

CSP. Common Sense Pathology

A REGULAR CASE-BASED SERIES ON PRACTICAL PATHOLOGY FOR GPs

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DNA

genetic testing

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DNA genetic testing

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Introduction

DNA genetic testing has been used in pathology practice for more than 20 years. Despite this, the general practitioner has not had much direct exposure to the DNA genetic test, which has usually been ordered by specialists in clinical genetics. This has come about because:

- (1) A relatively small number of laboratories, usually in the public health system, have been involved;
- (2) No Medicare reimbursement was available until 1998 when the first item number was issued for haemochromatosis (HFE) DNA testing. Today, only six DNA tests associated with venous thrombosis are funded through Medicare: haemochromatosis (HFE); fragile X mental retardation (FMR1); and four enzyme deficiencies (Factor V Leiden, antithrombin III, protein C and protein S), despite the fact that many hundreds of different DNA tests are undertaken;
- (3) On the whole, DNA genetic tests are mostly directed to relatively rare Mendelian genetic disorders to which GPs have little exposure; and
- (4) The necessary requirement for genetic counselling needs expertise, as well as resources, particularly time, to deal with the patient and family. It should be noted that genes (ie, DNA) are shared between family members, so that any DNA test result for a genetic disorder will automatically have implications for other family members.

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Utility of DNA genetic testing

Two unique properties of DNA set genetic DNA tests apart from other pathology tests:

(1) Any tissue can be used, including blood, tissue scrapings and hair follicles, since DNA is the same in all tissues in the body. This also allows DNA to be used for first trimester prenatal diagnosis, as well as testing for various genetic diseases; and

(2) DNA is the same from conception to death. This allows DNA testing to predict the onset of a genetic disorder years before signs or symptoms are present.

A DNA test can be used for many purposes, from confirming a clinical diagnosis or predicting a patient's response to treatment, to predicting the likelihood of developing a disease in the future. Five broad classes of DNA tests can be identified (see Table 1). Some DNA tests are straightforward, eg, diagnostic, while others are more complex, eg, screening and predictive DNA tests. The complexity arises because of the predictive

Table 1: Five classes of DNA tests

Class of DNA test	Explanation	Examples
Diagnostic	Confirms that the patient has a suspected disease. The DNA test in this circumstance is comparable to most other pathology tests, although a positive result has implications for family members.	A case of venous thrombosis in a young patient with no other predisposing factor should be investigated for an underlying cause, including the Factor V Leiden DNA mutation. Finding this mutation provides a possible cause for the venous thrombosis.
Prenatal	Detects a genetic disorder in the fetus or embryo.	In a fetus at risk of a genetic disorder, chorionic villus sampling or amniotic fluid sampling provides a source of fetal DNA. In some circumstances, pre-implantation genetic diagnosis allows a diagnosis to be made in the developing pre-embryo in association with IVF.
Screening	Investigates asymptomatic individuals or populations to determine carriers or those with a genetic predisposition.	Various screening options are available: i. Pregnancy screening; ii. Newborn screening; and iii. Community screening.
Predictive (pre-symptomatic*)	Predicts the development of a genetic disorder in advance of any signs or symptoms.	i. The DNA predictive test for Huntington's disease is used to identify whether an at-risk individual has inherited the mutant or normal gene from an affected parent; and ii. Individuals with familial breast cancer can be tested for BRCA1 and BRCA2 mutations to guide them in future treatment decisions, and identify risks in family members.
Therapeutic (pharmacogenetic)	DNA test involving drug metabolism pathways to predict a patient's response to treatment and so individualise drug dose.	Individuals exposed to anaesthetic agents can develop life-threatening complications such as malignant hyperthermia.

*For simplicity, the terms predictive and presymptomatic DNA testing will be considered as the same.



nature of these tests — there is potential for stigmatisation or discrimination that could arise because a person is clinically “normal”, but the DNA test has indicated a genetic abnormality that could become manifest in the patient or family members at a future date. Although a fifth class has been included (therapeutic, also called pharmacogenetic), this is still in the research phase. The same DNA test can be a diagnostic test in some circumstances (eg, confirming that a patient with a raised ferritin level has haemochromatosis), while at other times the DNA test is predictive (eg, testing the genetic status of an asymptomatic sibling of a patient with confirmed haemochromatosis).

Methodology

There are three components to a DNA test: preparing DNA; amplifying the gene of interest; and identifying a mutation in the gene.

Preparing DNA is simple and routine. There are now kits available that allow a drop of blood to be passed through a column — and out comes the DNA.

Amplifying the gene of interest is undertaken with a test called the polymerase chain reaction (PCR). PCR has revolutionised DNA testing and its impact has been confirmed by the awarding of a Nobel prize eight years after its discovery. It allows a small, specific segment of DNA to be amplified more than a million times.

The final step in the DNA test is to search the amplified DNA for a mutation. This is probably the most difficult of the three steps, and is undertaken with various approaches.

The doctor ordering a DNA test does not have to know the technical intricacies of the methodology. However, it is important to appreciate that a DNA test is also a pathology test and therefore subject to the usual constraints of testing (ie, sample mislabelling at collection or laboratory processing, other clerical errors, etc). Finally, the PCR technique is so powerful in amplifying DNA that it will also amplify any contaminating DNA. Therefore, a DNA test is not infallible. This is particularly important to appreciate with genetic DNA tests because, apart from the diagnostic class of test, all the other classes involve patients who are asymptomatic. Therefore, there are generally no clinical clues available to inform the doctor that the test result may not be correct.

My own laboratory recommends that any DNA test that is important and has implications for the patient and family members (most DNA tests would fit into this) should be repeated with a separate blood sample to avoid, at least, the potential for mislabelling and avoidable errors.

Haemochromatosis DNA testing

Of all the DNA genetic tests available, the one most likely to be ordered by the GP is the HFE test for haemochromatosis. HFE is the name of the haemochromatosis gene that was identified in 1996. Three mutations have been found in this gene and they are implicated to varying degrees with the development of clinical haemochromatosis. The three mutations are: Cys282Tyr (also written C282Y), His63Asp (H63D) and Ser65Cys (S65C) (see Table 2).

Although haemochromatosis is an autosomal, recessive genetic disorder (therefore both HFE genes need to be mutated before the disease can develop), its pathogenesis and natural history is not straightforward. Environmental factors (diet), sex (women are less likely to develop haemochromatosis because they lose iron at menstruation) and genetic factors all play a role in the development of clinical haemochromatosis with its varied clinical features. Therefore, it is important to distinguish genetic haemochromatosis (ie, an individual who is positive for the HFE mutations and so at risk of developing clinical haemochromatosis, but not guaranteed to do so because of the other factors involved) from the person who develops clinical or symptomatic haemochromatosis. It is necessary to appreciate this distinction when providing counselling (see cases below).

The indications for ordering haemochromatosis DNA tests are listed in Table 3. Note that if the test is to be funded through Medicare, it must satisfy certain prerequisites.



Table 2: Clinical significance of HFE mutations

Genotype (ie, DNA mutation status)	Phenotype (ie, clinical or laboratory features)
Heterozygosity for any of the three mutations	No clinical significance to the patient
Homozygous C282Y*	This is the usual mutation found in patients with clinical haemochromatosis. If present in a patient with features of haemochromatosis, it confirms the diagnosis.
Compound heterozygote for C282Y and H63D*	This is another combination associated with the development of clinical haemochromatosis, although the severity in this case is considerably less than what is seen with homozygous C282Y
Homozygous H63D and the S65C* mutation alone, or in combination with other HFE mutations	There is some doubt about the significance of these mutations in clinical haemochromatosis. It is best to seek expert advice about the implications of these test results for individual cases.

*Abbreviations: C282Y – cysteine is replaced by tyrosine at amino acid 282. Histidine is replaced by aspartic acid at position 63 and serine is replaced by cysteine at position 65.

Table 3: Indications for ordering HFE DNA testing

Class of test	Clinical or laboratory scenario	Medicare prerequisites
Diagnostic	Patient has clinical or laboratory features of haemochromatosis	The patient has an elevated transferrin saturation or elevated serum ferritin on testing of repeated specimens
Predictive	Testing first-degree family members who do not have clinical or laboratory features of haemochromatosis	The patient has first-degree relative with: i. Clinical haemochromatosis; ii. Homozygosity for C282Y, or a compound heterozygote for HFE mutations

Case study 1

A 50-year-old man of Celtic ethnic origin comes to see you because he is tired. You suspect haemochromatosis and order a serum ferritin, which is elevated at 2000 µg/L. This is confirmed on repeated testing. You next order a HFE DNA test, which shows the presence of homozygous C282Y.

Question 1: What are the implications of the HFE DNA test result for the patient?

The clinical suspicion has now been confirmed. Treatment should start. Liver biopsy is indicated only if it is necessary to assess the patient for liver damage. Specialist referral is indicated because life-long treatment will be needed. There remains a risk for complications, such as cirrhosis and hepatocellular carcinoma.

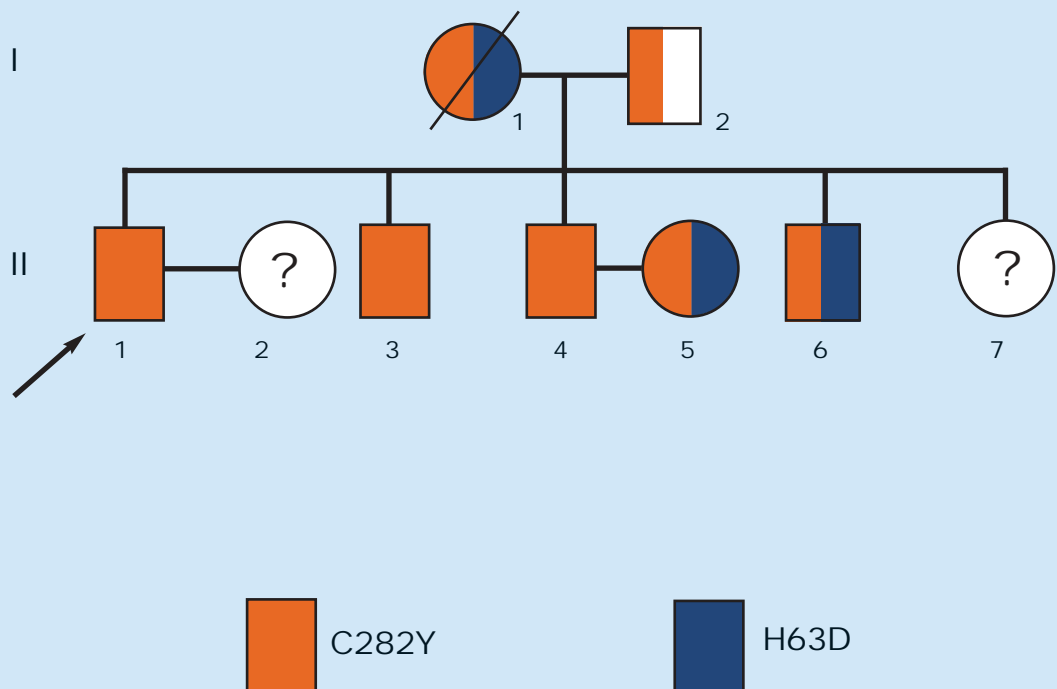


Question 2: What are the implications of the HFE DNA test for a patient's family?

There are several options here. Relatives could be screened with ferritin levels, or alternatively gene HFE testing. In this particular family (Figure 1), the patient with DNA proven clinical haemochromatosis who came to see you is II-1 (→). He has three brothers and one sister. After the family has agreed to predictive DNA testing, it is evident that two brothers (II-3 and II-4) have genetic haemochromatosis. These two should be followed carefully with regular estimations of serum ferritin. A third brother (II-6) is a compound heterozygote, and so at lesser risk, but should also be followed. One sister (II-7) has refused testing and so her genetic status is unknown, although she is at less risk of clinical haemochromatosis because of her sex. Incidental findings in this family include, firstly, II-5, the wife of II-4, also having genetic haemochromatosis. She needs her ferritin levels checked. All children of II-4 will have genetic haemochromatosis. They should be warned about this, especially the male children. A second finding is that I-2, the living father of II-1, is heterozygous for C282Y and therefore the deceased mother must have been a double heterozygote for C282Y and H63D. This can be concluded even without a DNA test (II-6 could have inherited the H63D mutation only from her) and she must also have been heterozygous for C282Y to have sons who are homozygous for this mutation.

Figure 1: Haemochromatosis family and DNA testing

Interestingly, DNA testing of II-5 would not have been funded by Medicare because this individual does not satisfy the prerequisites for an HFE DNA test (Table 2). The risks for genetic haemochromatosis in the children of II-1 (your patient) cannot be accurately determined without testing the mother (II-2), who is not eligible for Medicare benefits for an HFE DNA test. At present, all that can be said is that all children of II-1 are at least obligatory carriers of the C282Y mutation.





Case study 2

Another 50-year-old man, this time of Greek ethnic background, comes to see you a week later, presenting with tiredness. Again, you suspect haemochromatosis, but on this occasion the ferritin level is marginally raised, even on repeat testing. You order the HFE DNA test and the result comes back normal, ie, no mutation found.

What are the implications of this DNA test result for the patient?

Interpretation of this DNA result is more difficult because of the ethnic background of the patient. The C282Y mutation is more commonly found in those of north-western European background, so a negative finding in a patient of southern Mediterranean origin does not necessarily exclude genetic haemochromatosis. As with other DNA genetic tests, a positive result is much more meaningful than a negative one. Clinical haemochromatosis is not excluded by the DNA test and the patient requires further investigation.

Future challenges

Methodology: the methodologies of testing are improving continually, making DNA testing more versatile, and GPs will be faced with an increasing number of options for DNA tests.

Role of the GP and specialists in DNA testing: rare diseases, including those for which predictive testing is being sought, will continue to remain restricted to referral by genetics specialists. However, common diseases or those diseases requiring long-term follow-up require the GP to be involved in the DNA testing in some capacity.

Duty of care: this is an interesting medicolegal dilemma that remains unresolved. Because DNA is shared with other family members, where does the doctor's duty of care end in terms of advising or warning others about their risks? In the US, one judge has ruled that the doctor's duty of care stops with the patient and that advising the patient that the family is at risk is sufficient. However, this judgment was reversed on appeal.

Continuing education: information about new genes and their effects on health and wellbeing will continue to emerge as information gained from the Human Genome Project becomes translated into knowledge about how genes work. This will be a challenge for continuing education and, in the case of DNA-based genetics, it will be necessary to use Internet and computer-based resources to gain regular updates.

Sources

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