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Huntington's Disease: Prediction and Prevention [and Discussion]

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Huntington's disease: prediction and prevention

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The identification of a DNA restriction fragment length polymorphism closely linked to Huntington's disease on the short arm of chromosome 4 has for the first time allowed presymptomatic prediction to be undertaken in first-degree relatives at risk. The late and variable onset of this dominantly inherited disorder makes such prediction a powerful and potentially valuable aid in genetic counselling, but in the absence of effective therapy there are serious ethical reservations concerning such a predictive test.

The new developments have stimulated an active and informative debate among professionals and family members on whether and how predictive tests should be used. Guidelines have emerged which should be useful not only for Huntington's disease, but for other serious late-onset neurogenetic disorders. Meanwhile, studies in Wales and elsewhere have not only confirmed the original linkage but have excluded multi-locus heterogeneity as a significant problem. Genetic prediction for the individual at risk remains critically dependent on a suitable family structure, present in only a minority of families in Wales. A more feasible alternative for most families is prenatal exclusion, which can allow risk prediction for a pregnancy without altering the situation for the person at risk. This approach has already been applied in Wales; the experience gained will be useful in full prediction, which is currently being introduced.

Introduction

Huntington's disease (HD) one of the most severe and frequent human neurodegenerative disorders, has been well delineated, both clinically and pathologically, for many years. Its inherited nature was recognized by George Huntington in his original description of the disorder more than a century ago (Huntington 1872); it follows a typical autosomal dominant pattern, with penetrance of the gene close to 100% by old age but low during the years of reproductive life.

A characteristic pattern of neuronal degeneration affecting parts of the basal ganglia and cerebral cortex has been documented (Bruyn 1968); however, despite much research, no primary biochemical defect responsible has yet been identified (Comings 1981).

Because most patients with HD have inherited the abnormal gene from a parent, the disorder is potentially a preventable one by reducing the number of abnormal genes transmitted. Until recently, however, the only way this might be achieved has been by a general limitation of family size of those at risk. Not only is no primary biochemical abnormality known, but no secondary changes in cerebral structure, function or metabolism have been found which can reliably detect the presence of the HD gene in young individuals at genetic risk who might desire this information before making decisions on reproduction (Klawans *et al.* 1980).

The localization of the HD gene to a site on the short arm of chromosome 4 (Gusella *et al.*

1983) promises to change this situation radically, certainly in terms of genetic counselling for the individual at risk and possibly for the wider prevention of the disorder in the population.

This paper examines the implications of these new developments, as seen in a stable and well-studied population followed over a prolonged period.

Huntington's disease in south Wales

Wales, with a population of almost three million and an annual birth rate of just over 30000, forms a single health region, with a well-developed regional medical genetics service covering all parts of Wales, coordinated from the Medical Teaching Centre in the capital, Cardiff. Industrial south Wales forms a compact and densely populated area around Cardiff, with 1.7 million inhabitants. This area has been the subject of a systematic, prospective population study of HD for the past fifteen years (Walker *et al.* 1983; Harper 1986), not only aiming at total ascertainment in this specific geographical area, but also aiming to trace extended families, to provide practical medical and social support, and to offer non-directive genetic counselling to all those at significant risk of developing the disorder (Tyler *et al.* 1983).

A particular feature of this study has been the development of a genetic register for HD (Harper *et al.* 1982), whose form has been adapted with advances in computer technology (Sarfarazi *et al.* 1987), and which has served as the basis both for organizing the services provided and for monitoring trends in prevalence and birth rate in the population. Table 1 shows some of the basic data for south Wales from this register, updated to 1986; the prevalence represents a small but significant increase on previous published data from this area. This increase indicates that, even when ascertainment is considered complete, some HD families will be overlooked.

Table 1. Huntington's disease in south Wales: basic epidemiological data

(Figures updated to 1986.)

total individuals on register	2748
known kindreds with HD	150
living affected individuals	120
living individuals at risk (risk > 10%)	1307
disease prevalence	8.5×10^{-5}
heterozygote prevalence	30.5×10^{-5}

The prevalence of HD in south Wales (Walker *et al.* 1981) is among the highest recorded in European and North American populations, although to what extent this reflects intensity of study is uncertain. Most other population studies have given prevalence values of between 4 and 7 per 100000. Of more direct importance for the initiation and organization of preventive measures, however, are the figures shown in table 1 for the number of individuals at risk for the disorder. Only those whose age-adjusted risk exceeds 10% are shown in this estimate, all having a prior genetic risk of 50% or 25% (i.e. an affected parent or grandparent). Figure 1 shows the life table curves on which these risk estimates are based (Newcombe 1981). Because reproduction by those with recognized HD is rare, it is through these individuals that the gene will be predominantly transmitted. They will be the ones for whom genetic counselling is relevant, both in terms of individual decisions concerning childbearing and also in terms of future changes in prevalence of the disorder.

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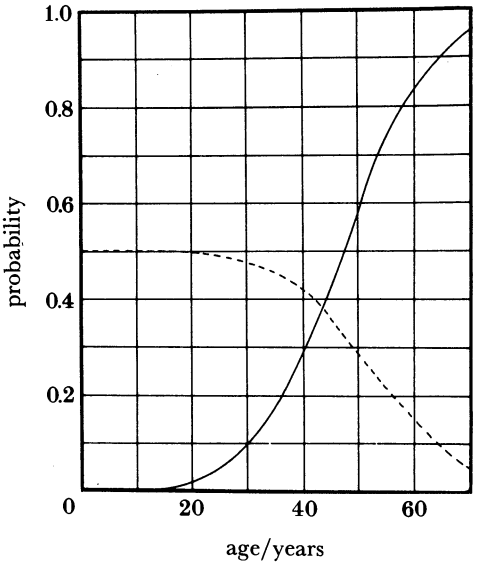

FIGURE 1. Age-related genetic risks in HD. The solid line represents the cumulative age of onset of HD modified by the life-table method of Newcombe (1981). The dotted line represents the chance that an individual born at 50% risk will develop HD if he or she is asymptomatic at a given age.

The principal aims of the south Wales study were to identify those family members at significant risk and to offer them accurate and non-directive genetic counselling before they embarked on childbearing. Until this study there was little information available on the attitudes of HD relatives to genetic counselling or on the way in which it actually affected their reproductive behaviour. Carter & Evans (Carter *et al.* 1979) showed a reduction in family size among those attending a genetic counselling clinic because of HD, but this was a highly selected and motivated group, not comparable to an entire population at risk. Successive analysis of the births in south Wales at risk for HD based on our register has shown a progressive decline over the past two decades, both in absolute numbers and when compared with the general population trend (Harper *et al.* 1979, 1981). Figure 2 shows the recently updated figures which confirm that the number of HD genes entering the population has more than halved during this period, even allowing for the additional ascertainment of cases and family members during the course of the study.

At first sight it might appear from these results that population prevention of Huntington's disease is being satisfactorily achieved by a programme of this type even in the complete absence of predictive tests for the gene. However, closer analysis suggests that this is not so. In the first place the fact that the HD births in the study area have declined does not necessarily indicate that genetic counselling or the study itself were responsible. This was recognized by the authors, who also noted that the decline antedated the onset of the study. Recent data collected from north Wales (Quarrell *et al.* 1988), where ascertainment and genetic counselling were (until recently) less systematic, have suggested a similar fall in birth rate for HD families in this area; this result indicates that wider social factors may be involved.

Even more important is the fact that genetic counselling for those at high risk, in the absence of any predictive tests, gives very limited options for the individuals involved. Essentially, the risk can either be accepted, with the consequence of high risk to offspring should the parent indeed later become affected; or the couple can limit their family or refrain from childbearing,

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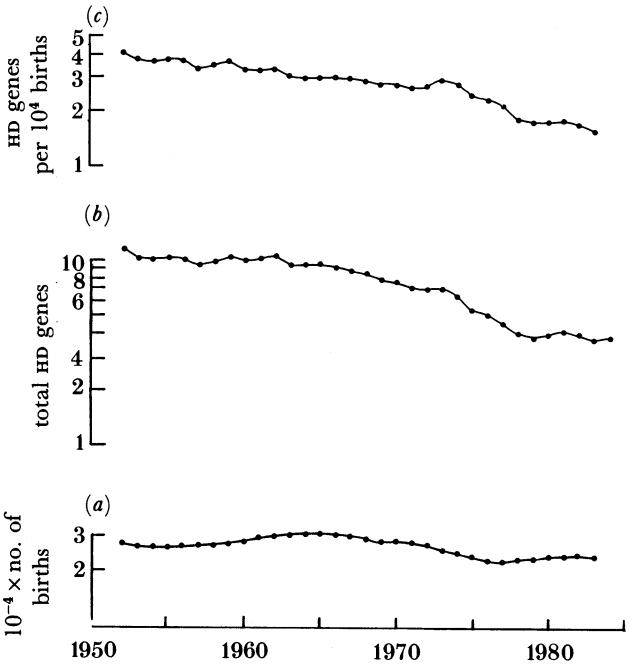

FIGURE 2. Trends in births at risk for HD. (*a*) The number of births that occurred in south Wales, expressed as a five-year moving average. (*b*) The estimated number of HD genes born into south Wales, expressed as a five-year moving average. (*c*) The estimated number of HD genes born per 10^4 population births.

only to find in later life that this was not really necessary if they remain free from the disorder. Thus the cost in terms of individual personal anxiety and suffering to family members is often a high one, regardless of decisions that are made. A reduction in future population prevalence, however desirable it may seem, is not the principal aim of genetic counselling, nor does it solve the problems of those individuals at risk for HD who wish to have children, but not to pass on the risk of the disorder.

For all these reasons, genetic counselling and prevention of HD can only be undertaken in a limited and imperfect manner in the absence of a method of predicting the presence of the gene. Nevertheless, a programme such as the one we have developed in south Wales has proved valuable, partly in allowing those at risk to have ready access to accurate genetic information and systematic support, but also in allowing the development of an organizational framework for family ascertainment and contact without which no future predictive test would be able to operate.

LOCALIZATION OF THE HD GENE

Although many attempts have been made to devise tests that would predict those relatives who would in future develop the disorder, all have been until very recently uniformly unsuccessful. However, it was recognized from an early stage that the detection of a marker gene close to the HD locus might provide such a prediction, and that such a marker would have the advantage of being independent of age. The following quotation from the paper of Julia Bell and J. B. S. Haldane (Bell & Haldane 1937), in which they described the first example of

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

genetic linkage in man (haemophilia and colour-blindness), published exactly 50 years ago, shows a remarkable prescience in the possible future application to HD.

> If, however, to take a possible example, an equally close linkage were found between the genes determining blood group membership and that determining Huntington's chorea, we should be able, in many cases, to predict which children of an affected person would develop the disease, and to advise on the desirability or otherwise of their marriage.

Attempts to use blood-group markers and other protein polymorphisms to map the HD gene were subsequently made, but proved uniformly negative; this is not a surprising result in view of the relative paucity of available markers in relation to the total human genome. More surprising was the rapid discovery of linkage when the first polymorphic DNA markers were tested (Gusella *et al.* 1983). The discovery that the probe G8 (locus D4S10), located near the end of the short arm of chromosome 4, showed close linkage to HD in two large kindreds, not only gave a conclusive localization of the HD gene, but also provided immediate evidence that this approach would be of clinical significance in prediction.

Analysis of our south Wales families, summarized in table 2, has confirmed that they show the same close linkage with this marker (Youngman *et al.* 1986); a study of more than 50 families worldwide (Haines *et al.* 1986) has shown no evidence of more than one locus, with the recombination fraction between marker and disease clearly less than 5%. The marker locus is a highly polymorphic one (table 3), with over 90% of individuals heterozygous for at least one of the restriction fragment length polymorphisms (RFLPs) that can be detected. The potentially predictive nature of the marker can be seen especially in families that are large and where one of the rarer alleles is transmitted with HD as seen in figure 3.

TABLE 2. HUNTINGTON'S DISEASE GENETIC LINKAGE DATA FOR PROBE G8 (LOCUS D4S10)

	south Wales[a]	total[b]
families studied	16	52
recombination fraction (θ_{max})	0.02	0.04
maximum lod score	17.6	75.3
95% confidence limits for θ	0.004–0.063	0.024–0.065

[a] Youngman *et al.* (1986). [b] Haines *et al.* (1986).

TABLE 3(*a*). POLYMORPHISMS AT THE D4S10 LOCUS (SOUTH WALES DATA)

	size of fragment/kb		allele frequency		no. of chromosomes
enzyme	allele 1	allele 2	allele 1	allele 2	tested
*Hin*dIII (site 1)	17.5	15.0	0.77	0.23	236
(site 2)	4.9	3.7	0.13	0.87	236
*Eco*R1	14.5	9.1	0.43	0.57	236
*Pst*I	5.5	2.4	0.86	0.14	110
*Nci*I	1.6	0.7	0.86	0.14	108
*Bgl*I	2.0	1.9	0.61	0.39	136

(*b*) *Hin*dIII HAPLOTYPES AT THE D4S10 LOCUS

	size of fragment/kb		frequency
	1	2	
A	17.5	3.7	0.68
B	17.5	4.9	0.09
C	15.0	3.7	0.19
D	15.0	4.9	0.04

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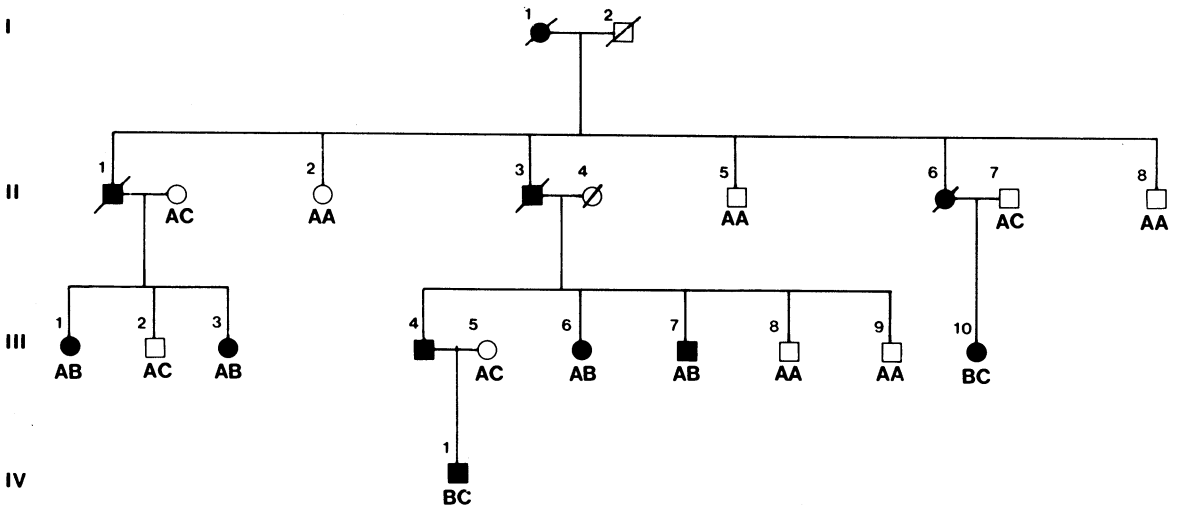

FIGURE 3. HD pedigree (family 8) showing DNA typing with the *Hin*dIII polymorphism of the G8 probe subclone pK082. All affected individuals have the 'B' haplotype. Unaffected individuals were over the age of 55 years.

This impression is to some extent misleading, because the structure of most HD families is far from ideal for prediction. In general a three-generation structure is required for prediction, so that at least one grandparent must be living for a fully accurate analysis; in a few families, the presence of an affected sibling may be an adequate substitute (figure 4). Study of families in south Wales (Harper *et al.* 1985) has shown that only a minority have this suitable structure (table 4), although the situation will alter in time as DNA is stored systematically on affected and elderly individuals who are likely to be dead at the time when descendants request prediction.

In addition, the use of predictive tests for a severe and currently untreatable disorder, such as HD, raises serious ethical issues, which have been under discussion since long before any linked marker was discovered, but which have intensified since it became clear that clinical

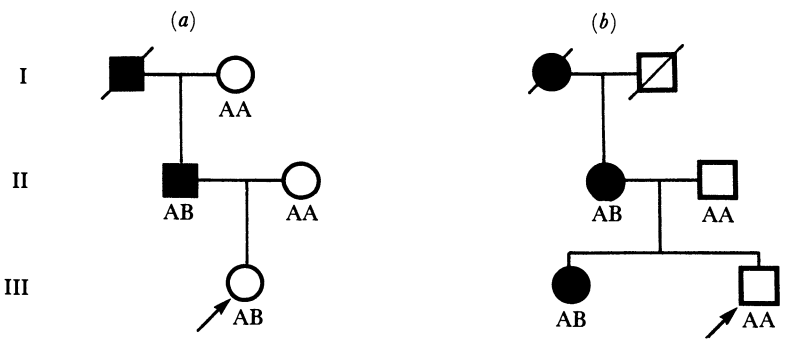

FIGURE 4. Family structure suitable for predictive tests. (*a*) Ideal three-generation structure. The affected parent in generation II is typed A, B. The unaffected parent in generation I is typed A, A; it is therefore certain that haplotype B and HD were inherited together. The consultand (arrow) in generation III was typed A, B; she is therefore predicted to develop HD. The only chance of error is if a crossover occurred between HD and the locus for G8 during meiosis. (*b*) A less ideal structure. No information is gained from generation I. The affected parent in generation II could have HD associated with either A or B. Information for the consultand (arrow) depends on DNA typing of the affected sister. There are now two meioses to consider, so she has a 10% chance of developing HD.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

TABLE 4. PROPORTION OF FAMILIES WITH HD SHOWING SUITABLE STRUCTURE FOR PREDICTION OR EXCLUSION TESTING

(Data from Harper & Sarfarazi (1985).)

	prediction for individual at risk (%)	'exclusion testing' in pregnancy (%)
individuals at 50% risk (age 16–45 years)	26	88
100 consecutive pregnancies at risk	22	89

application was imminent. The problems are outlined later, but because of them and because of the limited proportion of families for whom full prediction for the individual was applicable, our initial approach to prediction has been the somewhat different one of prenatal exclusion.

PRENATAL EXCLUSION TESTS FOR HD

Although for some individuals at risk for HD the value of a predictive test lies in removing uncertainty concerning their own status, for others the principal reason is to give the possibility of having children free from serious risk of the disorder. Prenatal diagnosis based on DNA analysis of first-trimester chorion biopsy is now applicable to any RFLP (Williamson *et al.* 1981), and would allow testing of a proportion of the small number of pregnancies occurring when a parent is already affected. A more generally applicable approach, which we have evaluated extensively during the past year, is that of pregnancy exclusion testing, as illustrated in figure 5. It can be seen that the third generation necessary for linkage prediction is here supplied by the pregnancy itself, so that there is no longer a necessity for a grandparent of the person at

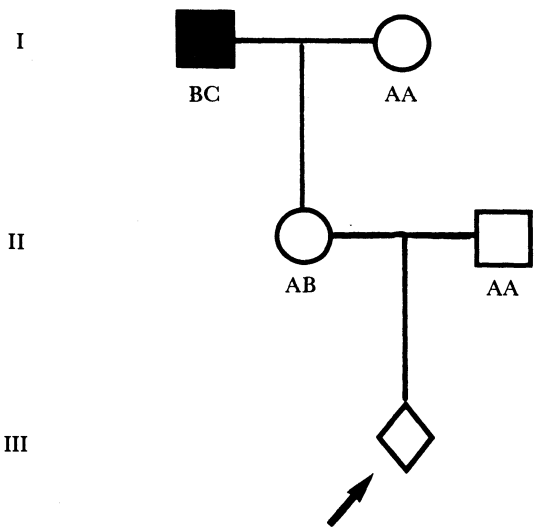

FIGURE 5. Family structure suitable for an exclusion test. This time the fetus forms the third generation. The parent at 50% risk in generation II inherited haplotype A from the unaffected grandparent to the fetus; therefore haplotype B is associated with a 50% risk. If the fetus types A, B then the risk to the fetus will increase from 25% to nearly 50%. If the fetus types A, A the risk to the fetus will decrease from 25% to 2.5%, allowing for the possibility of a crossover. The risk to the parent in generation II has not been altered.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

risk to be sampled. In the example shown, prenatal DNA analysis will show whether the fetus has inherited the marker allele from its unaffected or its affected grandparent. If the former is the case, HD can be considered excluded apart from the risk of recombination; if the latter, then the fetus has the same risk (apart from recombination) as the parent, i.e. close to 50%. In this situation it must be particularly be noted that the fetus is not being diagnosed as having HD; it merely has the same risk (50%) as its parent by virtue of its genotype, instead of the 25% risk in the absence of testing. Nor does the outcome of the prenatal typing affect the parental risk; this remains completely unaltered, a point that requires careful explanation and which is frequently not appreciated beforehand by either the family or by referring clinicians.

The data already shown in table 4 indicate that a proportion approaching 90% of couples in the south Wales population have a family structure appropriate for this form of prenatal testing, in contrast to the low proportion suitable for full prediction. When allowance is made for those who are not heterozygous or who have an identical genotype to their partner, the proportion is reduced to around 75% having a fully informative combination of genotypes.

We have now counselled and typed a series of couples requesting this form of testing in a future pregnancy (Quarrell *et al.* 1987), with results summarized in table 5. In this series 18

TABLE 5. REQUESTS FOR PREGNANCY EXCLUSION TESTS TO DECEMBER 1987

total requests	64
pregnancies	18
HD excluded	8
HD not excluded	6
chorion biopsy not undertaken	4

pregnancies have so far occurred, of which 14 have been tested on chorion biopsy. Six of these showed a risk to the pregnancy increased by the testing (i.e. HD not excluded) and in all cases termination of the pregnancy was requested. One such case is shown in figure 6. In the other eight HD was 'excluded' (i.e. the risk reduced to around 2.5%) and the pregnancies continued; in one case miscarriage occurred three weeks after chorion villus biopsy. Hayden *et al.* (1987*a*) have reported a case where a similar approach was used.

At this early stage it is difficult to assess what proportion of couples where a partner is at risk for HD will wish to avail themselves of the option of pregnancy exclusion testing. The fact that termination is required with only a 50% risk of the fetus being affected is a clear drawback; that the test does not alter the parental risks is seen by some as an advantage, but others would prefer in addition full prediction for themselves. At present the limitation of family structure means that pregnancy exclusion is going to be feasible in many families where full prediction is not, but this may alter if gene probes specific for or in strong allelic association with HD are developed in the future.

A practical advantage that we have found in embarking on a programme of pregnancy exclusion before full predictive testing is that it has allowed us to develop and evaluate a suitable organizational framework and counselling protocol which will in part be suitable for both approaches. The necessity for obtaining full family data, diagnostic and neuropathological confirmation, and checking the availability of essential family members, are all aspects which may give as much work and difficulty as the complexities of genotyping.

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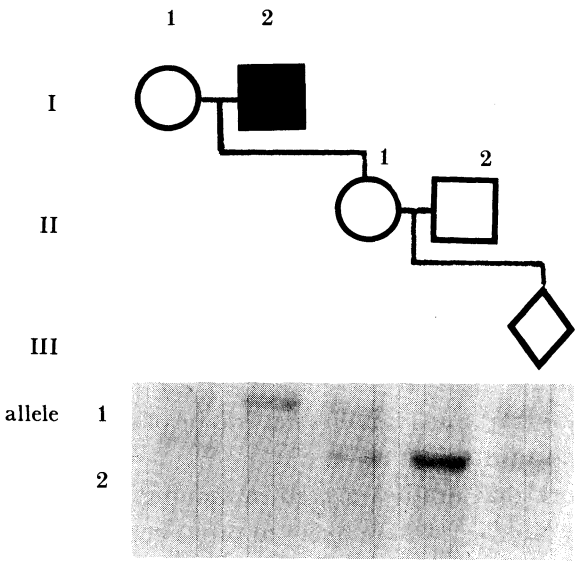

FIGURE 6. Result of one case in the series of exclusion tests in pregnancy. The polymorphism was identified with *Eco*R1 G8 subclone pK083. The parent in generation II typed 2, 1; the affected grandparent to the fetus typed 1, 1. Therefore the 50% risk is associated with genotype 1. This was inherited by the fetus and the pregnancy was terminated.

PREDICTION FOR THE INDIVIDUAL

No topic in medical genetics is fraught with as much emotion, and at times controversy, as the subject of prediction for the person at risk of developing HD. A clear discussion of the issues involved was written before localization of the gene (Thomas 1982), but subsequent to this development there has been considerable debate over the ethical and organizational aspects among both lay and professional groups. Several studies of attitudes to prediction among family members have also been carried out, again both before and after the localization of the gene (Quarrell *et al.* 1987; Kessler *et al.* 1987, Mastromauro *et al.* 1987; Meissen *et al.* 1987; Markel *et al.* 1987). These surveys have generally shown a proportion of one half to two thirds who state that they would wish predictive testing for themselves; pregnancy exclusion tests have received almost no consideration in such surveys.

There is general agreement that if prediction for individuals at risk of HD is to be undertaken, it should be done only under conditions that allow a combination of accurate laboratory analysis, careful analysis of diagnostic and pedigree data, as well as full and skilled pre- and post-test counselling and support. Such criteria clearly limit the establishment of predictive testing to a relatively small number of centres, and it is perhaps not surprising that it has taken more than three years from the first detection of genetic linkage to reach the stage where such prediction is actually being carried out. So far only a very small number of predictions have been undertaken, none reported in the scientific literature as yet. Because those centres involved are all undertaking detailed evaluations of the procedure and of those undergoing prediction, by using standardized protocols, it should become possible to recognize at least some of the factors that may prove to be associated with adverse reactions to prediction of abnormality and, we hope, to devise approaches that will minimize problems. At present the general attitude of those involved is one of considerable caution.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Progress towards isolating the HD gene

Although the localization of the HD locus is a major advance, it should be seen as only an interim step towards the goal of identifying the HD gene itself and characterising the abnormalities in it that are responsible for the disorder. This should not only allow fully accurate prediction within families, but also in some cases a specific diagnosis without the necessity for studying family members. Until very recently, progress in this direction was rather slow, in part the result of the initial linkage being so much closer than might have been expected in terms of numbers of markers analysed. From conventional family methods, the distinction between a recombination rate of around 3% and one that is clearly less than this is not easy, and may depend on the analysis of a few critical families showing recombination for one marker but not for another. Such families need to be of a complete and multigeneration structure that is rarely found in HD.

The principal lines of work that are being used in moving from gene localization to gene isolation are summarized in table 6, and are likely to prove complementary to each other. An obvious but necessary approach is the isolation of further and close markers on the appropriate part of chromosome 4; DNA libraries enriched or specific for all or part of chromosome 4 are being employed for this with, as a DNA source, either flow-sorted chromosomes or hybrid cell lines in which chromosome 4 is the principal human chromosome.

Table 6. Strategies for identifying the HD gene

close 'flanking' markers
pulsed-field electrophoresis
recognition of HTF islands
linkage disequilibrium
screening cDNA libraries
testing candidate genes

Cell lines showing deletion or rearrangement of parts of chromosome 4 form an additional resource for the localization of new DNA probes. A panel of such cell lines allows a probe to be assigned to a specific subregion, a procedure that allows identification of those probes likely to show linkage with HD and G8 among the larger number located in more remote parts of the chromosome that are not worth pursuing in terms of family studies.

Patients showing deletion of the terminal region of 4p show a characteristic dysmorphic syndrome, 'Wolf–Hirschhorn syndrome', characterized by facial and other skeletal abnormalities, as well as by mental retardation. The finding by Gusella and colleagues (1985) that such patients were hemizygous for the G8 probe, when from their parental genotypes they should have been heterozygous, provides a good example of how such a deletion syndrome can be useful in the mapping of gene markers.

At present the number of new DNA probes likely to be as closely or more closely linked to HD than G8 is limited despite a considerable amount of work; some have not yet been reported in full. Gusella's group has reported one clone, C4H, among a large number isolated from a chromosome 4 library, which maps physically to the region of 4p distal to G8 (Gilliam *et al.* 1987); Hayden *et al.* (1987*b*) have reported a probe, p8, derived from a conserved primate sequence; however, this appears to be less close to HD than probe G8 on physical mapping evidence. A third such clone, isolated by Youngman *et al.* (1988) from a flow-sorted

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

chromosome 4 library, also maps in this region. A further closely linked sequence, 674 (Smith *et al.* 1988) promises to be particularly useful diagnostically, owing to its high degree of polymorphism. Family studies are in progress on all these markers, so it is to be hoped that they may supplement and even supersede G8 in diagnostic use, as well as possibly giving a flanking marker for the HD gene. At present the likely order of loci (Gilliam *et al.* 1987) appears to show HD distal to G8 and the other closely linked sequences; this arrangement suggests an extremely terminal localization of HD that may well make isolation of a flanking marker difficult.

As mentioned before, distinction between the genetic distances and order of probes that are closely linked is extremely difficult by using family analysis alone. The use of other techniques is thus being pursued to isolate and to detect abnormalities in the HD gene itself. Pulsed field gel electrophoresis, by which large DNA fragments of 100000 to 2×10^6 base pairs can be separated in a manner comparable to that for smaller fragments with conventional electrophoresis, is one such approach (Schwartz *et al.* 1984). This technique is one such approach valuable in detecting gene deletion by alteration in fragment size, and has already been extensively used in constructing a map of the Duchenne muscular dystrophy region of the X chromosome (Burmeister *et al.* 1986). No results for HD are yet available, but analysis of the distal region of 4p is being actively studied.

Because new mutations for HD are known to be rare, it is possible that only a few or even a single HD mutation exists in each region; a search for deletions might thus prove unrewarding. In such a situation, however, closely linked probes should show strong linkage disequilibrium or allelic association, something already demonstrated for cystic fibrosis, where the number of new mutations also appears to be small (Estivill *et al.* 1987). When such an association is demonstrated, it should permit a marker haplotype relatively specific for HD to be identified.

A further development that will facilitate isolation of the HD gene is the recognition that specific genes, especially their 5′ ends, tend to be associated with unmethylated CpG-rich areas of DNA known as HTF islands (Bird 1986). Recognition of such an island will help to distinguish the site of a gene from surrounding non-functional DNA.

Finally, the accurate localization of the HD gene allows the testing of 'candidate genes' for the disorder. These may be genes of known important neurological function isolated during the course of other work or genes isolated from copy DNA (cDNA) libraries of normal or diseased brain tissue. Whatever their origin they can be rapidly assessed in relation to HD by showing whether they show an appropriate localization on chromosome 4. If not, they clearly cannot be the HD gene; if their localization is appropriate, then further work can be undertaken to resolve the question.

So far these and other molecular approaches are at an early stage in HD research, but experience with the 'reverse genetics' approach in other disorders, such as Duchenne muscular dystrophy, gives confidence that, either separately or in conjunction, they will give substantial progress towards isolation of the gene in the near future. This will then allow us to begin to analyse the molecular pathology of the disorder at both the DNA and subsequently the protein level. Once this stage is reached, the potential for therapy will cause our current attempts at prediction to be reassessed in a different light. Recent developments in cell transplantation into the brain, related to other degenerative disorders such as Parkinson's disease, may offer an alternative therapeutic approach (Madrazo *et al.* 1987). It seems possible that most individuals carrying the HD gene would wish to know this if there were even a small chance that the course of the disease could be delayed or modified by appropriate therapy. Such a development would

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

remove many of the current difficulties and anxieties associated with prediction, and would allow a unified approach to the prevention of HD in the genetically affected individual, no longer depending totally on avoiding transmission of the gene as at present.

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Discussion

S. V. Hodgson (*Guy's Hospital, London, U.K.*). In view of the apparent absence of heterogeneity in Huntington's disease, has Professor Harper found any clear evidence of a new mutation in any of his families?

P. S. Harper. No. Because of the late and variable onset, it is very difficult to prove a new mutation, but we have not encountered one although we have several isolated cases that could be. Certainly new mutations make up only a very small proportion of the total.

Anne L. McLaren, F.R.S. (*M.R.C. Mammalian Development Unit, University College London, U.K.*) I realize the great ethical problems in predictive testing. Does the Huntington Disease Society have a view on this topic; if so, what is it?

P. S. Harper. The lay societies in Britain, Continental Europe and America have all discussed this extensively and are broadly in agreement with each other and with professionals. All those involved agree that prediction requires caution, sensitivity and skill, and that mechanisms for support must be established to help particularly those predicted to carry the gene.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

J.-J. Cassiman (*Centre for Human Genetics, University of Leuven, Belgium*). The Huntington leagues on the Continent have worked closely with the genetics centres from the beginning. The lay groups feel strongly that the magnitude of diagnostic error should be less than 1% before the testing of individuals at risk is made available.

P. S. Harper. Everyone would agree that it is important for the margin of error to be as low as possible. However, a significant number of individuals wish for a test even in the knowledge that error from recombination may be as high as 5%. Also, one must not forget the possibility of error from other reasons, such as uncertain diagnosis or non-paternity, which may be of the same order as that due to recombination.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

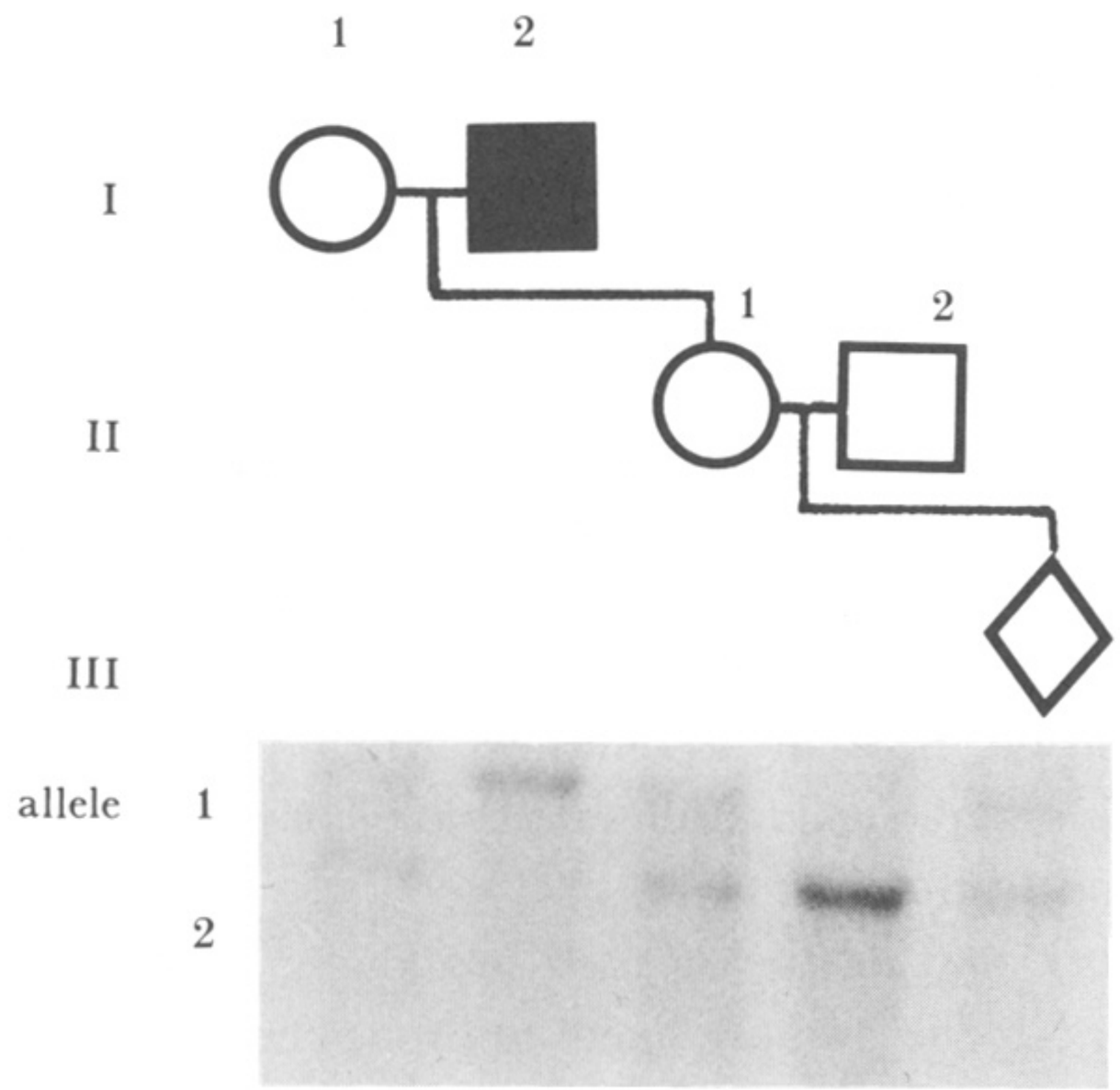

FIGURE 6. Result of one case in the series of exclusion tests in pregnancy. The polymorphism was identified with *Eco*R1 G8 subclone pK083. The parent in generation II typed 2, 1; the affected grandparent to the fetus typed 1, 1. Therefore the 50% risk is associated with genotype 1. This was inherited by the fetus and the pregnancy was terminated.