Good science equals good medicine: the role of next-generation diagnostics

Advances in clinical science are vital to the provision of healthcare overall and in forging significant improvements in the delivery of individual patient care. Here, Tony Sackville reports on the latest diagnostic innovations.

Innovative approaches in clinical diagnostics are at the heart of the drive to improve outcomes for patients in the key priority areas in healthcare. Sometimes new technology leads development, sometimes opportunities are created through a deeper scientific understanding of the underlying biology. In every case, better care should be the desired measure of success. Earlier detection, improved understanding of risk and the monitoring of treatment have previously been the drivers of change; more recently the selection of treatments personalised to individual patients has been the priority.

Earlier this year, GSTS Pathology held the inaugural one-day Innovation Academy event in London, where more than 100 laboratory scientists from Guy's and St Thomas' and King's College Hospitals NHS Foundation Trusts (partner trusts in GSTS Pathology) shared the latest diagnostic innovations in four areas: child health, keeping people healthy, infectious diseases and next-generation diagnostics. This short report outlines three examples of the innovative science covered in the last of these areas, highlighting recent innovations in pre-implantation genetic diagnosis (PGD) in *in vitro* fertilisation (IVF), improving the identification of familial endocrine cancer, and the future of chromosomal copy number variation (CNV) detection.

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PRE-IMPLANTATION GENETIC DIAGNOSIS

Dr Pamela Renwick (consultant clinical scientist in molecular genetics, Guy's Hospital) explained that the *Human Embryology and Fertilisation Act 1990* forbids the creation, storage or use of human embryos outside the body except under licence. One such licensed procedure is pre-implantation genetic diagnosis (PGD), an established reproduction option for couples at risk of conceiving a pregnancy affected by a genetically inherited disorder. New NHS Commissioning guidelines, published in April 2013, recommend that three cycles of PGD should be available for appropriate couples. *In vitro* fertilisation and intracytoplasmic sperm injection (ICSI) are used to create embryos to obtain material to test. Eight-cell embryos are tested three days after fertilisation, and are transferred to the mother, if suitable, on day five as a blastocyst.

Pre-implantation genetic diagnosis provides the opportunity for couples to avoid recurrent miscarriages, termination of pregnancy and the birth of an affected child. Disorders may include monogenic diseases such as cystic fibrosis and sickle cell disease, or chromosome rearrangements such as reciprocal and Robertsonian translocations or copy number variation (CNV). Pre-implantation genetic diagnosis is a sophisticated form of early prenatal diagnosis and there are only a few specialist centres around the world that can perform the procedure. Requiring multidisciplinary interaction from specialist teams in clinical genetics, genetics laboratories, embryology and reproductive medicine, PGD is a complex process.

The testing of DNA for PGD has evolved in

recent years, from simplex polymerase chain reaction (PCR) mutation, to fluorescence duplex PCR mutation and linked marker, to fluorescence multiplex PCR mutation and several markers. However, with more sophisticated technologies, such as whole gene amplification, yielding a 10^6-fold amplification, even coverage and good sequence representation, and microarrays providing more efficient methods for diagnostic clinicians, the potential for PGD to detect a wider range of genetically inherited disorders in pre-implanted embryos is impressive.

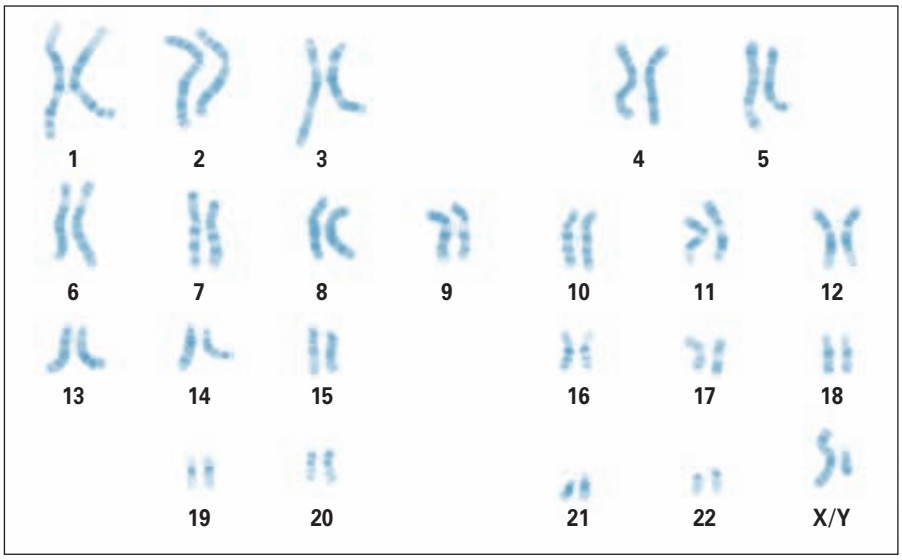
Importantly, PGD can also be used for diagnosis in an expanding range of genetic scenarios including late-onset diseases such as Alzheimer's, susceptibility to diseases such as breast cancer, profound disabilities such as deafness, and human leucocyte antigen (HLA) typing in saviour siblings, for example. Each year, PGD is performed for new scenarios previously not undertaken using prenatal diagnosis.

New developments in PGD at GSTS, and other leading centres, mean that the efficient and comprehensive testing of embryos to indicate development potential will yield greatly improved obstetric and perinatal outcomes. As processes become even more streamlined, PGD will have an increased ability to meet service demands as well as provide couples with valuable time to make life-changing decisions. For the next generation, the challenge is to provide a robust moral, ethical and legal framework for those fortunate enough to be involved in PGD.

FAMILIAL SCREENING FOR ENDOCRINE CANCERS

While some inherited genetic disorders can be detected prenatally, there are many that cannot. For some of these, familial screening can hold the key. Familial screening can be used to detect the risk of developing a disease or other related conditions. Dr Katharine Bates (senior clinical scientist in molecular pathology and clinical biochemistry, King's College Hospital) illustrated progress in this area with reference to work on phaeochromocytomas and paragangliomas (PPGLs).

Phaeochromocytomas and paragangliomas are rare catecholamine-producing tumours arising from adrenal and extra-adrenal chromaffin tissue which occur sporadically or as the result of germline mutations of several tumour susceptibility genes. They present clinically as a triad of sweating, headache and heart palpitations and have an annual incidence of 1.55–8 cases per million. Most PPGLs are benign but those that are malignant are more often caused by mutations in one of the PPGL genes. There are at least 10 genes recognised as responsible for hereditary PPGLs but it is estimated that PPGL gene mutation is the cause of a third of all PPGLs, benign or malignant.

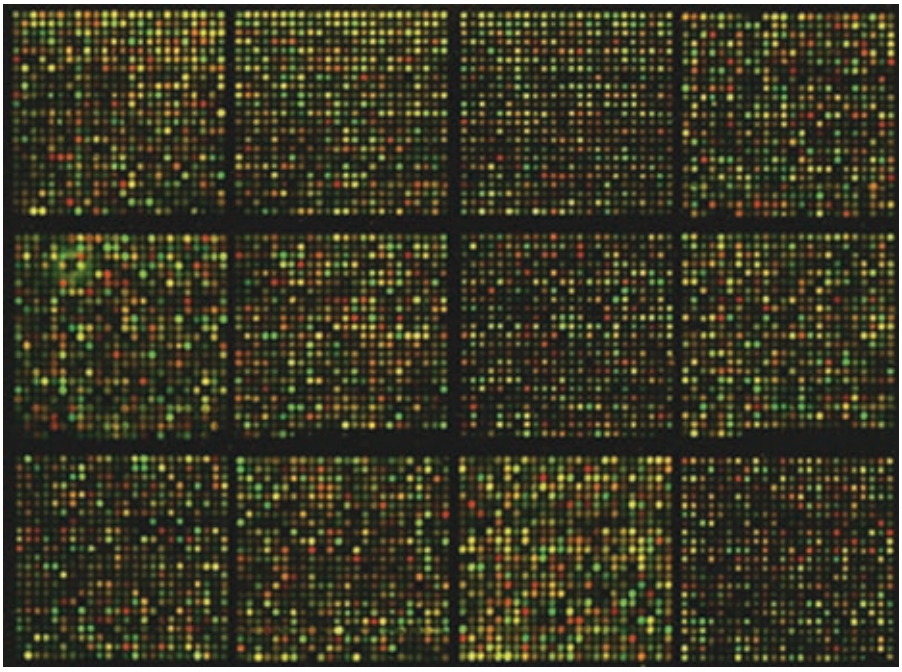


Karyotyping, such as the normal male example pictured, is being replaced by array comparative genomic hybridisation (CGH) for the diagnosis of genome-wide chromosome imbalance.

The implications of identifying a PPGL gene mutation are profound. Not only is it beneficial to the proband, but, due to the familial nature of the disease, close relatives can also be screened for early identification. An affected individual is often at greater risk of developing further phaeochromocytomas as well as other tumour types. Currently, the diagnostic method for PPGLs involves the measurement of catecholamines or their metabolites in samples of plasma or urine. However, this method does not provide further information about whether or not the identified PPGL is the result of an inherited endocrine tumour system (eg multiple endocrine neoplasia type 2). The well-established Sanger sequencing method is also less than ideal. It requires 500-bp PCR fragments and a day to prepare the sample. Forward and reverse directions must be performed separately and an ABI 31310xl genetic analyser takes one hour to run 16 samples.

Next-generation sequencing (NGS) has, in many applications, supplanted Sanger methodology. Also known as 'massively parallel sequencing' and 'deep sequencing', NGS methods include reversible termination, pyrosequencing and sequencing by oligonucleotide ligation and detection (SOLiD). The DNA molecules in NGS platforms are bound to a solid surface upon which they are amplified and sequenced *in situ*, whereas Sanger sequencing takes place in the liquid phase.

Unfortunately, NGS samples can take two days to prepare, and the technique requires additional bioinformatics power. It may also



Microarrays reflect the gene expression of an individual and provide a more efficient diagnostic approach.

yield higher error rates, although this can be compensated for by higher read depth. Overall, the advantages of NGS can be said to outweigh the disadvantages as it is less expensive to run, has higher throughput that enables the testing of multiple patients and multiple genes, and a greater ability to detect minor alleles.

Ongoing work at GSTS to develop NGS technologies will allow all the PPGL-causing genes to be screened, resulting in faster genetic diagnosis, reduced cost to the NHS, and improved patient care. This method will also identify rare genetic variants that otherwise are not easily classified as a pathogenic mutation or a benign polymorphism; thus, cases that may have been missed can be sent for further investigation.

CHROMOSOMAL COPY NUMBER VARIATION IN GENETIC DISEASE

Professor Caroline Ogilvie (consultant clinical scientist, Guy's and St Thomas' NHS Foundation Trust) discussed how genetic mutation and variation is not always inherited but can be the result of a 'random' process. For example, one particularly rich source of genetic structural variation, which can be inherited or appear *de novo*, is copy number variation (CNV), which is an alteration to the DNA of a genome that results in the cell having more or fewer than the normal complement of a section of DNA. Copy number variations correspond to relatively large regions of the genome, greater than 1000 bases, that have been deleted (fewer than the normal number) or duplicated (more than the normal number) on certain chromosomes.

Most individuals carry multiple small CNVs with no adverse effects; indeed, different combinations of CNVs may contribute towards individual phenotypic variation. However, they can be the cause of increased disease susceptibility and of a number of syndromic diseases. Copy number imbalance can be the cause of learning difficulties, developmental delay, dysmorphic features, autism, epilepsy and neurodisabilities, as well as a number of other conditions. Currently, relatively little is known about the contribution of CNVs to these conditions. *De novo* CNVs and those inherited from an affected parent are more likely to be pathogenic, while those inherited from an unaffected parent are more likely to be benign.

Dr Pamela Renwick is consultant clinical scientist in molecular genetics and head of molecular pre-implantation genetic diagnosis at Guy's Hospital.

Dr Katharine Bates is senior clinical scientist in molecular pathology and clinical biochemistry at King's College Hospital.

Traditionally, copy number imbalance has been detected by visual examination of fixed and stained chromosomes (a method known as karyotyping) and has a resolution of between 5 and 10 megabases. In 2008, Guy's Hospital was the first NHS laboratory to replace karyotyping with array comparative genomic hybridisation (CGH) for the diagnosis of genome-wide chromosome imbalance.

Array CGH compares a test sample of DNA with a reference sample by hybridisation of pooled test and reference DNA to short DNA sequences (oligonucleotides) fixed to a glass slide. This has a much improved resolution of around 120 kilobases, and can therefore uncover smaller CNVs and provide diagnoses for a larger number of patients. Innovative strategies were required in order to keep the costs of this test at the same level as karyotyping, resulting in no financial implications for the NHS. These included the introduction of robotics, setting the analytical resolution such that affordable inheritance testing could be achieved, and novel 'signal intensity' analysis to reduce the need for follow up.

Sequencing technologies, such as those described above, are in routine use for targeted mutation testing for certain monogenic diseases; in time, this sequencing-by-synthesis technology is likely to be used for whole-genome sequencing to replace array CGH, providing a common platform for molecular and cytogenic testing, as well as yielding a higher resolution with an increased number of more accurate diagnoses.

Exciting alternative sequencing methodologies are in development, such as semiconductor platforms and single-molecule sequencing using nanopore extrusion. However, the costs of whole-genome sequencing using currently available platforms are too high for routine diagnostic use, and, until these costs fall, array CGH will remain the method of choice for CNV detection.

CLINICAL SCIENCE: A POWERFUL DRIVER

Approaches such as PGD, familial screening and CNV detection enable choices to be made and treatment to commence as early as possible to maximise the potential for a positive impact on the patient pathway. The three areas covered in this report demonstrate new and improved ways quickly and reliably to identify individuals at risk of developing serious genetic diseases, and illustrate how clinical science is a powerful driver, not only in developing the provision of healthcare overall but also in forging significant improvements in the delivery of individual patient care. ■

Tony Sackville is head of sales and marketing at GSTS Pathology.
More information about GSTS Pathology and the Innovation Academy is available online (www.gsts.com)

About the Innovation Academy

As the UK's leading independent provider of pathology services, GSTS Pathology has launched the Innovation Academy as a new initiative to disseminate information to customers and colleagues in the NHS about how its scientific innovations and clinical services can improve patient care. In addition, the Innovation Academy is designed to create an environment that supports innovation, quality and the development of future scientific leaders within the partnership. GSTS Pathology provides reference testing services and a complete range of routine and specialist pathology tests and clinical support services. GSTS provides services to NHS organisations around the UK, to the private sector and to other public sector services in the UK, and to international clients.