss-of-function would make all cells arrest at metaphase (and therefore stop growing) at high temperature.

For full credit, the answer should include the idea of metaphase arrest (or at least mitotic arrest) at high or restrictive temperature. "Dead" at high temperature would get partial credit. "Mad" at high temperature would get 1 or 2 points.

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6. (30 points total) The *let-60* gene in *C. elegans* encodes the worm homolog of the human *RAS* protooncogene. Gain-of-function mutations in *let-60* result in a dominant "Multivulval" (Muv) phenotype due to excess proliferation of the cells that give rise to the vulva.

The genetic pathway below leading to vulva development has been worked out through extensive genetic analysis:

a) You want to find genes that can suppress the Muv phenotype of *let-60(gf)* mutations when they are targeted by RNAi. To do this, you put several *let-60(gf)* worms onto each of many different RNAi feeding plates, each containing *E. coli* bacteria expressing double-stranded RNA corresponding to a different *C. elegans* gene.
You find a number of RNAi plates where the *let-60(gf)* worms have either a normal vulva or no vulva. Which of the genes in the pathway above might behave this way? Explain your reasoning. (6 points)

The genes *lin-45, mek-2*, and *mpk-1* would be likely to act as suppressors of *let-60(gf)* in an RNAi based-screen, because the activity of these genes is required downstream of *let-60* to execute or upregulate vulval development. Loss of function of upstream genes (RNAi can only lead to loss-of-function) would not be expected to suppress a constitutively active *let-60* mutation. It's possible that some students might include *sem-5* on the list, and if they state explicitly that loss of *sem-5* might downregulate let-60, I'd give them partial or full credit (if they also get *lin-45*, *mek-2,* and *mpk-1*, but not if they include *lin-3* or *let-23*, since loss-of-function of these genes would only exacerbate the upregulation of *let-60*.)

b) You carry out a conventional (forward) genetic screen to find suppressors of *let-60(gf)* mutations. You mutagenize homozygous *let-60(gf)* Muv animals, allow them to self-fertilize for two generations, and identify a single suppressed F2 animal with a Vulvaless (Vul) phenotype.

How would you determine whether the Vulvaless phenotype is due to a new mutation in the *let-60* gene or to an extragenic suppressor mutation in another gene? Explain what type of cross(es) you might do and what phenotypes you would expect to see in what ratios in the F1 and F2 generations for both an intragenic and an extragenic suppressor. (Note: you should ignore for the purposes of this question that Vulvaless worms are difficult to cross). (6 points)

You would cross the suppressed animals to wild-type males and allow the F1 hermaphrodite progeny to self-fertilize. If the suppressor is a new mutation in the *let-60* gene, the F1 generation would probably all look wild-type (assuming that the new mutation is a recessive, loss-of-function mutation), and the F2 generation would include ¼ Vulvaless animals and ¾ wild-type animals. If the suppressor is extragenic, there are a number of possibilities. If you assume that the suppressor is recessive and unlinked to *let-60*, the F1 generation would include 50% Multivulval hermaphrodites, and the F2 generation would include ¼ Vul, 9/16 Muv, and 3/16 wild-type hermaphrodites (they may forget that the original mutation is dominant, so you can deduct just one point credit if they get: ¼ Vul, 3/16 Muv, and 9/16 wild-type). They might also assume that the suppressor is dominant (although this is less likely), which would give different answers – more Vul F2 animals and only 1/16 wild-type F2. If anyone does this, let me know.

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6. (continued)
You determine that the Vulvaless (Vul) phenotype in the suppressed *let-60(gf)* strain described in part b) is due to an extragenic suppressor, which you designate *Sup1*. You carry out crosses to eliminate the *let-60(gf)* mutation from your strain and find that your *Sup1* mutation gives a recessive Vul phenotype even when *let-60(gf)* is not there.

To map the new gene to a chromosome, you cross males homozygous for the *Sup1* mutation to the following mapping strain: *dpy-5 I; bli-2 II; unc-32 III*. You allow the F1 hermaphrodites to self-fertilize and you pick the Dpy (Dumpy), Bli (Blistered), and Unc (Uncoordinated) F2s to separate plates.

You observe the following numbers of Vul and nonVul animals of each of these phenotypes:

	Vul	nonVul
Dpy	22	98
Bli	3	74
Unc	16	51

c) Which chromosome is the *Sup1* gene most likely to be on? (4 points)

Chromosome *II*. *The Sup1 mutation shows good evidence of linkage to bli-2. The deviations in the ¼ ratio of Vul:nonVul animals in the other cases is likely to be due to small sample size.*

d) Approximately how far is the *Sup1* locus from the marker gene (*dpy-5, bli-2,* or *unc-32*) on the same chromosome? (4 points)

About 20 map units from *bli-2*.
(3/74 Bli animals were Bli Vul double-recombinants, so the map distance is (the square root of approx. 1/25), which is about 1/5, or 0.2, or 20 map units.)

e) If you cross the DpyVul F2 animals from the mapping cross above to wild-type males and allow the hermaphrodite cross-progeny to self-fertilize, what fraction of *their* progeny would have the following phenotypes: (4 points)

Since the two mutations are not on the same chromosome, they should segregate independently, and you should get:

Dpy nonVul? _____**3/16**_____

Vul nonDpy? _____**3/16**_____

Dpy Vul? _____**1/16**_____

wild-type? _____**9/16**_____

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f) Loss-of-function mutations in the *mpk-1* gene result in healthy but sterile animals (they cannot reproduce). If you construct a $P_{mpk\text{-}1}$*:gfp* fusion gene (a transgene in which GFP expression is driven by the *mpk-1* promoter) and introduce it into wild-type *C. elegans* by injection, where might you expect to see high levels of GFP fluorescence in the progeny of the injected animals? (6 points)

You would most likely see GFP fluorescence in the germline. (full-credit answers might also include "meiotic nuclei," "reproductive organs," or (unlikely) "the distal tip cell"). Answers like "in the vulval cells" should get partial (maybe half) credit – Vulvaless worms can actually reproduce fine.

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7. You carry out a screen for *C. elegans* mutants that produce severely reduced numbers of progeny due to abnormally low levels of cell proliferation in the *C. elegans* germline. You find several such mutants, which you call "*glp*" mutants (for *germline proliferation*). You map them to a number of different genes. One of your mutants, *glp-1*, turns out to be a dominant gain-of-function mutation in a phosphatase gene. Another mutant, *glp-2*, is a recessive loss-of-function mutation in a transcription factor gene. You screen for suppressors of *glp-1* and find an intragenic suppressor (*glp-1a*) that results in hyperproliferation of the germline tissue.

a) You consider three possible pathways that might control cell proliferation. In pathway (1), the GLP-1 phosphatase might control the activity of the GLP-2 transcription factor; in pathway (2), the GLP-2 transcription factor might regulate the expression of the GLP-1 phosphatase; and in pathway (3) the two genes might act independently to control cell proliferation. Draw three pathways (1, 2, and 3) consistent with all of the information above. (9 points)

The behavior of mutations in the genes indicates that the GLP-1 phosphatase inhibits proliferation, while the GLP-2 transcription factor promotes proliferation. Pathways consistent with this evidence are:

1. GLP-1 ⊣ GLP-2 → cell proliferation

2. GLP-2 ⊣ GLP-1 ⊣ cell proliferation

3. GLP-1 ⊣ cell proliferation; GLP-2 → cell proliferation

Both the order of the genes and the signs (arrow or bar) should be correct for full credit. If the order but not the sign is correct, you can give partial credit.

b) You want to test which of your pathways is most likely to be correct. Describe the experiment(s) you might do to figure this out, using any of the mutants that you already have. What outcome of the experiment(s) would lead to the conclusion that the phosphatase regulates the activity of the transcription factor? What outcome would lead to the conclusion that the transcription factor regulates the expression of the phosphatase gene? (15 points.)

You would make a strain of worms homozygous for both the *glp-1a* loss-of-function mutation and the *glp-2* loss-of-function mutation and look at the level of germline cell proliferation. If the germline shows low proliferation (or there are few progeny), then the phosphatase probably regulates the activity of the transcription factor, since you see the *glp-2* loss-of-function phenotype (and therefore *glp-2* is downstream of *glp-1*). On the other hand, if you see hyperproliferation, this is the *glp-1a* loss-of-function phenotype, so *glp-1* is downstream of *glp-2* (in other words, the transcription factor regulates the expression of the phosphatase).

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Question 8 (15 points)

The *balding* gene is autosomal and paternally imprinted. The two *balding* alleles produce two differently sized proteins that are easily distinguished by gel electrophoresis. One allele produces a full-length protein (FL), the other a truncated protein (T). Individuals expressing only the T allele become bald at a very early age.

John and Sarah have two children, a boy Tim and a girl Betty. Protein analysis shows that John expresses the FL form, Sarah expresses the T form, and both children express the FL form. John's dad expresses the FL form and John's mom expresses the T form. No information is available for Sarah's parents.

(a) Using only the above information, indicate the genotypes of the following individuals in Column 1. Alleles should be separated by a slash (e.g. T/T); if one or both alleles cannot be determined, use a question mark (e.g. T/?). In Column 2, indicate the phenotype of the individual; state "unaffected", "bald" (early balding), or "?"(unknown). (7 pts)

	Column 1 (genotype)		Column 2 (phenotype)	
John's mom	FL/T	(1 pt)	bald	(0.5 pt)
John's dad	FL/?	(0.5 pt)	WT	(0.5 pt)
John	FL/?	(0.5 pt)	WT	(0.5 pt)
Sarah	FL/T	(1 pt)	bald	(0.5 pt)
Tim (son)	FL/?	(0.5 pt)	WT	(0.5 pt)
Betty (daughter)	FL/?	(0.5 pt)	WT	(0.5 pt)

(b) Betty marries Alex and they have two children, a boy Xavier and a girl Jess. Alex and Xavier express the T form and Jess expresses the FL form. Considering all data, indicate the genotypes of the following individuals, using the same conventions as above. (4 pts)

John's mom	FL/T	(no extra credit)
John's dad	FL/T	(1 pt)
John	FL/T	(1 pt)
Sarah	FL/T	(1 pt)
Tim (son)	FL/?	(no extra credit)
Betty (daughter)	FL/T	(1 pt)

(c) What form(s) of the protein does John express in his germ cells? What form(s) does Sarah express in her germ cells? Briefly explain your answer. (4 pts)

John expresses neither of the proteins in his germ cells, because paternally imprinted genes are passed on from the father to the next generation in a silenced state. Paternally imprinted genes are not silenced in female germ cells; thus Sarah, who is heterozygous, passes on both the FL

and T genes in an active state. (Her germ cells carry only one balding allele, either FL or T, but whichever is present in any single germ cell will be expressed).

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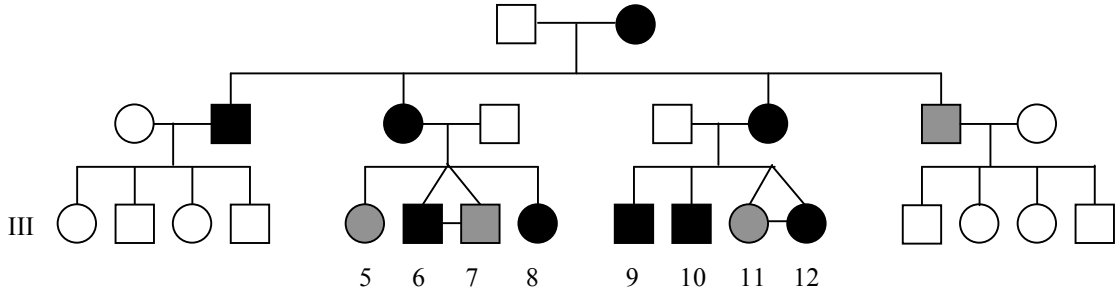
Question 9 (15 points)

In the human pedigree below, shading indicates individuals affected by a muscular degenerative disease. Black shading indicates severely affected individuals whereas gray shading indicates affected individuals with milder symptoms.



a) What type of inheritance is most consistent with this pedigree? Broadly speaking, what type of disease is typified by this type of pedigree and what feature(s) of the pedigree led you to this conclusion? (5 pts)

The disease shows non-Mendelian or maternal inheritance. Mitochondrial diseases are typified by this pedigree. Features of mitochondrial diseases are: (a) all children of affected mothers are affected (to some degree), (b) children of affected fathers are not affected, (c) heteroplasmy can lead to differences in disease severity, and (d) the disease affects tissues with high energy requirements.

b) There are identical twin boys in Generation III (6 and 7). State a plausible reason why one twin displays less severe symptoms than his identical twin and describe mechanistically how this could occur. (6 pts)

Identical twins have identical nuclear genomes, but their mitochondrial genomes can differ. Basic Concept: Twin 1 (III-6) has a greater ratio of mutant to wildtype mitochondrial genomes than Twin 2 (III-7) in tissues that are affected by the muscle degeneration disease.

Possible explanations (only one necessary):
(1) When the heteroplasmic zygote split into two to create identical twins, the mitochondrial genomes were segregated unequally, with Twin 1 (III-6) receiving a higher ratio of mutant to wildtype mitochondria than Twin 2 (III-7).
(2) The initial segregation of wildtype to mutant mitochondria was essentially equal, but during subsequent segregation of organelles during mitosis, more mutant mitochondria relative to wildtype mitochondria were inherited by cells giving rise to muscle (the cell type most affected by the disease) in Twin 1 (III-6) versus Twin 2 (III-7).

c) There are also identical twin girls in Generation III (11 and 12). Will III-11 and III-12 transmit the disease to their offspring? Why or why not? Will the twins' siblings transmit the disease to their offspring? Why or why not? (4 pts)

Because mitochondria are inherited from the mother and not from the father, the twin sisters (III-11 and III-12) will both pass the disease on to their offspring. (The severity of the disease my vary for the reasons stated in part b.) Conversely, the twins' brothers (III-9 and III-10) will not pass on the disease to any of their offspring.

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