Report of the MetBioNet Antenatal Diagnosis Workshop

Birmingham Childrens Hospital

6th November 2008

Attendance
19 people attended including representatives from all the major centres performing antenatal diagnosis.
The laboratories represented were:-
Sheffield Childrens Hospital
Guys Hospital, London
Great Ormond Street, Hospital, London
University Hospital of Wales, Cardiff
Willink Unit, RMCH, Manchester
Clinical Biochemistry, RMCH, Manchester
Bristol Royal Infirmary
Bristol Southmeade Hospital
Birmingham Childrens Hospital

Programme

1. ***Base Line Activity Data***

   George Gray (Birmingham) presented the results of a survey of all the centres involved in the antenatal diagnosis of inborn errors of metabolism.
   The questionnaire asked for data on the tests offered for antenatal diagnosis including the number performed and on what tissue or cell type they were performed. There were also questions on routine practice including whether audit was attempted, whether results on uncultured CVS were confirmed on culture, on how tests are selected and whether maternal contamination was excluded.

Workload
The workload figures showed laboratories split into three groups

a. Three laboratories with very high workloads,
b. Three with moderate workloads often specialising in a less broad range of diseases.
c. Four with a low workload usually specialising in a narrow group of diseases for which they were the national centre.

The most common disorders tested for were lysosomal storage diseases with I-cell disease top of the list whilst the most common non-lysosomal disorder was Smith-Lemli-Opitz Syndrome.

Sample Types
In terms of sample types analysed most laboratories offering lysosomal enzyme assays in uncultured CVS also offered them in cultured CVS and AF cells. However there were some anomalies that were discussed. In the case of many of the organic acid and amino acid disorders the test was only offered in cultured cells for technical issues relating to problems with analysis in uncultured CVS. In these cases early diagnosis would require defining the mutation in the relevant gene.
In terms of the other questions there was clearly a lot of variability between laboratories in their policies.

2 ***Discussion on Current Practice***

Marie Jackson (Guys Hospital, London) lead a discussion on the issues raised from these questions and other matters relating to our current practices. There was an active discussion and in some cases general agreement on certain matters (see below).

Local Laboratory Involvement in Metabolic Tests
The level of involvement of the laboratories in the organisation of antenatal diagnoses varied substantially. In some cases, if the test wasn't done in house, the requesting clinician was advised to deal directly with the laboratory performing the test and the home laboratory had no further involvement. However some laboratories had a closer involvement which varied from liaising with their local Cytogenetic and DNA laboratories, to offering a full coordinating service for the laboratory aspects of the test.

Test Selection

In terms of test selection some labs preferred a DNA to an enzyme test and some vice versa (assuming equal test reliability). In some cases it was felt that this decision was strongly influenced by the labs experience in that type of testing (i.e. enzyme or DNA). It was certainly felt that it was important to make Clinical Geneticists aware that a DNA test was, depending upon context, not necessarily the best test to do when a biochemical test was also available. The issue of whether to perform more than one test on a sample (eg DNA <u>and</u> enzyme) was discussed. Whilst there was a noted difference in practice it was generally agreed that was always useful to have a second test available should there be problems with the first line test.

Turnaround Times
It was agreed that in the case of uncultured CVS or amniotic fluid supernatant assays all tests should be done as soon as possible, preferably with 3 working days of sample receipt.

X-linked Recessive Conditions
It was agreed that normally, for X-linked recessive conditions, we would not perform analysis on female foetuses.

Confirmation on Cultured CVS cells
The practice of confirming an uncultured CVS result on cultured CVS cells varied between laboratories. Some laboratories always confirmed, if possible, and reported the result. Other laboratories confirmed but did not report the results unless there was a disparity whilst others did not confirm unless there were concerns about the original uncultured CVS cell result. It was, however, generally agreed that it was important to grow cultured CVS cells where possible in case there was a problem with the uncultured CVS analysis. It was agreed that if confirmation was to be done on cultured CVS cells it should be done as soon as possible so that any disparity detected can be clarified by amniocentesis.

It was agreed that, certainly for the lysosomal assays, it was important to measure enzyme activities in the parents to exclude pseudodeficiency states. Ideally this should be done around the time of diagnosis. Permission should also be obtained, at the same time, to store parental DNA as, in the event of a mutation(s) being defined in the affected child, this will have to be confirmed in the parents.
The issue of whether to report carrier status when DNA analysis is performed was discussed. Sarah Ball pointed out that current policy from the Joint Committee on Medical Genetics of the RCPath, RCP and BSHG states that there are no grounds for withholding carrier status (see Joint Committee on Medical Genetics meeting minutes of 25/01/07 para 13: http://www.bshg.org.uk/joint_committee/JCMG_minutes/jcmgJAN_07.pdf)

3 ***Presentations of Problem Cases***

There were presentations of cases where there were problems with the antenatal diagnosis:-

Heather Church (Willink Unit, Manchester) presented two cases were there were disparities between the results on cultured cells and non-cultured sources (uncultured CVS and leucocytes)

Simon Olpin (Sheffield) presented a case of a multiple acyl CoA dehydrogenase deficiency were the level of enzyme activity varied in different siblings with the same mutation which caused problems in an antenatal diagnosis.

Nigel Manning (Sheffield) reviewed the role of amniotic fluid supernatant analyses for antenatal diagnosis and the general opinion was that they were still very useful. He also presented a critical review of the use of quantitation of maternal urine methylmalonic acid concentrations to diagnose the disorder in the foetus.

Tim Hutchin (Birmingham) presented a case of an antenatal diagnosis for metachromatic leucodystrophy which has lead to the referring lab recommending DNA analysis in preference to enzyme assay for the antenatal diagnosis of this disorder. He also presented a case of atypical later-onset non-ketotic hyperglycinaemia which

lead his laboratory to offer enzyme analysis only in “classical” cases.

Marie Jackson (Guys Hospital, London) presented a problem with a case of I cell disease where there was considerable variability of enzyme activity between different cultured CVS cell lines. She also presented a case of Maple Syrup Urine Disease where there was variability between activities in different fragments of uncultured CVS villi in a CO2 release assay. Finally she reported an antenatal diagnosis for MPS1 where the uncultured CVS analysis was equivocal and the diagnosis had to be confirmed by DNA analysis. All these presentations gave us food for thought alerted us to the potential problems with patients, diseases and methods.

4 Maternal Cell Contamination

Sarah Ball (Birmingham) gave a presentation on the exclusion of Maternal Cell Contamination.
The results of the questionnaire showed that practices for this varied substantially. Certainly it was agreed that maternal contamination should be excluded if DNA analysis is performed or if the analysis is performed solely on cultured CVS cells. Sarah pointed out these services were available in all Regional Genetic Laboratories and in fact, as regards DNA analysis, were in the Good Practice Guidelines.
The problem was more complex with uncultured CVS cell analysis as in many cases the sample analysed was used up during the biochemical assay so one could never test the actual sample analysed for contamination. It was generally thought that enzyme analyses were less likely to be affected by low levels of contamination than a PCR based DNA test although what level was acceptable would vary from test to test. Certainly however it was important to consider it when interpreting uncultured CVS test results.

5 ***<u>Audit of Antenatal Diagnoses</u>***

George Gray (Birmingham) finished with a presentation on the system used at Birmingham Childrens Hospital for audit of antenatal diagnosis. He showed that it was possible to obtain a high rate of audit success with a simple database and agreed to supply a copy of the database (without patient information) if required. He also warned that audit is likely to become obligatory fairly soon.
The questionnaire showed there was considerable variability in practice. Whilst most labs have a record system but most do not actively chase audits unless there is concern. Some laboratories have had problems getting consent forms for analysis of the Products of Conception after a Termination of Pregnancy. There also have been problems of how to dispose of unused tissue.
It was generally agreed that there were issues about using cord blood as an audit specimen. These stemmed from concerns that the reference ranges for some plasma and leucocyte lysosomal enzymes in cord blood may differ substantially from postnatal blood and also the possibility of maternal cell contamination.

Conclusions and Recommendations

There was a general agreement for the following:-

1 It is very important to make Clinical Geneticists aware that a DNA test was, depending upon context, not necessarily the best test to do when a biochemical test was also available

2 All uncultured CVS or amniotic fluid supernatant assays should we done as soon as possible, preferably with a target reporting time of three (working or calendar) days from the date of sample receipt.

3 We should not normally perform analysis on female foetuses for X-linked recessive conditions.

4 It is important, where possible, to grow cultured CVS cells from the biopsy in case there is a problem with the uncultured CVS analysis.

5 If a confirmatory test is to be performed on cultured CVS cells it must be done as soon as possible so that any disparity detected can be clarified by amniocentesis.

6 For the lysosomal storage disorders it is essential to measure enzyme activities in the parents to exclude pseudodeficiency states.

7 Permission should also be obtained at the same time to store parental DNA as in the event of mutation(s) being defined in the affected child this will have to be confirmed in the parents.

8 Maternal contamination should be excluded if DNA analysis is performed or if the analysis is performed solely on cultured CVS cells.

9 There are concerns about using cord blood as an audit specimen when lysosomal enzyme or DNA analysis is to be performed.

10 Amniotic Fluid Supernatant assays still have a major role to play in antenatal diagnosis

George Gray 11/12/08