Genetics and Genomics in Medicine Chapter 7

Questions & Answers

Multiple Choice Questions

Question 7.1

Depending on the base position within as codon, the percentage of base changes that alter the interpretation of the codon vary remarkably. Which of the following statements, if any, is true?

a) 100% of all possible base changes to the first base cause an altered interpretation for the codon.
b) 100% of all possible changes to the second base cause an altered interpretation for the codon.
c) About 30% of all possible changes to the third base cause an altered interpretation for the codon.
d) Less than 10% of all possible changes to the third base cause an altered interpretation for the codon.

Answer 7.1

b) 100% of all possible changes to the second base cause an altered interpretation for the codon.
c) About 30% of all possible changes to the third base cause an altered interpretation for the codon.

Explanation 7.1

a) About 96% of single nucleotide change to the first base in an amino-acid specifying codon result in a change in the specified amino acid (but for certain arginine and leucine codons, first base position changes can be synonymous substitutions).

Question 7.2

Which of the following changes is i) a synonymous mutation, ii) a conservative substitution, iii) a nonconservative substitution, iv) a stop-loss mutation, v) a stop-gain mutation.

a) UGA → UCA
b) UAC → UAA
c) AGA → AAA
d) AGA → CGA
e) UGU → CGU

Answer 7.2

a) iv)
b) v)
c) ii)
d) i)
e) iii)

Question 7.3

Concerning disorders resulting from unstable expansion of tandem oligonucleotide repeats, which, if any of the following statements is false.

a) The expansions can occur in coding DNA in some cases, and in noncoding DNA in other cases.
b) The repeats are of a variable number of nucleotides (from three to six) in both coding DNA and noncoding DNA.
c) The expansions in noncoding DNA are generally much larger in size than those in coding DNA.
d) The expanded arrays in noncoding DNA always result in loss of function of the host gene or of a neighboring gene.

Answer 7.3

b) The repeats are of a variable number of nucleotides (from three to six) in both coding DNA and noncoding DNA.
d) The expanded arrays in noncoding DNA always result in loss of function of the host gene or of a neighboring gene.

Explanation 7.3

The repeats in coding DNA are trinucleotides that specify glutamine. Expanded arrays in noncoding DNA can cause loss of function, as in the case of fragile X syndrome and Friedrich ataxia but in some other disorders a mutant RNA is produced that has a novel function.

Question 7.4

Match the descriptions of a type of mutation given in a) to d) with one of the possible mutations listed in i) to iv).

Description	**Mutation**
a) A mutation that does not case a disease but that is unstable at mitosis and meiosis and	i) a post-zygotic (somatic) mutation ii) a missense mutation

can change into a pathogenic mutation.

b) A pathogenic mutation that is unstable at mitosis and meiosis and can result in progressively severe phenotypes.
c) A type of mutation that results in genetic mosaicism.
d) A class of mutation that is quite frequently associated with a gain of function.

iii) a dynamic mutation
iv) a premutation

Answer 7.4

a) iv)
b) iii)
c) i)
d) ii)

Question 7.5

Regarding mutations, which of the following statements, if any, is false?

a) A *de novo* mutation is one that has occurred post-zygotically.
b) Each of us has multiple genes where both the maternal and paternal alleles have inactivating mutations.
c) The vast majority of mutations in our DNA do not adversely affect gene expression.
d) The mitochondrial genome has a very high gene density and accordingly the mutation frequency in mtDNA is low.

Answer 7.5

a) A *de novo* mutation is one that has occurred post-zygotically.
d) The mitochondrial genome has a very high gene density and accordingly the mutation frequency in mtDNA is low.

Explanation 7.5

De novo mutations can occur at any stage, including within the zygote. Mutation frequency has nothing to do with gene density and the frequency of mutations in mtDNA exceeds that of mutations in nuclear DNA by a factor of about 10 or more. .

Question 7.6

Regarding chromosome abnormalities, which of the following statements, if any, is false?

a) A Robertsonian translocation is a common example of an aneuploidy.
b) Nondisjunction is a common cause of aneuploidy

c) Triploidy is most commonly caused by fertilization of an egg by a diploid sperm.
d) Tetraploidy is usually due to replication of the zygote's DNA without cell division.

Answer 7.6

a) A Robertsonian translocation is a common example of an aneuploidy.
c) Triploidy is most commonly caused by fertilization of an egg by a diploid sperm.

Explanation 7.6

A Robertsonian translocation is a common example of a structural chromosome abnormality, not an aneuploidy (which involves a change in chromosome number). Treiploidy is most commonly due to fertilization of an egg by two sperms.

Question 7.7

Interpret the following examples of chromosome karyotypes.

a) 47,XX,+mar.
b) 45,XY,der(13;14)(q10:q10) .
c) 46,XX,del(15)(q11q13).
d) 46,XY,t(3;17)(q26q23)

Answer 7.7

a) A cell from a female that contains a marker chromosome (an extra unidentified chromosome)
b) A male carrier of a Robertsonian translocation that has arisen via breakpoints on the short arms of chromosomes 13 and 14 (q0 is not a chromosome band; it means the centromere).
c) A female with an interstitial deletion on the long arm of chromosome 15 with breakpoints at q11 and q13.
d) A male with a balanced reciprocal translation with breakpoints at 3q26 and 17q23.

Question 7.8

Which, if any, of the following statements is true?

a) A person is said to be a chimera if he or she has two or more genetically different cells.
b) The diversity of immunoglobulins made by a person is due to genetic mosaicism.
c) Having cells that inactivate the paternal X and cells that inactivate the maternal X is an example of genetic mosaicism in female mammals.
d) A person with cell populations that are genetically different because they originated from two different zygotes is described as a genetic mosaic.

Answer 7.8

b) The diversity of immunoglobulins made by a person is due to genetic mosaicism.

Explanation 7.8

a) This person would be described as a genetic mosaic.
c) X-inactivation is an epigenetic phenomenon and so it would be difficult to describe the cellular mosaicism as an example of genetic mosaicism.
d) This person would be described as a chimera.

Question 7.9

Which, if any, of the following statements is false?

a) A null allele is one where the gene has been deleted or received an inactivating mutation causing complete loss of gene function.
b) Inactivating point mutations are a common cause of pathogenesis in recessively inherited disorders.
c) Inactivating point mutations are a common cause of pathogenesis in dominantly inherited disorders.
d) When a gain-of-function allele is known to be pathogenic in a single gene disorder, a heterozygote will always show disease symptoms.

Answer 7.9

d) When a gain-of-function allele is known to be pathogenic in a single gene disorder, a heterozygote will always show disease symptoms.

Explanation 7.9

d). It depends on the penetrance. In some cases there is a late age at onset of symptoms and so a person may appear perfectly healthy. c) many dominant disorders are due to haploinsufficency where one allele is a loss-of-function allele that is often an inactivating point mutation.

Question 7.10

Which, if any, of the following statements is false?

a) Gain-of-function mutations are often missense mutations.
b) A missense mutation that has a dominant-negative effect can often produce a greater loss of protein function than a null mutation.
c) Pathogenic gain-of-function and loss-of-function mutations in the same gene produce different phenotypes .
d) A mutant protein that antagonizes the wild type protein produced from the normal allele is known as a hypomorph.

Answer 7.10

d) A mutant protein that antagonizes the wild type protein produced from the normal allele is known as a hypomorph.

Explanation 7.10
Such a mutant protein is known as an antimorph

Fill in the Blanks Questions

Question 7.11

Fill in the blanks using numbers.

Depending on our ethnic background, each of us carries about ___1___or so mutations that would be expected to result in loss of gene function (with an average of ___2____ genes that are homozygously inactivated), plus about ____3____ missense variants that severely damage protein structure. When you factor in additional mutations in noncoding DNA, a normal person might be expected to have a total of over ____4____ damaging DNA variants.

Answer 7.11

1. 100. 2. 20. 3. 60. 4. 400.

Question 7.12

Fill in the blanks using single words or with one or two letters.

The most commonly used method in human chromosome banding is known as ___1___-banding, when the chromosomes are treated with trypsin and then stained with the ____2____ dye. The ____2____ dye binds preferentially to ___3____-rich regions in DNA and the staining produces a series of alternating ____4____ bands that are ____2___-positive and ___3____-rich and ____5____ bands that are ____2___-negative and ___6____-rich. The ____4____ bands have a generally ____7____ content of genes, whereas the ____5____ bands have a ____8____ content of genes.

Answer 7.12

1. G. 2. Giemsa. 3. AT. 4. dark. 5. light. 6. GC. 7. low. 8 high.

Question 7.13

Fill in the blanks using single words.

A person with two or more genetically different cell lines is described as a genetic ___1____. Because we have so many cells in our bodies everyone will have cells that are genetically different as a result of ___2____ mutation; each of us is a genetic ___1___. People who have cells that originated from different zygotes are described as ___3____. That can happen when a person is a recipient of organ or cell _____4____. It can also happen during pregnancy at the earliest stages of development when non-identical ____5____ ___6___ fuse, and, more

commonly at later stages of development, when there can be an exchange of cells between the ____7____ and the ___8___.

Answer 7.13
1. mosaic. 2. post-zygotic (or somatic). 3. chimeras. 4. transplantation. 5.twin. 6. embryos. 7. mother. 8 fetus.

Question 7.14

Fill in the blanks using single words.

Human mitochondrial DNA is transmitted exclusively by ____1_____. As well as transmitting chromosomes to the oocyte, sperm also transmit _____2____ but they are selectively ___3____ in the early embryo. Because mtDNA replication is independent of the cell cycle and there are many mtDNA molecules per cell, a population of mutant mtDNA molecules can co-exist in a cell with a population of normal mtDNA molecules, a state known as _____4_____. Because mtDNA replication is ____5____ and because ____6____ expansion of mutant DNAs is variable, the proportion of mutant to normal mtDNAs in a cell can ___7____ significantly between cells in the same individual.

Answer 7.14
1.females. 2. mitochondria. 3. destroyed. 4. heteroplasmy. 5. relaxed. 6. clonal. 7. vary.

Essay and List Questions

Question 7.15

Genetic variation can cause disease by causing a gene product to have an altered sequence of amino acids or ribonucleotides, or by altering the amount of gene product that is made. Describe the different ways in which genetic variation leads to a change in the amount of gene product.

Answer 7.15

- An inactivating point mutation that results in failure to make a product. That can be the consequence of a premature in-frame termination codon specified at the DNA level (nonsense mutation, frameshift mutation) or following a mutation that results in altered splicing, producing an aberrant mRNA that is subject to nonsense-mediated decay.
- A gene copy number change. That can mean whole gene deletion, gene duplication or sometimes gene amplification (in cancer cells).
- A mutation in a regulatory sequence that controls the expression of a gene (often causing down-regulation, but some point mutations may lead to over-expression) or the stability of a mRNA, for example.

Question 7.16

The genetic code that is used in our mitochondrial differs from the "universal" genetic code in the case of four codons. What are these codons and how does their interpretation differ between nuclear DNA and mitochondrial DNA?

Answer 7.16

Codon	**Nuclear DNA**	**Mitochondrial DNA**
AGA	Arg	STOP
AGG	Arg	STOP
AUA	Ile	Met
UGA	STOP	Trp

Question 7.17

Regarding chromosome nomenclature, explain the following terms:

a) distal

b) proximal
c) acentric chromosome
d) derivative chromosome

Answer 7.17

a) located distant, or more distant, from the centromere
b) located close, or closer, to the centromere
c) a rearranged chromosome that lacks a centromere
d) a structurally rearranged chromosome that continues to have a centromere

Question 7.18

Nonsynonymous mutations can be grouped into three classes. What are they?

Answer 7.18

1) Missense mutations. A codon specifying an amino acid is replaced by a codon that specifies a different amino acid
2) Stop-Gain (Nonsense) mutations. A codon specifying an amino acid is replaced by a stop codon
3) Stop-loss mutations. A stop codon is replaced by a codon specifying an amino acid.

Question 7.19

What is a synonymous substitution and when does it not mean a silent mutation?

Answer 7.19

The terms synonymous substitution and silent mutation have long been used interchangeably to mean a codon change that did not change the interpretation of the codons mutations. However, it has become apparent that some synonymous mutations are not neutral: they change the expression of a gene and can directly cause disease (usually by altering RNA splicing – see Figure 7.4B on Page 196 for an example). In modern times, therefore, the term synonymous substitution is preferred to silent mutation.

Question 7.20

Why should our mitochondrial genetic code be different from our nuclear genetic code?

Answer 7.20

Selection pressure is exerted to keep the genetic code constant, but the strength of that selection pressure depends on the number of different messenger RNAs where the genetic code is deployed in codon interpretation. Our nuclear genetic code is called the “universal” genetic code

because exactly the same genetic code is used in all cells across life. When a genetic code is used to make thousands of different proteins in a cell there is huge selection pressure to keep it constant – any change could be disastrous because very large number of proteins could have their sequences changed.
Over evolutionary time the genome in the ancestral cell that gave rise to our mitochondrial genome was progressively reduced so that now our mitochondrial genome is tiny – just over 16 kb – and makes just 13 different polypeptides. With a progressive change to just a very small number of coding DNA sequences, selection pressure to keep the code constant was gradually relaxed over time and the codon interpretation was allowed to change four codons (AGA, AGG, AUA, UGA).

Question 7.21

Amino acids can be divided into non-polar amino acids and polar amino acids. List the five different classes of polar amino acids.

Answer 7.21

1) Basic (possess a positively charged side chain): arginine, lysine, histidine
2) Acidic (possess a negatively charged side chain): aspartate; glutamate
3) Amide group-containing: asparagine, glutamine
4) Hydroxyl group-containing: serine, threonine, tyrosine
5) Sulfhydryl group-containing: cysteine (but not methionine which is a non-polar amino acid)

Question 7.22

Why should cysteine be the least mutable amino acid?

Answer 7.22

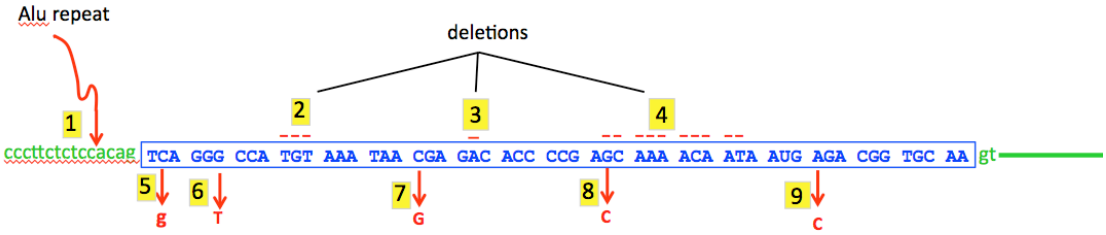
Many amino acids belong to classes of amino acids that have similar chemical properties such as serine and threonine or lysine and arginine, for example. Conservative substitutions in the codons for these amino acids can allow one amino acid to be replaced by another of the same chemical class, often with minimal effect to the working of a protein.
Cysteine has a unique role in allowing cross-linking within proteins through the formation of both intrachain and interchain disulphide bonds Not all cysteines in a protein engage in disulphide bonding but there is a special importance attached to those cysteines that do participate in disulphide bonding because there is no other amino acid that can perform this role – replacement by another amino acid could often abolish protein function. As a result, there is a very strong selection pressure to conserve cysteines to maintain protein function.

Explanation 7.22

See Figure 2.5 on page 130 for the example of disulphide bonds that are important in the conformation of the individual A and B chains of insulin and that also covalently link the A chain to the B chain.

Question 7.23

In the sequence below, the blue sequence represents an exon containing coding DNA near the beginning of a large gene and green lines and letters are flanking intron sequence. Nine mutations are shown: an insertion of an Alu repeat insertion plus three deletions at top and five single nucleotide substitutions below. Comment on the mutation class in each case and on its likely effect.



Answer 7.23

1) Insertion of an Alu repeat. The long pyrimidine tract of the splice acceptor sequence will be placed far from the expected exon-intron boundary and might be expected to inactivate a splice acceptor site, and might lead to exon skipping. If so it will produce a frameshift because the exon has 56 nucleotides, a number that is not a multiple of three.
2) Deletion of a single amino acid. In this case a cysteine is deleted, which may cause major protein structure difficulties if this *particular* cysteine is involved in disulphide bonding, or may affect protein function if the cysteine is important in some other way.
3) A single nucleotide deletion, causing a frameshift that introduces a premature termination codon after six amino acid-specifying codons. Expected to inactivate gene expression.
4) A frameshifting deletion (10 nucleotides long). Expected to inactivate gene expression.
5) A nonsense mutation. Expected to inactivate gene expression
6) A synonymous mutation. Likely to have no effect (unless it changes an exonic splice enhancer or suppressor)
7) A non-conservative amino acid substitution replacing arginine by glycine. Might be pathogenic because of the change from a positively charged amino acid to a neutral amino acid with the smallest possible side chain.
8) A conservative amino acid substitution that replaces a serine codon by a threonine codon. May have minimal effect.

9) Although the first base of the codon position has been substituted this is a synonymous mutation. Likely to have no effect.

Question 7.24

What is the major natural role of the nonsense-mediated decay mechanism in our cells?

Answer 7.24

It has a role in mRNA surveillance. Just like our cells have DNA surveillance mechanisms that check for DNA damage and then send signals that it should be repaired, there is a need to constantly monitor RNA integrity. Some of the major problems that can go wrong here are errors in RNA splicing and occasional mistakes made during transcription (the wrong bases can get incorporated by an RNA polymerase).

Splicing accidents may be quite common, and may, for example lead to retention of an intron sequence that will almost inevitably result in in-frame premature termination codons. In mammalian cells the primary nonsense-mediated decay (NMD) mechanism is splicing-dependent, and it can detect errors that result in in-frame premature termination codons other than in the last exon or within the 55 nucleotides or so from the end of the penultimate exon. NMD depends on the ribosome displacing exon-junction protein complexes that are naturally deposited by the splicing machinery at intervals on the mRNA, roughly about 20-24 nucleotides in advance of the end of each transcribed exon except the last exon (which often contains just untranslated sequence). In the case of an early in-frame premature termination codon the ribosome disengages from the mRNA without displacing the later exon-junction complexes and the mRNA will continue to have the exon-junction complexes attached to it. That usually sends a signal to the cell that the mRNA must be destroyed.

Question 7.25

What is a cryptic splice site? What are expected consequences of activation of i) a cryptic splice donor site located within an exon ii) a cryptic splice acceptor site within an intron?

Answer 7.25

A cryptic splice site is any sequence that is closely similar to the consensus splice acceptor and splice donor sequences, and that may very occasionally be used in splicing or be activated by a mutational change so that it is recognized by the RNA splicing machinery.

i) Activation of a cryptic splice donor site within an exon can result in altered splicing so that the exon is truncated at the 3’ end.
ii) Activation of a cryptic splice acceptor site within an intron can result in altered splicing so that the following exon is extended at the 5’ end.

Explanation 7.25
See Figure 7.4C on page 196.

Question 7.26

Certain sequence classes in our genome are particularly prone to mutations. List three examples and explain why they are so prone to mutations.

Answer 7.26

1) *Mitochondrial DNA*. The mutation rate in mtDNA is probably 10-fold or more greater than that in the nuclear genome. The mitochondrial genome is generally vulnerable to mutation for a variety of reasons. The great majority of reactive oxygen species are produced in mitochondria and cause damage to the mtDNA (which, unlike nuclear DNA, lacks a protective chromatin coat), and the efficiency of DNA repair is less for mitochondrial DNA than for nuclear DNA.
2) *Tandem mononucleotide/dinucleotide repeats*. Mostly, by chance, there are occasional long arrays of a single nucleotide or a dinucleotide repeat. When that happens there is a much higher chance of *replication slippage*: the DNA polymerase skips forward or backwards when copying this type of repeat to make a new DNA strand. That results in occasional insertions or deletions.

3) *Long tandem repeats*. Duplicated copies of sequences from hundreds of base pairs to megabases are vulnerable to change if the sequence copies show a very high degree of sequence identity that facilitates mispairing of chromatids. Resulting unequal crossover or unequal sister chromatid exchange produces chromatids with extra repeats or fewer repeats.

Question 7.27

The number of cell divisions needed to make human gametes differs extensively between men and women and also between different men. Explain these differences.

Answer 7.27

Both sperm and eggs are generated by two meiotic cell divisions but the number of preceding mitotic cell divisions are different. In females, all of the roughly 22 mitotic divisions required to get to the first meiotic cell are accomplished before birth, and indeed part of meiosis I has been completed by then but is suspended until activated by ovulation. No matter how old mothers are, a total of about 24 cell divisions separate zygote from egg cells.
Males are different because gametogenesis continues throughout adult life. About 30 cell divisions separate the zygote from the spermatogonial stem cell that is used to make the first sperm cells at the onset of puberty. From spermatogonial stem cell to gamete takes four mitotic

divisions and then two meiotic divisions. So, at the onset of male puberty gametes are formed that have gone through 30 + 4 + 2 divisions = 36 cell divisions. Thereafter, spermatogonial stem cells divide every 16 days or so (or about 23 times per year). Sperm from a man who had reached puberty 50 years earlier will have gone though (23 x 50) + 36 = 1186 cell divisions.

Question 7.28

Two groups of human disorders involve expansion of tandem trinucleotide repeats in coding DNA to give gene products with abnormally long polyglutamine or polyalanine repeats. The pathogenic mechanisms have rather different characteristics, however in the way that the trinucleotide repeats expand. In what ways do they differ?

Answer 7.28

Pathogenic polyalanine expansion involves modest expansions of arrays of consecutive alanine-specifying codons that occur in some human proteins. Pathogenesis occurs when the small non-polymorphic polyalanine tract (from 10 to 18 amino acids in normal individuals) expands by a small amount. For example in hand-foot genital syndrome normal individuals have a tract of 18 alanines that has expanded in affected individuals to between 24 and 26 alanines.
Unlike the non-polar polyalanine tracts, polyglutamine tracts are highly polar. Pathogenic polyglutamine expansion is different from polyalanine expansions in several additional respects. First, unlike polyalanine tracts, the polyglutamine tracts – also known as poly(Q) tracts – typically contain just one codon (CAG) and they are polymorphic in normal individuals (possibly as a result of replication slippage). Secondly, after a critical length is reached at the DNA level, the expanded alleles are meiotically and somatically unstable; after passing a certain critical length the extended long array then has a tendency to expand further and faster than the smaller arrays. That is, the poly(CAG) expansion is unstable and considerable lengths can be reached. For example, in spinocerebella ataxia type 7 normal individuals have a poly(Q) tract that has between 7 and 18 glutamines but in affected individuals the same tract has expanded to between 38 and 200 glutamines.

Question 7.29

Deletions, duplications and inversions in our DNA often result in interaction between tandem repeats, direct repeats, or inverted repeats. What are the essential differences between these repeat classes.

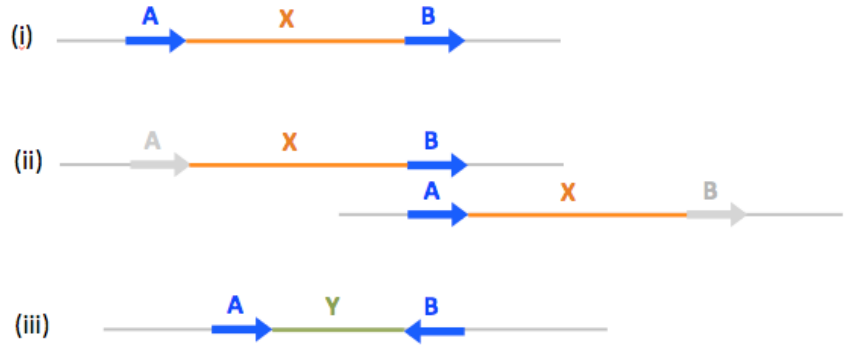
Answer 7.29

- Direct repeats means copies of a sequence that are orientated in the same direction on the same DNA molecule and, in practice, the meaning is reserved for repeats that are not adjacent to each other but are separated by an intervening DNA segment.

- Inverted repeats means copies of a sequence that are orientated in the opposite direction on the same DNA molecule and, in practice, the meaning is usually reserved for repeats that are not adjacent to each other but are separated by an intervening DNA segment.
- Tandem repeats means copies of a sequence that lie adjacent to each other on the same DNA molecule and not separated by an intervening DNA sequence. Almost always, the repeats are orientated in the same direction ("head-to-tail" configuration).

Question 7.30

Sequence exchange between two non-allelic copies of the same long sequence on chromosomal DNA molecules can have different consequences, depending on the positioning of the repeats that participate in sequence exchange. In (i) to (iii) imagine that there is sequence exchange between non-allelic sequence copies A and B shown by the blue arrows – the sequence exchange is between different chromatids of the same chromosome in the case of (ii). What types of sequence exchange might occur and what would be the likely outcome?



Answer 7.30

i) sequence exchange between direct repeats A and B could produce a chromatid with a deletion of sequence X; the other possible product would be a very small circular DNA containing a hybrid A/B sequence that would be lost after cell division

ii) the sequence exchange might involve crossover between the mismatched repeats that would produce one chromatid with a hybrid A/B sequence and a deletion of sequence X, plus one chromosome that has a duplication of sequence X plus a hybrid A/B sequence in addition to the normal A and B sequence copies. Another sequence exchange might take the form of a gene conversion where one of the interacting non-allelic repeats, A or B, is used as a template for replacing at least part of the sequence of the other matched repeat by a copy of itself.

iii) in the case of inverted repeats, alignment of A and B and crossover would result in an inversion of the intervening sequence Y.

Question 7.31

List three examples of single gene disorders that show an extremely limited range of point mutations and explain why there should be such mutational homogeneity.

Answer 7.31

1) Sickle cell anemia. The mutation is always a missense mutation that replaces a glutamine at position 6 in the β-globin chain by a valine. This special missense mutation results in a form of haemoglobin aggregation that causes erythrocytes to be deformed in a very specific way so that they have a crescent shape. The deformed erythrocytes have a much shorter life span and the body cannot replace dead erythrocytes fast enough, resulting in anemia, while aggregated haemoglobin fibers block small blood vessels causing hypoxic tissue damage. This missense mutation has come under positive selection in heterozygotes because the deformed erythrocytes make it more difficult for malarial pathogens to go though their life cycle in the body. The resulting heterozygote advantage had led to a high frequency for the disorder.
2) Steroid 21-hydroyxylase deficiency. About 99% of the pathogenesis is due to sequence exchanges between the normal 21-hydroxylase gene and a very closely related pseudogene, and virtually all pathogenic point mutations arise from gene conversion in which sequences are copied from regions of the pseudogene, introducing a copy of an inactivating mutation. There are only a very limited number of inactivating mutations in the pseudogene, and so the point mutation spectrum in 21-hydroxylase deficiency in this disorder is very limited.
3) Achondroplasia. The disease is always caused by single nucleotide substitution that replaces glycine at amino acid position 380 of the FGFR3 receptor protein by an arginine residue. There is a clear paternal age-effect in this disorder and transmission seems to occur exclusively from the paternal line, most likely as a result of selfish spermatogonial stem cell selection. That is, this specific type of missense mutation appears to result in a selective growth advantage on any spermatogonial stem cell that contains it so that the mutation reaches a high frequency.

Question 7.32

Match each of the genetic mechanisms a) to d) with one or more of the possible outcomes i) to iv).

Genetic mechanisms	**Possible outcomes**
a) sequence exchange between inverted repeats on a single chromatid.	i) Gene conversion
b) sequence exchange between non-allelic	ii) DNA deletion
	iii) DNA duplication

genes of a duplicated gene family on sister chromatids

c) sequence exchange between two identical direct repeats on a mtDNA molecule
d) sequence exchange between non-allelic genes of a duplicated gene family on nonsister chromatids

iv) An inversion

Answer 7.32

a) iv)
b) and iii)
c) ii) and iii)
d) i), ii) and iii)

Question 7.33

What is meant by aneuploidy, and how does it occur?

Answer 7.33

Aneuploidy is a class of chromosome abnormality where the chromosome set has too few chromosomes or has extra chromosomes. Aneuploid cells arise through two main mechanisms.

1) *Nondisjunction*. Here, paired chromosomes fail to separate (*disjoin*) during meiotic anaphase I and migrate to the same daughter cell, or sister chromatids fail to disjoin at either meiosis II or mitosis. Nondisjunction during meiosis produces gametes with either 22 or 24 chromosomes, which after fertilization with a normal gamete produce a trisomic or monosomic zygote. If nondisjunction occurs during mitosis, the individual is a mosaic with a mix of normal and aneuploid cells.
2) *Anaphase lag*. If a chromosome or chromatid is delayed in its movement during anaphase and lags behind the others it may fail to be incorporated into one of the two daughter nuclei. Chromosomes that do not enter a daughter cell nucleus are eventually degraded.

Question 7.34

Constitutional aneuploidy is occasionally viable in humans. Why should having fewer or extra copies of certain chromosomes be compatible with life, but not so in the case of other chromosomes?

Answer 7.34
The problem is dosage-sensitive genes, and it is most acute in the case of having fewer chromosomes. A small minority of our genes are dosage-sensitive. Autosomes have multiple dosage-sensitive genes, many of which are especially sensitive to haploinsufficiency, and the cumulative effects mean that no autosomal monosomy is viable. The dosage-sensitive genes are less sensitive to having three copies, however, and those autosomes with the smallest number of genes (and presumably the smallest number of dosage-sensitive genes) are the ones where trisomy is viable: chromosomes, 13, 18 and 21.
The sex chromosomes are different in two ways. First, there can be multiple X chromosomes, but all but one of the X chromosomes will be subject to X-inactivation, becoming highly condensed to form Barr bodies. Secondly, the Y has very few genes. As a result, gene dosage is less of a problem for sex chromosomes, and monosomy X is viable, as is having multiple sex chromosomes in various combinations.

Question 7.35
A chromosome has received two double-stranded breaks. What kinds of chromosome abnormalities can result?

Answer 7.35
If the two breaks occur on the same chromosome arm, there are two possibilities. First an *interstitial deletion* can result, after the central piece is excised and the two ends are fused at the breakpoints (the excised central fragment cannot be propagated because it lacks a centromere). Alternatively, a paracentric inversion results (the central piece, lacking a centromere, rotates and then is fused to the end pieces).
If the two breaks occur on different chromosome arms, deletion is not normally viable: the central fragment cannot be excised and lost as it contains the centromere. Instead, there are two viable alternatives. First, a pericentric inversion results (the central piece, containing a centromere, rotates and then is fused to the end pieces). More rarely, a ring chromosome can occur where the two ends of the central fragment fuse to form a circular chromosome.

Explanation 7.35
See Figure 7.11 on page 216.

Question 7.36
What are the characteristics of a Robertsonian translocation?

Answer 7.36
A Robertsonian translocation is an unbalanced translocation that occurs between the short arms of two chromosomes that represent one of the five human acrocentric chromosomes:

(chromosomes 13, 14, 15, 21 and 22) and results in a dicentric chromosome that is stable in mitosis. The short arms of these five human chromosomes have the same type of DNA organization: an array of about 30-40 tandem ribosomal DNA repeats (each containing sequences that specify 18S, 5.8S and 28S ribosomal RNAs), sandwiched between two large blocks of heterochromatin.
A Robertsonian translocation chromosome is stable in mitosis because the breakpoints are close to the centromere. That means it has two centromeres but the centromeres are so close to each other that they behave as one giant centromere. During its formation there will be loss of the distal sequences on both short arms, That is not a problem for the cell because much of the lost material is constitutive heterochromatin and the loss of 18S, 5.8S and 28S ribosomal RNA genes is not so significant given that another 8 chromosomes will be available in a diploid cell to provide between them about 250-300 copies of the ribosomal RNA genes.

Question 7.37

How does just a single loss-of-function allele cause a dominantly inherited disorder?

Answer 7.37

That happens occasionally if one allele at the disease locus has been epigenetically silenced, such as happens in the case of an imprinted gene locus. If the second allele receives an inactivating mutation that causes loss of function, no gene product will be made.
A more common situation occurs where the disease locus is one in which expression of the gene is very tightly regulated so that the gene makes just the correct amount of product - making just half the normal amount of product can result in disease (and making more of the normal product can often cause problems too). For such dosage-sensitive genes, therefore, a single loss-of-function allele can cause disease, a property known as haploinsufficiency.

Question 7.38

How can a missense mutation with a dominant-negative effect result in greater loss of protein function and more severe disease than a full length gene deletion at the same gene locus?

Answer 7.38

That can happen when the disease is due to insufficient quantities of a multisubunit protein where the protein consists of two different types of polypeptide, one of which is encoded by the gene in question, while the other polypeptide is made by a different gene. Collagens provide a useful example of proteins like this.
Take the case of type I procollagen. This protein is made up of a triple helix that contains two identical collagen chains encoded by the *COL1A1* gene at 17q21, plus one collagen chain encoded by the *COL1A2* gene at 7q21. A single gene deletion (or other inactivating mutation) in one allele at the *COL1A1* locus means that the output of polypeptide from the *COL1A1* locus

drops by 50%. As a result, the total amount of procollagen protein made will drop by 50% (the excess polypeptide chains made by *COL1A2* will be degraded). The 50% reduction in protein product leads to a mild form of osteogenesis imperfecta.
Now consider a missense mutation in one *COL1A1* locus that has a dominant negative effect because it causes disordered packaging of the triple helical chain. For simplicity, let us designate the polypeptide chains from *COLIA1* as A1N (normal) and A1M (mutant), and the polypeptide chain from *COLIA1* as A2, then there are four ways in which they can combine: A1N-A1N-A2; A1N-A1M-A2; A1M-A1N-A2; and A1M-A1M- A2. That is the packaging will be disrupted in three out of every four triple helices so that they are non-functional. Effectively, only 25% of the normal amount of the procollagen protein is made, resulting in the severe type IIA form of osteogeneis imperfecta.

Explanation 7.38
See Figure 7.17 on page 227 for an illustration.

Question 7.39
Give two examples of genes where loss-of-function and gain-of-function mutations result in different disease phenotypes.

Answer 7.39
1) *RET* (proto-oncogene)
   - loss-of-function mutations result in susceptibility to Hirschsprung's disease;
   - gain-of-function mutations result in medullary thyroid carcinoma/multiple endocrine neoplasia types 2A, 2B.
2) *AR* (androgen receptor gene)
   - loss-of-function mutations result in androgen-insensitivity syndrome, a
   - recessive form of pseudohermaphroditism (affected individuals have a 46,XY karyotype, but feminization occurs because their androgen receptors do not work normally and the end organs are insensitive to androgens)
   - gain-of-function mutations take the form of unstable CAG expansions resulting in an expanded polyglutamine tract in exon 1 so that the mutant protein and/or RNA becomes toxic to cells, causing degeneration of certain lower motor neurons to produce spinal and bulbar muscular atrophy.

Question 7.40
Give three examples of disorders where the pathogenesis can result from recurring large deletions and one where the pathogenesis can result from a recurring large duplication.

Answer 7.40

Angelman syndrome:	recurring 5 Mb deletion at paternal 15q11-q13
Prader-Willi syndrome:	recurring 5 Mb deletion at maternal 15q11-q13
DiGeorge syndrome:	recurring 1.5 or 3 Mb deletion at 22q11
Charcot-Marie Toot type 1A:	recurring 1.4 Mb duplication at 22q11

Explanation 7.40

See Table 7.12 on page 230 for other examples.

Question 7.41

In terms of its contribution to pathogenesis which of the following mutant proteins is the odd one out, and why is it the exception?

a) β-globin carrying the p.Glu6Val substitution .
b) PI*Z and PI*E mutant α_1-antitrypsins
c) mutant CFTR protein with the common p.Phe508del deletion.
d) mutant prion proteins.

Answer 7.41

c) mutant CFTR protein with the common p.Phe508del deletion.

Explanation 7.41

The p.Phe508del mutant undergoes aberrant protein folding that cannot be rectified by chaperones. The other mutant proteins are each involved in protein aggregation. Thus, in the homozygote the mutant β-globin binds to α-globin to form a mutant hemoglobin, HbS, When deoxygenated, HbS has a strong tendency to aggregate to form mutant haemoglobin fibers composed of 14 long strands of HbS tetramers which cause red blood cells to become deformed, and which block small blood vessels. The PI*Z and PI*E mutant α_1-antitrypsins are not processed and secreted from liver cells into the plasma as normal; but instead they aggregate in the endoplasmic reticulum of hepatocytes causing the hepatocytes to die, which can result in eventual cirrhosis of the liver. Mutant prion proteins can not only aggregate, but also induce normal copies of the prion protein to do so.

Question 7.42

Match each of the characteristics in a) to e) with the mutant alleles listed in (i) to (v).

Characteristic	**Mutant allele**
a) impaired protein processing and secretion.	i) the fibroblast growth factor receptor 3 p.Gly380Arg allele
b) aberrant protein aggregation.	ii) the Pittsburgh variant of α_1-antitrypsin
c) alteration of substrate specificity.	

d) Paternal transmission and paternal age-effect.
e) Aberrant protein folding.

iii) the CFTR p.Phe508del mutant
iv) the PI*Z α_1-antitrypsin allele
v) the β^S-globin allele

Answer 7.42

a) iv)
b) v)
c) ii)
d) i)
e) iii)

Question 7.43

The pathogenesis of α_1-antitrypsin deficiency due to the common missense mutants PI*S and PI*Z is due to a failure in protein processing and secretion, and to aberrant protein aggregation. Explain how.

Answer 7.43

α_1-antitrypsin is a plasma protein whose principal role is to regulate the levels of certain serine proteases that play important natural protective roles in cleaving unwanted proteins. The name, α_1-antitrypsin, is a bit of a misnomer because it blocks elastin more effectively than trypsin (elastase is secreted by neutrophils, the white blood cells that engulf bacteria).

There is a need to regulate proteins such as elastase because they can be over-produced and cause tissue damage, as when there is tissue inflammation and neutrophils over-produce elastase. As a result, α_1-antitrypsin needs to be produced in sufficient quantities to prevent sensitive tissues, such as lung tissue from damage by elastase over a lifetime.

In the case of the common mutant alleles PI*S and PI*Z, α_1-antitrypsin protein is still made in the liver but processing and secretion into plasma is impaired. As a result, ZZ homozygotes can only make 15% of the normal amount of plasma α_1-antitrypsin and SZ heterozygotes make 40% of the normal amounts of plasma α_1-antitrypsin. Affected individuals often develop emphysema, a form of chronic obstructive lung disease in which tissues needed to support the shape and function of the lungs are destroyed

A second feature is that the α_1-antitrypsin proteins that are not secreted but retained in the endoplasmic reticulum of hepatocytes aggregate to form inclusion bodies that cause hepatocytes to die and can result in eventual cirrhosis of the liver.

Question 7.44

“The pathogenesis of sickle cell anemia is due to aberrant protein aggregation. Explain how.

Answer 7.44

In the homozygote the mutant β^S-globin binds to α-globin to form a mutant hemoglobin, HbS. When deoxygenated, HbS has a strong tendency to aggregate to form mutant hemoglobin fibers composed of 14 long strands of HbS tetramers. The HbS fibers cause red blood cells to become deformed, giving the characteristic sickle cell shape. The mutant red blood cells do not live so long (their lifespan is on average just 10-20 days, compared to the 90-120 days for normal red blood cells). As a result, the body cannot make new red blood cells fast enough to replace the dead red blood cells and anemia results.

When the red blood cells die they release their HbS fibers and they can then block small blood vessels. That will disrupt oxygen transport to tissue causing tissue damage.

Question 7.45

The disease mechanism in prion protein diseases has sometimes been considered a type of epigenetic mechanism. On what basis?

Answer 7.45

In prion disease, a normal cellular form of prion is misfolded into an abnormal conformation that is prone to aggregation. The most striking characteristic of the mutant prion protein is that when it comes into contact with normal prion proteins it can induce them to switch conformation so that they, too, adopt the mutant structure. Thus, if our cells are exposed to abnormal prion proteins from an infected animal or person, the abnormal foreign prion proteins will induce normal host prion proteins to adopt the mutant prion structure.

Effectively, the abnormal prion protein structure can self-propagate by a form of replication that has nothing to do with nucleic acid sequences. Because the stable propagation of the mutant prion proteins has nothing to do with DNA sequence, the disease mechanism has sometimes been compared to classical epigenetic mechanisms that involve stable propagation of chromatin modifications.

Question 7.46

What are amyloid diseases? In what respects do neurodegenerative amyloid diseases resemble prion diseases?

Answer 7.46

Amyloid protein is the term used to describe a broad family of proteins that have a high content of β-sheets that make them prone to aggregation. Prions are a well-known subclass of amyloid proteins but in addition there are many other examples. Amyloid diseases are disorders in which amyloid proteins are implicated. The aggregation of amyloid proteins can occur outside of cells (such as in the case of prion proteins and β-amyloid), within nuclei (huntingtin), or within the cytoplasm (SOD1; Tau; and synuclein).

Some common amyloid diseases are not associated with neurodegeneration, such as type 2 diabetes (aggregates of serum amyloid A protein are found in the pancreatic islets of Langerhans). However, neurodegeneration is the most striking clinical characteristic of many amyloid diseases.
Alzheimer disease, Parkison disease, amyotrophic lateral sclerosis, and frontotemporal disease, resemble prion protein diseases in many ways and are sometimes classified as *prionoid* diseases. In these diseases direct involvement of the aggregated proteins in disease is supported from familial forms of these disorders in which mutations in the relevant gene promote the formation of amyloid protein.
There is no evidence from animal studies that the aggregated proteins in prionoid disorders are infectious like prion proteins. But there is quite strong evidence that the pathogenesis resembles prion protein disease in two respects. First, like prion proteins, misfolded amyloid proteins in these disorders can induce the formation of the amyloid state in the normal proteins so that they aggregate. Secondly, for several of the disorders there is strong evidence for cell-to-cell spreading of the disorder.

Question 7.47

Studies of single gene disorders have sought to draw correlations between the genotypes at a disease locus and the phenotype of the single gene but the genotype-phenotype correlations are often poor. Even within families there may be significant variability in the phenotype of affected members (who are expected or known to have the same genotypes at the disease locus). List three factors that can explain why that should be so.

Answer 7.47

1) *Modifier genes*. The disease locus in single gene disorders makes the predominant genetic contribution, but other modifier genes can be expected to also make a contribution. For example, the β-thalassemia phenotype can be exacerbated by genetic variation at other globin loci, such as the α- and γ-globin loci.
2) *Environmental factors*. They include exposure to certain chemicals and microbes, and dietary factors. For example, the phenotype in phenylketonuria is heavily dependent on the presence of dietary phenylalanine and the treatment for this disorder is based on a low-phenylalanine diet.
3) *Heteroplasmy in mitochondrial disorders*. Due to variability in the proportion of mutant and normal mtDNAs.