LETTERS TO THE EDITOR

Polymerase chain reaction (PCR) on fixed necropsy material

Te Meerman et al1 reported prenatal diagnosis of cystic fibrosis for a couple where the affected child had died by use of DNA markers in linkage disequilibrium with the CF locus. In many instances, however, where a person with a single gene disorder has died from or because of complications of their inherited disorder, surgical or necropsy formalin fixed, paraffin embedded histological specimens may be available. The report of PCR amplification, Southern blotting of restriction digests, and hybridisation using oligonucleotide probes to the β globin gene from such specimens2 prompted us to try and use PCR3 in circumstances where such material was the only potential source of DNA available.

A couple was referred for genetic counselling, having lost a child in the very early newborn period from surgical complications of meconium ileus. Surgical specimens obtained at the time of surgery were the only material available for DNA analysis from the child. DNA was extracted from blood samples from the parents by conventional means: 5μ paraffin embedded tissue sections of bowel were dewaxed, suspended in solution, and incubated for five days in 0.1 μg/ml Proteinase K. Subsequent extraction of DNA was by phenol/chloroform/isoamyl alcohol followed by chloroform/isoamyl alcohol. DNA from the blood samples and the tissue sections were incubated for 35 cycles of the polymerase chain reaction (PCR) using primers to the closely linked markers XV2.c, met H, CS.7, KM19, and pJ3.11 at the D7S23 locus. The reaction products were digested with TaqI, MspI, HhaI, and PstI to detect the respective restriction fragment length polymorphisms.

The genotypes for both parents and the affected child are fully informative for prenatal diagnosis with CS.7/HhaI (figure) given the caveat that not all children with meconium ileus necessarily have cystic fibrosis.

It is of interest that all the DNA sequences amplified from the tissue section satisfactorily apart from that for KM19. The DNA sequence that one is attempting to amplify with the primers for KM19 is longer than those with the other primers used and there may be a limit to the average length of intact DNA in histological samples processed in this manner.

The use of the polymerase chain reaction to look at DNA sequences in surgical or necropsy material will have important routine and research applications.

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1 Te Meerman GJ, ten Kate LP, Cobben JM, von Essen T, Buys CHCM, Haley D. Prenatal diagnosis of cystic fibrosis where single affected child has died. Lancet 1988;i:745.
2 Impraim GC, Saiki RK, Erlich HA,


Huntington's disease testing: what and what not to tell

Millan et al5 may be getting on to somewhat of a slippery slope in imagining that they might 'leak' good news about the patient at risk, but withhold bad news, that they happen upon in prenatal exclusion studies for Huntington's disease. In the case of good news, the couple are getting a bonus beyond what they had expected. Great for them: but others in the same boat, through informal and formal Huntington family links, might well get to learn about their happy situation. Those who don't get, or hadn't previously got, good news—and whose 'contract' had been just for testing for prenatal exclusion—may well draw an unfortunate conclusion about their own status. The moral of the story is that, for the greater good, explicit contracts should be made and they should be stuck to.

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Options for prenatal testing for Huntington's disease using linked DNA probes

Fahy et al discuss four approaches to prenatal testing for Huntington's disease (HD), which depend on the risk status and desires of the parent at risk. The first is the familiar prenatal exclusion testing option,2-5 where the parent at risk seeks to minimise the chance of passing the HD gene to the fetus without changing his or her own risk status. The second and fourth approaches are examples of standard prenatal diagnosis for couples where the at risk partner's status has been clarified either by presymptomatic testing or by early signs of the disease itself. In the third approach, termed 'exclusion-definitive' testing, the initial prenatal exclusion test determines subsequent action. If the fetus has not inherited the HD gene, no further action is taken, the usual practice in prenatal exclusion testing. However, if transmission of the HD gene to the fetus cannot be excluded, a definitive test on the fetus is offered. The attraction of this approach is that it avoids termination of pregnancies where the fetus has a 50% chance of being normal. The disadvantage is that the parent at risk can no longer remain unaware of his or her own risk status.

We have recently been involved in several cases that lead us to propose another approach to prenatal testing for HD. There are two situations which differ slightly. In the first, the parent at risk is homozygous for all linked DNA markers and the HD chromosome cannot be distinguished from the normal chromosome. The usual procedure would be to advise the mother that no prenatal exclusion test is possible and that her child will have a 25% risk of inheriting HD. However, it sometimes happens that linkage data are available for other members of the family, perhaps acquired through research programmes or because someone else in the family has enrolled in presymptomatic testing. If these data indicate that the consultant does not carry the HD gene, we believe that it might be good clinical management to provide this information even when it represents an unsolicited presymptomatic test.

In the second situation, represented in the figure, heterozygosity of markers in the consultand makes it easy to do a prenatal exclusion test on the fetus. Again, it is possible that other family data may be available that substantially alter the risks of the mother transmitting the HD gene. For example, in the family shown in the figure, the HD gene is segregating with the 17:5 kb fragment at the D4S10 locus,6 and the consultand has not inherited this allele. Thus, if she were to have a chorionic villus biopsy, a 17:5/15:0 genotype in the fetus would carry a risk of about 4% of HD. A 17:5/17:5 genotype would carry a risk of only 0.16%.

Against these risks must be set the risk related to the procedure of chorionic villus sampling (CVS), which is of the order of 2%.

In our centre the established presymptomatic protocol requires that additional linkage data acquired through research be regarded as confidential. We would therefore carry out the CVS and then advise the mother of a low risk of HD in the fetus. However, we have recently become dissatisfied at this rather mechanical attitude to the problem. It seems to us that the decision as to whether to proceed with a CVS when fetal risks and procedure risks are nearly matched is one that must be taken by the parents and not by their medical advisers. Thus, in this situation, we are also inclined to provide an unsolicited presymptomatic test to the consultand.

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Pedigree illustrating the problem of prenatal exclusion testing when the consultand's carrier risk is known to be low. RFLPs at HindIII (site 1) for the D4S10 locus are shown.