Questions and Answers for Genetics and Genomics in Medicine Chapter 11

Question 1

As a genetic test, indirect linkage analyses have mostly been supplanted by direct mutation screening. When are they still sometimes useful? What are the possible drawbacks of using linkage analyses to predict the inheritance of disease alleles, and how can they be minimized?

Answer

When a test has to be done quickly and involves mutation scanning of a complicated gene, indirect linkage analyses are often done. For example, mutations in the *PKHD1* gene cause autosomal recessive polycystic kidney disease. Mutation scanning of the 66 exons in this gene takes considerable time for most diagnostic laboratories, and if there is a question of time pressure (a woman at, say, 15 weeks pregnant, having had a previously affected child whose DNA had not been tested) it may be much easier to carry out indirect linkage analyses, assuming that samples can be obtained from relevant family members.

Linkage analyses with a single linked marker are inevitably inaccurate because of recombination between the marker and disease loci. That can be minimized by using several markers including very closely linked (and sometimes intragenic) markers, including both proximal and distal markers.

Question 2

The table below shows the peak areas for individual markers tested by quantitative fluorescence PCR to assess the possibility of a human trisomy. Numbers within the peak area row are quantitative estimates of the fluorescence recorded for individual peaks at the corresponding marker locus (numbers are separated by a colon if there are two or more peaks). Give an interpretation of the results.

Marker	***D13S268***	***D13S634***	***D13S797***	***D18S386***	***D18S391***	***D18S535***	***D21S11***	***D21S1411***	***D21S1446***
Peak area	12790: 12596	32165	15695: 13894	51670	12557: 28261	31052: 14941	7911: 8267	41294	11098: 10786

Answer

Three of the markers (*D13S634*, *D18S386*, and *D21S1411*) are homozygous and uninformative. Of the remaining markers, the two on chromosome 13 (*D13S268* and *D13S797*) show two alleles with an approximate 1:1 ratio in the areas of the two peaks, as do the two other markers on chromosome 21 (*D21S11* and *D21S1446*). However, the remaining markers on chromosome 18 (*D18S391* and *D18S535*) show two alleles with peak areas that have a ratio of close to 2:1. The interpretation of trisomy 18 would be more secure if other markers on chromosome 18 could confirm the findings, especially if a locus were demonstrated to have three alleles of different sizes that would appear as three peaks.

Question 3
The amplification refractory mutation system (ARMS) is a type of genetic test. What is it used for, and what is the principle on which it is based?

Answer
ARMS is basically an allele-specific PCR assay that is used to distinguish between a normal allele and a mutant allele that differ by a single nucleotide change. It depends on a crucial requirement during the DNA synthesis stage of PCR: there must be perfect base matching at the 3' end nucleotide of a PCR primer. Two alleles can therefore be distinguished by designing oligonucleotide primers to hybridize to the DNA template with the 3' end nucleotide of the primer positioned opposite a nucleotide that differs between the two alleles, and with the sequences of the primers differing at the 3' end nucleotide.

Question 4
Genetic testing for cytosine methylation is important in some diseases. What kind of diseases are tested, and what form does the testing take?

Answer
Diseases in which cytosine methylation contributes to disease are those in which alleles are epigenetically silenced, including cancers, imprinting disorders, and certain other diseases in which expanded repeats are subject to silencing.

Testing for altered cytosine methylation often involves treating DNA with sodium bisulfite, which under carefully controlled conditions converts unmethylated cytosines to uracils; these are converted to thymines during the DNA replication cycles of a subsequent PCR reaction. Methylated cytosines are unaffected, and when sequences containing methylated cytosines are amplified by PCR, the methyl groups are not propagated. In this manner, sequencing of PCR products from amplified regions of sodium bisulfite-treated DNA gives thymines where there was previously an unmethylated cytosine, and cytosines where there was previously a methylated cytosine.

Other approaches use methylation-sensitive restriction enzymes; that is, restriction enzymes that will cleave a specific sequence containing a CpG only if it is unmethylated. For example, the enzyme *Hha*I cuts DNA at the sequence CCGG unless the second cystosine is methylated.

Question 5
What is meant by genetic screening? Illustrate your answer by giving examples of genetic screening with different objectives.

Answer
Genetic testing has traditionally mostly been carried out on affected individuals and their close relatives. Genetic screening, however, describes genetic testing that is carried out at the level of communities and populations and is focused on testing individuals for the presence of a clinically

relevant genetic variant that may cause them to develop symptoms of a serious genetic disorder at a later stage or that they may transmit to future generations. Examples include the following:

- Maternal screening for fetal aneuploidy: notably for trisomies, with the option of termination of pregnancy for a trisomic fetus.
- Newborn screening: screening babies for highly penetrant genetic variants that cause very serious disorders in which early clinical intervention can make a substantial difference, such as phenylketonuria.
- Carrier screening: for communities in which there is a high frequency of a serious recessive disorder. By identifying asymptomatic carriers at an early stage, prenatal diagnosis follow-up can be directed to couples in which both individuals have previously been identified as carriers.

Question 6

How is noninvasive prenatal genetic testing carried out, and how does it compare with standard invasive prenatal genetic testing?

Answer

Standard invasive prenatal genetic testing usually involves the transabdominal retrieval of material for testing, either chorionic villus tissue at an early stage, or amniotic fluid from later pregnancies; the procedures carry a risk of about 1% of miscarriage. Noninvasive prenatal testing involves simply collecting a sample of blood cells from the pregnant woman, and separating and discarding the cells to leave plasma. That is possible because the plasma contains short fragments of DNA that have originated from fetal cells (placental cells undergoing apoptosis) as well as from maternal cells that have been degraded by apoptosis or necrosis.

Massively parallel genome DNA sequencing is so sensitive that it can distinguish fetal sequences from maternal sequences simply by counting the parental haplotypes; maternal haplotypes that are transmitted to the fetus will originate from both maternal and fetal cells and be at significant excess over maternal haplotypes that are not transmitted to the fetus (which can originate only from maternal cells).

Question 7

What is meant by variants of uncertain clinical significance and incidental findings, and why have they increasingly become a problem in genetic testing?

Answer

According to the proportion of the genome that is investigated in genetic scanning, multiple genetic variants can be identified. In the past, when the extent of testing was limited to a tiny fraction of the genome, this was not a problem. More recently, however, as genetic testing is increasingly being extended to exomes and whole genomes, huge numbers of genetic variants are being uncovered. The challenge is to interpret the variants and, as required, to test potentially promising ones to identify those involved in pathogenesis.

The problems are twofold. First, some genetic variants are not readily identified as being pathogenic (missense mutations, mutations in noncoding DNA); when there are hundreds or thousands of genetic variants to sift through, this can be troublesome because there may be many variants whose clinical effects are difficult to predict (variants of uncertain clinical significance). Second, genomewide mutation scanning can incidentally identify potentially pathogenic mutations that are unrelated to the medical reason for ordering the test (incidental findings). For example, genetic variants associated with susceptibility to a late-onset disease may be found in a child whose genome had been sequenced to identify the cause of some pediatric disorder. That raises ethical questions regarding the extent to which the genetic variants found in such a wide mutation scan should be disclosed.