Genetics and Genomics in Medicine Chapter 11

Questions & Answers

Multiple Choice Questions

Question 11.1

Interpret the following DNA and amino acid variants:

a) g.410_411insC
b) p.Gly418*
c) c.*62A>T
d) c.142+4C>T

Answer 11.1

a) C inserted between nucleotides 410 and 411
b) The codon specifying glycine at amino acid position 418 has been replaced by a stop codon.
c) The A at position 62 in the 3' untranslated region has been replaced by a T
d) Replacement of C by T at the fourth nucleotide within the intron that follows nucleotide 142 in the cDNA sequence

Question 11.2

Interpret the following DNA and amino acid variants:

a) c.*15A>G
b) p.Asp522del
c) c.-22T>G
d) c.121-6C>A

Answer 11.2

a) Replacement of A by G at nucleotide position 15 in the 3' untranslated region
b) Deletion of aspartate at amino acid position 522.
c) Replacement of T by G at nucleotide position -22 within the 5' untranslated region (the A of the triplet ATG that specifies the initiator codon is counted as +1)
d) Replacement of C by A at the sixth nucleotide within the intron that precedes nucleotide 121 in the cDNA sequence

Question 11.3

Concerning prenatal diagnosis, which, if any, of the following statements is false?

a) It relies on surgical procedures to recover fetal tissues from a pregnant woman.
b) Chorion villus biopsies are typically taken around 16 weeks of gestation.
c) Amniotic fluid samples allow culturing of fetal cells for cytogenetic analyses as well as allowing DNA analyses.
d) There is always a small excess risk of miscarriage.

Answer 11.3

a) It relies on surgical procedures to recover fetal tissues from a pregnant woman.
b) Chorion villus biopsies are typically taken around 16 weeks of gestation.
d) There is always a small excess risk of miscarriage.

Explanation 11.3

Noninvasive techniques that do not involve surgery (and the associated excess risk of miscarriage) are increasingly being adopted. Instead of analysing fetal cells samples of maternal plasma are analysed (they contain small amounts of fetal DNA from fetal cells that had undergone apoptosis). Chorion villus biopsies are typically carried out in the first trimester.

Question 11.4

Concerning preimplantation genetic diagnosis, which, if any, of the following statements is incorrect?

a) It is carried out within the context of assisted reproduction.
b) The analyses always involve genotyping just a single cell and so are technically difficult.
c) Sometime a single blastomere is analysed from the embryo.
d) Sometimes a polar body is analysed to infer the genotype of the embryo.

Answer 11.4

b) The analyses always involve genotyping just a single cell and so are technically difficult.
d) Sometimes a polar body is analysed to infer the genotype of the embryo.

Explanation 11.4

Quite often the embryo is cultured to the late blastocyst stage and a few cells are taken for analysis from the trophectoderm (which will give rise to extra-embryonic membranes). The analyses are still technically difficult, but less so than when trying to analyze a single blastomere. A polar body is a cell formed by one of the two asymmetric cell divisions in female meiosis and can be used to infer the genotype of the *oocyte* (when there is a risk of a maternal transmission of a harmful genetic variant).

Question 11.5

Concerning genetic screening, which, if any, of the following statements is false?

a) Genetic screening is carried out primarily in communities and populations
b) In carrier screening the motivation is to identify carriers of a mutant allele for a severe autosomal recessive disorder that has a high prevalence in the community or population.
c) In most cases of pregnancy screening the motivation is to identify whether a fetus carries a genetic variant associated with a harmful single gene disorder.
d) In newborn screening the motivation is often to target early treatment for serious disorders for which early intervention can make a significant difference.

Answer 11.5

c) In most cases of pregnancy screening the motivation is to identify whether a fetus carries a genetic variant associated with a harmful single gene disorder.

Explanation 11.5

In most cases of pregnancy screening the motivation is to identify maternal age-dependent aneuploidy in the fetus, notably trisomy 21.

Question 11.6

Match the disorder a) to h) with the likelihood that it would be a focus for a genetic procedure listed in i) to vi)

Disorder

a) Huntington disease
b) Trisomy 21
c) phenylketonuria
d) β-thalassemia
e) familial hypercholesterolemia
f) polycystic kidney disease
g) breast cancer due to *BRCA1*
h) Tay-Sachs disease.

Genetic procedure

i) carrier screening
ii) prenatal diagnosis
iii) pre-symptomatic testing
iv) predictive testing
v) pregnancy screening
vi) neonatal screening

Answer 11.6

a) iii)
b) v)
c) vi)
d) i)
e) iii)

f) ii)
g) iv)
h) i)

Fill in the Blanks Questions

Question 11.7

Fill in the blanks below with single words.

Traditional _____1_____ diagnosis has involved _____2_____ surgical procedures in which a sample of fetal cells is recovered and analysed. There have been two major approaches. In one case a sample is taken from the _____3____ (the outermost extra-embryonic ____4_____). Typically this sample is taken in the ____5_____ trimester of pregnancy. In the other case, a sample of _____6_____ fluid is taken that will contain cells from the _____7_____ , an inner extra-embryonic ____4_____. This procedure, called ____8_____ is taken at, or close to, 16 weeks of gestation; it provides fetal cells that can be processed to allow ____9_____ analyses as well as DNA analyses. Because of the surgical procedures involved there is a small excess risk of ____10_____. As a result, there has been a trend to develop non-_____2_____ _____1_____ diagnosis. That has been fuelled by _____11_____ _____12_____ sequencing of DNA from maternal ____13____.

Answer 11.7

1. prenatal. 2. invasive. 3. chorion. 4. membrane. 5. first. 6. amniotic. 7. amnion. 8. amniocentesis. 9. cytogenetic. 10. miscarriage. 11. massively, 12. parallel. 13. plasma.

Question 11.8

Fill in the blanks below with single words.

_____1_____ genetic diagnosis is carried out in the contact of assisted reproduction. It can be technically challenging because quite often analysis is carried out on a ____2_____ cell, that may be from the early stage _____3____ (the stage at which the embryo consists of just a very few cells that are individually called ____4_____). Another alternative, that can be used when the mother is at risk of transmitting a harmful genetic variant, is to infer the genotype of the _____5_____ by analysing a ____6____ ____7_____ (a cell that is created by one of the asymmetric cell divisions in female ____8_____). Because of technical difficulties in analysing a _____2_____ cell, some centers prefer to analyse _____9_____ cells from a late-stage _____3____.

Answer 11.8

1. preimplantation. 2. single. 3. blastocyst. 4. blastomeres. 5. oocyte. 6. polar. 7. body. 8. meiosis. 9. several.

Essay, Listing, and Matching Questions

Question 11.9

What is the basis of the ACCE framework for genetic testing?

Answer 11.9

The ACCE framework was established by the Evaluation of Genomic Applications in Practice and Prevention initiative of the US Centers for Disease Control and Prevention. It gets its name from four aspects of how the test performs, as follows:

- **A**nalytical validity: how well does the test assay measure what it claims to measure? This has two key performance indicators: the *sensitivity* of the test assay (the proportion of all people with the condition who are correctly identified as such by the test assay); and the *specificity* (the proportion of all people who do not have the condition and who are correctly identified as such by the test assay).
- **C**linical validity: how well does the test predict the projected health outcome?
- **C**linical utility: how useful is the test result?
- **E**thical validity: how well does the test meet the expected ethical standards?

Question 11.10

A genetic test for a marker M was carried out in 500 people affected by disease X and showed that 480 of the affected individuals typed positive for the marker. In a suitably matched set of 1000 healthy controls, just 80 people typed positive for marker.

i) Establish the sensitivity and specificity of the test.
ii) Work out the false positive rate and the positive predictive value
iii) Work out the false negative rate and the negative predictive value.

Answer 11.10

The working hypothesis is that there is a strong correlation between marker M and the disease. (If the correlation were to be absolute, the specificity and the sensitivity would each be 100% because everyone who had the disease would type positive for marker M, and everyone who was normal would test negative for marker M).

i) Sensitivity = the number of affected people testing positive/total number of affected people who have been tested = 480/500 = 96%
   Specificity = the number of control individuals testing negative/total number of control individuals = 920/1000 = 92%

ii) False positive rate = the number of control people testing positive/total number of people testing positive = 80/560 = 14.3%
Positive predictive value = 1 – false positive rate = 85.7%

iii) False negative rate = the number of affected people testing negative/total number of people testing negative = 20/940 = 2.1%
Negative predictive value = 1 – false negative rate = 97.9%

Question 11.11

Mutation scanning and mutation testing can be used to identify pathogenic mutations. What is the essential difference between these two approaches?

Answer 11.11

In mutation scanning, the object is to seek out *any* candidate pathogenic mutations in a defined region of DNA (usually an exon or gene), or even in an exome or genome, without having prior knowledge of the nature of the pathogenic mutation(s). For example, DNA sequencing assays can identify changes in a desired DNA region, exome or genome. By looking at how the DNA sequence differs from reference sequences candidate pathogenic mutations can be identified, such as obviously inactivating mutations in coding DNA in the case of recessive disorders.
In mutation testing, the object is to identify a *defined* DNA change *at a specific location* in the DNA, usually a well-studied mutation. For example, we might wish to test a cystic fibrosis patient for the p.Phe508del mutation in the *CFTR* gene.

Question 11.12

Give an outline of the different laboratory approaches to testing for aneuploidies and discuss their relative merits.

Answer 11.12

In the past, the primary approach was cytogenetic analysis using metaphase or prometaphase Giemsa chromosome banding. That approach, which might still be used for identifying triploidy and tetraploidy, is disadvantaged by the significant time and effort it takes and is not amenable to automation.
More recently, fast automated or semi-automated DNA methods have been developed that can be used for this purpose. Array comparative genome hybridization (arrayCGH) uses panels of oligonucleotide probes from defined unique sequences across each of our chromosomes and involves hybridization with a mix of genomic DNA from a test sample and a normal control that have been labelled with different fluorophores to screen for differential fluorescence ratios that would identify the loss or gain of chromosomes. This approach is comparatively expensive and is more suited to scanning for unknown copy number variation across the genome.

The most popular approach is to use quantitative fluorescence PCR. This approach is widely used to screen for the viable autosomal trisomies (trisomy 13, 18, 21) and the sex chromosome aneuploidies. It is fast, largely automated and inexpensive.

Question 11.13

Describe the principles of arrayCGH and its applications in a diagnostic DNA laboratory.

Answer 11.13

In a diagnostic DNA laboratory, the object of arrayCGH is to screen a test sample of genomic DNA for evidence of copy number variation of a large region of DNA (tens of kilobases to tens of megabases). To do this, the test sample is labeled with a fluorophore (often the cyanine CY3) and then mixed with a control genomic DNA sample that has been labeled with a different fluorophore (often CY5). The labeled DNA mixture is denatured and allowed to hybridize to a panel of long single-stranded oligonucleotides that represent unique sequences at known locations from regular intervals on each chromosome and that have been fixed at specific grid positions on a microarray. At each individual grid position on the microarray there will be many identical copies of just one oligonucleotide probe fixed to the array that can bind any complementary sequences in the mixture of labeled DNAs.

If a region of DNA in the test sample has sustained a heterozygous deletion, the fluorescence emitted by the fluorophore in the test sample DNA might therefore be expected to be one-half that of the control sample's fluorophore; if there has been a duplication, the expected ratio is 3:2. The fluorescence ratios for each oligonucleotide marker is then plotted against the position of the marker on a linear chromosome scale so that large deletions and duplications can be identified (because a series of consecutive markers will have aberrant fluorescence ratios at the site of deletions/duplications.

Question 11.14

Why is there still a need for conventional karyotyping using chromosome banding techniques?

Answer 11.14

Conventional karyotyping using chromosome banding is nevertheless still clinically important. That is so because molecular genetic methods such as array CGH are not suited to detecting balanced chromosome rearrangements in which there is no net gain or loss of DNA. Balanced translocations and inversions would normally be invisible to these methods, but they can be detected by chromosome banding.

Question 11.15

What is involved in chromosome fluorescence in situ hybridization?

Answer 11.15

In chromosome FISH (fluorescence *in situ* hybridization) preparations of metaphase or prometaphase chromosomes (where the chromosomes are slightly less contracted than in metaphase and show more detail) are first obtained from donated cells. In the case of preparing metaphase chromosomes from a blood sample, for example, white blood cells are separated and placed in a rich culture medium laced with phytohemagglutinin and allowed to grow for 48-72 hours, then treated with colcemid, a spindle-disrupting agent to produce cells that are arrested at metaphase.

The metaphase or prometaphase chromosome preparations are fixed by air-drying on microscopic slides, and treated with a denaturing agent so that the DNA is separated into single strands that can then hybridize with a fluorescently labeled probe or probes. The locations of the fluorescent signals are recorded against a background stain, such as DAPI, that binds to all DNA sequences. With a unique sequence as the labeled probe, twin fluorescence signals may often be seen at one chromosomal location, representing the contributions made by hybridizing sequences on sister chromatids.

Question 11.16

What are the main uses of chromosome fluorescence in situ hybridization in a genetic service laboratory?

Answer 11.16

Chromosome FISH is often used to confirm regions of chromosome duplication or deletion that have been suggested by other screening methods, such as array CGH or that are known to be strongly associate with a specific condition, such as large deletions on chromosome 22 in the case of DiGeorge syndrome. It can also be used to screen for the amplification of specific oncogenes that are associated with particular types of cancer, such as amplification of the *MYCN* gene in neuroblastoma.

Another major application is in detecting translocations, notably acquired translocations that are common in cancer. (Balanced translocations cannot be identified by array CGH because there is no significant loss or gain of DNA sequences). Recurrent translocations are associated with certain types of cancer; often, the translocation involves breakages in specific genes, producing hybrid genes that are inappropriately expressed. For example, translocations involving the *BCR* gene and the *ABL1* oncogene are common in chronic myeloid leukemia.

Question 11.17

What is involved in Southern blot hybridization?

Answer 11.17

DNA samples, almost always genomic DNA samples, are treated with a suitable restriction endonuclease that cleaves the DNA into double-stranded fragments of a manageable size. The DNA fragments are separated according to size by agarose gel electrophoresis (DNA is negatively charged and will migrate towards the positive electrode through the porous agarose gel; small fragments move faster through the gel because there is less frictional resistance than with the larger fragments).

After electrophoresis, the gel is immersed in strong alkaline solution and the resulting denatured DNA is transferred by capillary action to a nylon membrane that is placed in contact with the gel ("blotting"). The individual DNA fragments become immobilized on the membrane at positions which are a faithful record of the size separation achieved by agarose gel electrophoresis. Subsequently, the immobilized single-stranded test sample DNA sequences are allowed to associate with labeled single-stranded probe DNA. The probe will bind only to highly related DNA sequences in the test sample DNA, and their position on the membrane can be related back to the original gel in order to estimate their size.

Question 11.18

What are the main applications of Southern blot-hydridization as an assay in a genetics service laboratory?

Answer 11.18

The main applications are to scan for large-scale DNA changes that are too big to be readily identified by PCR. Some types of unstable expansions of short oligonucleotide repeats can be quite large. For example, the expanded arrays of CTG or CCTG repeats in myotonic dystrophy can extend to many thousands of repeats. Another example would be testing the array size of D4Z4 repeats in facioscapulohumeral dystrophy where the array size of large D4Z4 repeats can be a contributory factor to pathogenesis.

Question 11.19

The triplet repeat-primed PCR assay is commonly used in analysing samples from individuals with unstable trinucleotide repeat expansions. What is involved in this method?

Answer 11.19

Standard PCR assays are often used to follow modest expansions in oligonucleotide repeats, including those associated with CAG codon expansions in various neurodegenerative disorders such as Huntington disease. For larger expansions, Southern blot hybridization assays have been used, but increasingly a modified PCR reaction is the preferred method. The triplet repeat-primed PCR (TP-PCR assay) uses an external primer (which hybridizes to a sequence flanking the oligonucleotide repeat array) plus a primer that can hybridize to target sequences within the

oligonucleotide repeat array as well as outside it. Because of the tandem repetition, the internal primer can hybridize to multiple possible binding sites within the array, producing a series of peaks of increasing size. Increased sizes are apparent in affected individuals when referenced against controls.

Question 11.20

Multiplex ligation-dependent probe amplifaction (MLPA) is an important technology used in genetics research and genetic testing. What uses is it put to in a genetics service laboratory and what is the basis of the method.

Answer 11.20

Applications. The most frequent application of MLPA in genetics is to scan for copy number variation of exons, notably those producing deletions and duplications. The genes that underlie certain single gene disorders, such as the dystrophin gene, are especially liable to internal deletions and duplications that can result in translational frameshifts and/or remove valuable functional sequences.

MLPA as a single reaction. The basis of the method is that for a target sequence whose copy number is to be tested (such as an individual exon), a pair of probes is designed that (i) will hybridize to adjacent sequences within a specific target (such as an individual exon) and that (ii) carry unique end sequences *not present in the genome*. The aim is to use DNA ligase to seal the left and right probes to give a continuous sequence flanked by the unique non-genome end sequences, and then to amplify the continuous sequence by using primers complementary in sequence to the unique non-genome end sequences.

The point of having a pair of "half-probes" each with a unique non-genome sequence at one end only is to ensure that PCR cannot take place unless both half-probes have *hybridized* (at adjacent positions) to the correct target sequence. Then they can be ligated and PCR can work on the full-length *hybridized probe* only. The amount of PCR product will then reflect the amount of hybridized probe which in turn will reflect the copy number of the target sequence.

MLPA as a multiplex reaction. In practice, it is usual to use probe pairs for multiple different target sequences (such as multiple exons within a gene) that are simultaneously hybridized to their target sequences and ligated to form continuous sequences that are then simultaneously amplified in a multiplex reaction. To do that one of the two "half-probes" also carries a "stuffer" fragment whose length varies from one pair of probes to the next. That is, the point of the stuffer fragment is simply to provide a spacer sequence whose length can be varied. This can ensure different sizes of the PCR products from a multiplex reaction (in which multiple probe sets are used simultaneously) so that the products can be readily separated by capillary gel electrophoresis.

Question 11.21

Explain the principles underlying target-sequence enrichment from a complex nucleic acid population.

Answer 11.21

As well as forming the basis of diverse assays, the specificity of nucleic acid hybridization can also be exploited as a way of *capturing* nucleic acid sequences of interest from a complex starting population. To do that, a panel of probes is designed to represent the types of sequences that we wish to capture, and used as baits to capture complementary sequences from within a complex starting population, such a total genomic DNA.

The probe population may be fixed on microarrays, but solution hybridization has come to be the preferred approach. It uses *biotinylated* probes (carrying a covalently linked biotin group at one end) that can bind specifically to streptavidin-coated magnetized beads (the vitamin biotin and the bacterial protein streptavidin happen to have an extraordinary binding affinity).

To carry out the capture, the complex nucleic acid population is first cut into small fragments and then adaptor sequences are ligated to the ends of the fragments. The fragments are denatured and then mixed with the magnetized beads that have biotinylated probes bound to the outer streptavidin molecules. While bound to the magnetized beads, the oligonucleotides capture complementary sequences from within the complex nucleic acid population. The beads containing the oligonucleotides and their captured target complementary sequences are removed by using a magnet. Thereafter, the captured sequences are eluted by raising the temperature (to break the hydrogen bonds holding them to the biotinylated probes), and then are PCR-amplified using primers that are specific for the adaptor sequences.

Question 11.22

List four different ways in which a known, specific single nucleotide mutation can be detected within a defined exon.

Answer 11.22

1) Amplification refractory mutation system (ARMS)
2) Oligonucleotide ligation assay
3) Pyrosequencing
4) DNA sequencing

Question 11.23

What is meant by cascade testing?

Answer 11.23

Cascade testing means testing of relatives after the identification of a genetic condition in a family. The relatives might be at risk of going on to develop the same single-gene disorder (predictive testing). Unaffected relatives may also be at risk of transmitting a disorder if they carry a harmful allele (heterozygote carriers in recessive disorders, non-penetrance in dominant disorders) or a balanced translocation.

The family and the genetic counselor need to consider different issues. How important is it for the relatives to be made aware of the information on the basis of the severity of the condition and the level of risk of a relative developing the condition, or having a child with the condition? How might the information change things? How easy will it be for family members to pass information on to relatives?

Take the example of a child with multiple malformations and developmental delay who has inherited unbalanced chromosome translocation products from a parent with a balanced translocation. The translocation will be explained to the parents along with information about their future pregnancies, and also the possibility of other family members carrying the same balanced translocation. In addition to addressing questions from the couple about the risk to future pregnancies and about their child's future, health professionals need to consider which additional family members should be contacted who might have the same balanced translocation (and be at risk of producing children with unbalanced translocation products), and how to go about this. The same principles apply to cascade testing for carriers of autosomal or X-linked recessive disorders.

Question 11.24

Prenatal diagnosis involves analysing samples originating from fetal cells (either recovered directly from fetal tissue, or from maternal blood samples). What are the aims of these procedures?

Answer 11.24

Couples who have a family history of a serious genetic disorder usually want to know whether they are at risk themselves of having an affected child. If they are at risk, they might choose not to have children but to adopt instead. More frequently, they wish to have children.

Occasionally, such a couple will choose to have a child in a way that circumvents the genetic risk: by egg or sperm donation, or by preimplantation diagnosis so that only healthy embryos are selected, as described below. More commonly, the couple opt for natural conception and prenatal diagnosis in which the fetus is tested to see if it carries the harmful genetic abnormality, or not. Couples who request this type of diagnosis usually wish to terminate the pregnancy if the fetus is affected. Genetic counseling and risk assessment is important in prenatal diagnosis.

Accurate genetic testing is possible for single-gene disorders in which the major genetic variant contributing to disease has been identified in an affected family member. Prenatal diagnosis is also often carried out in situations in which there is an increased risk of transmitting a

chromosomal aneuploidy (advanced maternal age is an important risk factor). Or one parent might have been identified as a carrier of a balanced translocation, and there is a risk that a fetus with unbalanced translocation products might be viable but have severe problem.

Question 11.25

Traditional prenatal diagnosis has typically meant analysing samples that have been recovered from the developing fetus by some type of invasive procedure. What is involved in these procedures?

Answer 11.25

In the context of prenatal diagnosis, invasive procedures mean surgical collection of fetal tissue, usually by transabdominal approaches guides by ultrasound. Sometimes, a sample is taken from the chorion (the outermost extra-embryonic membrane), and fetal DNA can be isolated from the cells obtained. The sample can be taken any time in the pregnancy from 11 weeks onward, but typically in the first trimester (to allow the possibility of early termination of pregnancy). This procedure is associated with a roughly excess risk of miscarriage.

Amniocentesis is the other major alternative sampling method and also has a small risk of miscarriage. A sample of amniotic fluid is taken at, or close to, 16 weeks of gestation; it provides fetal cells that are processed to give either chromosome preparations to check for chromosome abnormalities, or fetal DNA samples for analysis.

Question 11.26

What is involved in preimplantation genetic diagnosis, and why is it carried out?

Answer 11.26

Preimplantation genetic diagnosis (PGD) is carried out in the context of assisted reproduction (*in vitro* fertilization). Standard assisted reproduction techniques are used to obtain embryos for testing: ovarian stimulation (to produce eggs that are then collected under sedation), addition of sperm, and assessment of the *in vitro* fertilization and of the embryos produced.

Sometimes a single cell (blastomere) is removed from the very early embryo for testing. In that case embryos are grown in culture to reach the 6–10-cell stage; a small hole is made in the zona pellucida and a single cell is removed through the hole for testing. Despite the loss of one cell for analysis, the embryo will go on to develop normally. For technical reasons, some centers prefer instead to allow development to continue to the late blastocyst stage whereupon a few cells taken from the outer trophectoderm (which will give rise to extra-embryonic membranes). Another alternative is to test instead polar bodies (the small cells formed during the two asymmetric cell divisions in female meiosis). By analyzing a polar body it is possible to infer the genotype of an oocyte, which would be relevant in cases where the mother was at risk of transmitting a harmful genetic variant).

There are two broad categories of preimplantation genetic testing: diagnosis and screening. Diagnosis applies to couples who are at risk of transmitting a specific genetic abnormality: one or both parents have previously been shown to carry a harmful mutation or chromosome abnormality that the test is designed to identify. By contrast, screening is performed on couples who may have difficulty in conceiving but have no *known* genetic abnormality. Here, the embryo is screened for the presence of any chromosomal aneuploidy. In both cases, the object is to implant normal embryos only, to avoid the birth of an affected child (in diagnostic cases) or to improve the pregnancy success rate (in screening cases).
For preimplantation genetic diagnosis, prior identification of mutant alleles in one or both parents allows a test in which one or more relevant DNA regions in the DNA from the biopsy are PCR-amplified and sequenced. (If, for any reason, there is difficulty in identifying or assaying a parental mutation, indirect genetic linkage tests can be conducted using a well-established set of polymorphic markers that span the disease gene locus). Occasionally, the test seeks to identify the transmission of a chromosomal abnormality, and involves interphase FISH.
The process of achieving a pregnancy also becomes medicalized (with potential side effects associated with ovarian hyperstimulation). Additionally, the likelihood of a successful pregnancy outcome is quite low: it is only about 1 in 5 at the start of an IVF treatment cycle (some- times no embryos are suitable for transfer, depending on the number of eggs fertilized, and the number and quality of unaffected embryos), but increases to 1 in 3 after embryo transfer.

Question 11.27

With reference to genetic screening, what is the primary motivation for a) pregnancy screening, b) newborn screening, and c) carrier screening, and what types of disorders are involved?

Answer 11.27

a) *Pregnancy screening.* The motivation for the test is to identify whether or not a fetus carries a harmful genetic variant or chromosome abnormality (associated with a severe disorder) in women who are at high risk of transmitting this type of variant/ genetic variant or chromosome abnormality. In most cases, the aim will be to terminate the pregnancy. The disorders can sometimes be serious single gene disorders that occur with high frequency in some communities but often the screening is for aneuploidies, notably trisomy 21 in the case of elderly women who are at high risk of producing a child with trisomy 21.
b) *Newborn screening*. The major motivation is to target early treatment in serious disorders for which early intervention can make a substantial difference and may lead to disease prevention. Genetic screening of newborns began with certain metabolic disorders, such as phenylketonuria, and this class of disorder is still a major focus.
c) *Carrier screening*. The motivation is to identify carriers of a mutant allele for a severe autosomal recessive disorder that has a high prevalence in the community or population, such as in the case of β-thalassemia in many populations. The ultimate aim is disease

prevention: a couple who have both been identified as carriers by the screen can subsequently elect to have prenatal diagnosis and termination of pregnancy for affected fetuses.

Question 11.28

In cancer testing biomarkers based on DNA variants or gene expression profiles are increasingly important. List four types of role that biomarkers can have in cancer testing and illustrate your answer with examples.

Answer 11.28

1) *Diagnostic role*. Identification of the *BCR-ABL1* fusion gene in chronic myelogenous leukemia.
2) *Predictive role*. Identification of gene amplification in the case of the *HER2* oncogene in breast cancer allows prediction of a poor response to anti-HER2 antibody treatment.
3) *Prognostic role*. Identification of *TP53* mutations on chronic myelogenous leukemia or of *BRAF* mutations in metastatic colorectal cancer indicate a poor outcome in both cases.
4) *Disease monitoring role*. Detection of the *BCR-ABL1* fusion gene can be used as a measure of the extent to which residual cancer cells persist after treatment

Question 11.29

Give a summary of the principal anticipated benefits and challenges that might be expected from widespread clinical genome sequencing.

Answer 11.29

- *Benefits.* A major benefit will be that the guesswork can be taken out of drug doses (even now, prescriptions for drugs such as warfarin are usually routinely made on a one-size fits all approach; knowing a person's genome sequence will allow more optimal dosing according to the profile of variants in the key drug-metabolizing enzymes). In the case of infectious disease, there is the prospect of rapid-response sequencing of the genomes of individual pathogen strains within hours of disease outbreaks, allowing rapid, innovative responses to epidemics across the globe.
- *Challenges.* The development of massively parallel genome sequencing has meant that the bottleneck has moved from data acquisition to data analysis. The sheer volume of sequencing data poses challenges for how to both store the data safely and provide a means of rapid ready access to the data while at the sane time maintaining data security and confidentiality.

  When even highly trained geneticists struggle to understand current genome sequencing outputs reports, how can we expect physicians to make sense of them? There is a long way to go, and significant challenges in working out out what all the genetic variants

mean, which will require very significant development of bioinformatics capabilities allied with functional studies.

Question 11.30

What are the main ethical arguments against genetic manipulation of the germ line?

Answer 11.30

Germ-line gene therapy involves making a genetic change to germ-line cells that can be transmitted down the generations. Technical difficulties persist with the efficiency and accuracy of genetic modification of human cells, but even if the technology could be improved so that it was risk-free, and the initial treatment were done with informed consent, later generations would be given no choice in the matter.

If germ-line gene therapy were ever to be attempted, it would most probably be done by genetic manipulation of a preimplantation embryo. A particularly compelling argument against germ-line gene therapy involving genetic modification of the nuclear genome is that it is simply not necessary. Candidate couples would most probably have dominant or recessive Mendelian disorders (recurrence risk 50% and 25%, respectively). Given a dish containing half a dozen IVF embryos from the couple, it would seem bizarre to select the affected ones and then subject them to an uncertain procedure of genetic manipulation, rather than simply select the 50% or 75% of unaffected ones for reimplantation.

Question 11.31

Two important ethical principles that relate to genetic testing in children are the principle of beneficence and the "right to an open future". Explain what is meant by these principles.

Answer 11.31

1) As applied to genetic testing in children, the ethical principle of beneficence requires that any genetic testing of children must be of benefit to the child, rather than be dictated by the needs or wishes of adult relatives. It should test for clinically relevant and *actionable* genetic variants only. That is, if the test identifies a clinically relevant genetic variant there must be some type of clinical intervention that can then be applied to provide benefit to the child.
2) The "right to an open future" is an ethical principle that respects an individual's autonomy. It requires that any information obtained on non-actionable genetic variants which confer susceptibility to late-onset disorders should not be disclosed before the child becomes an adult and subsequently wishes to obtain that information.

Question 11.32

Two ethical controversies relating to preimplantation genetic diagnosis involve sex selection and HLA selection. What are the issues involved?

Answer 11.32

1) *Sex selection*. Screening for Y-linked markers allows the sex of a preimplantation embryo to be identified. Because of cultural preferences for male children in some societies, this can result in biased sex selection when male embryos are preferentially selected for implantation.
2) *HLA selection.* HLA selection occurs when an embryo is selected for implantation to produce a child that can provide HLA-matched transplant tissue to save the life of a sick brother or sister. Some highly publicized cases of HLA selection involve a transplant of stem cells from cord blood to repopulate the bone marrow. This would do no harm to the baby—it would be different if it were proposed that the child should donate a kidney. It is understandable that parents might wish to do all that they can to help their affected child, but knowing that he or she was born primarily to act as a cell donor might harm the psychosocial development of the unaffected child.