Prenatal exclusion testing for Huntington's disease: a problem of too much information

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SUMMARY At eight weeks of pregnancy a couple were informed that the prospective father's mother had died of Huntington's disease (HD). There were no living affected members in the immediate family to confirm the diagnosis. By inspection of the local genetic register, it was established that it was indeed HD segregating in the extended family.

Genotyping of the prospective mother and father, the father's unaffected father, and his unaffected maternal grandmother was carried out using a battery of polymorphic DNA markers, including a new probe which has a very low recombination rate with the HD locus. Analysis of DNA from a chorionic villus sample taken at 10 weeks of pregnancy showed that the fetus must have inherited a chromosome from its father's affected mother. Its risk of developing HD was 47%. If the genotype of the unaffected maternal grandmother was taken into account, the risk was reduced to 42%. Neither risk was considered acceptable by the prospective parents and the pregnancy was terminated at 12 weeks' gestation. Prospects for future pregnancies are good, with a 50% chance of having a child whose risk of inheriting the HD gene is less than 1.5%.

In retrospect it was noted that although genotyping of the maternal grandmother had refined the fetal risk, it had also nearly contributed to an inadvertent and unwanted predictive test for HD on the father. This case makes the point that in prenatal exclusion testing, linkage information must be generated with considerable care.

The general principles of prenatal exclusion testing for Huntington's disease (HD), using linked DNA markers, have been outlined by Harper.¹ The test may be applied to pregnancies where either the father or the mother has a 50% risk of developing HD, because he or she has an affected parent. The purpose of the test is to minimise (or 'exclude') the chance of the HD gene being passed to the fetus, and to do this without changing the status of the parent at risk. If no prenatal exclusion can be made, the couple would be counselled to consider an early abortion in the hope that in a subsequent pregnancy their luck would change.

In the examples of prenatal testing for HD which have been reported,^{2,3} probes were used at the *D4S10* locus which has a recombination rate with

the HD locus of about 5%.² Recently, Gilliam *et al*⁴ have described new DNA markers at a locus (*D4S43*) within 0 to 1.5 cM of the genetic defect. A conservative estimate of recombination for these probes is 2%.

In the case reported here, the risk of HD was only discovered when the mother was already pregnant. Furthermore, there were no living affected members in the immediate family, so that phase had to be established rapidly from unaffected relatives. After genotyping a chorionic villus sample with probes from the *D4S10* and *D4S43* loci, the chance of the fetus inheriting the HD gene could not be excluded, and the pregnancy was terminated.

Patients and methods

The proband (figure, III.2) is a woman in her late twenties, who discovered when she was eight weeks

pregnant that her husband's mother had died of HD in 1981. This woman (II.1) had been in hospital since her son was quite young and he was unaware of the family history of HD. Information from the family and from examination of our genetic registers showed that the family was part of a large kindred in which HD was segregating, but that because of the compounding effect of recombination the nearest affected living relative (a first cousin of II.1) was unlikely to be useful in linkage analysis.

FIGURE Genotypes of the family in which HD was segregating. Affected members are shaded. Haplotypes could not be established in the R7/HindIII system for II.2 or III.1 and are indicated by a slash.

As it seemed possible that a prenatal exclusion of the HD gene could be achieved by analysis of the unaffected members of the immediate family, a transcervical chorionic villus biopsy was carried out at 10 weeks' gestation by ultrasound dating. At the same time blood samples were taken from the prospective father and mother, the father's father (II.2), and his grandmother (I.2).

DNA was extracted from blood and from the CVS by standard methods.⁵ As only enough DNA was available from the villus sample for two or three enzymatic digestions, initial analyses were carried out on the blood samples using the probes and enzymes listed in the table. From these data it was apparent that full information could be obtained on the CVS using a combination of pK083 and EcoRI digestion and pS7 and TaqI digestion.

Risks were calculated using the program LINKAGE.⁷ The recombination fraction between the D4S10 and HD loci was taken as 5%,² and between the D4S43 and HD loci as 2%.⁴ The probability of III.1 showing clinical signs of HD at the age of 25 was estimated as 5%.⁸ Allele frequencies were as shown in the table. The order of loci was assumed to be HD-D4S43-D4S10.⁴

Results

Genotypes found in the blood samples of the four members of the family are shown in the figure. Each of the four was homozygous for the 5.5 kb band in the R7/PstI system, and this marker was therefore discarded. The at risk father (III.1) was heterozygous at site 2 of the R7/HindIII system and also for the pK083/EcoRI and S7/TaqI systems. However, the HindIII digestion of the chorionic villus sample failed, so that fetal analysis was based on the last two systems.

The fetus was homozygous for both the 14.5 kb band detected by pK083/EcoRI and the 2.3 kb band detected by S7/TaqI. Thus, the chromosome inherited from the at risk father bears haplotype 14.5, 2.3. This chromosome must come from the affected grandmother (II.1), since it is not present in

TABLE Polymorphisms used in this study.

Locus	Probe	Enzyme	Fragment size (kb)		Allele frequency		Polymorphism information (PIC)*
			Allele 1	Allele 2	Allele 1	Allele 2	
D4S10	pR7	HindIII site 1	17.5	15.0	0.75	0.25	0.305
		HindIII site 2	4.9	3.7	0.13	0.87	0.201
	pR7	PstI	5.5	2.4	0.86	0.14	0.212
	pK083	EcoRI	14.5	9.1	0.5	0.5	0.375
D4S43	pS7	TaqI	3.4	2.3	0.19	0.81	0.26

*Calculated as in reference 6.

the unaffected grandfather (II.2). On this basis, and ignoring recombination, the fetus had the same 50% risk as its father of inheriting the HD gene. The risk was modified to 47% when the effects of recombination and allele frequency were included in the calculation.

If the genotype of the great grandmother (I.2) is considered, it can be seen that she also carries a chromosome with haplotype 14.5, 2.3. This modifies to some extent the risk of HD in the fetus. When the possibilities of recombination between the two different marker loci and the HD gene are included in the calculation, and allowance made for allele frequencies, the final risk to the fetus is 42%.

Discussion

The case presented here shows that it is possible to carry out rapid prenatal exclusion testing for HD even when there are no affected subjects in the immediate family available for genotyping with linked DNA markers. It was obviously important to be able to confirm that it was indeed HD segregating in this family, since this fact had been concealed from the at risk father until after his wife became pregnant. The availability of a genetic register,⁹ containing the details of many Scottish HD families, allowed this key factor to be established.

Although HD could not be excluded in the fetus, and although the pregnancy was terminated, in subsequent pregnancies the couple will have a 50% chance of producing a child with a very low risk of carrying the HD gene. For fetal genotypes 14.5/9.1, 3.4/2.3 or 9.1/9.1, 3.4/2.3 the risks will be 1.0% and 1.4%, respectively. This indicates the advantage of using a DNA probe with a very low recombination rate with the HD gene.

The analyses carried out here had to be undertaken in some haste. In retrospect we realise that it was a mistake to have genotyped blood from the great grandmother (I.2). Although it allowed the risk to the fetus to be refined somewhat, it did not increase the chance of excluding transmission of the HD gene. Furthermore, if the fetal genotype had turned out to be 14.5/14.5, 3.4/2.3, we would have inadvertently shown that (barring recombination) the at risk father was actually carrying the HD gene. Such information was not sought by the father and our possession of it would have created a considerable problem in genetic counselling. Fortunately, now that the haplotype of the father's at risk chromosome has been shown to be 14.5, 2.3, this possibility cannot arise in subsequent pregnancies. There are a number of lessons from this experience

for those involved in prenatal testing for HD.

(1) It is obviously possible to complete DNA analyses on high risk families rapidly and to make predictions with confidence, provided that one is sure that no error in the original diagnosis has been made. The availability of a genetic register is critical; our register connected the limited pedigree shown in the figure to a large kindred with at least eight confirmed cases of HD.

(2) It is also important, whatever the degree of haste involved, not to acquire more linkage information than the minimum necessary. If the father had turned out to be affected we would probably have withheld that information, but would have been very uncomfortable in so doing. If, on the other hand, the father had been unaffected, we would almost certainly have communicated the good news.

(3) Now that a new generation of probes is available,^{4, 10} tightly linked to the HD gene, those at the *D4S10* locus should be phased out as soon as possible.

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