The Royal College of Pathologists of Australasia

Faculty of Science
Sample Medical Genomics Examination Questions and Model Answers

Medical Genomics Part I Written Examination

Question

The following question refers to the pedigree of a Huntington disease family (Figure 1).

a) If testing was performed on the unaffected male (ii.1 - red arrow) what is the most likely result and what principles does this illustrate?

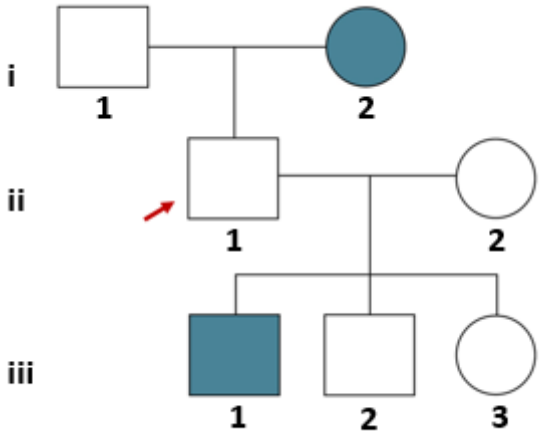


Figure 1. Pedigree

b) Explain the characteristics of Huntington disease (HD). How is it diagnosed?

Figure 2 shows genotyping results of six patients tested for HD.

c) Classify the alleles for each patient below (ie. normal, intermediate, HD-causing etc.) Write a clinical interpretation for patient 1 and patient 3

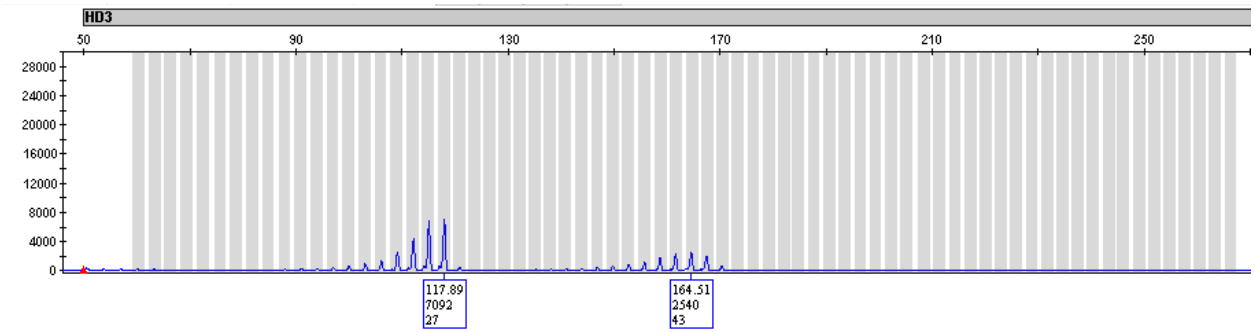
Figure 2 Key:

Top number – Size

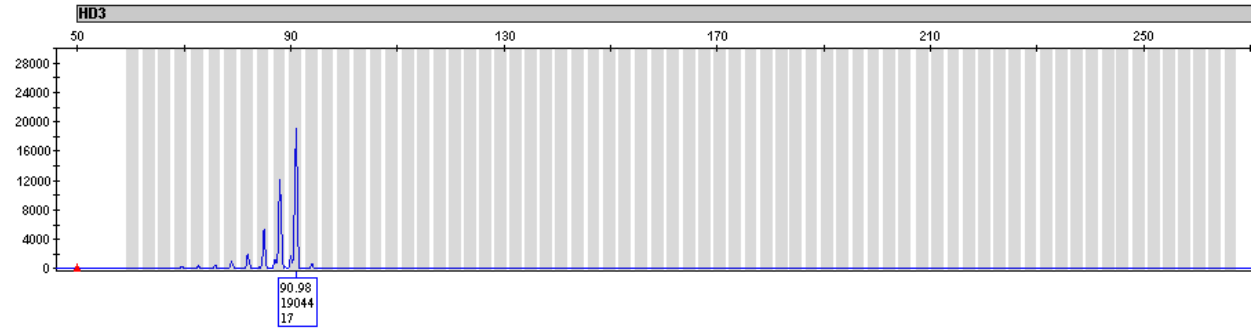
Middle number – Height

Bottom number – Repeat number

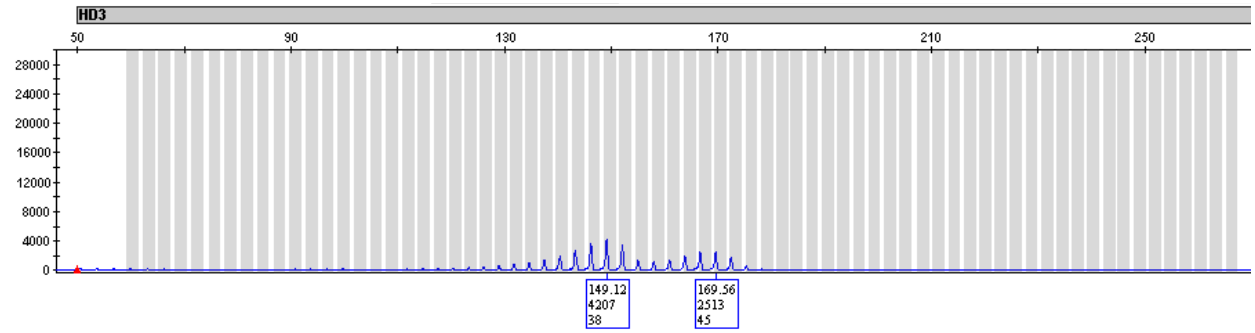
Patient 1



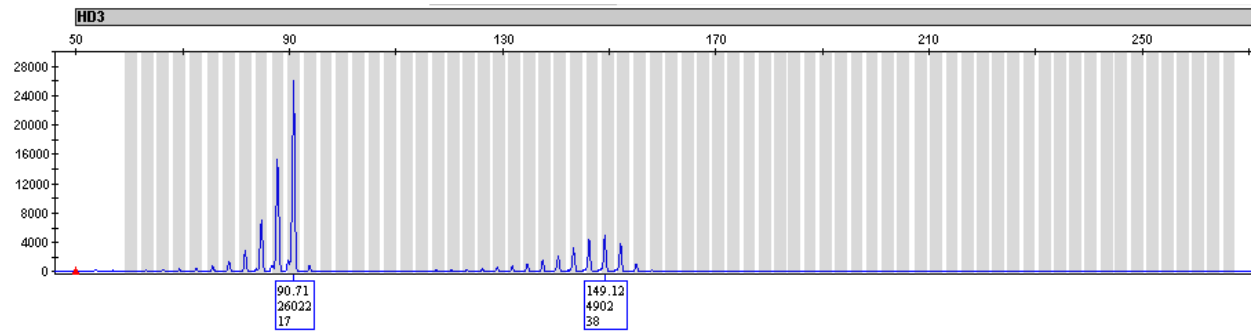
Patient 2



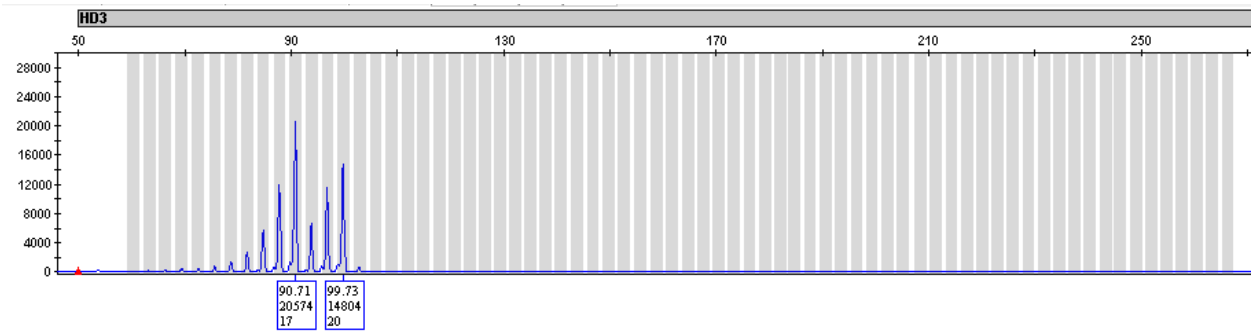
Patient 3



Patient 4



Patient 5



Patient 6

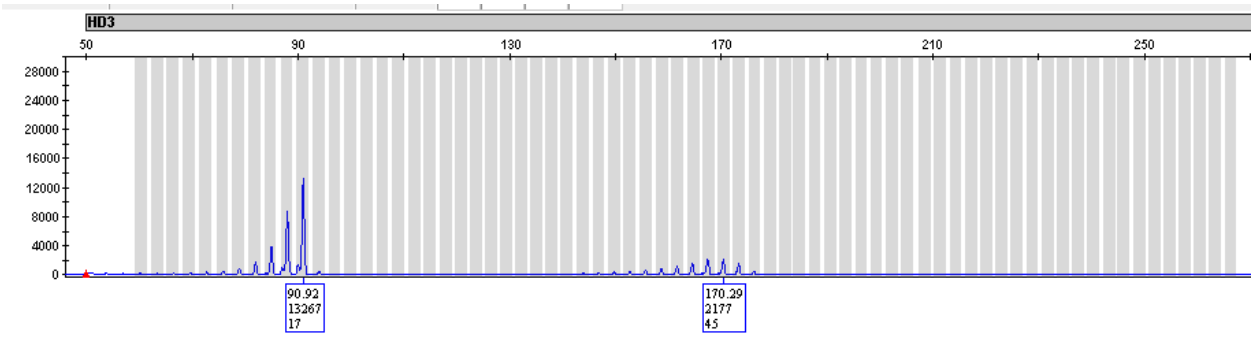


Figure 2. Genotyping results for HD patients.

d) In relation to the risks for HD, write an interpretation for each of the scenarios below (1-5) and provide any recommendation for further testing.

1. heterozygous normal alleles
2. homozygous normal alleles
3. intermediate allele/mutable normal
4. HD reduced penetrance allele
5. HD full penetrance allele

For each scenario, indicate whether you would offer prenatal testing and why?

Answer

a) AD with reduced penetrance, unaffected male is a carrier of the mutation.

b) HD is a progressive disorder of motor, cognitive, and psychiatric disturbances. The mean age of onset is 35 to 44 years and the median survival time is 15 to 18 years after onset. The diagnosis of HD rests on positive family history, characteristic clinical findings, and the detection of an expansion of 36 or more CAG trinucleotide repeats in *HTT* gene.

c) Patient 1: 27,43 (mutable normal and affected range)
Patient 2: 17 ? homozygous normal range (to be confirmed with another assay)
Patient 3: 38,45 (reduced penetrance and affected range)
Patient 4: 17,38 (normal and reduced penetrance range)
Patient 5: 17,20 (normal range)
Patient 6: 17,45 (normal and expanded range)

Clinical intepretation:
Patient 1: This patient has an expanded allele in the *HTT* gene, which confirms the diagnosis of Huntington's Disease. This patient also has a normal allele that falls within the 27-35 repeat range which has a possibility of expansion into the HD affected range during transmission (particularly paternal) to offspring. Therefore, this patient's children have >50% risk of becoming affected with HD. Testing is available to at-risk adult relatives to confirm the diagnosis or for pre-symptomatic testing.

Patient 3: This individual is expected to develop HD. All offspring of this individual would be at risk of inheriting an HD allele due to having a 50% risk of inheriting the fully penetrant HD allele and a 50% risk of inheriting the allele of reduced penetrance which has the possibility of expanding into the fully penetrant affected range during transmission. This result also has implications for siblings as well as other relatives on both maternal and paternal sides of the family. Testing is available to at risk adult relatives to confirm the diagnosis or for pre-symptomatic testing.

d)

1. not at risk of developing HD.
2. require further testing to rule out risk of HD eg. TP-PCR, southern Blot.
3. individual with an allele in this range is not at risk of developing symptoms of HD, but because of instability in the CAG tract, may be at risk of having a child with an allele in the HD-causing range.
4. individual with an allele in this range is at risk for HD but may not develop symptoms.
5. individuals will develop HD with great certainty.

Prenatal testing offered to: 3, 4 and 5 - high risk to offspring.

Medical Genomics Part I Oral Examination

Question

This question refers to Non-invasive prenatal testing (NIPT).

a) What are the differences between diagnostic tests and screening tests? Is NIPT a diagnostic or a screening test?

Your laboratory offers NIPT. You have been asked for advice on an NIPT result by your local maternofetal medicine service. Read the patient scenario below and answer the following questions.

Patient Scenario

A 28-year-old female presented for NIPS at 15 weeks gestation, with clinical indication stating "maternal anxiety". The NIPT report from your laboratory states "High risk for trisomy 13". Subsequent ultrasonography at 16 weeks gestation is reported as "essentially normal". The MFM specialist is puzzled "as we can usually pick up trisomy 13 on a scan", and would like advice.

b) Discuss possible underlying explanations for this patient's results.

c) Of these possibilities, what is the most likely explanation?

d) Are there any investigations you could carry out within your laboratory that may be useful in this situation?

e) The MFM specialist asks about what to do next for this patient. What options could be considered, and what factors should be taken into account when deciding on the best option?

Answer

a)

i. Diagnostic tests are carried out when there is a relatively high prior probability of a condition (e.g. a clinical suspicion), and the test is done to confirm (or exclude) that condition.
ii. Screening tests are generally carried out on patients where there is a lower prior probability/suspicion of the disorder (e.g. a broad patient population). The test is done to identify patients who are at increased risk of the condition, to determine who would potentially benefit from a diagnostic test.
iii. In general, screening tests have high sensitivity for the condition in question (and therefore a low false negative rate) but may have lower specificity (false positives are more acceptable), as the diagnostic test should be offered to patients with high risk on the screening test.

Other characteristics of a good screening test include (WHO/'Wilsons Criteria'):

- Condition should be an important health problem;
- There should be an intervention/treatment for the condition;
- Facilities for diagnosis and treatment should be available;

- There should be a latent/asymptomatic/early stage of the disease;
- There should be a diagnostic test for the condition;
- The screening test should be acceptable to the population;
- The natural history of the disease should be understood;
- The total cost of finding a case should be economically balanced with respect to medical expenditure as a whole.

iv. Diagnostic tests must have high sensitivity and specificity, as they are used to confirm/exclude a diagnosis. In the case of NIPT, the follow-up diagnostic test would be either CVS or amniocentesis (with the caveat for CVS that it too examines placental not fetal cells).

v. NIPT is a screening test (it is also called NIPS, with S standing for 'screening') as it has high but not 100% sensitivity and specificity for the targeted chromosomal abnormalities.
   - Sensitivity is highest for T21 in most NIPT tests, and lowest for T13 and sex chromosome aneuploidy. As the prior probability is typically low for the tested conditions, NIPT's negative predictive value (NPV) is very high.
   - Specificity is >99% for most NIPTs for the targeted chromosomes, but as the prior probability is typically low for the tested conditions, PPV is not as high and is more variable than NPV.

b)

First, note that NIPT tests cfDNA released from the trophoblast of the placenta. With that in mind:

i. The NIPT result may accurately reflect the placental and fetal genotype, but the scan result may be 'false negative' (unusual for trisomy 13 at 16 weeks, but possible);

ii. The NIPT result may reflect the placental genotype, but the fetal genotype may be normal or low-level mosaic – i.e. confined placental mosaicism (CPM I or CPM III);

iii. There may have been an error in the NIPT result – either pre-analytical (sample error), analytical (e.g. specimen swap, run failure) or post-analytical (e.g. transcription error in the report);

iv. NIPT methods are typically based on counting statistics, and the patient's T13 counts may have been at the extreme upper end of the normal range leading to a false positive result (statistical outlier);

v. The patient or fetus may have a large CNV involving chromosome 13, or one of the chromosomes used for normalization in the NIPT algorithm;

vi. There may be maternal mosacisim for T13 e.g. due to maternal malignancy.

c)

i. CPM is a relatively common phenomenon and would be the most likely explanation for these results. NIPT PPV in most studies for T13 is approximately 50%.

ii. The next most likely would probably be 'false negative' scan.

iii. The others are rare (or in the case of lab error, should be rare if the correct laboratory procedures are in place).

d)

i. Go back and check the result for this sample – is it a transcription error.

ii. Check both run and sample QC metrics pass the required thresholds.

iii. Check internal quality control samples gave expected result(s).

iv. Trace tube transfers as much as possible. Start with the collected blood (if still available/stored) to check the correct patient details are labelled on the blood tube.

Follow through all tube transfer steps, which should be traceable in your lab information system/lab records to determine whether there is a possibility of sample swap error at plasma isolation, cfDNA extraction, or during the NIPT assay.

e)

i. Suggest they could consider repeat NIPT, but this is unlikely to give a different result (if there is no indication of a pre-analytical, analytical or post-analytical error as per the investigation above).
ii. One option would be no further testing, depending on the patient's level of concern about T13, and whether they would consider termination of pregnancy if the fetus had T13.
iii. Suggest they could consider other screening tests – discuss whether cFTS was carried out at 11-13 weeks (information not given in question) and if so was risk of T13 elevated? Also consider possibility of second trimester serum screen (quad test). These tests would be non-invasive, and a low risk result would be reassuring (especially as PPV a priori for T13 is approximately 50%, and in a 28-year-old with normal 16-week USS would be lower than this). The availability of second trimester screen may be limited, depending on location.
iv. Another option in addition to 'conventional' screening tests (or instead of) would be detailed ultrasonographic examination for evidence of trisomy 13 at 18-20 week scan. This is again non-invasive, but would require waiting another 2 weeks to obtain the information – depends on level of parental concern/anxiety.
v. The only definitive "answer" would be a diagnostic test, which in this patient would be an amniocentesis, as the patient is 16 weeks gestation (could note that in some ways this is 'better' than a CVS as a follow-up to NIPT, as it directly samples cells of fetal rather than placental origin). The downside of this is the small risk of procedure-related miscarriage, which current estimates suggest is 1/500-1/1000 (versus the often quoted 1/200).
vi. Which of these options is "best" (no further testing, alternative screening modality, detailed structural USS or amniocentesis) depends on the patient's views on T13 (e.g. whether they would consider termination), their views on risk (given the PPV is likely to be low in their circumstances) and their level of concern about getting a result "now" (e.g. second trimester screen or amnio) versus waiting (e.g. 18-week USS).