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House of Lords Science and Technology Committee

Sub-Committee II enquiry into Genomic Medicine

Evidence from the Foundation for Genomics and Population Health (the "PHG Foundation") and the Joint Committee on Medical Genetics (the "JCMG")

regarding the use of Cell-free Fetal DNA (and RNA) for Non-Invasive Prenatal Diagnosis

Evidence addressing specific policy points:

- *What is the state of the science? What new developments are there? What is the rate of change?*
- *Who is taking the lead in the consideration and co-ordination of research and the development of new technologies?*
- *What is the role of industry?*
- *Are there any regulatory gaps?*

Key points

- Cell free fetal DNA (and RNA) is present in the maternal circulation during pregnancy and can be used for non-invasive prenatal diagnosis of specific genetic conditions.
- Applications include: the determination of fetal sex and diagnosis of certain single gene disorders in pregnancies at high risk of an inherited condition, typing of fetal blood groups (such as Rhesus D, C, c, E and Kell) in order to improve pregnancy management, and potentially routine use for Down syndrome testing of all pregnancies.
- Determination of fetal sex and Rhesus D typing in high risk women are already being used in UK clinical practice at a few specialist centres within a limited setting.
- This technology potentially offers safer, earlier and easier antenatal testing relative to current standard practice, which raises a number of specific ethical and social issues, including equity of access, patient autonomy and informed consent.
- There are a number of non-clinical applications of non-invasive prenatal diagnosis (NIPD), including fetal sex determination for social reasons and paternity testing, which will require special consideration and potential regulation.
- A number of companies (both direct-to-consumer and professional service laboratories) are already offering NIPD, primarily within the USA for fetal sex determination.
- The technology is developing rapidly in this area, and the timeframe for moving the technique out of a research setting and into full clinical implementation within the NHS will be within the next 3-5 years.
- Public awareness of this technique is increasing rapidly and clinicians are already coming under pressure to provide it, so in order to safeguard public trust and manage expectations, it is important to keep the public informed about progress and aware of potential barriers to its adoption within the NHS.
- Clinical and laboratory evaluations are essential to establish test performance and clinical utility within specific clinical pathways before NIPD can be responsibly offered by the NHS.
- Research is ongoing in the UK, and numerous professional and patient groups are involved collaboratively to ensure the timely, ethical and effective implementation of NIPD within the NHS.
- This technology provides an exemplar of the development, evaluation and implementation of new genetic technologies into health care and will provide significant challenges associated with the re-modelling of current prenatal antenatal healthcare pathways, i.e. screening and testing.

1. Introduction

1.1 The PHG Foundation is the successor body to the Public Health Genetics Unit, established in 1997.

1.2 The JCMG is an intercollegiate committee of The Royal College of Physicians, The British Society for Human Genetics and The Royal College of Pathologists.

1.3 Following a request from the JCMG in 2007, the PHG Foundation established an expert UK working group to review the use of cell-free fetal nucleic acids (DNA and RNA) for the purpose of non-invasive prenatal diagnosis, and produce a strategy for implementation of this technology for different applications within UK clinical services.

1.4 This working group comprises representatives from numerous statutory bodies and professional bodies including the National Screening Committee, several of the Royal Colleges (including midwives, general practitioners, obstetricians and gynaecologists, and clinical geneticists) the UK Genetic Testing Network, the Human Genetics Commission, regional and national genetics reference laboratories, as well as patient organisations (namely the Genetics Interest Group and Antenatal Results and Choices).

2. Scientific Status

2.1 Cell-free fetal DNA ("cffDNA") circulating in the maternal blood was discovered by Dennis Lo and colleagues at the University of Oxford. It derives from the placenta, and can be detected in the mother's blood from 5 weeks gestation. The amount of cffDNA present represents 3-6% of the total cell-free DNA in the mother's blood (the rest being maternal in origin) and increases during gestation; it is comprehensively cleared from the maternal circulation within an hour of birth.

2.2 The prospect of a non-invasive test using a maternal blood sample which can be used for prenatal diagnosis of specific genetic conditions is very attractive. It would be safer than current invasive methods of prenatal diagnosis, such as amniocentesis and chorionic villus sampling, which carry a procedural risk of miscarriage of around 1% and can only be performed from 11 weeks gestation by specialist clinicians. In contrast, cffDNA testing poses no direct risk to the health of the fetus, and may be performed earlier in pregnancy from around 7 weeks gestation.

2.3 Due to the relatively small proportion of cell-free DNA of fetal origin in the maternal blood, applications for NIPD are currently primarily limited to those in which paternally inherited genetic sequences can be detected – for example, DNA originating from the Y chromosome of male fetuses (which would not otherwise be present in female blood). It should also be noted that, although the entire fetal genome is present in the maternal blood, it is broken up into short fragments of DNA (generally less than 300 base pairs), so there is an inherent limitation on the size of mutations that can be detected using cffDNA.

2.4 A number of highly sensitive methods are also being rapidly developed to compare the relative proportions of a particular genetic sequence, eliminating the need to detect only paternal sequences explicitly. Early reports suggest that these technologies could be used to detect conditions which result from the presence or absence of whole chromosomes, including Down syndrome, and may also be sensitive enough to detect other clinically significant chromosomal imbalances.

2.5 Cell-free fetal mRNA ("cffRNA") has also been detected in the mother's blood, derived from genes that are expressed in the placenta. Since a small subset of genes are actively expressed only during fetal development, cffRNA also offers an attractive target for NIPD and diagnosis of Down syndrome has been reported as a potential clinical application.

3. Clinical applications and current status in the UK

3.1 There are broadly four potential applications for which cell-free fetal nucleic acids (DNA or RNA) can be used for the purpose of NIPD. The status of development of these applications varies enormously in terms of the level of technical development and extent of clinical evaluation. The first two below are applicable to a small population of pregnancies at risk of an inherited genetic disorder, whilst the last two could affect routine antenatal care for all pregnant women.

3.2 The first application is the determination of fetal sex in families at risk of a sex-linked disease, by detecting genes present on the Y chromosome in male fetuses. This is primarily used for the purpose of reproductive choice in families with rare X-linked inherited disorders, such as Duchenne muscular dystrophy, where only males are affected. NIPD can be used to determine the sex of a fetus from 7 weeks and only pregnancies with a male fetus will then require an invasive diagnostic test to determine whether the specific disease-causing mutation has been inherited. It can also be used in the management of pregnancies affected by certain endocrine disorders, such as congenital adrenal hyperplasia, where early identification of fetal sex enables women carrying female fetuses to continue with dexamethasone treatment, but those carrying a male fetus can stop the medication. This application is currently available in the UK following referral by a geneticist or obstetrician. A recent audit of the use of this technique in the UK (~160 cases) revealed that the test is 98% accurate when offered after 7 weeks, resulting in a 45% reduction in the number of invasive tests.

3.3 The second application is for detecting specific single gene disorders in families with a high risk of an inherited disorder, often caused by a single point mutation. This application is currently limited to paternal disease causing mutations that are not present in the maternal genome, including the diagnosis of paternally inherited dominant diseases or mutations occurring de novo, such as achondroplasia, and ruling out a diagnosis of recessive diseases in which the paternal mutation differs from the maternal one ("compound heterozygotes") such as cystic fibrosis. Currently this application is accessed via specialist clinical genetics services and is only available on an *ad hoc* basis from a very limited number of laboratories within the UK, and considerable further development of the techniques is required.

3.4 The third application is the determination of fetal Rhesus D blood group status in Rhesus D-negative women, by detecting the paternally inherited *RHD* gene. Currently this is done in those women at high risk of haemolytic disease of the newborn, either because of a rising antibody titre or a previous affected pregnancy. The potential for routine fetal *RHD* typing in all D-negative women booking for antenatal care is being explored in a study funded by the NIHR. Rhesus D-negative women are currently given immunoprophylaxis (anti-D therapy) at 28 and 34 weeks gestation, to prevent possible sensitisation to a Rhesus D-positive fetus and reduce the risk of potentially life threatening haemolytic disease of the newborn in subsequent pregnancies. Early confirmation of fetal *RHD* status would allow those women carrying a D-negative fetus to avoid unnecessary anti-D administration with its concomitant exposure to human blood products.

3.5 The fourth application is the detection of fetal aneuploidy (presence of an excess chromosome), particularly Down syndrome ("trisomy 21"). This application is currently the least well developed, although preliminary results suggest that this test could be highly accurate and larger scale trials are planned in the USA next year. There are still several different techniques under investigation, including high throughput 'shotgun' sequencing of fetal DNA and detection of fetal-specific RNA derived from the chromosome of interest. It is currently unclear how the test will affect the current Down syndrome screening programme, which is offered to all pregnant women in the UK (ca. 700,000 *per annum*), of whom over 30,000 seek invasive diagnostic testing. Depending upon the accuracy of the test, it could potentially complement or replace either the current biochemical screening tests and/or the invasive diagnostic test which in turn will have a significant impact on the recruitment, re-training and retention of clinical scientists and technologists currently providing these services.

4. Wider issues

4.1 The improved safety, earlier detection and relative ease of testing using cffDNA technology raises specific ethical and social implications, through an increase in the volume of diagnostic testing, should NIPD become universally available. One of the major ethical challenges to implementing NIPD is safeguarding patient autonomy and informed choice, which will require significant professional and

public education as well as clear policy and guidelines for good clinical practice. Managing expectations and ensuring equity of access will also be critical to the implementation of NIPD in the NHS.

4.2 Whilst the introduction of NIPD for families at risk of inherited genetic diseases could be regarded as a replacement technology, and therefore be seen to raise few new ethical issues, there are concerns over the use of NIPD for conditions for which prenatal diagnosis is not currently offered. Appropriate laboratory and clinical evaluations are critical before the test can be offered routinely, both to establish the test parameters (sensitivity and specificity) and its clinical utility within a particular clinical care pathway.

4.3 The introduction of NIPD to routine antenatal care raises a number of specific issues, dependent upon how the test is ultimately applied. If cffRNA were to replace the current Down syndrome screening programme, for example, the move from a multistep process of risk stratification to a single-step diagnostic test may have serious implications for informed choice, and possibly result in 'routinisation' of antenatal testing. The single step blood test, if sufficiently robust and appropriately validated, could eventually prevent the loss of several hundred essentially normal fetuses, which are currently miscarried due to invasive testing.

4.4 There are also a number of potential non-clinical applications of NIPD, including sex determination (and selection) for social reasons and paternity testing. These raise the question of how liberal (or conservative) UK society should become regarding reproductive choice. In particular, the extreme population skewing that has occurred as a result of fetal sex selection in countries such as India and China, has raised concerns about the use of cffDNA testing for fetal sex.

4.5 A full economic assessment of this technique has not yet been performed for any application, though it is underway for fetal *RHD* typing. However, a cffDNA test currently costs around £250, which is likely to be cheaper than invasive diagnostic testing but more expensive than the routine Down syndrome screening tests. It is currently believed that fetal sexing using cffDNA in families at risk of an inherited genetic disorder is approximately cost neutral.

4.6 A number of companies in the USA are already offering NIPD for fetal sex determination on a direct-to-consumer basis, and more companies are currently developing NIPD for other applications. The majority of these services can be accessed via the internet and potential demand from the UK is difficult to gauge. The US diagnostics company Sequenom® is the major provider in this area, and holds an exclusive licence to most of the key patents. Sequenom® has already launched a fetal Rhesus D laboratory testing service and is aiming to launch a Down syndrome screening test based on cffRNA in 2009 (physician referral only) in the US.

4.7 Implementation of the technology within routine antenatal care will require an integrated approach from a variety of stakeholders across multiple disciplines. Since the technology has the potential to offer a diagnostic test within a screening setting, existing clinical pathways will have to be modified and, in the short term, public expectations managed. Statutory authorities such as the National Screening Committee and the Human Genetics Commission are likely to be involved. Public access to this technology via the internet (without the mediation of a health professional) highlights a current regulatory gap (shared by other types of testing such as genetic susceptibility testing) and raises questions about the extent to which the NHS will need capacity to support those independently accessing such services.

5. Leadership, Implementation and Future Research in the UK

5.1 NIPD using cell-free fetal nucleic acids is already available commercially for a limited number of applications, and is likely to become more widespread within the relatively short time-frame of 3-5 years. Further research and evaluation of these techniques is therefore imperative.

5.2 In March 2004, the Special Non-Invasive Advances in Fetal and Neonatal Evaluation Network ("SAFE") was funded under EU Framework 6 for 5 years to encourage multidisciplinary collaboration. This network includes numerous investigators from the UK and has been instrumental in the development and evaluation of NIPD.

5.3 A number of research projects are ongoing and starting in the UK, including an audit of fetal sex determination using cffDNA, and the evaluation of routine early Rhesus D testing in non-sensitised women. An application has also been submitted to fund an integrated and collaborative approach to development of the technique over the next 5 years, for which a final decision is expected imminently.

5.4 The expert working group, commissioned by the JCMG and facilitated by and represented here by the PHG Foundation, will present its findings in early 2009. The Report will review the current scientific and clinical status of cffDNA technology, explore the ethical, legal and social implications of non-invasive prenatal diagnosis, and make recommendations for its timely and effective implementation within the NHS. Major recommendations will include development and implementation of appropriate clinical pathways, laboratory standardisation and infrastructure development, continuing professional oversight, and formal evaluation and long-term monitoring of prenatal testing. The Report will also highlight the urgent need for professional education (particularly for midwives, obstetricians and GPs) and public engagement.

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