Proceed with care: direct predictive testing for Huntington disease

This paper describes protocols for direct predictive testing for adult and prenatal asessment devised on the basis of the experience of the Canadian Collaborative Study on Predictive Testing (CCSPT). The results provided during the last eight years include >400 tests using DNA linkage analysis, and 416 using direct measurement of lysis, linkage who <1% with follow testing came up and 416 using direct measurement of the CAG expansion in the HD gene. Eighty-six percent of requests for direct predictive testing came from persons who had not previously received results using linkage studies. (Of the previously tested individuals who subsequently requested a direct test, only one received a contradictory result, when a prior risk estimate of 85% was reduced to <1% on direct testing). In devising their current protocol, a questionnaire was sent to patients who had participated in the predictive testing programme previously. Patients indicated that the self-assessment questionnaires were of great value, but there is no objective evaluation of this. Patients also indicated that a minimum of two counselling sessions should be offered before the results, with follow up counselling available at “regular intervals”. The protocol which the Canadians use is very similar in format to that in general use in the UK, but over a considerably compressed time scale. Counselling is done by two professionals, typically a geneticist working with a genetic counsellor, psychologist, or social worker, and patients are encouraged to bring a support person to counselling sessions. If a patient has never been seen before, they are offered an introductory session before formal enrolment in predictive testing. At the first session, the patient fills in the questionnaire, undergoes a neurological examination (having stated beforehand whether or not they wish to be informed of any abnormal signs), receives information about HD and the predictive test, and then signs the consent form. Presumably (although this is not stated explicitly in the text) the blood sample is also taken at this session, as the second session is scheduled for only three weeks later, that session being just one to two weeks before the results session. The professionals counselling the patients have no knowledge of the DNA results during the second session, as they are held in the laboratory until the results session. At the second session the patient is counselled again about the test and prepared for the results session. After the results session (which is one month after the first session), all patients are offered a further appointment two weeks later, but subsequent follow up depends on the result, implying that the result influences the follow up required, contrary to other studies. For patients who inherited the HD gene, personal interviews at six months and one year are recommended. For patients who have not inherited the gene, follow up telephone contact occurs at six months and a personal interview at one year. This protocol raises many questions. There are several published studies indicating that the way in which people are offered tests influences their participation in a testing programme. If there is a time interval between receiving the information and having the blood sample taken, participation rates fall. It is important that the Canadians can show that their policy of taking the blood sample early in the testing programme is not influencing patients’ decisions to avoid testing. It would also be helpful to establish that patients feel that a three week interval between the first and second counselling sessions is long enough to consider all the emotional and practical issues involved (including enough time to arrange insurance policies, etc.). The authors state that they inform people that they may withdraw from the testing programme at any time, but it might be helpful to pull out if your result is already known to others in the laboratory. The authors also state that their results can only be disclosed to other parties with the express written consent of the patient, but what would happen if the patient had decided against receiving the result, and a relative subsequently requested it before entering into a predictive testing programme himself or herself? The original patient might feel under pressure to change his or her mind and release the result. The paper does not include a figure for the dropout rate from the testing programme, but this is very important, as it could be compared with that seen in other studies. Follow up by the geneticists appears to cease after one year, so studies to evaluate how the patients who have been tested cope in the longer term will be difficult. Other interesting points emerge as well. Patients at 25% risk may be offered exclusion testing in the first instance, if the intervening parent does not wish to be tested, and only if that does not exclude HD do they consider proceeding to direct testing. The authors found one unaffected, unrelated spouse with a CAG repeat length of 39, with no history of HD over two generations. Why this sample was tested in the first place is unclear, but the authors quote an incidence rate of 1 in 1959 chromosomes of this happening. Presumably this person has a significant chance of developing HD, but counselling in these circumstances would be very difficult. The Canadian protocol is not to report the size of the CAG expansion, despite the inverse correlation between age at onset and repeat length, because the confidence intervals for prediction are very broad. Patients with suspected HD who do not have a confirmed clinical family history of the illness are not rare, and this study suggested that at least 3% of families may represent new mutations. However, among symptomatic persons with a negative family history, 17/56 (30%) did not have a CAG repeat length in the affected range. Some of these may turn out to have deuterconceptullodolysian atrophy (DRPLA), another triplet repeat disease which can mimic HD, and for which direct testing is now possible. Overall, there is an urgent need to collect data from all the various testing protocols in use worldwide, so that programmes can be modified as appropriate. The rate at which quite large numbers of people are tested already makes this work particularly urgent, and the title of this report is certainly appropriate: proceed with care.

FRANCES FLINTER

A molecular approach to the stratification of cardiovascular risk in families with Marfan syndrome

All families with classic Marfan’s syndrome that have been studied so far have shown a consistent linkage between fibrillin (FBN1) and the disease phenotype. However, it is not uncommon for persons to have a Marfanoid phenotype, sometimes inherited as a family characteristic, without any of the major manifestations that commonly occur in the condition. This paper is of considerable clinical interest as it reports three newly characterised intragenic microsatellite polymorphisms within the FBN1 gene that will further improve linkage analysis for this condition. Not only does this report come at a time when FBN1 mutations have been proving elusive, but it goes on to show the usefulness of the polymorphic markers for presymptomatic diagnosis. Among the families studied were two in whom some family members showed either classic Marfan’s syndrome or a milder but closely related phenotype. In one family, the copy of the fibrillin gene that cosegregated with classic Marfan’s syndrome was not inherited by family members with the milder phenotype. A new frameshift mutation in the fibrillin gene, present in the proband of the second family, was not found among other family members with a milder phenotype. Associated limited linkage analysis in the family showed cosegregation of the milder phenotype with a fibrillin intragenic haplotype which the proband had not inherited. Interestingly, these milder phenotypes, previously diagnosed clinically as Marfan’s syndrome, were not associated with aortic involvement. They are also consistent with an earlier report of a large French kindred with incomplete, artypall, and variable manifestations of Marfan’s syndrome which does not show linkage to FBN1. The report highlights the need for a review of the current diagnostic criteria for Marfan’s syndrome. While the study supports the use of a linkage method for presymptomatic diagnosis of Marfan’s syndrome, an associated editorial points out the question of heterogeneity has not been fully resolved.

DAVID RAVINE

Ultrasound measurement of placental thickness to detect pregnancies affected by homozygous α-thalassaemia-1

In south east Asia homozygous α-thalassaemia 1 is the commonest cause of hydrops fetalis. The infants do not survive beyond the early neonatal period and usually die before birth. The placenta becomes large and oedematous in
affected pregnancies. There is a significant maternal morbidity associated with such fe-
tuses from pre-eclampsia and antepartum haemorrhage so that there is a need for early
diagnosis on clinical grounds leaving aside
parental request because of a genetic risk.
Prenatal diagnosis is available by DNA ana-
lysis or by examination of fetal haemoglobin.
In this report placental thickness was assessed in
231 pregnant women at risk of having fetuses affected with α thalassaemia 1, by
transabdominal ultrasound scan at 10 to 21 weeks’ gestation, when the mothers presented
for invasive procedures for prenatal diagnosis.
A cut off of mean placental thickness plus
2 SD was used. At gestations of less than 12
weeks this had poor sensitivity, but after this
gestation sensitivity was improved although
there were two false negative results (out of
184). Placental sizes were said to be large in
these cases despite normal thicknesses. It is
suggested that serial placental thickness meas-
ures could be used as an alternative to invasive
procedures for prenatal diagnosis of α thal-
assaemia 1. The relative lateness of diagnosis
by this method and the possible occurrence of
both false negative and positive results makes
it unlikely to supersede current diagnostic
methods. The authors suggest that in areas
with a high incidence of this condition
facilities for invasive prenatal diagnostic pro-
cedures may be limited and there may be a
use for this method in such places. However,
there will be a need for skilled ultra-
sonographers and suitable equipment if such
a service is to be reliable.

JILL CLAYTON-SMITH

Genomic organization of the Btk gene and
exon scanning for mutations in
patients with X-linked agamma-
globulinemia
Hagemann TL, Chen Y, Rosen FS,
Screening of genomic DNA to identify
mutations in the gene for Bruton’s
tyrosine kinase
Conley ME, Fitz-Hilgenberg ME,
Cleveland JL, Parolini O, Roherer J.

These papers describe the identification of
mutations causing Bruton type X linked
agammaglobulinemia (XLAGGA) by
genome scanning using SSCP analysis and
PCR primers spanning all 19 exons of the
Bruton’s tyrosine kinase gene. Hagemann et al describe the
exon/intron structure of the gene for the
first time. In the majority (12/14 and 25/30)
of cases analysed, mutations were identified.
They included single base substitutions, small
deletions and insertions resulting in pre-
mature stop codons, and point mutations
resulting in amino acid substitutions. No de-
letions larger than a few bases were found.
All the mutations were family specific apart
from those in exon 15, suggesting a mutation
hot spot. Conley et al gauged the severity of the
phenotype associated with the mutation in
15 of their cases. They also confirmed
linkage to Xq22 or lack of transcription of
Btk in the five cases in which no mutation
was identified. Clinical diagnosis of this
condition is not always straightforward as 30
to 50% of cases have no family history. There
is some variability in phenotype, and about
10% of cases are females suggesting the pres-
ence of a phenocopy caused by an autosomal
gene. The battery of PCR primers described
in these papers will facilitate confirmation of
diagnosis of XLAGGA and ascertainment of
carrier status and are a considerable advance
on Southern and Northern blotting tech-
niques which can only identify a minority of
mutations.

ANGELA BARNICOAT

Maternal mild hyperphenylalaninemia: results of treated and untreated
pregnancies in two sisters

Maternal phenylketonuria (PKU) has been
associated with microcephaly, mental re-
tardation, congenital heart disease, and intra-
uterine growth retardation in the fetus.
There is evidence to suggest that treatment
with a phenylalanine restricted diet during
pregnancy may alleviate adverse fetal effects,
particularly if the diet is started before con-
ception. Many people with a raised serum
phenylalanine level have less severe disorders than
PKU. This is known as mild hyper-
phenylalaninaemia (MHP) and is allelic to
classical PKU. MHP, when the blood
phenylalanine does not exceed 600 μmol/l, is
often regarded as a benign disorder as mas-
ternal phenylalanine levels of this magnitude
are not considered high enough to cause
either maternal or fetal effects. In this paper
by Levy et al, the outcomes of treated and
untreated pregnancies in two sisters with
MHP are compared. The authors conclude
that there have been no fetal or maternal
adverse effects because of MHP in this family
as both of the mothers and their three off-
spring had IQs within the normal range and
no malformations. However, this is only a
single family and as the note at the top of the
paper states, “The conclusions in this article
do not necessarily represent the conclusions
of the Maternal PKU Collaborative Study.”
In fact data from Denmark regarding off-
spring whose mothers had blood phenyl-
alanine >400μmol/l, showed lower median
values for birth weight, head circumference,
and IQ than those with levels <400μmol/l, al-
though both fell within the normal range. It is
also of interest that the offspring in this report
who were all shown to be heterozygotes had
higher IQ levels than the mothers. Therefore,
although the main point of this report tells us
otherwise, perhaps one should be more wary
about regarding MHP as a benign disorder.

ANGELA BARNICOAT

The apolipoprotein E alleles as major
susceptibility factors for Creutzfeldt-
Jakob disease
Amouyel P, Vidal O, Launay JM,
Laplanche JL, for French Research Group
on Epidemiology of Human Spongiform
Encephalopathies. Lancet 1994;344:
1310-1.

Creutzfeldt-Jakob disease (CJD) is a mainly
sporadic subacute spongiform encephalo-
pathy with about 10% of cases being in-
herited as a dominant condition. The
prion protein gene (encoded by the host) has
been shown to have predisposing polymorphisms
and pathological mutations in cases of CJD.
An abnormal prion protein product ac-
culates in the brain of affected people.
Apolipoprotein E (APOE) is produced by
astrocytes in the central nervous system and is
thought to be involved in lipid metabolism
for repair and growth in the brain. There are
three common forms of APOE coded for by
specific alleles, one of which, e4, has been
shown to be associated with Alzheimer’s dis-
case. In this study 61 patients with probable
or definite CJD were genotyped for APOE by
a restriction fragment length polymorphism.
Cases of CJD were shown to be more likely
to have e4 APOE alleles than controls.
This observation was made in both sporadic cases
and in those with mutations in the prion
protein gene (16 cases). The relative risk of
CJD was computed between subjects with at
least one APOE e4 allele and subjects with
none; it ranged between 1.8 and 4.2 de-
pending on the control group used. The fre-
cency of APOE e4 allele bearers and the
relative risk of CJD was the same in the
sporadic group of CJD cases as in a subgroup
with a specific mutation in the prion protein
gene, although numbers in this group were
small (11 cases). A variation in disease dur-
atation was also noted depending on APOE
genotype, with an increase in duration of
illness in e2 allele carriers. There are other
factors which may account for this ob-
servation, but it persisted in some groups
even when the known confounding factors of
age of onset and polymorphism in the prion
protein gene were controlled for. APOE e4 is
shown to be a risk factor for CJD as well as
Alzheimer’s disease. APOE e2 increases the
duration of CJD in patients over 65 years and
has also been shown to have a protective
effect against Alzheimer’s disease. The
authors suggest that the isofoms of APOE may
interact with prion protein produced in CJD
to produce protein of an abnormal con-
formation. Genetic factors in the host in-
cluding APOE alleles are important in the
natural history of CJD, but as Will notes in the
associated Commentary there is yet little
information on variation in the infectious
agent in human spongiform en-
cephalopathies.