Cell-free fetal DNA for fetal sex determination in sex-linked genetic disorders

Guidance for commissioners and public health

April 2012

---

1 Introduction

Analysis of cell free fetal DNA (cffDNA) in the maternal plasma to undertake prenatal genetic testing is a ground-breaking new technology. It enables earlier determination of fetal sex for pregnant women at high risk for conditions that are related to the sex of the infant. Early determination of fetal sex allows up to 50% of women to avoid an invasive diagnostic test in X-linked genetic disorders, when only male fetuses are at risk, and congenital adrenal hyperplasia (CAH), a metabolic condition where female fetuses are at risk of virilisation.

This document covers the use of non-invasive prenatal diagnosis (NIPD) **as a diagnostic test to replace some current invasive testing** in two particular contexts: pregnancies at risk of serious X-linked genetic disorders (excluding haemophilia) and CAH. These contexts rely only on the ability to determine the sex of the fetus and do not currently extend to definitive diagnosis of the condition in question. However, it should be noted that these are only the first vanguard of applications for NIPD.

The document was drawn up by the PHG Foundation in collaboration with the RAPID team and the NHS National Genetics Education and Development Centre (NGEDC) following initial consultation with participants at the Implementation Sub-group of the RAPID workshop in November 2010.

Acceptability to patients

There is little doubt that NIPD is popular with patients as an alternative to invasive testing because it poses no risk to the fetus or mother and may be done earlier in pregnancy. As part of the RAPID programme work is being done to determine how the use of the tests can be optimized, in particular by identifying attitudes and anxieties about the test, information needs, and support for test provision as a high quality element within the antenatal care pathway (Lewis 2012a & b).

2 Aims of this document

Non-invasive prenatal diagnosis for fetal sex determination using cffDNA in the maternal plasma has been available in the UK on a research basis in several NHS laboratories. This document has been produced as part of the NIHR funded RAPID programme for applied research to provide guidance and information to commissioners on the implementation of NIPD for fetal sex determination within the specialised medical genetic and fetal medicine services in the specific contexts of antenatal testing for serious X-linked disorders and CAH.

It is suggested that commissioners and public health specialists use this document and supporting information indicated in the document and available in up to date versions on associated websites to engage with the relevant services and agree how NIPD will be provided to their population.

3 Commissioning background

NIPD should be commissioned to determine fetal sex early in pregnancies with known familial risk, thereby reducing the number of invasive procedures required. Commissioning of this service is placed within the general commissioning background of medical genetics services and fetal medicine. NIPD tests could be ordered either by Clinical Genetics or Fetal Medicine Units. Relevant elements are contained within the following specialised definitions sets:

- **Specialised Services for Women's Health - Definition No. 4.**

Section A on Fetal Medicine services covers: detailed assessment of fetuses at risk of malformations or dysmorphic syndromes; and management planning including offering further investigations (***including invasive diagnostic tests***, parental tests, further imaging) as well as consultation about the implications and outlook for the baby and possible invasive therapeutic procedures

- **Medical Genetic Services (all ages) - Definition No.20,**

Section 4.2 on core clinical genetics services includes appropriate follow-up to prevent the occurrence of, or complications of, a disorder, eg ***pre-natal care and testing***, anticipatory care
Section 4.3 on laboratory genetic services includes prenatal testing for single gene disorders

It is estimated that the implementation of NIPD for earlier sex determination within these services is approximately cost-neutral (Hill et al 2011a). There will be a requirement for altered patterns of funding since the costs to medical genetics of NIPD laboratory testing must be offset by decreased costs of sample collection through invasive procedures that fall within the fetal medicine service and a decrease in the number of CVS samples that require analysis.

4 Context

Cell free fetal DNA in maternal blood is placental in origin and consists of short fragments of DNA rather than whole chromosomes and can be detected from 4 weeks gestation. This technology has an advantage over invasive methods such as amniocentesis and chorionic villous sampling, which can only be undertaken from 11 weeks gestation and carry an approximately 1% risk of miscarriage associated with the procedure.

The clinical determination of fetal sex by examining cell free fetal DNA in the maternal plasma is based on the ability to detect or exclude specific DNA sequences from the Y chromosome of the male fetus. This is useful for fetuses known to be at high risk of a sex-linked disorder and for management of pregnancies for which the fetus is at risk of CAH, a condition in which female fetuses may become virilised through the overproduction of androgens.

Sex-linked disorders

Sex-linked disorders occur with a frequency of about 5 per 10,000 live births. They include conditions such as Duchenne muscular dystrophy and haemophilia and are inherited through the female line, but typically affect only males. At the present time NIPD for fetal sex determination has only been approved by the UKGTN for serious X-linked disorders (excluding haemophilia) and CAH. In many pregnancies at risk of haemophilia information regarding fetal sex is required to determine perinatal management and can therefore be done later in pregnancy using ultrasound. Thus, currently haemophilia has been excluded pending further evaluation (ongoing as part of the RAPID programme).

Congenital adrenal hyperplasia

The prevalence of CAH in the UK is approximately 1 per 10,000 (from population needs and genetics services 1993 HMSO Dd DH004322 6/93). Classic CAH is inherited in an autosomal recessive pattern and is due to deficiency of the enzyme 21-hydroxylase. One of the effects of this is prenatal exposure to high levels of androgens at critical stages of sexual development, which can result in virilisation of the external genitalia of females. Administration of dexamethasone from six weeks gestation is effective in reducing this virilisation. As well as avoiding invasive testing, early determination of fetal sex using NIPD means that the duration of dexamethasone treatment for women carrying male fetuses can be significantly reduced, potentially reducing risks of its possible adverse effects on fetal brain development as well as maternal side effects.

5 The evaluation of cell-free DNA for fetal sex determination in sex-linked disorders and CAH

The use of the technology has been extensively evaluated through the RAPID NIHR Programme and the PROOF audit (Prospective Register Of Outcomes of Free fetal DNA testing)(Hill et al 2011b). The main findings are as follows:

(a) **Fetal sex determination is highly accurate when performed in NHS service laboratories using stringent reporting criteria**.

(b) **Ultrasound should be performed before the NIPD test to confirm the gestation and exclude the presence of multiple fetuses or an embryonic sac.**

(c) **Parents should be advised of the possibility that repeat testing may be required to resolve inconclusive results and the small risk of discordant results. Because of the very small risk of the wrong sex being predicted the care pathways recommend fetal sex is confirmed using ultrasound after 12 weeks gestation.**

An audit of the use of free fetal DNA for fetal sexing was carried out in two phases between 1 April 2006 and 31 March 2009. In the second phase, which stipulated stringent reporting criteria and testing after 7 weeks gestation it was possible to issue a report in more than 95% of cases. The concordancy rate, indicating

pregnancies in which the correct sex was predicted, was 99.5% (CI 98.2-99.9) 401/403 pregnancies.

(d) **Non invasive prenatal diagnosis has changed clinical practice reducing the need for invasive testing**

The clinical utility of NIPD was shown in the PROOF audit as only 41.1% of women at risk of affected pregnancies with X-linked conditions (excluding haemophilia) and 30.5% at risk of CAH underwent invasive testing. This confirms previous findings that, for X-linked disorders, most women carrying female fetuses choose to avoid CVS while the majority carrying male fetuses underwent invasive testing, as, for the latter, there is still a need to determine whether the male fetus has actually inherited the condition through the specific mutation detection on chorionic villi.

(e) **Overall costs of NIPD were offset by the smaller proportion of women who required invasive testing and, as a result, NIPD is no more expensive than traditional invasive testing.**

An economic analysis compared incremental cost of NIPD for fetal sexing with traditional invasive testing for two representative conditions. Main costs were those of NIPD testing itself, CVS (sampling and molecular testing) scans and pregnancy outcomes. The differences in mean costs were small for NIPD versus IPD for DMD, mean difference (-£87, 95% CI -£303 to +£131) and CAH (-£193 95% -£301 to +£84). Testing costs for NIPD were offset by fewer women requiring invasive testing (Hill et al 2011a).

6 Commissioning pathways that include cell free fetal DNA

Offer and provision of NIPD should always be in the context of specialised genetics services

The offer and use of NIPD should be within the context of care pathways for management of women whose pregnancy is at known risk of a serious X-linked condition or of CAH. Detailed care pathways and best practice guidance have been drawn up by the RAPID group, in collaboration with the UKGTN gene dossier working group, and agreed through consultation with the wider community and are available on the RAPID website and in appendix A.

Women for whom NIPD may be appropriate will usually already be under the care of the clinical genetics team or occasionally another specialist team (e.g. metabolic medicine or endocrinology). **The disease causing mutation will normally already have been characterised, as this is a necessary precondition for informative prenatal testing, and the woman should already have an antenatal care plan for antenatal diagnosis.** If this is not the case, an urgent referral should be made to the regional genetics services.

Provision of the testing itself should be through one of the UK accredited laboratories offering cell free fetal DNA testing

As part of the RAPID programme, Gene Dossiers have been approved by UKGTN. These include separate submissions for NIPD for serious sex-linked conditions (excluding haemophilia) and for CAH. These dossiers provide evidence in support of the evaluations of test accuracy, clinical utility and cost and set out criteria for testing, cost and expected activity (UKGTN website-http://www.ukgtn.nhs.uk – What's new). Importantly, the acceptance by UKGTN signals to commissioners that these tests are clinically appropriate and should be provided on an equitable basis for the population.

Provision of NIPD in the UK by UKGTN member laboratories provides assurance to commissioners that quality standards will be met. A list of all UKGTN current providers is given on the UKGTN website (as above).

Volume and cost of testing (UK wide)

The following estimates were provided to the UKGTN within the Gene Dossier submissions. These are based on the data collected for the annual CMGS audit and therefore only reflect cases referred for invasive diagnostic testing. It is possible that activity might increase given that the first part of testing (fetal sexing by NIPD) can now be done avoiding the risk of miscarriage associated with invasive diagnostic testing.

Condition	**Expected activity per annum**	**Cost per test**	**Total cost**
Serious X-linked conditions	280[1]	£275	£77,000
CAH	30	£275	£8,250

The costs associated with the samples requiring analysis of cffDNA will be offset by the reduction in CVS samples that require testing and by cost savings in the fetal medicine units due to the expected reduction in the number of invasive procedures required.

Note 1- these numbers are based on the experience of current activity requests. Note that the expected birth prevalence of serious X-linked conditions is 350 (based on a rate of 0.5 per 1000 and 700,000 births in UK each year).

Ensuring quality

(a) *Laboratory quality control*

If NIPD is to be offered as a national service it will be essential that a quality assessment scheme be developed. In collaboration with UK NEQAS and NGRL (Manchester) a pilot quality assessment scheme has been run with 4 laboratories as part of the RAPID programme. This is currently being scaled up to include units in Europe who offer NIPD. Further information on current progress with EQA can be obtained through the RAPID and Eurogentest2 websites.

(b) *Communication and consent*

One of the major challenges of NIPD will be to support the informed choice of women to undergo prenatal testing. Given the relative safety of NIPD, it is argued that women may more readily consent to prenatal testing, possibly without having fully weighed up the implications of possible test results. In the specific circumstances covered in this current guidance, the use of NIPD for serious X-linked genetic disorders will be in the context of fetal medicine services and specialised genetics services and includes a pre-agreed antenatal testing plan where possible. The regional genetics standards for counselling and consent will thus apply to this shared care between clinical genetics and fetal medicine departments. Specific patient and health professional information, and training competencies are being developed as part of the RAPID programme. These must be in place prior to a NIPD service being commissioned to provide NHS services.

It should be noted, however, that the possible wider future use of NIPD as part of antenatal diagnosis and/or screening (for example, for Down's syndrome) will require separate consideration of how the necessary levels of informed consent can be achieved within the context of the routine antenatal setting.

(c) *Education of public*

Public education and engagement is a vital aspect of service provision for NIPD and must contain a clear and accurate message about what the technology can do and also its limitations.

It is vital that commissioners should work with service providers and other relevant professional bodies and organisations such as the Royal Colleges, the National Screening Committee, NHS Information resources and patient organisations to present a consistent and up to date message.

At present these information resources are gathered and moderated on the RAPID website.

(d) *Education of professionals*

It will be essential that NHS service professionals involved in NIPD build on their existing expertise in providing antenatal screening and diagnosis by acquiring new skills and knowledge. This will include those directly involved in provision and those in other areas related to the care pathways. The additional workforce competences for professionals providing a NIPD service have been identified by the NGEDC through the RAPID programme. Commissioners should have assurance that the necessary training of professionals involved in a proposed NIPD service has been completed prior to the service being commissioned.

For relevant professionals, the additional necessary competences include:

- Know the local care pathways for NIPD
- Have an awareness and working knowledge of the genetic conditions occurring within their clinical area for which NIPD may be offered
- Appreciate that although the assay is performed on a blood sample, the information obtained is likely to be equivalent to that obtained by invasive tests

- Be able to discuss potential results obtained by NIPD and their implications, and thereby obtain informed consent

NGEDC has produced resources to support health professionals in acquiring the necessary competences, including specific education packages for health professional groups which contain information and explanations about NIPD, and the types of genetic conditions for which the tests are available. Further information about resources is available on the NGEDC and RAPID websites.

Commissioners should determine with local providers how education relevant to the provision of NIPD within the whole pathway context will be commissioned, delivered and evaluated.

Monitoring and audit

The introduction of NIPD for fetal sex determination in X-linked disorders and CAH should be undertaken within the strict parameters outlined in the paragraphs above. Commissioners will wish to ensure that these are observed, that trends in use of NIPD are observed and that outcomes are measured. Important elements areas are:

- The number and range of conditions for which NIPD is used
- Adherence to agreed care pathways, in particular the source of referrals for NIPD
- That the technical aspects of testing satisfy EQA standards
- The clinical utility of testing
- The availability and quality of educational and public outreach materials
- Evidence for the professional competence of relevant health professionals

Commissioners may wish to consider whether national coordination of this monitoring may be advantageous.

7 Potential future developments

Currently undergoing development are applications for single gene other than sex-linked disorders (e.g. achondroplasia, sickle cell disease and beta thalassaemia) where it will be necessary to identify the mutant alleles inherited by the fetus. A further development is use in aneuploidies (abnormalities of chromosome number), particularly Down's syndrome, where it is necessary to determine relative quantities of chromosomal material.

Thus, whilst the use of NIPD as set out in this document may seem at present to have clear boundaries and a fairly limited scope, this is inevitably increasing as the use of NIPD has the potential to greatly improve the quality of prenatal testing services more widely. However, it must be introduced in the most effective and cost-effective way and with appropriate supporting resources to ensure a high quality service. It will be important also that, over time, the conditions for which it is used are monitored and short and longer term outcomes evaluated.

8 Conclusion

The availability of NIPD using cell free fetal DNA has enabled an improvement in the quality of NHS prenatal testing for pregnancies where the fetus is at risk of a serious X-linked condition (excluding haemophilia) or CAH for no extra cost to health service providers, and with the benefit to women of avoiding invasive testing and risk of losing a normal baby. These tests are favoured by pregnant women in at-risk groups and have general public support. Implementation of this service within the context of specialised medical genetics and fetal medicine services is recommended on a national basis. This document summarises current evidence and guidance and points to development of supportive resources that will ensure commissioning and provision of high quality services.

Commissioners should note that these applications are expected to signal a general trend towards non-invasive prenatal diagnosis as relevant technologies are developed. Embedding current applications in high quality services will ensure that the service is best prepared to incorporate future developments.

9 References

Hill M, Taffinder S, Chitty LS, Morris S. Incremental cost of non-invasive prenatal diagnosis versus invasive prenatal diagnosis of fetal sex in England. Prenat Diagn. 2011a 31(3):267-73.

Hill M, Finning K, Martin P, Hogg J, Meaney C, Norbury G, Daniels G, Chitty LS. Non-invasive prenatal determination of fetal sex: translating research into clinical practice. *Clin Genet* 2011b. 80:68-75

Hill M, Compton C, Lewis C, Skirton H, Chitty LS. Determination of foetal sex in pregnancies at risk of haemophilia: a qualitative study exploring the clinical practices and attitudes of health professionals in the United Kingdom. Haemophilia. 2012 Sep 23. 18(4):575-83.

Lewis C, Hill M, Skirton H, Chitty LS. Fetal sex determination using cell free fetal DNA: Service users' experiences of and preferences for service delivery. Eur J Hum Genet. 2012a Mar 28. [Epub ahead of print]

Lewis C, Hill M, Skirton H and Chitty LS. Non-invasive prenatal diagnosis for fetal sexing – what is the value for service users? Prenat Diagn. 2012b May 10. [Epub ahead of print]

Hill M, Lewis C, Jenkins L, Allen S, Elles R, Chitty LS. Implementing non-invasive prenatal fetal sex determination using cell free fetal DNA in the United Kingdom. Expet Opin Biol Ther. 2012 Suppl 1:S119-26.

Useful websites:

www.rapid.nhs.uk

www.phgfoundation.org

www.ukgtn.nhs.uk

10 Acknowledgements

This guidance presents independent research commissioned by the National Institute for Health Research (NIHR) under RAPID Programme Grant RP-PG-0707-10107. The views expressed in this publication are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.

Authors:

Dr Hilary Burton, PHG Foundation*
Professor Farndon, NGEDC
Ms Jacquie Westwood, UKGTN
Professor Lyn Chitty, UCL Institute of Child Health

Correspondence
hilary.burton@phgfoundation.org

This guidance should be reviewed when new evidence likely to have a material effect on it becomes available

Appendix A

Best practice guidelines for non-invasive prenatal diagnosis to determine fetal sex for known carriers of X-linked conditions excluding haemophilia.

Background

Fetal DNA is required for prenatal genetic testing. Traditionally invasive testing by amniocentesis or chorionic villus sampling has been required to obtain fetal DNA. These procedures carry a small but significant (around 1%) risk of miscarriage. In 1997 cell free fetal DNA (cffDNA) was identified in the maternal circulation. This is present from early pregnancy, is rapidly cleared from the circulation after delivery, but only constitutes a small proportion of cell free circulating DNA, the majority being from the mother (For review see RCOG 2009). cffDNA can be used as an alternative source of fetal DNA for prenatal testing for genes not present in the mother and has been used extensively in the UK and Europe for fetal sex determination (Finning and Chitty 2008, Hill et al 2010). Testing requires a simple blood test rather than an invasive procedure and thus avoids the risk of miscarriage.

This care pathway, describes an innovative method for fetal sex determination based on cffDNA which will massively improve equity of access and the quality of care offered to women whose pregnancy is at risk of a serious sex-linked condition by removing the need for invasive testing, and thus potential iatrogenic pregnancy loss for around 50%. An economic analysis has shown that there are no cost implications for service providers as testing in these circumstances has been shown to be cost-neutral (Hill et al. Manuscript submitted August 2010). There has been a thorough audit of this service as currently offered by NHS genetics service laboratories in the UK demonstrating a high sensitivity and specificity (Hill et al 2010). A pilot NEQAS scheme has been established (Schlecht et al 2010) and educational materials for health professionals, information and competencies are being developed by the National Genetics Education and Development Centre.

Additional care pathway information

1. Known carrier of X-linked disorder

- NIPD should be offered to known carriers of serious X-linked conditions. The majority of these women will already be under the care of the clinical genetics team and will have had a care plan for antenatal diagnosis discussed. Occasionally women who are known carriers of an X-linked metabolic or immunological disease will be under the care of the metabolic of immunological teams as practice varies with locality, but these women will also have a care plan for antenatal diagnosis in place.
- In the majority of cases the disease causing mutation will have been previously been characterised.

Red flag: Anyone presenting to obstetrics or GP without previous genetics or other relevant health professional care within the NHS should be given an urgent referral to the local regional genetics team.

2. Offer of fetal sexing by NIPD

- When a known carrier of a serious X-linked condition becomes pregnant contact is made with the genetics team, either directly or through their GP or obstetrician.
- The genetics team speak to the woman about NIPD at an appointment or by phone.
    - Discuss how the woman would like to receive results (genetics appointment, phone call or letter)
    - Discuss how NIPD performed, accuracy of NIPD and possible occurrence of inconclusive results.
    - Describe need for dating scan and for confirmation of gender by ultrasound.
    - Discuss option of amniocentesis/chorionic villus sampling if male identified.
- The woman will be referred to have a dating scan and the genetics team will organise for blood to be taken after 7 weeks gestation as confirmed by scan.

5. Dating scan

- Ultrasound scan performed at the woman's local maternity unit to establish gestation and confirm singleton pregnancy.

6. Discuss options with parents – when NIPD is declined

- Discuss options of invasive testing or standard antenatal care.

7. NIPD blood test

- Blood can be taken any time after 7 weeks (confirmed by dating scan). There is no upper limit of gestation for NIPD.
- A second blood sample may be required for testing for pregnancies below 9 weeks gestation. Please refer to the testing pathways of the specific laboratories providing this test.
- Blood can be taken wherever convenient for the woman - genetics appointment, GP or obstetrician/midwife appointment.
- Blood should arrive in the laboratory within 48 hours of sampling.
- The laboratory performing NIPD is notified to ensure rapid sample processing on arrival.
- Sexing test is performed with a target turnaround time of 3 days and report faxed to a named genetic counsellor or consultant.

9. Standard antenatal care

- Standard antenatal care should include the offer of Down syndrome screening according to local practise.

14. Confirm gender by ultrasound

- When NIPD indicates a female fetus an ultrasound scan performed at the woman's local maternity unit is used to confirm gender. Repeat scans may be needed.

15. Discuss options with parents - when the NIPD result is inconclusive

- When NIPD gives an inconclusive result parents should be offered the options of repeating the NIPD or having an invasive test.

16. Amniocentesis / chorionic villus sampling

- Discuss with the woman how she would like to receive results (genetics appointment, phone call or letter)

- Advise woman:

    - to rest for a day or two after the procedure, avoiding lifting, bending or stretching where possible
    - that she may experience discomfort in the lower abdomen after the procedure, which can be relieved with simple analgesics, eg. Paracetamol
    - to contact her GP if she has a pyrexia (there is a slight risk of infection with invasive procedures), losing either fresh blood or water type loss (not urine) from the vagina, losing any discharge with an offensive odour from the vagina, severe lower abdominal pain, feeling generally unwell or decreased fetal movement (after amniocentesis only where some women have already experienced fetal movements)
    - that she will not require Down syndrome screening if the pregnancy continues as the fetal cells obtained following the invasive test will also be sent for cytogenetic analysis.

Amniocentesis:

- procedure is suitable from 15+0 weeks of pregnancy
- is performed under continuous ultrasound guidance
- the procedure involves inserting a fine needle through the abdomen into the womb and then into the amniotic sac and aspirating a sample of amniotic fluid (fluid surrounding baby)
- at the laboratory, DNA testing is performed to identify the presence/absence of the mutation and in addition maternal cell contamination of the fetal sample is excluded. Fetal cells extracted from the amniotic fluid are examined; the number, arrangement and shape of the fetal chromosomes are checked
- about 1 in 100 samples proves to be inadequate
- the risk of miscarriage associated with amniocentesis is 1 fetal loss in 100 procedures

Chorionic villus sampling (CVS):

- procedure is usually performed from 11+0-12+6 weeks of pregnancy
- usually performed in a fetal medicine unit by a specialist trained in fetal medicine
- two types of CVS - Transcervical (TC) and Transabdominal (TA) **a) Transcervical (TC)**The procedure involves inserting a fine plastic catheter in the vagina, through the cervix and into the placenta (whilst simultaneously undertaking an abdominal scan). A small amount of placental tissue is then aspirated and sent for cytogenetic investigation. This is rarely performed **b)Transabdominal (TA)** Similar to an amniocentesis, this procedure is usually undertaken in up to 13+6 weeks but can be performed later in pregnancy. The procedure involves inserting a fine needle through the abdomen, into the womb and into the placenta and then aspirating a small amount of placental tissue for analysis
- at the laboratory, DNA testing is performed to identify the presence/absence of the mutation and in addition maternal cell contamination of the fetal sample is excluded. Fetal cells extracted from the CVS are examined; the number, arrangement and shape of the fetal chromosomes are checked
- about 2 in 100 samples prove to be inadequate
- the fetal loss rate associated with a TA CVS is 2 in 100 procedures. The risk associated with TC is slightly higher

18. Standard antenatal care

- Standard antenatal care should include the offer of Down syndrome screening according to local practise.

21. Standard antenatal care

- Standard antenatal care should include the offer of Down syndrome screening according to local practise.

25. Standard antenatal care

- Standard antennal care does not include offer of Down syndrome screening at this point as karyotyping is done on chorionic villi or amniocytes.

26. Discuss options with parents – when an X-linked mutation is identified

- Provide information and support to the woman whose pregnancy is affected by the X-linked condition.

References

Amniocentesis test - information for parents. 2009. NHS Fetal Anomaly Screening Programme

Chorionic villus sampling (CVS) - information for parents. 2009. NHS Fetal Anomaly Screening Programme

Finning KM, Chitty LS. Non-invasive fetal sex determination: Impact on clinical practice.

Semin Fetal Neonatal Med 2008;13:69–75.

Hill M, Finning KM, Martin P, Hogg J, Meaney C, Norbury G, Daniels G and Chitty LS. Non-invasive prenatal determination of fetal sex: translating research into clinical practice. *Clin Gen*. 2010; *In press*.

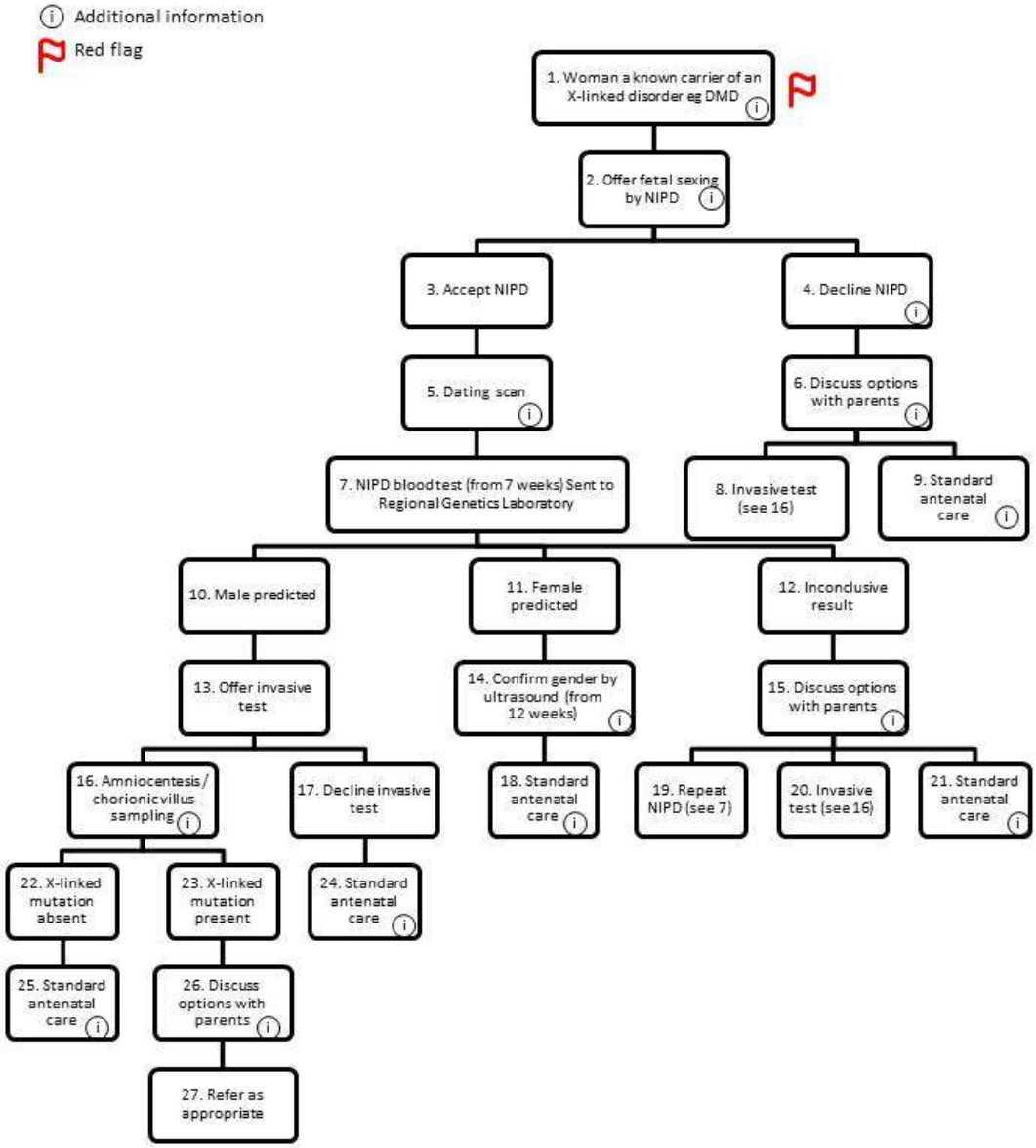
Hill M, Taffinder S, Chitty LSand Morris S. Incremental cost of non-invasive prenatal diagnosis versus invasive prenatal diagnosis of fetal sex in England. *Submitted to Prenatal Diagnosis August 2010*

Royal College of Obstetricians and Gynaecologists (RCOG). Noninvasive prenatal diagnosis using cell-free DNA in maternal blood. *Scientific Advisory Committee Opinion Paper* 15 June 2009.

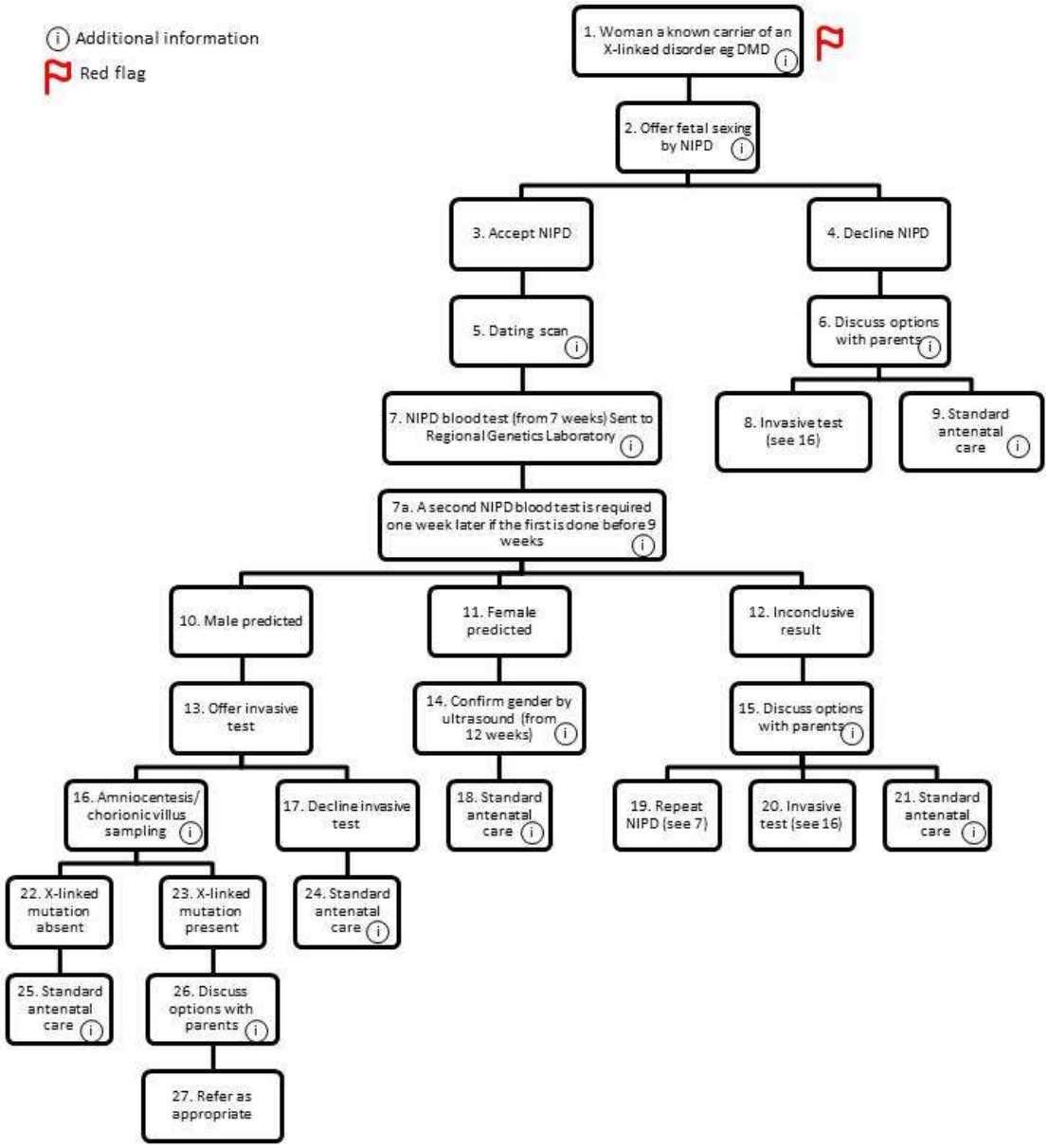
Schlecht HB, Charlesworth N, Ramsden SC, Elles RG and Deans Z. Non-invasive prenatal fetal sex determination – service experience and quality assurance. *J Med Genet* 2010; 47 Supplement 1 S72.

MANCHESTER CARE PATHWAY

Fetal sexing by non invasive prenatal diagnosis for serious X-linked conditions eg. Duchene muscular dystrophy (DMD) excluding haemophilia



BIRMINGHAM AND GREAT ORMOND STREET CARE PATHWAY
Fetal sexing by non invasive prenatal diagnosis for serious X-linked conditions
eg. Duchene muscular dystrophy (DMD) excluding haemophilia



Best practice guidelines for non-invasive prenatal diagnosis to determine fetal sex for known carriers of congenital adrenal hyperplasia (CAH).

Background

Fetal DNA is required for prenatal genetic testing. Traditionally invasive testing by amniocentesis or chorionic villus sampling has been required to obtain fetal DNA. These procedures carry a small but significant (around 1%) risk of miscarriage. In 1997 cell free fetal DNA (cffDNA) was identified in the maternal circulation. This is present from early pregnancy, is rapidly cleared from the circulation after delivery, but only constitutes a small proportion of cell free circulating DNA, the majority being from the mother (For review see RCOG 2009). cffDNA can be used as an alternative source of fetal DNA for prenatal testing for genes not present in the mother and has been used extensively in the UK and Europe for fetal sex determination (Finning and Chitty 2008, Hill et al 2010). Testing requires a simple blood test rather than an invasive procedure and thus avoids the risk of miscarriage.

This care pathway, describes an innovative method for fetal sex determination based on cffDNA which will massively improve equity of access and the quality of care offered to women whose pregnancy is at risk of congenital adrenal hyperplasia by removing the need for invasive testing, and thus potential iatrogenic pregnancy loss for around 50%. An economic analysis has shown that there are no cost implications for service providers as testing in these circumstances has been shown to be cost-neutral (Hill et al. Prenatal Diagnosis submitted August 2010). There has been a thorough audit of this service as currently offered by NHS genetics service laboratories in the UK demonstrating a high sensitivity and specificity (Hill et al 2010). A pilot NEQAS scheme has been established (Schlecht et al 2010) and educational materials for health professionals, information and competencies are being developed by the National Genetics Education and Development Centre.

Additional care pathway information

1. Pregnancy known to be at risk of CAH

- NIPD should be offered to couples when both are known carriers of CAH.
- In the majority of cases the disease causing mutation will have been previously been characterised.
- The majority of these women will already be under the care of the clinical genetics team and will have had a care plan for antenatal diagnosis discussed. Occasionally women whose pregnancy is known to be at risk of CAH will be under the care of the endocrinology team as practice varies with locality, but these women will also have a care plan for antenatal diagnosis in place.
- Prior counselling of known carriers will have included discussion of the possibility of prenatal treatment with dexamethasone, including dose and timing of treatment, potential side effects, and referral for maternal investigation from an appropriately experienced endocrinologist if necessary.
- Confirmation of fetal gender is important for guiding dexamethasone treatment. Standard practise is for the continuation of dexamethasone only when there is confirmation of an affected female fetus. Women known to be carrying a female fetus who decline invasive testing should be referred back to their genetics or endocrinology team for further discussion of management.

Red flag: Anyone presenting to obstetrics or GP without a previous genetics appointment should be given an urgent referral to genetics for counselling and initiation of urgent molecular testing if appropriate.

2. Steroid treatment

- When a known carrier of CAH becomes pregnant steroid treatment should be offered by the obstetrics or genetics team according to the previously determined care plan.
- Any woman without a suitable plan for dexamethasone administration should be referred urgently to the genetics team.

3. Offer of fetal sexing by NIPD

- When a known carrier of CAH becomes pregnant contact is made with the genetics team, either directly or through their GP or obstetrician.
- The genetics team speak to the woman about NIPD at an appointment or by phone.
    - Discuss how the woman would like to receive results (genetics appointment, phone call or letter)
    - Discuss how NIPD performed, accuracy of NIPD and possible occurrence of inconclusive results.
    - Describe need for dating scan and for confirmation of gender by ultrasound.
    - Discuss option of amniocentesis/chorionic villus sampling if female identified.
- Parents must be made aware that an invasive test will be required to confirm sex if NIPD is unable to confirm a male fetus as ultrasound is unreliable in this condition because of the risk of virilisation of female external genitalia.
- The woman will be referred to have a dating scan and the genetics team will organise for blood to be taken after 7 weeks gestation as confirmed by scan.

6. Dating scan

- Ultrasound scan performed at the woman's local maternity unit to establish gestation and confirm singleton pregnancy.

7. Discuss options with parents – when NIPD is declined

- Discuss options of invasive testing or standard antenatal care.

8. NIPD blood test

- Blood can be taken any time after 7 weeks (confirmed by dating scan). There is no upper limit on gestation for NIPD.
- A second blood sample may be required for testing for pregnancies below 9 weeks gestation. Please refer to the testing pathways of the specific laboratories providing this test.
- Blood can be taken wherever convenient for the woman - genetics appointment, GP or obstetrician/midwife appointment.
- Blood should arrive in the laboratory within 48 hours of sampling.
- The laboratory performing NIPD is notified to ensure rapid sample processing on arrival.
- Sexing test is performed with a target turnaround time of 3 days and report faxed to a named genetic counsellor or consultant.

9. Standard antenatal care

- If invasive testing has been declined and the woman is on dexamethasone the treatment options regarding continuing or sopping treatment should be discussed.
- Standard antenatal care should include the offer of Down syndrome screening according to local practise.

17. Confirm gender by ultrasound

- When NIPD predicts a male fetus an ultrasound scan performed at the woman's local maternity unit is used to confirm gender. Caution should be used in interpretation as a severely virilised female may be difficult to distinguish from a male fetus both on antenatal ultrasound and in the newborn period. Repeat scans may be needed.

19. Discuss options with parents - when the NIPD result is inconclusive

- When NIPD gives an inconclusive result parents should be offered the options of repeating the NIPD or having an invasive test.

21. Amniocentesis / chorionic villus sampling

- Discuss with the woman how she would like to receive results (genetics appointment, phone call or letter)

- Advise woman:

    - to rest for a day or two after the procedure, avoiding lifting, bending or stretching where possible
    - that she may experience discomfort in the lower abdomen after the procedure, which can be relieved with simple analgesics, eg. Paracetamol
    - to contact her GP if she has a pyrexia (there is a slight risk of infection with invasive procedures), losing either fresh blood or water type loss (not urine) from the vagina, losing any discharge with an offensive odour from the vagina, severe lower abdominal pain, feeling generally unwell or decreased fetal movement (after amniocentesis only where some women have already experienced fetal movements)
    - that she will not require Down syndrome screening if the pregnancy continues as the fetal cells obtained following the invasive test will also be sent for cytogenetic analysis.

Amniocentesis:

- procedure is suitable from 15+0 weeks of pregnancy
- is performed under continuous ultrasound guidance
- the procedure involves inserting a fine needle through the abdomen into the womb and then into the amniotic sac and aspirating a sample of amniotic fluid (fluid surrounding baby)
- at the laboratory, DNA testing is performed to identify the presence/absence of the mutation and in addition maternal cell contamination of the fetal sample is excluded. Fetal cells extracted from the amniotic fluid are examined; the number, arrangement and shape of the fetal chromosomes are checked
- about 1 in 100 samples proves to be inadequate
- the risk of miscarriage associated with amniocentesis is 1 fetal loss in 100 procedures

Chorionic villus sampling (CVS):

- procedure is usually performed from 11+0-12+6 weeks of pregnancy
- usually performed in a fetal medicine unit by a specialist trained in fetal medicine
- two types of CVS - Transcervical (TC) and Transabdominal (TA) **a) Transcervical (TC)**The procedure involves inserting a fine plastic catheter in the vagina, through the cervix and into the placenta (whilst simultaneously undertaking an abdominal scan). A small amount of placental tissue is then aspirated and sent for cytogenetic investigation. This is rarely performed **b)Transabdominal (TA)** Similar to an amniocentesis, this procedure is usually undertaken in up to 13+6 weeks but can be performed later in pregnancy. The procedure involves inserting a fine needle through the abdomen, into the womb and into the placenta and then aspirating a small amount of placental tissue for analysis
- at the laboratory, DNA testing is performed to identify the presence/absence of the mutation and in addition maternal cell contamination of the fetal sample is excluded. Fetal cells extracted from the CVS are examined; the number, arrangement and shape of the fetal chromosomes are checked
- about 2 in 100 samples prove to be inadequate
- the fetal loss rate associated with a TA CVS is 2 in 100 procedures. The risk associated with TC is slightly higher

22. Decline invasive test

- Refer back to genetics team for further discussion regarding management including cessation of dexamethasone.

25. Standard antenatal care

- Standard antenatal care should include the offer of Down syndrome screening according to local practise.

31. Standard antenatal care

- Standard antenatal care should include the offer of Down syndrome screening according to local practise.

32. Discuss options with parents - when CAH is confirmed

- Provide information and support to the woman whose pregnancy is affected by CAH.

33. Standard antenatal care

- Standard antenatal care does not include offer of Down syndrome screening at this point as karyotyping is done on chorionic villi or amniocytes.

References

Amniocentesis test - information for parents. 2009. NHS Fetal Anomaly Screening Programme

Chorionic villus sampling (CVS) - information for parents. 2009. NHS Fetal Anomaly Screening Programme

Finning KM, Chitty LS. Non-invasive fetal sex determination: Impact on clinical practice.

Semin Fetal Neonatal Med 2008;13:69–75.

Hill M, Finning KM, Martin P, Hogg J, Meaney C, Norbury G, Daniels G and Chitty LS. Non-invasive prenatal determination of fetal sex: translating research into clinical practice. *Clin Gen*. 2010; *In press*.

Hill M, Taffinder S, Chitty LSand Morris S. Incremental cost of non-invasive prenatal diagnosis versus invasive prenatal diagnosis of fetal sex in England. *Submitted to Prenatal Diagnosis August 2010*

Royal College of Obstetricians and Gynaecologists (RCOG). Noninvasive prenatal diagnosis using cell-free DNA in maternal blood. *Scientific Advisory Committee Opinion Paper* 15 June 2009.

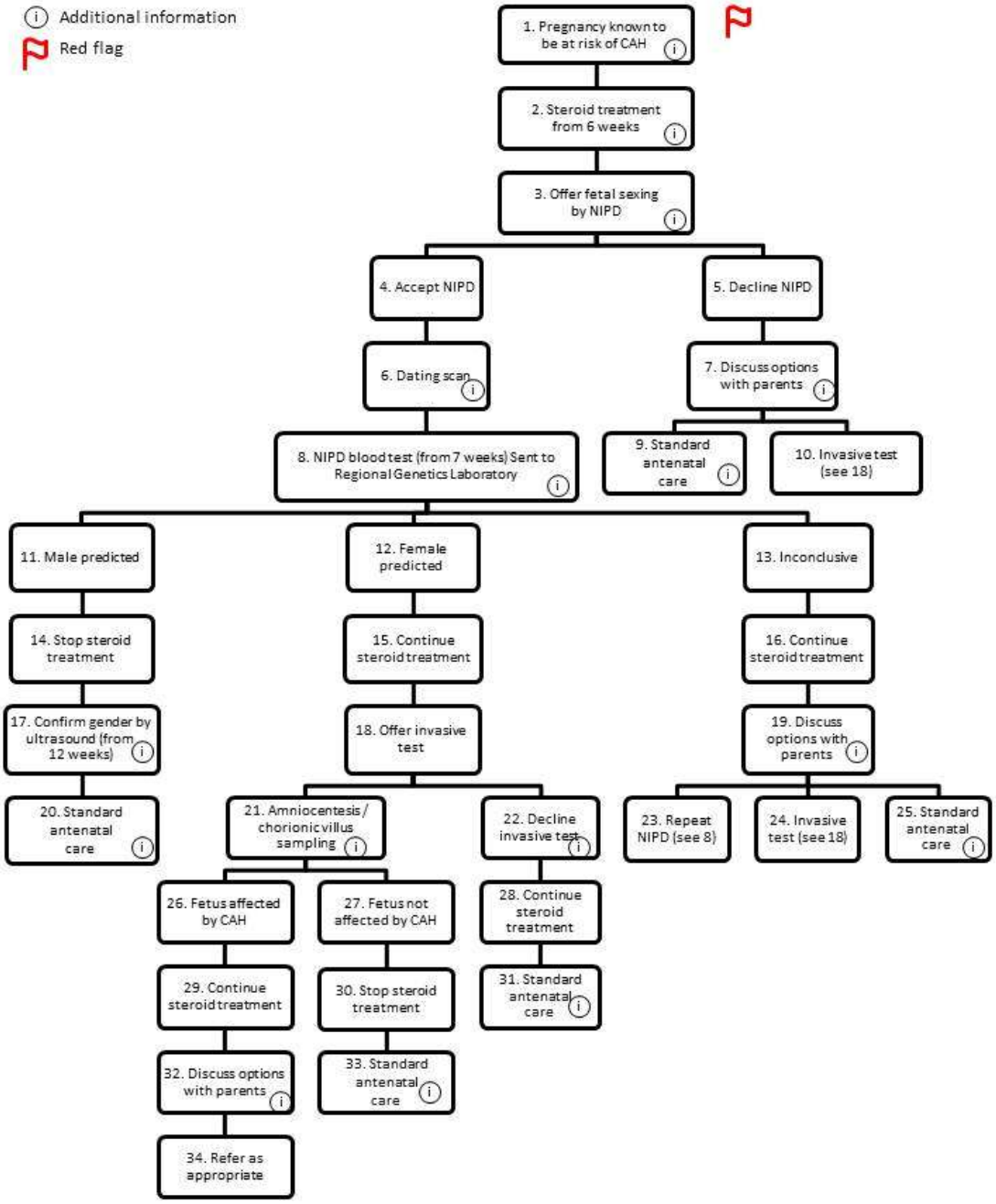
Schlecht HB, Charlesworth N, Ramsden SC, Elles RG and Deans Z. Non-invasive prenatal fetal sex determination – service experience and quality assurance. *J Med Genet* 2010; 47 Supplement 1 S72.

NHS

UK Genetic Testing Network

MANCHESTER CARE PATHWAY

Fetal sexing by non invasive prenatal diagnosis for Congenital adrenal hyperplasia (CAH)



BIRMINGHAM AND GREAT ORMOND STREET CARE PATHWAY
Fetal sexing by non invasive prenatal diagnosis for Congenital adrenal hyperplasia (CAH)