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1 INTRODUCTION

Direct genetic testing, until recently, consisted of either an examination of whole stained chromosomes (cytogenetics) or a DNA test which characterises a specific DNA sequence (molecular genetics). The genotyping microarray is a new genetic testing method that can replicate, combine and enhance the capability of these two established genetic testing methodologies.

The great potential of microarray technology is its ability to perform many genetic tests simultaneously on one genetic sample. The high throughput, high resolution testing is not only more efficient than performing many separate tests (and potentially more cost effective), it also allows changes (mutations) to be found that are currently not detected. In the context of mutation screening, rather than screening, for example, for the five most common mutations that cause a disorder in a population, a whole gene can be screened in one experiment, thereby allowing multiple and rare deleterious mutations to be found routinely. In the cytogenetic context, smaller chromosomal changes, which may still cause a large physical effect, can be detected more readily than at present.

Microarrays are miniature, systematic arrangements of molecules (probes) on a solid substrate. They are often called ‘chips’ for their superficial similarity to computer microchips. The molecules on a microarray comprise any one of a number of different biological materials, but are generally DNA-based for genetic studies. The DNA to be tested is extracted from blood or tissue samples and allowed to attach to the DNA probes on the array. When the test sample binds to the matching DNA probe ‘spot’ on the array, a signal (usually fluorescence) is detected by a scanner or array reader. The data must undergo statistical analysis before it can be used to make predictions for diagnosis or treatment. Currently, the result is usually confirmed using another method before being accepted as accurate.

Microarrays were first developed for use in cancer research. They were used to measure the expression levels of various types of RNA (the intermediate between DNA instructions and protein product). The amount of RNA produced tends to reflect the amount of protein produced by a cell or tissue, and can also give an indication of how many copies of a particular gene are active or present. Changes in expression or gene copy number may drive cancer development. Microarrays may be used for typing tumours to determine what might be the most effective method of treatment, if any, with fewer side effects. The focus of this section, however, will be on the use of microarrays in determining DNA sequence variation (genotyping).

Genotyping microarrays have many high throughput uses and more are being developed over time. Current uses include microorganism identification (including the SARS virus as a corona virus); detection of antibiotic resistance; testing for, and of, genetically modified organisms; finding new drug targets; and tissue typing for
blood transfusion. Within the field of pharmacogenetics, there is now a cytochrome P450 microarray ‘chip’ to indicate how quickly an individual’s liver might process various chemicals including pharmaceuticals (and this is now FDA approved).

Microarrays comprise a new technology, which is proving to be very good for diagnostic genetic testing where there is a phenotype to explain. Their use is now moving towards genetic screening however, where there are more implications for clinicians and others wanting to use the technology.

This chapter aims to explore some of the current, albeit new, uses of microarrays in human genetic testing and the potential uses of the technology in the short to medium term. This section sets the context for the following, more detailed, examination of prenatal testing using microarrays.

2 THE BASIS OF GENETIC DISEASE

For more detailed information on the molecular basis of human genetic disease, consult a general genetics text.

DNA is the substance that holds the genetic instructions for making proteins and other types of biological materials. DNA is condensed into structures called chromosomes. DNA is made up of four principal nucleotides: adenosine (A); cytidine (C); guanosine (G); and thymidine (T). C and G nucleotides and A and T nucleotides specifically pair up to form double-stranded DNA. DNA encodes not only the instructions for the specific protein sequence (coding DNA) but also the regulatory instructions for when, where and how much protein to make (non-coding DNA). Figure 1 illustrates how double-stranded DNA is compacted into chromosomes.

![Figure 1: DNA to chromosomes](Image modified from Qui, 2006.)
Genetic disorders and syndromes can be caused by as little as a change in one nucleotide of DNA or as much as the deletion or duplication of a whole chromosome. Microarrays can be used to screen for both chromosomal changes and molecular mutations.

2.1 Smaller molecular variations and the HapMap

A number of types of small molecular change (mutation) may result in a disorder. These range from a single change in a nucleotide, which may alter a crucial amino acid in a protein, through to variously sized small deletions and insertions, which may change the protein sequence or even introduce a premature ‘end-of-protein’ (stop) signal. The manner in which some of these changes may modify the function of a gene is illustrated in Table 1.

| Normal allele | THE ONE BIG FLY HAD ONE RED EYE. |
| Missense | THQ ONE BIG FLY HAD ONE RED EYE. |
| Nonsense | THE ONE BIG. |
| Frameshift | THE ONE QBI GFLYHA DON ERE DEY E. |
| Deletion | THE ONE BIG HAD ONE RED EYE. |
| Insertion | THE ONE BIG WET FLY HAD ONE RED EYE. |
| Inversion | THE ONE BIG YLF HAD ONE BIG EYE. |
| Duplication | THE ONE BIG FLY FLY HAD ONE RED EYE. |
| Expanding mutation | |
| Generation 1 | THE ONE BIG FLY HAD ONE RED EYE. |
| Generation 2 | THE ONE BIG FLY FLY FLY HAD ONE RED EYE. |
| Generation 3 | THE ONE BIG FLY FLY FLY FLY HAD ONE RED EYE. |

Table 1: Types of molecular mutations. These gene ‘sentence’ examples illustrate the effects of various types of molecular mutation on the meaning or function of the sentence (gene). The site of the mutation or the actual change is underlined.

Source: Modified from Lewis, 2003

This variation in the genome may cause single-gene (monogenic) disorders, and this type of variation is often called a mutation. However, most of the variation in the genome causes no change to the function of the gene or consequent protein at all, and the effect may be neutral or even, occasionally, advantageous; variation that is not strongly linked to disease is often called polymorphism.
Single nucleotide polymorphisms (SNPs) are relatively common in the genome. They may occur anywhere in the genome and the majority are thought to be close to neutral in effect. Some SNPs, however, may be linked to increased risk of a particular disorder.

Smallish blocks of chromosomes have been shown to ‘travel together’ when they are passed down a family line over many, many generations. The polymorphisms that travel together are easier to determine in newer populations (such as from the Pacific Islands) than in older populations (such as from Africa), as there has been less time for the chromosomal blocks to break up. The more generations down which a chromosome block is passed, the more likely it is to be broken up.

Included in these blocks of chromosomes is the normal variation (polymorphism) that you would expect to find in any genome. In order to characterise this variation quickly, the HapMap project has analysed more than four million single nucleotide polymorphisms in four population groups from Asia, Africa and Europe. They have been able to determine which polymorphisms tend to travel together. By looking at one polymorphism in a particular group (a tag), researchers can deduce which other polymorphisms are there with it. Using this information, a genome can quickly and economically be scanned to give good information about which other polymorphisms are present. Researchers are now trying to associate these polymorphic tags to complex conditions and traits.

2.2 Larger chromosomal variations

Changes in the number and structure of chromosomes are of most interest at the prenatal and childhood testing stages. These changes produce a state called aneuploidy, where there are extra or missing whole chromosomes. An extra or missing portion of a chromosome is known as a segmental aneuploidy.

Changes in the copy number of whole chromosomes commonly affect the viability of pregnancies. Most large deletions and duplications typically miscarry early in gestation. Some fetuses with specific types of aneuploidy can survive pregnancy but, even in these types, many will miscarry before term.

Duplication of whole chromosomes 13 (Patau syndrome), 18 (Edwards syndrome), 21 (Down syndrome), X and Y (several syndromes including Klinefelter syndrome), are the most common aneuploidies found in newborns and children, although Patau and Edward syndrome are typically fatal within a few days to a few months of birth. The only viable whole-chromosome deletion is of the X sex chromosome (Turner’s syndrome).
Segmental deletions or duplications (of parts) of chromosomes also cause various conditions or syndromes, e.g. Cri-du-chat and Williams syndrome. Children diagnosed with developmental delay are often found to have small chromosomal perturbations. Segmental aneuploidies are less likely to be lethal because fewer genes are affected.

Changes (up or down) in expression of multiple genes can have multiple physical effects. The wide-ranging effects of large and smaller changes to chromosomes are the result of relative changes in expression of hundreds or even thousands of genes. Many of these gene expression changes will have no effect; but, generally, the expression of enough essential genes is affected to cause many body systems to be affected also.

Parts of chromosomes may also be moved around from their ‘home’ chromosome through a number of mechanisms, to produce a state known as translocation. Sometimes the translocation is a direct swap of genetic material between two chromosomes. This exchange is known as a reciprocal translocation. In other situations, the long arms of two chromosomes merge, as do the respective short arms, creating two new chromosomes (Robertsonian translocation). Some translocations cause disorders because two areas of the genome are abnormally brought together, destroying functional genes, changing the expression pattern of genes or creating new genes. They may also be harmless to the carrier (as the total complement of chromosomes has generally not changed). Translocations do cause fertility problems, however, because some of the eggs or sperm created in a carrier are chromosomally unbalanced, in turn creating embryos with segmental aneuploidies.

The last main group of chromosomal rearrangements are inversions. An inversion is where a segment of DNA is swapped in orientation. This may be completely neutral (or even advantageous) or may cause some of the problems seen with translocations, such as the elimination of a functional gene, the change of the expression pattern of a gene or the creation of new genes.
**Figure 2:** Metaphase chromosome spread. Cells are grown in the laboratory, and then stopped at the metaphase stage of cell division, when the chromosomes are condensed and easily seen. Chromosomes are freed from the cells and stained with a dye to give the characteristic ‘banding’ pattern and to make them more visible. This karyogram shows a male karyotype, with one X and one Y chromosome.

**Source:** Image sourced from Leiden University Medical Center

### WHAT ARE CHROMOSOMES?

All genetic material (DNA) in the nucleus is wound up into structures called chromosomes. The DNA is coiled around on itself (like a twisted rubber band) and around a protein scaffold. This enables a large amount of DNA to be reduced in size so as to fit into a cell.

Humans typically have twenty-two chromosome pairs and two sex chromosomes (either an X and a Y or two Xs): forty-six chromosomes in all.

The area where like chromosomes pair up during replication is known as the centromere. The centromere is characterised by repeated DNA sequences and may be found anywhere from the middle to one end of the chromosome.

The two ends of each chromosome are known as the telomeres. They are also repetitive DNA sequences.

Chromosome arms extend from the centromere to a telomere. The short arm of a chromosome is known as the p arm (an abbreviation of petite) and the long arm is known as the q arm.
2.3 Uniparental disomy and imprinting

Uniparental disomy is the presence of two copies of one chromosome from only one parent. There is a number of mechanisms whereby this situation can occur, the most common thought to be loss of an extra chromosome from an embryo where there are three copies (trisomy). This situation may cause disease in two ways. If the chromosomes present have a recessive, disease-causing mutation then two copies of the mutation result in a disorder. The other way in which uniparental disomy causes disease is when it involves imprinted genes.

Approximately fifty-three imprinted genes in forty-one ‘groups’ are known in humans. With imprinted genes, only one of the two copies of the gene is expressed, according to which parent it was inherited from. If the gene is only active on the paternally inherited chromosome, for example, then a loss of the paternal copy of the gene can result in a disorder. This is one of the mechanisms through which changes to genes close to SNRPN cause Prader-Willi syndrome. Deletions in or of the imprinted gene from the relevant parent are another common mechanism for imprinting disorders.

2.4 Copy number variation

Until recently, only very large and very small changes in the genome have been studied in detail. Researchers are now beginning to explore medium-size (submicroscopic) chromosomal differences (1 000 to 50 000 nucleotides) that turn out to be common variations within and between populations. These medium-size gains and losses of copy number are known as copy number variation (CNV). Recent studies show that CNV may affect up to 12 per cent of the whole genome, far more than previously anticipated. Any two humans, however, will only vary at a small number of the approximately 1500 chromosomal locations characterised so far.

Many CNVs are likely to be harmless, but some are linked to developmental delay and others to complex disorders such as Alzheimer’s and Parkinson’s disease.

The existence of this extensive variation both complicates and simplifies the use of genetic testing. On the one hand, we now know there is a large amount of CNV that is not linked to medical disorders. This knowledge does complicate interpretation of genetic testing results, as we cannot necessarily distinguish between neutral or disease-causing variations. On the other hand, now that we know of its existence, we can and have begun to characterise the extent of the CNV and can record the variation, how commonly it is found and possible effects on phenotype in large databases. This in turn will improve interpretation of genetic testing results. Increased genetic testing of this type and reporting of results to these databases will also increase the body of knowledge around what may (or may not) cause or be associated with various conditions.
3 WHAT ARE GENOTYPING MICROARRAYS?

3.1 Principles of microarrays

Single-stranded DNA can bind to another single-stranded DNA molecule with complementary sequence, to form the double-stranded DNA helix. The method of binding or base pairing has been likened to a zip that can be joined or separated.

All DNA microarrays comprise a solid support with specific pieces of DNA (probe) attached to it in a regular and defined manner. When (single-stranded) test DNA is added to the array, the probe DNA on the array is chemically ‘attracted’ to the test DNA with the ‘opposite’ (complementary) sequence. Any two pieces of exactly complementary DNA ‘fasten’ together (hybridise) to form double-stranded DNA and can be detected through a variety of means.

If the sequence doesn’t match exactly (particularly for short pieces of probe DNA), then there is no hybridisation and no signal to analyse. Both hybridisation and non-hybridisation data give useful information about the test DNA being analysed. If the sequence, or the size of the DNA probe fragments, is altered, the arrays become adaptable for specific purposes.

The solid support is generally a microscope slide-size piece of glass (approximately 75 mm x 26 mm) although there are many commercial companies with proprietary designs now making their own microarrays for sale. DNA is ‘printed’ onto the glass in discrete microscopic spots of down to approximately one nanometre. The DNA probes are firmly attached to the solid support by one of a number of methods and are difficult to remove under experimental conditions.

Short fragments of DNA (oligonucleotides) for arrays can be synthesised in the laboratory. Longer fragments of DNA must be prepared from existing pieces of cloned DNA. Oligonucleotides can also be build up, one nucleotide at a time, on to the solid support of the array. A variation on a glass slide support is miniature beads, onto which specific oligonucleotides are synthesised. The beads are then mixed with other beads sporting differing sequences and are poured onto a small tray support. The position of each type of bead, held on the tray by electrostatic forces, is defined before it leaves the factory.

Microarrays have a varying number of spots of DNA, depending on what they are being used for. Some microarray sets being commercially produced can contain up to 500 000 separate spots.

Each individual spot can contain DNA with a unique sequence. In practice, however, there is some redundancy (repetition) to serve as an internal check. If two or more spots with the same DNA probe sequence give similar results in an experiment or test, and are on different areas of an array, then one can be more confident of that result.
3.2 Oligo-based microarrays

Oligonucleotide-based arrays are probably the most versatile type of array. Short oligonucleotides (approximately twenty-five nucleotides long) are synthesised in the laboratory and can be designed to hybridise to any DNA sequence of interest. They are sensitive enough to be able to detect one mismatch in twenty-five nucleotides.

Oligo-microarrays can be used in two principal ways. First, they can be used to scan a chromosome or genome; for example, by using known mutations as a design basis or using tag SNPs from the HapMap project.

The second method is to ‘resequence’ a gene or small genomic area. This is done by preparing oligonucleotides that match every possible option at each nucleotide position. A large series of overlapping oligonucleotides is produced; each oligonucleotide sequence is shifted one nucleotide along from the last. For each nucleotide position in a genomic area of interest, all four alternatives (A, C, G or T) can be included in the microarray. This gives massive redundancy, as each nucleotide can be interrogated by up to two hundred different probes. They can also be used to detect an insertion or deletion of a nucleotide(s), if no genomic DNA binds to any of the four alternatives. This use may be suitable for mutation screening for monogenic disorders such as cystic fibrosis.

**Figure 3:** A resequencing microarray. A portion of the fluorescence pattern of the microarray (left) has been magnified on the right. Each column in the right hand picture represents four similar oligonucleotides, with a variation at just one nucleotide position. The top row has the A nucleotide alternative in that position; the second row, the C nucleotide; the third row, the G; and the last row, the T. Using this information, DNA sequence can be read off the microarray from the position of the fluorescent spot.

*Source:* Maitra et al., 2004
3.3 Array CGH (aCGH)

This technique is described more fully in the chapter by Mildred Cho, ‘Array Comparative Genomic Hybridisation (aCGH): An Analysis of the Current Technology and its Future in Prenatal Diagnosis’.

Comparative genomic hybridisation (CGH) is sometimes used in diagnostics and prenatal sample screening. In this technique, the test (e.g. prenatal) and control (‘normal’) DNA samples are separately labelled with different-coloured fluorescent dyes, then combined in equal quantities. The combined DNA is hybridised to a traditional chromosome spread and gain or loss of DNA copy number is shown by differences in the relative fluorescence of each dye.

The modified microarray version involves hybridisation of the combined DNA to a microarray. Instead of hybridising to a chromosome spread, the test and control DNA are hybridised to smaller pieces of human DNA. Many of these human DNA clones are bacterial artificial chromosomes (BACs), as they are replicated in bacteria to make many copies for use on the microarrays; but there are also other types of clone system used.

In prenatal testing, the choice of BAC DNA spotted onto the microarray is currently focused on those areas that are known to be more commonly affected by aneuploidy (unbalanced number of chromosomes) or to cause specific disorders. The majority of these involve deletions. The combined test and normal DNA will hybridise to BACs that share the same (or similar) DNA sequence. If there is a large deletion in the test DNA, then it will not bind to the BAC that is equivalent to the missing DNA. The normal sample should hybridise, however, resulting in a difference in fluorescence for that spot. If there is a duplication, the test signal will be greater than the ‘normal’ signal.

The use of larger pieces of DNA means that this type of testing is directed at large deletions. Smaller deletions or duplications (less than 80 000 nucleotides) will not be detected.

The newest Baylor College of Medicine prenatal microarray has 1475 different clones, although many overlap to ensure test integrity. In addition to looking for specific chromosomal disorders, the centromeres and telomeres are closely covered, as loss or gain of material in these regions can cause developmental delay and other clinical effects.
4 RECENT AND FUTURE USES OF MICROARRAYS

It is difficult to predict how microarrays might be used in the future. Some current and up-and-coming uses are discussed in this section.

4.1 Prenatal screening

*This technique is described more fully in the chapter by Mildred Cho, ‘Array Comparative Genomic Hybridisation (aCGH): An Analysis of the Current Technology and its Future in Prenatal Diagnosis’.*

There are two current methods for obtaining prenatal tissue for genetic testing. Amniocentesis is by far the most common; amniotic fluid samples are taken between fifteen and twenty weeks of gestation. Chorionic villus sampling is performed earlier than this (ten to twelve weeks of gestation) and involves taking a small sample of part of the placenta.

The difference in timing between the two sampling techniques suggests that different arrays could be used. As chorionic villus samples are from earlier in pregnancy, they may still carry major perturbations that will cause a miscarriage later in the pregnancy but before amniocentesis sampling. Having said this, the major chromosomal perturbations that could cause this outcome are likely to be detected by current microarrays anyway.

Due to the novelty of aCGH as a screening method, fetal samples are karyotyped in the traditional manner, as a backup test and potentially for confirmation of any abnormalities found. Fluorescent *in situ* hybridisation (FISH) is another technique used to confirm abnormal results.

The presence of copy number variation (CNV) in the genome means that all ‘abnormalities’ are then looked for in parental blood samples. If the change is found in an apparently functional adult, then the CNV is reported as a neutral variant, unless there are other suspicious circumstances.

Many microarrays used for screening are generated ‘in-house’ and are known as ‘home-brew’ arrays, particularly those used for array CGH (aCGH).

Non-invasive sampling of fetal cells or DNA in the maternal blood stream is still in its infancy. If the fetal cells or genetic material can eventually be isolated reliably from maternal material, then microarrays may play a significant role in prenatal screening.
4.2 Preimplantation genetic diagnosis (PGD)

Microarrays offer more comprehensive screening, of aneuploid embryos in particular, than current technologies. The increased sensitivity from using microarrays means that many more aneuploidies can be detected, hopefully leading to improved rates of implantation and successful pregnancy. The current limit on how many chromosomes can be screened in preimplantation genetic diagnosis (PGD) (approximately eleven chromosomes at best) is postulated as the reason why aneuploidy screening (preimplantation genetic screening) is less successful than it intuitively ought to be. Current techniques only detect the presence or absence of a small part of any one chromosome so segmental aneuploidies are generally not detected either.

Use of microarrays in PGD has been published in a small number of papers, but they are research papers only. Routine implementation of this technique is dependent on a reliable method of whole genome amplification (WGA, see following information box).

Aside from WGA, the other impediment to successful implementation of microarray screening of embryos is mosaicism. Many embryos apparently have some cells with a normal chromosomal complement and some that are aneuploid. Which cell, in the eight-cell embryo, is biopsied is a matter of chance and can lead to a situation where some ‘mostly normal’ embryos are discarded as being aneuploid and vice versa. There is also recent evidence that some mosaic embryos can ‘correct’ themselves and at a later embryonic stage be effectively chromosomally ‘normal’. Whilst this issue does not prevent successful PGD, it reduces the chances of success by reducing the number of healthy embryos for transfer.

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**WHOLE GENOME AMPLIFICATION (WGA)**

In PGD, there is only a very small amount of genetic material to analyse: that which can be freed from a single cell. To have any chance of genetic analysis (using current technology), the DNA from the single cells must be copied many times over. For the technique to be useful in microarray analysis, the whole genome must be copied evenly so that there is the same amount of DNA, proportionally, that there was in the original cell. If, for some technical reason, a part of the genome is copied more frequently (or less frequently) than the rest of the genome, then the microarray result may show aneuploidy where there is none.

Whilst techniques for copying DNA are improving, there are significant problems in reliable amplification giving accurate results. Current WGA techniques merely exacerbate the problems that exist in amplifying a small section of DNA for genetic testing.
4.3 Newborn screening

DNA screening and profiling of newborns is discussed in more detail in the chapter ‘Newborn screening: Present and future’.

The use of microarrays has been suggested for the screening of newborns for early onset disorders. Current technologies use indirect measurements for genetic disorders, such as metabolic molecules from a blood sample. Metabolic newborn screening (as it is known) is effective in detecting early onset disorders as the disorders manifest within two or three days of birth. There is now some discussion around extending screening to include direct DNA screening.

Genetic technologies such as microarrays would be useful as the testing can be targeted to specific early disorders. However, when compared to metabolic screening in which there is an actual medical condition manifesting itself, there remains much uncertainty in interpretation and use of these results. Some disorders have a variable manifestation, even when two individuals carry the same causative mutations. Some mutations are so rare that the possible effects are unknown, with respect to medical symptoms. With other disorders, not all genetic causes are known and some newborns may miss out on crucial early treatment.

Genetic profiling of newborns is a possibility in the future; although in one recent study it was dismissed due to cost and technological barriers. There are also many ethical and policy issues that need to be addressed before this use of microarrays is implemented.

4.4 Childhood and adult testing

The subject of genetic testing of children is comprehensively covered in the report by Deborah Lawson.

Apart from the potential for newborn screening or profiling, the current use of microarrays in children is for diagnostics.

Microarray testing is very efficient, as many tests can be performed simultaneously; but all the issues associated with genetic testing in children are amplified by this efficiency. Unless the child has symptoms that are consistent with loss or gain of genetic material (i.e. aneuploidy), the question then becomes, ‘Which tests do we perform on this sample?’ The answer usually depends on the symptoms exhibited; but, for commercial efficiency, most microarrays would include many more genes and mutations than are needed for a specific child. Thus, considerably more information is obtained than is needed. Production of a wide variety of customised microarrays is not yet viable.
The oligonucleotide-based resequencing microarrays have a great deal of potential for carrier screening and susceptibility screening in adults.

In the United States, there is a national programme offering parents-to-be screening for carrier status for common cystic fibrosis (CF) mutations. If both parents are found to be carriers of a CF mutation, prenatal testing of the fetus (or embryos) can then be offered. Only twenty-three of the ~1500 described mutations are used for screening. This selective screening leaves a small risk that both parents may still be carriers yet one or both may get a negative result. If all known mutations can be put on a single microarray, then this risk is negligible. On the other hand, there have already been documented issues with clinicians or parents misunderstanding the implications of results from the current small screening panel. Again, the issues of interpretation and understanding could be magnified with an expanded screening panel.

For disorders such as heritable breast cancer, some laboratories sequence the entire BRCA 1 and 2 genes. Other laboratories screen only for common mutations associated with this condition, at least initially. A great deal of time could be saved if these two genes could be resequenced on a microarray, in their entirety; rare mutations could then easily be detected, rather than requiring the use of a two or three tier screening process.

The field of pharmacogenetics is already putting microarrays to use. There is an FDA-approved microarray that screens the cytochrome p450 gene, to deduce how substances, including pharmaceuticals, are processed in the liver. Drug dosages can be altered according to whether someone is a ‘fast’ or ‘slow’ processor of drugs such as chemotherapies or warfarin. A comprehensive blood-typing microarray is also in development for frequent users of blood transfusions. The more closely matched a blood transfusion, the better the result and the less likely a person is to develop an immune response to donor blood.

Expression profiles have been used to differentiate and categorise apparently similar cancer subtypes, by comparing those who respond to a specific treatment and those who have a poor long-term prognosis, ‘apparently reflecting the variation in tumour proliferation rate, host response and differentiation state of the tumour’. Detected changes in expression levels (up or down) have been used to find molecular markers for response to therapeutic treatments (before and after treatment), which can then be used in developing treatments.

In the future, genotyping microarrays could be designed to assess genetic risk of complex disease, such as heart disease, diabetes, Alzheimer disease and other late onset disorders. The microarrays could only assess the genetic risk, however, and complex disorders have significant environmental influences.
4.5 Limitations

Despite the many positive possibilities regarding microarrays in clinical genetics, there remain some negatives, although not all are unique. Some are mentioned later in this chapter; others, such as interpretation of ambiguous results, are covered in the chapter by Mildred Cho, ‘Array Comparative Genomic Hybridisation (aCGH): An Analysis of the Current Technology and its Future in Prenatal Diagnosis’.

Despite the glib descriptions of how the technology works, microarrays remain a technically demanding technique. There are issues of reproducibility as well as interpretation of results. This is discussed more fully in the next section.

The technology is currently expensive, microarrays costing approximately NZ$1000 per chip. Labour, materials for processing samples and, in particular, the machinery needed to complete the entire process involve additional cost.

Current limits placed on genetic testing, in the form of licensing of patented information, are also a problem with microarray testing. Obtaining permission to licence an individual gene test and/or prohibitively expensive licensing fees are currently affecting diagnostic uses of microarrays.

Microarrays cannot detect changes in the entire set of chromosomes (whole ploidy). If, for example, there are three copies of each chromosome in a prenatal sample, then the relative amount of DNA will be the same and will be reported as ‘normal’. The limits of the sensitivity of microarrays are also being explored in respect of mosaicism. Mosaicism refers to the situation in which some parts of the body contain one set of genetic information and cells in other parts of the body contain another set, the result of a late mutation event. In the example of the trisomy 21 (Down syndrome) mosaicism, some cells will have three copies of chromosome 21 and some cells will have two copies. Depending on when and where the mutation event occurs (e.g. the loss of an extra chromosome 21), the tissues affected will vary from person to person and those with mosaicism will have different levels of ability. Microarrays are thought to be able to detect as little as 30 per cent or less mosaicism in a tissue sample. This does not mean that mosaicism will be detected, however, as it depends which tissue is examined for the changes.”
5 THE NEW ZEALAND SITUATION

In New Zealand, microarrays are currently used only in research laboratories. Some of this research usage is in the context of screening for targets for clinical applications or trials, an excellent example being finding clinically relevant targets for cancer profiling.

Clinical services are certainly not using microarrays in genotyping or any other application as yet; although some laboratories have apparently been investigating use of aCGH for diagnostic applications. The fact that only one laboratory in the United States is commercially offering aCGH for prenatal screening suggests that this use is some way off in New Zealand.

5.1 Molecular genetic testing in New Zealand, 2003

The 2003 report has also been reviewed by Deborah Lawson in the section of her report entitled, ‘Genetic Testing of Competent Minors’.

There is no apparent systemic overview of genetic testing in New Zealand and the introduction of new testing technologies appears to be ad hoc. There have been a number of reports since 1995 on the subject in the New Zealand context but there appears to have been no progress concerning implementation.

Genetic services in New Zealand are divided into the Northern (upper North Island) and Central and Southern (the rest of New Zealand) Regional Genetic Services. These groups are separately funded and have nominal support and supervision by ‘host’ District Health Boards (DHBs), Auckland and Capital Coast DHBs respectively. There are four principal cytogenetics laboratories (in Auckland, Hamilton, Wellington and Christchurch) and two main molecular laboratories (Auckland and Christchurch, with limited services in Wellington) offering genetic testing services, also under the aegis of local DHBs. Most testing is referred through genetic services, with some referred through various specialities (including obstetrics and paediatrics) and a limited amount through primary health services such as general practitioners.

The 2003 report reviews the current molecular genetic testing situation in New Zealand and finds it wanting. It points to a lack of criteria for assessment of clinical validity and utility of tests; a lack of testing capacity due to funding and personnel shortages; and a lack of coherence in and possible duplication of services offered by laboratories. It also makes suggestions with regard to consent, use of different testing methodologies, appropriateness of testing and the need for education of both medical professionals and the public.

Since then, there has been little overt progress; although it seems that DHB New Zealand (which co-ordinates the individual regional DHBs) ‘has now set up an expert group to prepare for the improvement to genetic services’.
6 CONCLUSIONS

The use of microarrays is still predominantly in the research sector. There has been some movement into the clinical testing and diagnostics arena internationally, but the eventual utility in clinical screening remains to be seen. The diagnostic aspect of microarrays has been enthusiastically reported in the clinical and scientific literature and remains one of the most likely uses of the technology as the cost comes down.

There is still a technology block for use of microarrays with preimplantation genetic diagnosis (PGD) for aneuploidy screening in the form of whole genome amplification. If this problem can be overcome, microarrays could foreseeably make a positive difference to implantation rates and reduce miscarriage rates for those who choose to use PGD for this purpose. PGD requires, however, that IVF be used to generate embryos for testing. It is therefore unlikely that it will ever be used outside fertility clinics and, even then, only for a subset of clients. Future use remains debatable.

As cost comes down, microarray technologies will likely supersede the existing cytogenetic technologies as a first-line prenatal test. Arrays are faster and potentially offer more detailed screening for disorder-causing chromosomal changes. This does not preclude simultaneous karyotyping as a method for confirming any larger abnormalities, or use of other techniques for later confirmation of an abnormal result. All cytogenetic results should be confirmed, preferably by using another method. As knowledge increases regarding the effects of medium to large chromosomal changes, (ironically) through increased testing as well as new research data, uncertainty about the seriousness of particular changes will be reduced.

There is, however, still the difficulty of explaining the technology and results to a lay audience, and a number of other ethical issues have been raised around the use of microarrays for prenatal screening. In addition, microarray use in prenatal screening currently requires an invasive procedure to obtain fetal material for testing. Again, unless there are developments in non-invasive testing, this technology will be limited to those women already undergoing amniocentesis or chorionic villus sampling.

A debate exists regarding how much genetic information is useful and the advantages and disadvantages of selective versus whole genome screening. There is a corollary with the use of whole-body CAT and MRI scans for simple health ‘check ups’. Abnormalities may be detected that have no effect on the quality of life; but, because they have been found, they are investigated or treated unnecessarily. The more targeted the microarrays to specific clinical questions, the less likely this is to occur.

Genetic services in New Zealand are currently stretched. Introduction of routine use of microarrays would require a substantial investment, not only in technology and laboratory staffing, but also in clinical genetics and counselling personnel.
Implementation of new testing technologies in New Zealand currently appears to be driven by the clinical testing laboratories, on a cost recovery basis. There appears to be no national strategy for monitoring and introducing new techniques and technologies. Whilst this is not necessarily a negative, it may preclude a national push for the introduction of new genetic testing tools; particularly if this were to be based in a single laboratory, in competition with others. In addition, private genetic testing services have not been established in New Zealand or Australia. This may or may not affect whether laboratories offer new services. The promised follow-up to the 2003 report on molecular genetic testing in New Zealand is apparently being carried out by DHBNZ. We await their report with some anticipation.

Beyond microarray technologies, rapid whole genome sequencing is being touted as the next revolution in genetic testing. It is likely that rapid whole genome sequencing will become viable in the medium to long-term future. This technology reveals the ultimate genetic information: the exact sequence of the genomic DNA. This information is superior to the limited data from microarrays, although it is likely to need more interpretation. It is unlikely, however, to detect ploidy changes, such as trisomies, without additional analysis. Detection of chromosomal copy number variation (CNV) is the principal driver of most current and future PGD, prenatal and diagnostic testing.

ENDNOTES

1 Mutation screening could be for carrier detection, diagnostics, prenatal testing or risk analysis for a couple.
5 Currently being developed by the Bloodgen consortium: BBC News, ‘DNA blood test “could save lives”’. Available at: news.bbc.co.uk/1/hi/health/5335930.stm (last accessed 1 June 2007); Bloodgen consortium, ‘Bloodgen: Blood grouping and genotyping’. Available at: http://www.bloodgen.com/ (last accessed 1 June 2007).
8 A nonsense mutation is where the three ‘letters’ (nucleotides) that code for an amino acid are converted to a stop signal by a single change to one of them.
10 Polymorphism is generally classed as that variation which reaches approximately 1 per cent frequency in a population.
Robertsonian translocations mainly occur in chromosomes that have the centromere at one end of the chromosome. The commonly involved acrocentric chromosomes are 13, 14, 21 and 22. The chromosome created from the two short arms is often lost, leaving forty-five chromosomes.


Leiden University Medical Center, ‘Cytogenetics’. Available at: www.lumc.nl/4080/ CYTO/ patientcare_CYTO.html (last accessed 1 June 2007).


An example of these databases is the Decipher database of genomic variants: Sanger Institute, 'DatabasE of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources'. Available at: http://decipher.sanger.ac.uk/ (last accessed 1 June 2007).


Signature Genomics, 'Detection rates table'. Available at: http://www.signaturegenomics.com/detection_rates.html (last accessed 1 June 2007); Baylor College of Medicine, 'Disorder table v 6.2'. Available at: http://www.bcm.edu/cma/table.htm (last accessed 1 June 2007).


Currently being developed by the Bloodgen consortium: BBC News, ‘DNA blood test “could save lives”’. Available at: news.bbc.co.uk/1/hi/health/5335930.stm (last accessed 1 June 2007); Bloodgen consortium, 'Bloodgen: Blood grouping and genotyping'. Available at: http://www.bloodgen.com/ (last accessed 1 June 2007).

37 Kerruish, N., Personal communication, July 2006.
41 These issues are more comprehensively discussed in Cho, M., ‘Array Comparative Genomic Hybridisation (aCGH): An Analysis of the Current Technology and its Future in Prenatal Diagnosis’.