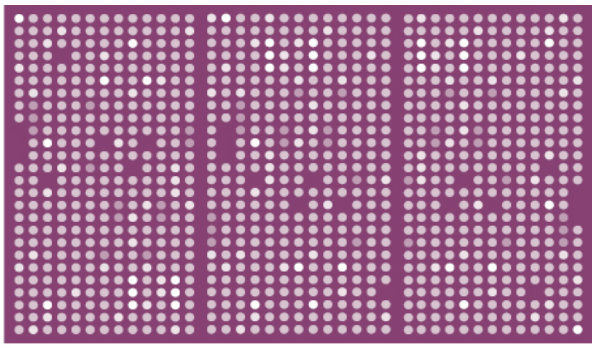
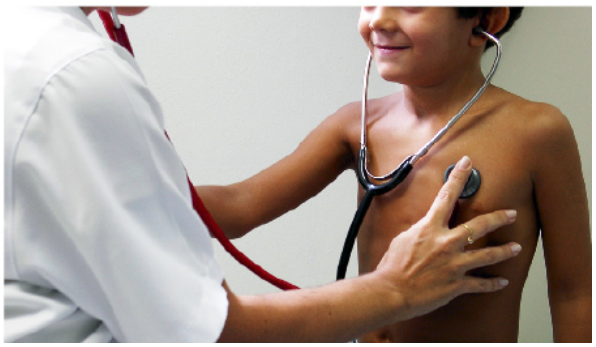
Evaluation of the use of array comparative genomic hybridisation in the diagnosis of learning disability

Report of a UK Genetic Testing Network Working Party





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August 2006

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The report can be downloaded from the PHGU website: **www.phgu.org.uk**

Acknowledgements

The project team would like to express their thanks to all members of the Working Group who shared so freely of their expertise and to the laboratories who participated in the survey and cost analysis. Thanks are also due to the UK Genetics Testing Network for funding the meetings.

The report is published by the Public Health Genetics Unit, a core facility of Cambridge Genetics Knowledge Park

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Executive Summary

Introduction

Microarray based comparative genomic hybridisation (array CGH) is a new technology with uses developing in various diagnostic areas within the NHS. One important context is the investigation of chromosomal amplifications, deletions and rearrangements that can be aetiological factors in learning disability (LD). A Working Group set up at the request of the UKGTN set out to evaluate the use of array CGH in this context and to make recommendations to the UKGTN on the introduction of this new technology into routine clinical practice.

Chaired by Hilary Burton, the Working Group met five times between July 2005 and April 2006. The Report is drafted in ten chapters including: a description of the technology; epidemiology; clinical context; the value of a genetic diagnosis (parental and clinical aspects); a survey of developmental work on array CGH being undertaken currently in UK laboratories; formal evaluation of tests including systematic review and meta-analysis; economic cost analysis; final discussion and recommendations.

Main findings

The technology: Array CGH allows the rapid detection of copy number changes across the entire genome at high resolution. Although a very powerful technique, it has the drawback that it can miss balanced translocations and mosaicism, and will also detect new variations of unknown significance. As a laboratory system, array CGH will eventually lend itself to automation and high throughput.

Thirteen laboratories across the UK completed a questionnaire indicating that they were currently developing the technology, with 11 having invested in their own equipment, mostly from NHS White Paper monies. The laboratories had varying experience. Most were testing the technology to validate known cases. Four laboratories were testing cases in which a chromosomal abnormality was suspected on clinical grounds but a diagnosis had not been reached after routine cytogenetic analysis (karyotype +/- FISH or MLPA[1]). The average yield was 25%, representing a rate of 14/55 for the four laboratories.

The laboratories have formed themselves into a microarray support group. Their main concerns were detection and work-up for polymorphisms or mutations of unknown significance, expense in time, money and training, complexity to implement, paucity of reliable commercial kits and standardisation of software.

The utility of a genetic diagnosis: Seeking a diagnosis about the cause of learning disability is part of clinical assessment and investigation. A recent review of 16 studies worldwide found that chromosomal abnormalities were present, on average, in 16.1% of individuals with LD (range 4.0 to 34.1%). These may be associated with dysmorphic features, congenital abnormalities and growth problems.

Parents value a genetic diagnosis for many reasons, most notably: the provision of an explanation and name for the problem, sometimes improving access to educational special needs services; information about the condition including prognosis; reproductive planning; and to access support groups. It can also help to optimise clinical support by identifying and providing information on known syndromes and providing associated information on genes affected and thus predicting possible effects. These two capacities are greatly enhanced by the use of databases such as DECIPHER[2] (a resource which records information about chromosomal rearrangements linked to phenotypic descriptions and genome mapping). A detailed diagnosis may allow provision of precise information on which genetic advice about risk to other family members and/or the offer of prenatal genetic testing can be made. Finally, it may end a long term quest for a diagnosis that can involve a large number of costly investigations and appointments over many years.

Systematic review and meta-analysis: This included two separate areas: the diagnosis of known abnormalities and the investigation of hitherto unknown abnormalities. **It was important to maintain these as distinct areas because of fundamental differences in the ways we conceptualised the evaluation.** (A consideration of these methods will be the subject of a separate methodological paper).

A total of 449 citations were retrieved and seven studies were eventually included in each category. It was found that array CGH was capable of identifying existing abnormalities with a high sensitivity (100% in four studies); the main shortcomings were in array design and it was concluded that arrays must include appropriate clones that relate to the genetic abnormalities in the most common phenotypes.

For unknown syndromes, meta-analysis showed that the overall diagnostic yield in patients in whom conventional cytogenetic analysis (karyotype +/- FISH or MLPA) was negative and who have fulfilled clinical criteria is about 13%. The number of variants of probably non-causal origin is likely in the region of 5-10% of the total sample.

Cost analysis: Costing was undertaken by the Oxford Genetics Knowledge Park using a micro-costing approach with information from three laboratories (Oxford, Birmingham and Edinburgh). The same approaches were used to cost karyotyping and array CGH. The total average cost for the array testing was £892 per sample, with a range between £377 and £1,135. The main cost driver for this total was the array component at £563, which included an average of £500 per array, plus £63 for array preparation. The second largest cost category was Hospital Trust on-costs at £148, followed by labeling at £78. In comparison, the cost of karyotyping was £117 although this does not take into account the follow-up investigations (such as FISH, telomeres and MLPA) which are frequently required. An analysis of these will constitute a cost-effectiveness study to be undertaken later this year.

Discussion

Array CGH is an effective adjunct to the investigation of learning disability. At present it is used, almost exclusively, in cases where karyotype is normal and second stage testing by FISH or MLPA in clinically selected cases is also normal. Array CGH does not require prior knowledge of specific areas of chromosomes and so can be used for further investigation looking for unknown chromosomal rearrangements. The use of clinical criteria to select cases for further testing after karyotype approximately doubles the diagnostic yield. However, these criteria are by no means 100% sensitive or specific and undoubtedly positive cases are lost in the group not

further investigated. Additionally, the large size of the group who do not fulfil criteria (often mild to moderate learning disability without dysmorphism) may mean that, in absolute terms current laboratory regimes risk missing many cases.

The overall additional yield from array CGH depends on the proportion of cases selected for further investigation. In the one cohort study available (de Vries et al.[3]), where 44% of cases were selected for MLPA the respective yield from the various stages (excluding Down syndrome) was: karyotype 4.8%, MLPA 2.0% and array CGH 1.8%, giving a total yield of 8.2%. We modelled the effect of using array CGH on all patients, making best estimates of the diagnostic yield in unselected cases, and estimate that the maximum yield that could be achieved using karyotype and a sequence of MLPA or FISH and then array CGH on all patients with negative results would be around 14% (excluding Down syndrome). However, this represents an inefficient use of resources, as, under this model, at least 86% of patients would have karyotype, FISH or MLPA and array CGH.

Thus, in the longer term, for maximum effectiveness and efficiency, we would suggest that the use of array CGH as a first line investigation should be considered. However, this requires further understanding of the possible disadvantages of this strategy, including, particularly, the numbers of false positives (identification of non-causal variants) and the amount and nature of further clinical and laboratory work needed to interpret these correctly. In general, this involves interrogation of databases, and genetic testing and clinical review of parents to determine whether the abnormality was inherited or arose *de novo*.

It also depends critically on the cost of the investigation; when the cost of arrays is reduced the use of this investigation on all patients may become a more viable option. There is some evidence that the cost of arrays has already fallen since the cost analysis was undertaken and there would be further price reductions with large volume orders.

Conclusion

Array CGH is a powerful new technology with such potential that experts believe it will inevitably become a prime tool in pathological diagnosis across a range of clinical areas. Our review has shown that it is an effective adjunct to the investigation of learning disability and is already being tested and used in this clinical area in 13 laboratories across the UK. Services face a number of barriers before it can be used more widely. The high cost of arrays is the main limiting factor but of importance also are the complexity of development of arrays and software, integration into the service, standardisation and quality assurance of the systems.

In the longer term, the advantages of array CGH in providing higher resolution and the ability to diagnose hitherto unrecognised syndromes may best be achieved by using the technique on all patients instead of the current regime of karyotype +/- FISH or MLPA +/- array CGH. Elimination of a large number of these current routine investigations will offset the high cost to some extent, whilst higher volume contracts should provide an incentive to price reduction. However, the technology is not proven in this context; in particular, the clinical and laboratory work in following up eventual false positives and the consequences of these findings have not been investigated.

Recommendations

We make the following recommendations:

1) Array CGH should continue to be available as an adjunct to routine laboratory cytogenetic analysis for investigation of cases of children with learning disability. Cases will usually be referred for array CGH following assessment by a clinical geneticist and application of appropriate selection criteria (modified from Shaw-Smith C. *et al.*[4]). As the cost of array CGH decreases consideration should be given to the affordability of increasing the proportion of patients having further investigation beyond karyotype in order to minimise missed diagnosis.

2) Means should be explored that will allow the revenue costs of array CGH testing already being performed in genetics laboratories to be met.

3) Work should continue to optimise the technology to ensure maximum sensitivity for known syndromes and genome wide screening (in proportion to gene density) and to minimise the incidence of false positives.

4) A multi-centre prospective cohort trial of array CGH should be undertaken to compare a cohort of patients managed by the current cytogenetic routine analysis, with one in which all patients receive an initial array CGH investigation. The trial should cover investigation of different platforms, potential selection criteria, clinical and social impact, different centres, economic aspects, laboratory and clinical follow-up for positives and negatives, implications for education and training of laboratory staff, and implications for information and education for parents.

5) A quality control system for array CGH should be devised and incorporated into the NEQAS system.

6) Geneticists should record findings from array CGH (in terms of genotype and phenotype for novel abnormalities) in a suitable database such as DECIPHER, to facilitate information sharing.

7) Cost-effectiveness analysis to explore the real cost of current LD investigations (ie frequency with which karyotyping, multiple FISH, telomere and MLPA tests are performed and cost of these) should be undertaken.

Endnotes

1 MLPA Multiplex Ligation-dependent Probe Amplification

2 DECIPHER DatabasE of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources. http://www.sanger.ac.uk/PostGenomics/decipher/

3 de Vries BB, White SM, Knight SJ, Regan R, Homfray T, Young ID et al. Clinical studies on submicroscopic subtelomeric rearrangements: a checklist. J Med Genet 200; 38 (3):145-50.

4 Shaw-Smith C, Redon R, Rickman L, Rio M, Willatt L, Fiegler H et al. Microarray based comparative genomic hybridisation (array CGH) detects submicroscopic chromosomal deletions and duplications in patients with learning disability/mental retardation and dysmorphic features. J Med Genet 2004; 41(4):241-8.

Chapter 1 Introduction and background

1.1 Introduction

Microarray based comparative genomic hybridisation (array CGH) is a new technology thought to show great potential in UK diagnostic laboratories. The technology looks for genomic gains or losses on chromosomes and provides greater resolution than current cytogenetic techniques. Moreover it can cover the whole genome and is able to look for previously unknown abnormalities.

Array CGH shows promise in many diagnostic areas within the NHS, including, for example, tumour gene expression analysis which provides better characterisation of disease and enhanced rationale for treatment. Many UK laboratories have recently invested in the technology supported in this by White Paper monies. However, the technology has not been fully developed and evaluated within the NHS context for any of its applications.

One of the clinical contexts suggested for its use is learning disability diagnosis. Several UK genetics laboratories are undertaking developmental work with array CGH in this context but a decision has not been made to recommend their use in routine clinical practice.

The UKGTN considered the development of these technologies in the context of learning disability at its meeting in early 2005 and reinforced the view that further appraisal should be undertaken before consideration of when or how they might be introduced into routine clinical practice. As a result, the Public Health Genetics Unit was asked to take this forward, involving experts and stakeholders from the genetics reference laboratories, the clinical genetics community and others.

Whilst focusing on the clinical context of learning disability, the review group tried to maintain an awareness of the likely use of array CGH in a wide range of other contexts and, where appropriate, to find out and comment on the likely 'fit' with other work.

1.2 Aims of the working group

The aims and objectives of the Working Group were set out as follows:

Aims

1 To evaluate the use of array CGH in the determination of chromosomal abnormalities for use in clinical practice using learning disability as an initial paradigm.

2 To assemble and consider the evidence and make recommendations to the UKGTN on :

a) Whether array CGH testing should be introduced into clinical practice. If so, for which patient group/s, in what clinical context, with what quality control, and with what supporting systems and safeguards. Such systems would be likely to include education for laboratory and clinical professionals, the development of educational and information resources for use with patients and families, and the use of international databases to provide information and build up knowledge on genotype-phenotype correlations; OR

b) Whether array CGH testing should be introduced into clinical practice as a pilot project or projects in the context of further research and evaluations. If so, to recommend what this research and evaluation should address OR

c) Whether array CGH testing in learning disability needs further research or technical development before it should be introduced into clinical practice - in which case to make recommendations on the key research questions OR

d) That array CGH testing should not be developed at the present time in the context of learning disability.

Objectives

1 To evaluate array CGH under the headings of:

a) Definition of the disorder/setting

b) Analytical validity

c) Clinical validity

d) Clinical utility (including wider psychological and social issues)

e) Economics

f) Feasibility

g) ELSI

2 To engage with laboratories and clinical services with an interest in this area to ensure general understanding of progress across the UK and ownership of the findings.

3 To write a report with recommendations for the UKGTN by March 2006. The Report would focus on the use of array CGH in the clinical context of learning disability, but would draw attention to learning that was applicable to the wider clinical context.

1.3 Method

The work was led by Dr Hilary Burton, Consultant in Public Health Medicine at the Public Health Genetics Unit (PHGU) in Cambridge and supported by a team from the PHGU. A Steering Group provided expertise and guidance on laboratory, clinical, and economic aspects and from the parent and voluntary organisation point of view. The PHGU provided expertise in epidemiology and critical appraisal as well as the organisation and administration of the work programme. A complete list of participants is given in Appendix 1.

The steering group met five times between July 2005 and April 2006. The meetings were used for the expert stakeholders to:

a) Provide information on the key issues

b) Design the detailed review work to be undertaken

c) Assist with and review progress

d) Consider and comment upon the emerging findings

e) Decide on the main recommendations

f) Comment on and assist in the writing of the final report

The PHGU undertook a detailed systematic review of clinical validity and obtained evidence from previous Cambridge Genetics Knowledge Park work on the clinical utility with particular emphasis on it's work on the value of a diagnosis to parents. The PHGU also undertook a survey of developmental work on array CGH being undertaken currently in UK laboratories. The Oxford Genetics Knowledge Park carried out the economic appraisal of array CGH. The PHGU wrote the report including an overview of the technology and background epidemiology of genetics and learning disability.

1.4 The report

The report is set out in three main sections:

The background includes an overview of the expert stakeholder perspectives on the use of array CGH in the context of LD, it's possible advantages and disadvantages and the issues that would arise in implementation; further consideration of the technology itself; the epidemiological and clinical context of learning disability; current views on the value of a genetic diagnosis from the parent and clinical perspectives.

In the findings we set out:

a) Progress in UK laboratories including the results of our survey of laboratories undertaking array CGH of the current progress in developing the technology and some discussion of the main issues that will arise in the provision of array CGH testing

b) Formal evaluation of array CGH in the context of LD diagnosis

c) Economic evaluation

In the conclusion and recommendations we bring together our main findings, discuss some of the implications and make recommendations to the UKGTN.

1.5 Background

At the beginning of the process, the stakeholder group was used to gather initial perspectives on array CGH and to set out some of the key issues that would need to be addressed in the working group and afterwards.

The patient perspective

In principle, patient groups such as *Unique* and affected individuals and families, especially those whose children are suspected of having a chromosome disorder but remain undiagnosed by conventional chromosome analysis, are in favour of the introduction of array CGH. They are conscious, however, of the potential of new technologies to increase the complexity of the clinical and social situation for individual families and of the implications for the delivery of high quality genetic counselling and risk analysis. In particular, difficulties in effectively communicating and explaining detailed information about very subtle chromosomal abnormalities with possibly unknown clinical significance and with complex nomenclature were highlighted. They are also mindful of the dangers of misinterpreting highly complex test results and of ensuring families fully understand the limitations of testing. Furthermore, concerns have been raised about ensuring the continued availability of existing testing methodologies like karyotyping and FISH

for relevant chromosome disorders such as balanced rearrangements. Unique members urge the need for clarity and honesty over the communication of test results, including the limitations of information derived from new forms of diagnostic testing.

The clinician perspective

Understanding the cause of a disease or condition is a central tenet of medical practice, with a diagnosis considered beneficial, even where therapeutic interventions are not available. Clinicians and cytogeneticists are thought to be generally enthusiastic about array CGH as a diagnostic tool for chromosomal abnormalities linked to learning disability. Their main concerns were issues of cost and quality assurance, criteria for patient testing, the stage at which they should be tested, and who should handle referrals for testing. Gate-keeping would be increasingly important as the potential applications of array CGH grow and it would need to be decided who would be the most appropriate gate-keepers for these specialised services, whether clinical geneticists or others such as community paediatricians with a special interest in the area.

The main clinical problems would be of interpretation in clinical practice, since there are wide gaps in the understanding of gene function and interaction. All the test positive families would need careful genetic counselling and assessments to establish whether the test result is causal, to ensure that the results were properly explained to families and to avoid giving families with polymorphic structural variations the mistaken impression that they have a genetic defect. These costs would need to be incorporated into any plan for implementation and there would also be important implications for education of health professionals and the wider public.

Laboratory perspective

Through White Paper monies and other sources a number of laboratories invested in the necessary equipment and have performed clinical validations; a number are now moving towards providing array CGH as a clinical service.

Laboratories face a number of technical and other issues in developing this work. These include:

- The lack of standardised over the counter arrays
- The need for a systematic and coordinated approach
- Expense of capital equipment, consumables and obsolescence
- Potential high numbers referred for testing
- False positives
- Need to link clinical and cytogenetic information
- Use of the technology for a wider range of applications
- Possible move to centralisation of more specialised testing

Commissioners and managers perspective

From the perspective of NHS providers, a case would need to be made to fund array CGH in learning disability in terms of value for money. This would require an assessment of whether array CGH made the best use of limited resources in testing for learning disability compared with standard care, that is, karyotyping. In practice, most new health care interventions are slightly or much more expensive than existing technologies. If arrays were initially more expensive than karyotyping, this would not necessarily be a problem if the arrays actually picked up more cases and hence avoided unnecessary referrals for additional tests, thus saving costs elsewhere. It is therefore important that a strong evidence base is compiled to inform

commissioners whether adopting array CGH for their population represents good value for money.

Chapter 2 Array CGH: the technology

2.1 Diagnostic technologies

Diagnosis of specific chromosomal causes of learning disability has been possible since the 1970s, when karyotype analysis for banding was introduced; this involves the staining of full sets of chromosomes from the individual in question to reveal characteristic bands. Expert visual inspection of these banding patterns under the light microscope can reveal the presence of large-scale chromosomal abnormalities including deletion or duplication of large chromosomal segments with a resolution of 3-5 Mb. To visualise chromosomes, cells must be actively growing in culture before being halted at the metaphase stage and banded. The most common form of staining to reveal chromosomal bands is called G-banding, but this technique cannot identify sub-microscopic abnormalities.

A major step forward came in the 1980s with the introduction of DNA hybridisation-based methods and fluorescent detection systems. This permitted the identification of specific sub-microscopic chromosomal abnormalities by the use of fluorescence in situ hybridisation (FISH). For example, if a particular genetic abnormality is suspected on the basis of clinical features, then a fluorescently labelled probe for that specific region of the chromosome can reveal the presence or absence of the corresponding region by binding to it. In addition to FISH diagnosis, detection of copy number changes at much higher resolutions than possible by banding analysis was facilitated by the use of comparative genomic hybridisation (CGH), initially as a stand-alone technique but more recently in combination with microarray technology.

2.2 Microarrays

DNA microarrays are relatively new systems that allow very rapid (automated) simultaneous analysis of thousands of different DNA sequences. Although different platforms vary, in essence they are all solid surface (e.g. glass or silicon) chips on which multiple, different, short, single-stranded (ss) DNA probe sequences have been immobilised in an ordered fashion at precisely defined points; these arrays are sometimes also referred to as 'gene chips'. The location of each spot on the array can be used to identify the sequence present. One key feature of microarrays is the ability to array many thousands of different sequences within a tiny area, yet still to resolve fluorescent signals from each individual one via advanced high-resolution scanning and detection systems. Microarrays also lend themselves to high-throughput analyses, because the process can be largely automated.

The technology relies on the ability of complementary single-stranded DNA sequences to bind together, or hybridise, by forming base pairs. By passing fluorescently labelled ssDNA from a sample of interest over the microarray surface, it is possible to determine the type and quantity of specific sequences present in the sample; DNA that is complementary to probe sequences on the array surface will bind to them. Fluorescence at these points, following laser excitation of the array, reveals hybridisation, which can be correlated with the exact probe sequence known to be present at that precise position on the array. The degree of fluorescence can be used as a relative measure of abundance of the corresponding DNA sequence in a sample.

Initially, microarrays were made using large-insert genomic clones (BACs, bacterial artificial chromosomes); they may now use DNA, cDNA (single stranded DNA produced from RNA templates by a process termed 'reverse transcription') or oligonucleotide (a short fragment of a single-stranded DNA that is typically 5 to 50 nucleotides long) probe sequences. These may be

referred to as genomic arrays, expression or gene expression arrays and oligo arrays, respectively. The advent of industrial techniques for synthesising high-density oligonucleotide arrays directly on to the solid surface chips has broadened their potential applications.

2.3 Comparative genomic hybridisation

Comparative genomic hybridisation (CGH) is a method for the identification of copy-number changes (amplifications and deletions) within the genome. The central procedure relies on the labelling of reference (normal) and test genomic DNA samples with different fluorochromes or fluorophores, (tags that emit fluorescent light at different wavelengths), most usually red (e.g. Texas red) and green (e.g. Fluorescein). The labelled ssDNA samples are mixed and applied to immobilised normal ssDNA, and fluorescence detected using laser excitation.

Where there is no change in sequence copy number in the test sample, there will be equal binding of test and reference sample DNA, equal amounts of green and red fluorescence and a net emission of yellow light. For sequences where there has been amplification in the test sample, there will be more green than red fluorescence and an overall green emission; conversely, deletions will result in a reduced level of green fluorescence relative to the red fluorescence from the reference sample, and a net red light emission.

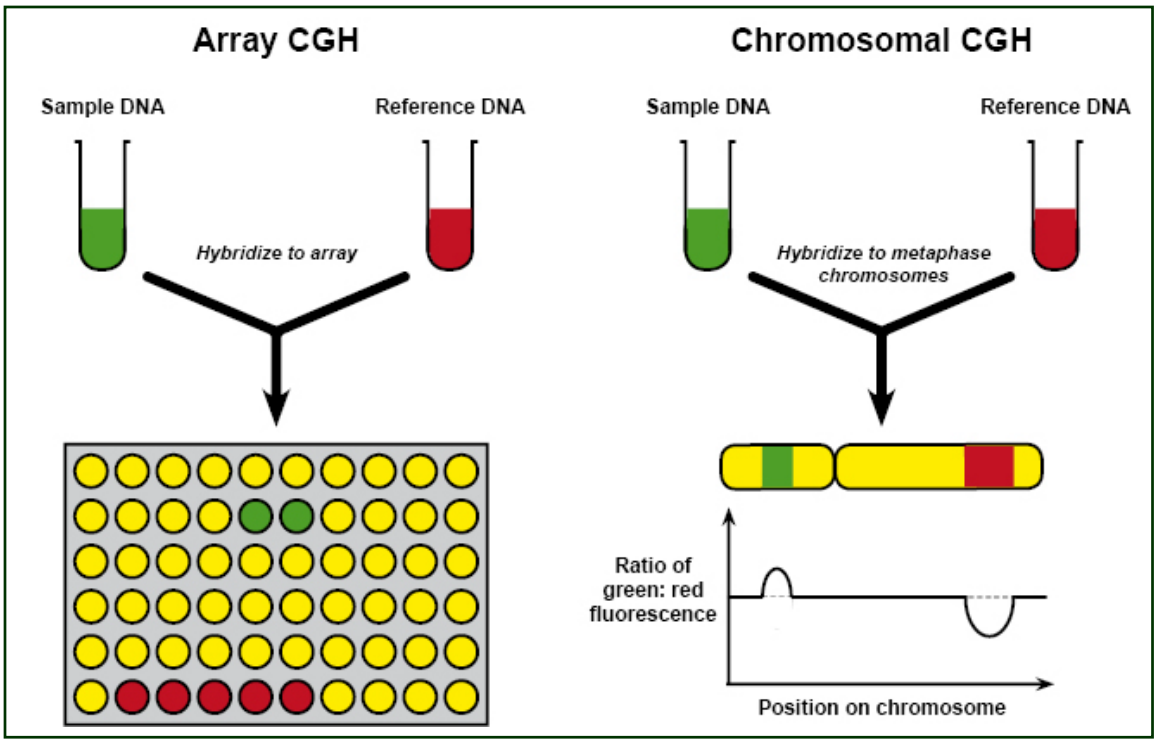
2.4 Array CGH

Originally, CGH was performed on whole metaphase chromosomes (chromosomal CGH), but this approach only permits the detection of relatively large copy-number changes with a resolution of ~10Mb using conventional CGH and 3-4 Mb using High Resolution-CGH (HR-CGH) in which reference control samples are used to improve the performance of conventional CGH. In contrast, microarray comparative genomic hybridisation, or array CGH, combines the principle of CGH with microarray technology to allow not only the identification and measurement of changes in DNA sequence copy number, but also the simultaneous mapping of these sites within the genomic sequence. Because a microarray can contain thousands of individual DNA probes, or reporter sequences, representing the complete genome (with partial or complete sequence information), hybridisation at a specific spot provides a much more precise indication of the site of aberrations in genomic sequence than a band on a chromosome could do, yet within a single experiment.

Fluorescence ratio imaging uses automated digital analysis of the images produced on laser excitation of the hybridised arrays to produce fluorescence ratio profiles, outputs with peaks and troughs representing areas of DNA amplification and deletion in the test sample correlated with genomic position, as defined by the probe sequences (see Figure 2.1). As with any microarray-based experiment, the relative levels of fluorescence can also be used to determine the degree of amplification.

The resolution of array CGH depends on the number of probe sequences on each chip, how long they are and how widely they are spaced throughout the genome. Currently, a typical resolution is 1Mb which is 3-5 times the resolution of karyotype. However, still higher resolution tiling path genomic arrays are already in development; tiling path arrays span chromosomes with overlapping reporter sequences, making coverage of the genome much more extensive (but requiring more complex data analysis to produce results).

Figure 2.1



(Public Health Genetics Unit 2006)

Genomic arrays are arrays of short, representative DNA sequences sampled from throughout the entire genome, which allow high-resolution detection of small chromosomal changes throughout the genome. These arrays are useful for genome-wide scans for possible cytogenetic abnormalities.

Targeted arrays comprise a series of reporter probes, each of which is specific to a particular chromosomal region, or known genetic abnormality. Coverage is typically smaller than the whole-genome CGH arrays, but resolution is maximized and they are a useful tool for confirming the presence or absence of the selected genetic features specified by the array. Their application is more specific, being used to investigate conditions in which there is reason to suspect the presence of a characterised copy number abnormality at a particular point or points, for example to make a correlation between genotype and clinical phenotype.

Conventional FISH may be targeted at single loci based on clinical suspicion of a particular syndrome (e.g. DiGeorge) or targeted at all 42 chromosome ends if there is a more general suspicion of a chromosomal abnormality. Multiplex ligation-dependent probe amplification (MLPA), is a technique that detects copy number variation at multiple different sequences to high resolution, and is appropriate for high throughput of samples. Because of the reduced costs, many laboratories are already switching from conventional FISH to MLPA for both kinds of targeted FISH testing.

2.5 Array CGH in practical terms

Although cell culture is not required, array CGH requires that DNA of good quality is extracted. Automated hybridisation chambers can help to standardise the essential hybridisation stage when the test DNA is hybridised to the DNA Chips that are mounted on glass slides. Hybridisation is usually performed overnight as a minimum and for up to 48 hours for some applications. The hybridised slides are scanned (frequently with scanners containing lasers) and the results captured by proprietary software. The data analysis stage is vital so that the software can automatically call significant or suspicious test results without the need to manually trawl through the considerable amount of data produced from each array CGH analysis. Most BAC arrays require a dye swap in which the analysis is re-run with the fluorescent labels reversed to improve the accuracy of the results.

2.6 Other applications of array CGH

The current and future applications of microarray technology extend beyond genetic diagnostics to a range of other fields, most notably oncology. Array CGH is used to identify genomic gains and losses in tumour samples, which can inform diagnosis and prognosis by allowing a more accurate, molecular-level identification of the nature of the tumour than histological examination alone permits.

2.7 The advantages and disadvantages of array CGH

Microarray CGH allows the rapid detection of copy number changes across the entire genome at high resolution. It does not require prior knowledge of specific areas of the chromosome or chromosomes to target via the use of particular probes, although it is also possible to use targeted arrays, which are the equivalent of multiple independent FISH analyses on a single chip.

However, there are limitations to the use of array CGH, notably the technique's inability to detect certain forms of chromosomal abnormality, such as balanced translocations or mosaicism. Translocation refers to a process whereby there is a reciprocal transfer of segments between different chromosomes, resulting in rearranged chromosomes that are composed of parts of other chromosomes. Where there has been a reciprocal exchange of material between two chromosomes, there is no obvious net gain of chromosomal material, and this is referred to as a balanced translocation; although there may be no net change in sequence copy number within the genome, the inappropriate juxtaposition of chromosomal sections can nevertheless have detrimental consequences for the individual. Chromosomal mosaicism is a phenomenon where different cells from the same individual may have different chromosomal compositions. Typically, some cells will have normal chromosomes and others will share a specific abnormality, although in rarer cases it is possible to have two or more different abnormal chromosomal sets within one individual, seriously complicating the deduction of underlying genetic causes of pathology.

A further drawback to the use of array CGH for the detection of chromosomal abnormalities associated with learning disability is the potential for identifying novel copy number variations that may not, in fact, be causal. Even if a variant is present in an affected individual but absent from the 'normal' parental genomes, it does not necessarily follow that it is a pathogenic change, and may rather represent an innocuous copy number polymorphism (i.e. normal variation). There is emerging evidence that DNA copy number polymorphisms may represent a major source of genome variation between different humans, although probes for array CGH generally avoid the use of sequences that hybridise to multiple genomic locations and are probably to some extent shielded from the detection of large-scale copy number variations. However, the

appropriate interpretation of findings from array CGH is nevertheless a skilled process and requires communication between specialists in order to associate apparent abnormalities with specific clinical features.

To facilitate this information sharing, a number of international databases have been established. DECIPHER (DatabasE of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources) is a resource for recording clinical information about chromosomal microdeletions/duplications/insertions, translocations and inversions linked to phenotypic descriptions and genome mapping. This database is intended to increase medical and scientific knowledge about chromosomal microdeletions/duplications with a view to improving medical care and genetic advice for affected families and facilitating research into genes that affect human development and health.

Other pertinent databases include: the Toronto-based Database of Genomic Variants, intended to provide a comprehensive summary of human large-scale genomic variants and a control data resource for studies aiming to correlate such variation with phenotypic data; ECARUCA (European Cytogeneticists Association Register of Unbalanced Chromosome Aberrations) a European database that covers both common and rare chromosome aberrations and already contains over 4,000 entries and brings together cytogenetic, molecular and clinical data; and "The Chromosome Anomaly Collection", a UK database that includes unbalanced chromosome abnormalities and variants with and without phenotypic effect that are not currently included in the other databases listed.

DECIPHER: http://www.sanger.ac.uk/PostGenomics/decipher/

Database of Genomic Variants: http://projects.tcag.ca/variation/

ECARUCA: http://www.ecaruca.net/

"The Chromosome Anomaly Collection" :
http://www.som.soton.ac.uk/research/geneticsdiv/Anomaly%20Register/

Chapter 3 Epidemiology of learning disability

3.1 Definition

Learning disability is a serious and lifelong condition characterised by the impairment of cognitive and adaptive skills. It represents a significant challenge to public health, being one of the few clinically important disorders with an aetiology that remains poorly understood. Historically and internationally the condition is known by many terms, including mental retardation, mental or intellectual handicap, intellectual impairment, intellectually challenged, learning problems, learning difficulty and learning, mental or intellectual disability. The term learning disability (LD) was adopted by the UK Department of Health in 1991 and is the term used throughout this document.

LD can be defined as a significant impairment of cognitive and adaptive functions, with onset before 18 years of age. Diagnostic criteria from the two principal international standards are given in Table 3.1. Both employ a locally relevant intelligence quotient (IQ), providing a means of classifying LD using a quantitative trait.

Table 3.1 Diagnostic criteria applied to LD according to the DSM-IV and ICD-10

Diagnostic and Statistical Manual for Mental Disorders (DSM-IV)
Definition of LD
▪ IQ <70 on the basis of an individually administered IQ test
▪ Dysfunction or impairment in >2 areas of: communication, self-care, home living, social/interpersonal skills, use of community resources, self direction, functional academic skills, work, leisure, health and safety
▪ Onset during childhood
Coding of LD (using IQ) #
▪ Mild: 50-55 to ~70
▪ Moderate: 35-40 to 50-55
▪ Severe: 20-25 to 35-40
▪ Profound: <20 or 25
International Statistical Classification of Diseases and Related Health Problems (ICD-10) (1)
"LD is a condition of arrested or incomplete development of the mind, which is especially characterised by impairment of skills manifested during the developmental period, contributing to the overall level of intelligence – i.e. cognitive, language, motor and social abilities."

Note: there is some overlap between coding categories.

3.2 Prevalence estimates of LD in the UK

The reported prevalence of LD (of any severity) varies substantially across studies and may be influenced by factors such as heterogeneity of definitions, clinical assessment tools, study design and population demographics. The World Health Organisation (WHO) has estimated the

prevalence of LD in industrialised countries to be around 3% (2), that is to say approximately 30 in 1,000 living people have a learning disability at any one time.

In the United Kingdom (UK), LD prevalence is sometimes reported as the 'ascertained' prevalence, which refers to the number of cases officially reported to the authorities. The true prevalence, that is the proportion of individuals with LD in the population regardless of whether they require services, can be estimated using population-based sampling methods. Here we review international and UK estimates of prevalence of severe and mild/moderate LD separately.

Severe learning disability (SLD)

A literature review of prevalence studies published up to 1995 found only small variations in the prevalence of SLD between studies, with an average of 3.8 per 1,000 (3). The review included several individual studies investigating the prevalence of SLD in regions of the UK, predominantly among young and teenage children. These are listed in Table 3.2, a table derived from Roeleveld et al. who undertook a review of the prevalence of learning disability in 1997. Prevalences ranged from 2.6 to 4.9 per 1,000. A more recent review of learning disability published in the Lancet in 2003 quoted frequency of IQs of lower than 50 of about 0.3 - 0.5%, and tends to vary less between populations (4).

A white paper published by the Department of Health (DH) has estimated the number of people living with SLD in England to be 210,000 (approximately 3.5 per 1,000), comprising 120,000 adults, 65,000 children and young people (<20 years of age), and 25,000 elderly people (5).

Table 3.2 Selected studies detailing the prevalence of severe learning disability in children and young people in the UK

Country and study year	Study population	Study size	Age (years)	SLD (per 1,000)	Ref
England					
1981	Regional register and survey (Oxfordshire)	81,401	5-14	4.1	(6)
1978-1980	Cohort 1958-1963 (London)	56,140	15-20	3.6	(7)
1972-1972	Cohort 1965-1967 (Hertfordshire)	46,960	7-9	3.1	(8)
1970-1971	Local register (London)	23,500	5-14	4.0	(9)
1961	Regional register (Salford)	38,750	5-19	2.6	(10)
1960	Local registers (Middlesex)	297,100	5-14	3.3	(11)
Scotland and Northern Ireland					
1980	Cohort 1961-1969 (Northern Ireland)	298,433	11-19	3.6	(12)
1969	National register (Northern Ireland)	±375,500	5-19	4.1	(13)

1963	National register (Northern Ireland)	358,850	5-19	3.7	(14)
1962-1964	Cohort 1950-1956 (Edinburgh)	39,498	7-14	4.9	(15)
1962	Cohort 1952-1954 (Aberdeen)	8,274	8-10	3.7	(16)

Table amended from Roeleveld et al. 1997 (3).

There is evidence that the prevalence of SLD diagnoses is age-dependent, with age-specific prevalence rates increasing to around 5.0 per 1,000 at age 15-19 before dropping to 1.0 per 1,000 above the age of 60 (3). This trend may have arisen due to a combination of non-diagnosis of SLD in the first few years of life, a higher than average mortality rate among persons with SLD, and due to flaws in registers and research methodology. The prevalence of SLD has also been shown to vary between the genders, with a male-to-female ratio indicating a 20% excess of SLD in males (3).

Mild/Moderate learning disability (MLD)

Obtaining true prevalence estimates of MLD is complicated by similar factors to those outlined above for SLD. However, inherent problems associated with case ascertainment mean that prevalence estimates for MLD should be treated with caution, and often under-estimate the true figure. The DH estimates the lower prevalence limit of MLD in England to be about 25 per 1,000 population, placing the total number of persons with MLD at around 1.2 million (5). In addition the prevalence in children of school age has been estimated to be 29.8 per 1,000 (3). Prevalence estimates for MLD show some variability between studies and tend to be higher than estimates for SLD. It is unclear whether the range of prevalence estimates for MLD represents some true underlying variation or whether it has arisen as a result of non-comparable populations, problems with case ascertainment, or whether the findings are simply due to chance.

The prevalence of MLD appears to vary according to gender, with an excess of males among MLD cases, ranging between 1.4-1.8:1 (17,18). There also appears to be a positive association between the prevalence of MLD and lower socioeconomic class and/or parental occupation. The explanations for this observation are numerous and it is likely that both poor environmental and social conditions, combined with suboptimal access to health care services and exposure to pathogens may play a role in the aetiology of MLD in this population sub-group.

3.3 Aetiology of learning disability

The occurrence of LD is influenced by genetic, environmental, infectious, and perinatal factors and a definitive cause cannot be identified for up to half of all cases (Table 3). Although comparison of results from clinical studies should be treated with caution, the results from several studies have shown that between 4-40% of all cases of LD may have a genetic basis (Table 3.3) (19,20). Exposure to environmental neurotoxins, such as lead and methyl-mercury poisoning, thalidomide, valproic acid, and alcohol may account for up to 13% of all cases of LD. The proportion of cases for which a definitive cause is identified also varies according to the severity of the LD, with approximately 30% and 70% of causes remaining unidentified in SLD and MLD, respectively (21).

Table 3.3 Causes of LD identified in literature surveys

	% of learning disability (LD)
Chromosomal abnormalities	4 – 28
Recognisable syndromes	3 – 7
Known monogenic conditions	3 – 9
Structural CNS abnormalities	7 – 17
Complications of prematurity	2 – 10
Environmental/teratogenic causes	5 – 13
Familial multi-factorial LD	3 – 12
Unique monogenic syndromes	1 – 5
Metabolic/endocrine causes	1 – 5
Unexplained	30 – 50

Table amended from Curry et al. (19).

Genetic causes of LD

Both genetic and environmental factors influence the aetiology of LD and differences in study design, case ascertainment, and variations in the sensitivity of molecular diagnostic assays make determining the contribution of genetic abnormalities problematic. However, advances in cytogenetic and molecular techniques are enabling the identification of an increasing number of genetic abnormalities associated with LD.

Genetic factors have been estimated to be the main cause of LD in around half of all SLD and around 15% of patients presenting with MLD (22). A recent review of 16 studies worldwide found that chromosomal abnormalities were present, on average, in 16.1% of individuals with LD (range 4.0-34.1%) (20). Learning disability can also be caused by defects in specific genes such as Fragile X or Rett syndrome.

The range of genetic causes of LD and their diagnosis

Some learning disability syndromes, such as Down syndrome or Turner syndrome are due to abnormalities of whole chromosomes and are visible using the light microscope. Other syndromes arise from deletion of a gene or a cluster of genes that are contiguously arranged along a chromosome. The Cri du Chat syndrome (Deletion 5p) and the Wolf Hirschorn syndrome (Deletion 4p) are good examples. These were identified based on the finding that part of one of the chromosomes was missing and, again, may be identified by light microscopy. More recently smaller deletions have been observed in syndromes that had not been previously associated with chromosome abnormalities (e.g. Prader-Willi and Williams). The size of deletions observed in some of these syndromes can vary from one individual to another and in many cases the deletion is not visible on routine chromosome analysis. The ability to detect such deletions depends on resolving the power of the chromosome analysis and, while resolution has improved over the last 30 years, deletions involving as many as several hundred genes may still go undetected by routine methods. FISH and techniques such as MLPA help us

to visualise sub-microscopic chromosome deletions and even deletions of single genes located on specific chromosomes.

Box 3.1 Williams syndrome: an example of a contagious gene syndrome

Typical facial features, occasional hypercalcaemia in infancy, supravalvular aortic stenosis and moderate to severe mental retardation. Irides often have a striking stellate pattern. The behaviour of the child is friendly and loquacious reflecting the greater preservation of verbal IQ, vocabulary and social use of language and auditory memory. In contrast there is poor visual motor integration and attention deficit disorder.

Causal deletions involving 7q11.2 have been found in 95% of patients with Williams syndrome. These typically involve the elastin gene (ELN) and the nearby LIM kinase-1 and RFC2 genes. Other genes may also be involved. The syndrome is truly a contiguous gene syndrome as deletion of elastin gene alone and mutations in ELN result in isolated supravalvular aortic stenosis without the other features.

Uniparental disomy, where both chromosomes of a pair are inherited exclusively from one parent is a further underlying cause of learning disability and can be associated with other features (e.g. short stature-maternal disomy 14 and polyhydramnios and narrow thorax - paternal disomy 14). Mosaicism, defined as the presence of two or more cell populations derived from the same conceptus that have subsequently acquired a genetic difference post-conception, can give rise to a number of phenotypes which involve learning disability.

Finally, there are learning disability syndromes caused by defects in specific genes. For example, Fragile X is a condition associated with triplet repeat expansions in the FRAX gene and Rett syndrome caused by mutations in the MECP2 gene. These conditions are diagnosed by molecular genetic techniques.

Table 3.4 provides a listing of learning disability syndromes with their underlying abnormality, grouped according to the main method required for diagnosis. This is a table of "selected" chromosome abnormalities. There are over forty recurrent micro-deletions and more, such as the 3q29 and the 17p11.2, are emerging as a result of research involving the array CGH technique. For details see DECIPHER website at:

https://enigma.sanger.ac.uk/perl/PostGenomics/decipher/manager?action=syndromes

Table 3.4 Examples of genetic abnormalities associated with LD

Syndrome	Genetic abnormality
Chromosomal aneuploidies	
Detectable by light microscopy	
Down	Trisomy of chromosome 21
Triple X syndrome	XXX
Turner	Monosomy X chromosome
May be detectable by light microscopy	
Monosomy 1p	del(1)(36.3)

Wolf-Hirschorn	del(4)(p16)
Cri du Chat	del(5)(p15)
Smith-Magenis	del(17)(p11.2)
Usually detectable by FISH only	
Williams-Beuren	del(7)(q11.23)
Prader-Willi	del(15)(q11q13)pat
Angelman	del(15)(q11q13)mat
Rubinstein-Taybi	del(16)(p13.3)
Miller-Dieker	del(17)(p13.3)
DiGeorge (velocardiofacial/VCF)	del(22)(q11.2)
Sub-telomeric abnormalities	Most telomeres
Uniparental disomy: not detectable by microscopy	
Short stature Precocious puberty Hydrocephalus	UPD(14)mat
Polyhydramnios Narrow thorax	UPD(14)pat
Prader-Willi	UPD(15)mat
Angelman	UPD(15)pat
Mosaic chromosome abnormalities	
Pallister-Killian	Tetrasomy 12p (mosaic)
Mosaic Trisomy 8	Mosaicism for Trisomy 8
Monogenic disorders detectable by molecular genetic techniques	
Fragile X syndrome	Frax gene triplet repeat expansion
Rett syndrome	Mutations in MECP2 gene
Duchenne Muscular Dystrophy	Mutation in DMD gene in boys

Overall frequency of LD due to genetic causes

Although each individual syndrome is relatively rare, together they comprise a substantial proportion of children with LD and about 0.5% of the general population of children (23). The birth prevalence of Down syndrome was 1.67 per 1,000 live born children in the UK in 1996 (24). Fragile X syndrome is more commonly diagnosed in males than females, with an estimated prevalence of 1 in 4,000 males and 1 in 7,000 females (25). The 22q11 deletion syndrome is one of the most common genetic syndromes associated with LD, and may occur at a higher prevalence than Fragile X, with an estimated 1 in 3,000 children affected (26).

3.4 Conclusions

The DH estimate that the number of persons with SLD may increase by up to 1% per annum over the next 15 years due, in part, to increases in life expectancies and decreases in mortality rates of affected individuals, and an increase in reporting rates among children (4). Advances in the field of molecular cytogenetics may be expected to lead to the genetic basis of an increasing number of LD syndromes being determined. As such, the increasing ability to correctly diagnose the specific cause of LD will produce positive benefits in terms of accurate risk prediction and communication, and allow an improved understanding of the aetiology of LD.

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Chapter 4 Clinical context

4.1 Overview

Array CGH as a new technology needs to be placed within the overall clinical context of the recognition, investigation and continuing management and care for the individual with learning disability and/or developmental delay and the provision of support and advice for the parents and family, who will often be the main providers of care.

The sequence of evaluation differs across the country and in different health service organisational system. One example, which builds on international guidance (1) and regional expertise, is that set out in the East Anglian guidance for professionals: *A guide to the investigation of children with developmental delay in East Anglia* (2). The guidance, devised by an expert group of clinical and laboratory geneticists and paediatricians, and including paediatric neurology and community paediatrics, provides an expert viewpoint of good practice. Fundamentally it shows that seeking a genetic diagnosis for a child is part of a sequence of assessment and investigation that must follow different pathways depending on findings at each stage. Elaboration of these guidelines with associated references is also available on the same website.

The investigations that might be undertaken for the adult with learning disability will differ. Because of the distress that might be caused by undertaking diagnostic tests and the need for informed consent, the reason for investigation at this stage must be explicit and should be explored carefully with patient, carers and family.

4.2 Stages in the investigation

Concerns over developmental progress might be raised by parents, in the course of developmental checks by health visitors or in the context of a child's education. The child will usually be referred to a paediatrician. The paediatrician undertakes a careful history, including family history and examination of the child with assessment of growth, (especially height, weight and head) and development and may involve other professionals in this process. He/she will exclude the possibility of chronic illness as the cause both clinically and through a baseline haematology and biochemical profile.

The parents may be offered tests to try to determine the cause. The guidance recommends that testing can be guided by clinical features, but even in the absence of these, investigations can still be positive. Children with significant developmental delay, at whatever age, should be considered for investigation.

Following this initial examination and assessment a sequential series of investigations is recommended. The clinician should consider the possible value of each of the following investigations - the guidance recommends the particular circumstances in which they should be offered (see Table 4.1). Importantly, it should be noted that chromosome analysis is thought to be the investigation with the highest yield and is recommended for all children even those who do not have dysmorphic features.

Table 4.1 Investigations that should be considered initially in all patients

Chromosome analysis. Karyotype and specific FISH tests for submicroscopic microdeletions (e.g. Williams, 22q11 syndrome) can also be requested.
Fragile X analysis - this is the commonest cause of inherited learning disability, but remains a rare disorder. It has fairly non-specific features and is difficult to diagnose on clinical grounds so it is therefore offered to all children with developmental delay.
Creatine kinase in boys - some boys with Duchenne muscular dystrophy present with speech delay and delayed motor milestones and/or global delay
Thyroid function tests – children born in the UK should have been tested for congenital hypothyroidism on the neonatal Guthrie spot. If this result was normal (need confirmation), unless there are clinical signs suggestive of hypothyroidism, repeat investigation is not required.
Amino and organic acids – inborn errors of metabolism are individually rare, but may present with non-specific features e.g. developmental delay and/or failure to thrive. Plasma & urine samples should be arranged if there is developmental regression, episodic decompensation, parental consanguinity, a family history or physical examination findings consistent with a metabolic disorder e.g. microcephaly, macrocephaly, hepato-splenomegaly. 'Non-specific' abnormalities are more common than true diagnoses.
Urine glycosaminoglycans (mucopolysaccharidoses) – in children with developmental regression, glue ear, coarse features, macrocephaly.
Ophthalmological opinion – especially if there is concern regarding vision, eye signs e.g. nystagmus or neurological signs e.g. microcephaly.
Audiology assessment – especially if there is speech delay or concern regarding hearing.
Consider **congenital infection** – in children with intrauterine growth retardation, microcephaly and eye/hearing signs. Requires comparison of maternal booking and current maternal serology. Useful for children up to ~18/12 of age.

Following consideration of these basic tests, Table 4.2 lists further investigations that may be arranged in particular clinical circumstances.

Table 4.2 Investigations that should be considered in particular clinical circumstances

Telomeres – where routine chromosome analysis is normal but a chromosome abnormality is suspected.
Cranial MRI scan – MRI scanning in young children with developmental delay requires day case admission to hospital and sedation or anaesthesia. It is indicated in children with microcephaly, macrocephaly, neurological signs, (e.g. hemiplegia, nystagmus, optic atrophy), seizures and unusual facial features (e.g. spacing of eyes). The diagnostic yield in normally grown children who have no neurological signs is very low.
Myotonic dystrophy – in children with motor or global delay with floppiness, history of poor suck and poor feeding in infancy or weakness and fatigue in childhood or a family history of myotonic dystrophy.
Angelman/Prader-Willi syndrome - children with seizures and no/very little speech (AS), infants with floppiness or young children with obesity (PWS).
Creatine kinase in girls (muscle disorders) – if significant delay in motor milestones with/without associated global delay.
Lactate (mitochondrial disorders) – there is usually multisystem involvement. Key features include; growth retardation, visual/hearing impairment, abnormal MRI findings.
Radiographs (X-rays) – if there are features suggestive of skeletal involvement. If delay is associated with macrocephaly and tall stature, an X-ray of the L wrist can be helpful to assess bone age.

Finally, Table 4.3 lists specialist investigations that would only usually be arranged in conjunction with specialist services, in the light of particular clinical findings.

Table 4.3 Specialised investigations which may be arranged in conjunction with specialist services

MECP2 analysis (Rett syndrome) – only in girls with features consistent with Rett syndrome.
7-deyhdrocholesterol (Smith-Lemli-Opitz syndrome) – in children with microcephaly, 2,3 syndactyly, cleft palate, congenital heart defect.
VLCFA's (paroxysmal disorders) – in children with hypotonia, delayed closure of the anterior fontanelle and multisystem involvement.
Electrophoresis of transferrin isoforms (congenital disorders of glycosylation) – children with multisystem involvement e.g. lipodystrophy.
White cell enzymes (**lysosomal storage disease**) – in children with hepatomegaly, coarse features and/or regression.
Acyl carnitines (fatty acid oxidation disorders) – in children with a tendency to fasting hypoglycaemia, prolonged failure to thrive, hypotonia or cardiomyopathy.

4.3 Current clinical practice

It should be noted that the guidelines described above are an example, devised by an expert group and used only in East Anglia at present. There are no guidelines for the UK as a whole, and it is likely that the process of investigation is highly variable.

The Steering Group sought evidence from the community paediatrician member (Dr Moira Pinkney) on current and recent practice in East Suffolk (Ipswich) for investigating this group of children. This is described in Box 4.1 below.

Box 4.1 Current clinical practice in investigation of developmental delay and learning disability

The East Suffolk Community Paediatric service receives referrals for children with developmental delay and significant learning disability; children with additional health and physical problems are often seen initially by acute paediatricians. Younger children are normally assessed using one of the standardised methods such as the Griffiths Mental Development scale. Significant delay would lead to a multidisciplinary assessment and, with parental agreement, investigation for an underlying cause. The tests performed would depend on the clinical scenario but would usually include karyotype and molecular genetic screening for Fragile X.

In 2005 Dr Pinkney undertook an audit of the investigation of children aged 3 to 19 years old with severe learning difficulty in the two Special Schools for such children in East Suffolk. There were 146 children giving a prevalence of severe learning difficulty of just over 3 per 1,000. Sixty (41%) of the children had a definite diagnosis or explanation for the cause of their learning disability – see list below.

Reason for severe learning difficulty	**Number of children**
Down syndrome	16
Fragile X syndrome	2
Angelman's syndrome	3
Williams syndrome	2
Other chromosomal disorder	8
Complications of prematurity	13
Perinatal asphyxia	4
Acquired brain injury	5
Other recognised cause *	7
Total	**60**

(* Triple C syndrome, lissencephaly, spina bifida, congenital toxoplasmosis, mucopolysaccharidosis, Rett syndrome, tuberous sclerosis)

80% of the remaining 86 children without a clear diagnosis had had chromosome analysis, some as long ago as 1989. Four had a similarly affected family member. Many had undergone cranial ultrasound as infants or CT or MRI scans when older but these investigations very rarely contributed to the diagnosis. Children who had been premature, growth retarded at birth, suffered from neonatal complications or had early onset epilepsy often had not had karyotypes checked.

Over half of the children with no recognisable cause for their learning disability were described as suffering from autism; epilepsy was also very common in the group without a diagnosis.

These results reflect levels of investigation in Suffolk; in some areas of the UK where there are no clear pathways for investigation of children with severe learning disability, the rate of clear diagnosis is lower. For example, in a recent study of the completeness of genetic investigations of 110 children attending special schools in Edinburgh (3) there was an existing diagnosis for 23.6%. In the remaining group, chromosome analysis had been performed in 34.5% and Fragile X analysis in 19% with 15.8% having had both investigations. Over half of this group had an affected first, second or third degree relative with 87% of these high risk cases having had

incomplete investigations. As a result 55% of the high-risk cases had further investigations and chromosomal abnormalities were found in three patients (13.6%).

Over the UK as a whole there may be a problem of under-investigation, both currently and in the past. In addition, children who have been investigated some time ago will not have had the benefit of newer techniques and, under certain circumstances, such as where there is risk to other family members, there may be a question of re-investigating. Certainly current investigation of children and the provision of more sophisticated tests should be done in the context of widely recognised and implemented guidelines.

4.4 The place of array CGH

From this guidance, it can be seen that the investigation of the child with learning disability is a complex process, highly dependent on the knowledge, skill and experience of the clinicians involved. It might include, at various stages, general practitioner, paediatrician, paediatric neurologist, geneticist and a range of other specialists.

At present, it includes, routinely, an analysis of karyotype with the possibility of going on to specific FISH tests or telomeres where indicated. However, the guidance illustrates the other branch points that investigation might follow in specific clinical circumstances, looking for example for the possibility of thyroid dysfunction, metabolic disorders, muscular dystrophy or congenital infection even at the initial stages of investigation. Subsequently, specialist assessment, for example by a paediatric neurologist or geneticist will influence other branch points of the decision tree: for example MRI scanning for the child with neurological signs or seizures, or specific genetic or metabolic tests where particular syndromes are suspected.

At present, array CGH is almost entirely used in a research setting as an additional test to look for chromosomal abnormalities which are strongly suspected on clinical grounds and where routine cytogenetic tests (karyotype +/- FISH) have proved negative.

4.5 Investigating learning disability - talking to parents about genetics

The possible introduction of further diagnostic techniques in the investigation of LD further accentuates the need to communicate well with parents. In Chapter 5 we look in detail at the value of a genetic diagnosis to parents and their families. From work undertaken at Cambridge Genetics Knowledge Park we know that achieving a diagnosis can be beneficial if well handled. Such good handling includes:

- Understanding the needs of individual patients and parents including unanswered questions
- Determining with them why a diagnosis could be beneficial
- Discussing and deciding what tests will be done, at least in general terms. It is particularly important for them to understand that some of the tests offered are genetic tests
- Helping them to understand what a genetic diagnosis might mean for them and their family and preparing them for this (e.g. carrier diagnosis)
- Preparing them for the fact that it might not be possible to make a diagnosis or that abnormalities might be found where we do not know if they are causal
- Following good professional practice in breaking bad news
- Providing follow-up genetic support to provide further information

A helpful leaflet for parents is provided on CGKP website (4).

Array CGH may provide particular problems in giving information about chromosomal variations for which we have little knowledge of clinical significance, i.e. whether it is thought to be a cause of the LD, or providing information about the syndrome. For this reason it will be important:

- Not to use too high a resolution so that small insertions of deletions which might not be significant are not detected
- To follow protocols for trying to determine causality (including looking for abnormality in parents - see Chapter 7)
- To ensure that abnormalities are logged on a database such as DECIPHER together with a description of the phenotype, so that, on a worldwide basis, intelligence is gathered on the genotype/phenotype relationship

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Chapter 5 Clinical utility: the value of a genetic diagnosis

5.1 Impairment, disability and handicap

The determination of a specific genetic abnormality as a likely cause for learning disability will not lead to a "cure" for the problem. Indeed, to look so hard for a medical "cause" for learning disability, for some individual and professionals, particularly those involved in the provision of social support and care, is sometimes said to be a distraction from the task in hand - that of ensuring that the child or adult is supported to make the best of his or her limited ability and is able to undertake a role in society with the minimum handicap. This requires a thorough and wide-ranging assessment of levels of ability across the full breadth of domains and implementation of the necessary support systems.

However, whilst supporting the disability is vital, to simply take the "social" viewpoint may lead us to miss out on some of the real advantage of understanding the genetic abnormality (impairment) that is the root cause of the disability - knowledge that can lead to health (including social and psychological) benefits for the individual and family.

Because there are so many different diagnoses (conditions) that might be the end point of genetic diagnosis in learning disability the literature does not include evidence on the value of a genetic diagnosis in learning disability in broad terms. Our evidence therefore comes from two main areas:

- Parents, carers and people with a learning disability through a report, *Parents as Partners,* compiled by the Cambridge Genetics Knowledge Park (CGKP) as part of a multi-disciplinary project on learning disability (1).
- Professionals - from clinicians involved in specialist genetics and paediatrics. This was developed from the Working Group, from the work of the CGKP project group on learning disability and from the literature; it uses case histories to illustrate some of the main domains.

It should be said that seeking a diagnosis is a central tenet of medical practice. Its value in itself is not usually questioned and, indeed, diagnosis and the provision of information is already a main outcome of the genetic service in general and the learning disability work in particular.

5.2 The viewpoint of parents, carers and people with learning disability

The purpose of providing a diagnosis is primarily in response to the needs of those with learning disabilities, their parents and carers.

In 2004/5 the Cambridge Genetics Knowledge Park, as part of a multi-disciplinary project on learning disability, undertook a major project to investigate these needs and the extent to which they were fulfilled during the process of seeking a genetic diagnosis. The resulting report Parents as Partners leaves us in little doubt about three things:

- The life-changing situation parents face in caring for a child with learning difficulties
- The importance they attach (and the huge emotional and practical investment they are prepared to make) in finding a diagnosis
- The benefit that can be achieved through a diagnosis, especially if well handled. The corollary of this is that poorly handled genetic diagnosis can be detrimental

"Parents as partners" the CGKP project

The value of a genetic diagnosis to families was investigated through an online discussion forum, and through interviews and focus groups lead by the Project group.

The process was consultative aimed at understanding parent and carer perspectives of genetic testing for, and diagnosis of, learning disability. It started with a broad set of general questions, refining or expanding the questions through consultation and deliberation; then feeding questions and responses back to participants. The input methods for involvement are fully described in the report and are detailed in Box 5.1.

Box 5.1 Methods for the Parents as Partners Public Involvement Project

Interviews. Interviews were conducted with 38 participants; two with a genetic condition, the remainder parents and carers. The interviews were conducted in systematic semi-structured narrative style aimed at getting participants to describe their personal experience of investigation and diagnosis.

Forum. An online, moderated forum provided a platform for 46 participants to discuss experiences in an anonymous group. Participants were invited to discuss advantages/disadvantages of genetic diagnosis, communication of diagnosis, services and support, and any other areas of concern.

Discussion groups. The final stage of the project was to take the feedback from face to face, telephone and online consultations to two discussion groups. Groups were asked to review consultation work, make suggestions and 'brainstorm' possible guidance.

Feedback and review. The process was iterative; draft documents were sent out to all participants in each of the above-mentioned groups (interview participants, forums, discussion groups) for feedback. They were then forwarded to a wide range of key stakeholder group's relevant experts.

Reasons for seeking a genetic diagnosis

According to participants, the most important reason for a family to wish to obtain a diagnosis is to gain knowledge about the condition. The most common reasons this knowledge is seen as important are set out below

Just to know

The most frequently repeated basis for seeking a diagnosis was also the most non-descript. That is, parents 'just need to know' what is causing the anomalous behaviour. Simply wanting to know should not be underestimated nor put down to mere curiosity; it is a genuine and profound emotional and intellectual need shared by almost all the parents of children with learning disability no matter what their experience or what the condition. One parent stated, 'uncertainty is the worst torture of all'.

> *"While not knowing in the early days allowed us a period of normality with our baby, later on we reached a point where we simply needed to know more about why he was not developing and what the future would bring. It was a very unsettling time because I was using every waking hour of the day to try and 'reach' my son. I was clutching at straws"*

Even though parents may recognise their child has a disability or learning difficulty/disability, there may still be uncertainty if it will ever improve or whether the child will 'grow out of it'. Parents can spend a long time worrying if and when the situation will improve. These parents seek a diagnosis to find some closure on the matter

There are also peripheral reasons why knowing may be important to some parents. Many want assurance that they are not 'making up the condition' and that their concerns have been realistic and valid. Many parents have unfortunately found that, without a diagnosis, some people – be they family, outsiders or even health professionals – do not take them seriously. Hence, parents often seek reassurance in a diagnosis that the condition is not a result of their own behaviour or parenting.

Having a name for the condition is also important to parents, particularly if the child is not obviously disabled. Parents who have a child who has no named diagnosis often state that 'it would be much easier to say she has X syndrome rather than having to explain her disability to everyone I meet'.

To understand the future needs of the child

Parents often seek a diagnosis in the hope that it will allow them to predict what the child will be like in the future, by comparing them with others who have the same condition. Participants who have been through the process are very realistic in stressing that a diagnosis never allows you to completely or adequately predict how a child will progress, how the disability will express itself or indeed all the potential problems a child will face. However, they do agree that it helps provide an insight into some of the future needs of the child. This allows parents to "anticipate potential problems and get something in place earlier rather than later" and "know what the best case scenarios are likely to be and aim to make these possible."

> *"The diagnosis was worth getting although traumatic – we have been able to re-plan our lives and drive our lives more pro-actively."*

To plan for further children

For many families a learning disability automatically equates to a genetic condition, whether or not it actually is the result of one. This can cause concern about future family planning, and has meant that some members of the family have chosen not to have children. Parents may also have other children who might be affected by the condition and whom they want tested. Finally, knowing about the condition allows parents to plan for the needs of other siblings or family members, because the disability will create specific needs for them too.

> *"We … know that it has happened because of a genetic mutation… Therefore our other children should hopefully go on to have families of their own without the additional risk of this particular condition."*

To find others affected by the condition and access support groups

For many parents having a child with a learning disability can be a very isolating experience. They are unable to share the experience of parenting with their peers because their child has different needs and behaviour to others. Having others to talk to and share experience of learning disability can be extremely important. Families may seek a diagnosis, in part, to find other families to identify with and speak to.

To help the child find services and support

One of the prime reasons that families seek a genetic diagnosis is in the hope of accessing increased support from services such as education and social services. They also see it as important for obtaining an early and accurate statement of special educational needs. Work by *Unique,* the voluntary organisation that supports families with rare chromosomal disorders, supports the view that families do, indeed, receive increased support once there is a formal diagnosis, although this is not invariable.

> *"E is a beautiful articulate tall elegant looking child – people look at you and think 'what is your problem?' Because she is compliant, well behaved, non-disruptive behaviour [sic] she slips through the net. A diagnosis and statement brings recognition and support that don't come naturally to a child like her."*

The impacts of the diagnosis

The above include the reasons for trying to obtain a genetic diagnosis. When a genetic diagnosis is provided, parents describe experiencing a huge set of emotions including the whole range of relief, shock, anxiety, anger, guilt, grief - sometimes an affirmation of their inner knowledge that something was wrong with their child, sometimes a loss of hope that this is not something that he or she will "grow out of". Once the immediate emotions have subsided, parents and carers have a chance to consider the impacts of the diagnosis. We asked parents to describe some of the advantages and disadvantages of genetic testing for learning disability. Some of the most common responses are outlined in Box 5.2 below.

Box 5.2 Parental experiences of advantages and disadvantages of a genetic diagnosis

Advantages	Disadvantages
May provide a name for what is wrong	There might not be a diagnosis
Can help put you in contact with a supportive community	There might not be a community to connect to if the disorder is very rare
May help you find out more about the disorder	There might not be any recognised medical treatment regime for the named condition
Other family members can be tested to see if they are affected	It may cause stress for your immediate and wider family
Can help long term planning for your child's and your family's future	It could impact on relationships with the extended family
Can alleviate concerns that developmental delays are the parent's or family's fault	It may make the future look more bleak
Can mean that others become more sympathetic/supportive	Having a named condition can sometimes result in negative stereotypes and labels
You may be able to access more/improved services	It may cause problems with securing insurance cover
It can allow parents to find a degree of 'closure'	In a worse case scenario it may impact on medical services

Overall, however, all parents thought that, on balance, genetic diagnosis was beneficial. However, if handled badly or if they were unprepared, it could be detrimental. Some information that helps parents understand and consider the impact of seeking a genetic diagnosis is given in the leaflet available on the CGKP website (see reference chapter 4). The document *Parents as Partners* also contains some outline guidance for consideration by professionals written by parents.

5.3 The clinical viewpoint

A precise genetic diagnosis can also help professionals to manage the individual better or provide more support or tailored information to the parents and family. Because there is a range of diagnoses achievable, we explored the general features of a genetic diagnosis and illustrate these with a number of clinical histories, included as an appendix to this chapter.

The clinical benefits of achieving a specific diagnosis include:

Aetiology: To establish a specific cause and allay concerns about other possible causes such as events during pregnancy (see case history 2).

To gain an understanding of the condition and possible prognosis: Case history 2 illustrates how a 7 year old boy with speech delay, learning disabilities and some physical abnormalities was diagnosed with 3q29 microdeletion syndrome. Descriptions such as the one below (Box 5.3) from the DECIPHER database can help clinicians and those managing him to consider the spectrum of possible features that might need surveillance and management.

Box 5.3 Description of 3q29 microdeletion syndrome on DECIPHER database

> *The clinical phenotype is variable despite an almost identical deletion size. It includes mild/moderate mental retardation with mildly dysmorphic facial features (long and narrow face, short philtrum and high nasal bridge). Of the 6 reported patients, additional features e.g. autism, ataxia, chest-wall deformity and long, tapering fingers were found in at least two patients in Willatt's series (DECIPHER database).*

Further characterisation of known abnormalities: Small chromosomal duplications are difficult to visualise on routine microscopy, and are detected less commonly than microdeletions, yet are expected to occur at a similar frequency as they arise by a similar mechanism of non-homologous crossing-over. Our knowledge of microduplication syndromes is therefore currently limited. The improved detection of duplications by array CGH has significantly increased our knowledge of the phenotypes and natural history of the duplication phenotypes. It is clear that some of these occur relatively commonly e.g. 22q11, 7q11, yet the majority of these were being missed on routine analysis. The ability of array CGH to size these duplications with more accuracy than routine karyotyping has also aided our understanding of these disorders. Array CGH can also be used to clarify known unbalanced or apparently balanced cytogenetic abnormalities.

To guide optimal management: for example it can enhance medical evaluation by enabling the clinician to undertake a targeted evaluation (e.g. with monosomy 1p36 this would include specific evaluation for hearing and visual impairment, palatal abnormalities, and cardiomyopathy or structural cardiac abnormalities in children).

Surveillance: it can help target a programme of surveillance and vigilance (e.g. in Down syndrome would include monitoring for cardiac disease, celiac disease and thyroid function, and vigilance for arthritis, atlanto-axial subluxation, diabetes mellitus, leukaemia, obstructive sleep apnoea and seizures).

To predict the clinical consequence based on an understanding of the genes involved. The ability to do this will depend on the use of databases such as DECIPHER which gives professional access to knowledge of which genes are affected and what they do (see Box 5.4).

Box 5.4 The Decipher database

The DECIPHER database allows clinicians to:

'Visualise the known genes and putative genes and expressed-sequence tags (ESTs) within the deleted/duplicated region
Print out a report, including an ideogram of the chromosomal location of the microdeletion/duplication or inversion'

For example, the entry on 3q29 microdeletion syndrome lists 21 genes that might be affected in this condition with links to information and references about their known functions.

To provide genetic advice. The ability to define more precisely the molecular abnormality can also help the geneticist to give more accurate advice about the possibility of recurrence or risk to the extended family. Thus, for example, a balanced parental translocation substantially increases the risk of an unbalanced event in the family, whereas if the abnormality is found to have arisen *de novo* and the parental karyotypes are normal, then the risks of recurrence are very low. It might also lead to the possibility of antenatal testing for the parents if they wished to have further children (case histories 1, 4).

Providing emotional, social and practical support for the family. Knowing that a child has a rare disorder can lead to a referral to support organisations where they can meet other parents of children with the same or a similar condition. This is useful for emotional, social and practical support over the years as they care for the child (case history 2).

To end the "diagnostic odyssey"

Case history 2 illustrates how a child may have a very large number of negative investigations over the years as parents and professionals seek an explanation and help to manage learning disorders and associated problems. In this case the final positive diagnosis was achieved after at least seven years of tests that included complex genetic and biochemical testing, as well as radiological examination of the subject and a sibling. In other cases investigation might even include tests such as MRI scanning that, in children, require admission to hospital and general anaesthesia.

Scientific benefits. Results from array CGH can provide potential insight into disease mechanisms and hence might lead to the development of therapeutic interventions (case history 3).

5.4 Reference

1. Gogarty B. Parents as a partners. A report and guidelines on the investigation of children with developmental delay; by parents, for professionals. Cambridge Genetics Knowledge Park, 2006. http://www.phgu.org.uk/pages/work/serv_projects.htm#learning

Appendix to Chapter 5: Case Histories

Case history 1

A 12 year old presented with microcephaly, severe learning disability and mild dysmorphic facial features. A duplication spanning five clones in 1p35.11-12 was identified by array CGH. This duplication was confirmed by quantitative FISH analysis and similar studies in the parents indicated that this finding was apparently a de novo event and was therefore likely to be the underlying cause of the patient's phenotype. As it has arisen de novo the parents could be advised that it was unlikely to happen again.

Case history 2

Patient A had been under the care of a general paediatrican since three years of age when he had been noted to have speech delay. When he started at mainstream school he required extra help and had difficulties with his behaviour. His brother had had similar problems and his mother had also struggled at school.

He was investigated by the paediatrican who checked chromosomes, Fragile X, creatine kinase and urine amino and organic acids, which were all normal. He was kept under 6 monthly paediatric review.

He was referred to the genetic clinic at the age of 7 years. Blood was taken for 22q11 FISH which was normal. He had a further review appointment a year later then failed to attend two further appointments which had been made for him. Three years later he was re-referred because he was having more difficulty at school with learning and behaviour. The paediatrician suspected a genetic problem because of his family history. FISH for 5p deletion and telomeres were done because his features suggested a chromosome abnormality.

Results were normal. A skeletal survey was carried out because of a slim build, pectus deformity and contractures. His brother was being investigated by another paediatrician and had also had a battery of blood tests and a chest X-ray.

He was considered a suitable candidate for the Cambridge CGH study and array CGH revealed a 3q29 microdeletion which was present in both his brother and his mother. Other members of the extended family are currently being investigated.

Finding the deletion provided:

- *An explanation for the learning disability*
- *An explanation for the family history*
- *Help for the school in dealing with his learning and behaviour problems*
- *Possibility of providing accurate genetic counselling to affected family members*

- *Now able to stop doing further investigations to try and find a cause for his problems*
- *Family pleased that a cause has been identified and that there is some genetic literature on it and a support group for individuals with rare chromosome disorders*
- *Mother feels she may have another pregnancy now that she knows the cause and may consider a prenatal test*

Case history 3

Baby C presented at birth with bilateral retinal dysplasia. His parents were second cousins and were healthy. A diagnosis of Norrie disease was made clinically by the ophthalmologists. (Norrie disease is an X linked inherited disorder of the retina also associated with progressive hearing loss, developmental delay and mild to moderate learning disabilities and a variety of other systemic abnormalities). Norrie mutation testing was carried out but was normal so the diagnosis could not be confirmed. He had a metabolic screen for causes of congenital cataracts and routine chromosome analysis and results were normal. Two other genes involved with retinal dysplasia were screened and no mutations were found. He remained under regular review by the paediatrician and was noted to have delay with his development. At first this was attributed to his visual impairment. At follow-up in the genetic eye clinic, however, he was noted to be microcephalic and dysmorphic and another cause for his problems was considered. An MRI scan was carried out but was normal. Baby C was noted to have hearing loss.

Baby C was considered to be a good candidate for CGH studies. CGH revealed a large duplication of chromosome 5 which had been missed on routine analysis. This had occurred de novo. There was also a single clone imbalance of chromosome 7 which was not confirmed on further testing and therefore not thought to be of significance.

- *The chromosome imbalance most likely represents the cause of his problems and X linked and recessive conditions could be ruled out*
- *There was no need to undergo more diagnostic investigations*
- *The case may provide useful information for localisation of genes for retinal dysplasia*

Case history 4

A boy presented shortly after birth with a hiatus hernia, pyloric stenosis, a heart murmur, hypospadias and dysmorphic features and a genetic opinion was sought. This was the parents' first baby and there was no family history of note. Birth weight was relatively high at 4 kg and the baby had low muscle tone. A number of investigations were arranged including chromosome analysis, metabolic screen, renal scan, cranial ultrasound scan, ophthalmologic opinion and hearing test. All of these were normal. As time went on it became clear that the child had significant developmental delay and microcephaly. A chromosome abnormality was still suspected and telomeric FISH was carried out and this was normal. In view of the dysmorphic features 7OH cholesterol was checked and mutation analysis of three X linked mental retardation genes carried out. All of these gave normal results. In all, the child was seen on seven occasions in the genetic clinic and on a regular basis in the paediatric clinic. His details were presented at the regional and UK dysmorphology meetings. He was invited to a specially convened joint dysmorphology clinic to see a team of four expert dysmorphologists. The consensus of opinion in each case was that the cause of his problems was probably chromosomal but that an X-linked disorder could not be ruled out. Many professionals were involved with this child and wrote to the genetic department on a regular basis to ask for information about his condition. When the child was nine years old his mother consented to participation in the Cambridge CGH study. A deletion of 8 clones on chromosome 8 was identified. This was not present in the mother. On reviewing the original slides it could still not be visualised but was confirmed by FISH. At this stage the father was unavailable for study.

- *A chromosome abnormality was suspected in this child from birth but could not be confirmed until CGH was carried out.*
- *An X-linked mental retardation syndrome was also considered as a possible diagnosis, so recurrence risk for a future pregnancy had been guarded. Confirmation of a chromosome abnormality on the child which was not present in the mother suggested that recurrence risk was likely to be low for her future children. Recurrence risks had been a major issue for this family, and the parents had not dared to have further children until the cause of the child's problems had been elucidated.*
- *This child had been extensively investigated at great expense in terms of genetic investigations and clinic visits.*
- *The family had instigated many genetic and paediatric reviews whilst the diagnosis was still unknown. The frequency of their review appointments has now been decreased.*
- *The various professionals involved with the child and his school have been made aware of the diagnosis of a chromosome deletion.*

Chapter 6 Array CGH in UK genetics laboratories

In this chapter we set out the current situation in the UK with regard to funding, current developmental work and progress and issues arising as a result of our questionnaire to laboratories.

6.1 Department of Health funding

In 2003 a number of laboratories received funding under the Genetics White Paper to develop microarray work. This included funding for both equipment and consumables. The laboratories funded included Leeds, Manchester, Oxford, Great Ormond Street, Kennedy Galton, Cambridge and Birmingham. In addition two laboratories (Sheffield and Liverpool) also received funding to develop microarray, from the local trust and from research funds respectively. All laboratories except Liverpool were investigating array CGH in the context of learning disability. Birmingham in addition is developing microarray in the diagnosis of chronic lymphatic leukaemia and Liverpool in solid tumours.

At the request of DH the funded laboratories also developed a support group lead by Professor Nick Cross at the Salisbury National Genetic Reference Laboratories (NGRL) to help coordinate the work and bring on those with less experience. This support group has also moved on to consideration of quality assurance and quality control for the technology.

6.2 UK Genetics Services/Sanger Institute collaborative research study

Several UK clinical and laboratory genetics centres collaborated with the Sanger Institute on a study led by Charles Shaw-Smith (University of Cambridge Department of Medical Genetics) and Nigel Carter (Wellcome Trust Sanger Institute). These centres included Cambridge, Manchester, Southampton, Liverpool, Nottingham, Great Ormond Street and Newcastle. In keeping with previously published work from this and other centres, the data from this study strongly supported the utility of array CGH in clinical diagnosis in patients with learning disability, and for the participating centres, the study has provided a springboard to address the issues of clinical implementation.

6.3 The laboratory survey

A survey was undertaken of UK cytogenetics laboratories to find out what progress was being made in developing array CGH in clinical practice with particular reference to learning disability. The survey sought information on their reasons for wishing to develop array CGH, the type of equipment and consumables in use, the current applications with referral arrangements, use of the system by other groups, capital, consumable and staff costs and use of databases. Importantly, laboratories were also asked to provide results of tests already undertaken in the context of learning disability, their opinions on centralised testing and their views on the advantages and disadvantages of array CGH.

A questionnaire with accompanying letter was sent by email to all cytogenetic laboratories and all members of the UK microarray. Reminders were sent by Dr John Barber (Laboratory member on Steering Group) in December 2005 and January 2006. The findings are detailed in the following sections.

Responses

Full questionnaires were returned by 14 laboratories as shown in Table 6.1 below. These are listed according to region and with their population served. No responses were received from laboratories in Northeast or Northern Ireland. All laboratories who responded were using array CGH except Bristol, which was planning to introduce this from 06/07. Details of those providing responses are given in Appendix 2. A further three laboratories (Royal Marsden, Barts and The London NHS Trust, and Dundee) contacted us to say that they were not using microarray in the context of learning disability.

Table 6.1 Laboratories undertaking array CGH who responded to survey

Region	Laboratory	Population served (million)	Using array CGH
Northwest	Manchester	5.5	Yes
Yorkshire and Humberside	Sheffield	1.8	Yes
	Leeds	3.75	Yes
East Midlands	Nottingham	2.2	Yes
West Midlands	Birmingham	5	Yes
Eastern	Cambridge	2	Yes
London and Southeast	North East London (GOSH)	4	Yes
	Cytogenetics, Guy's Hospital	4	Yes
	North West Thames (Kennedy Galton Centre)	3.6	Yes
Southwest	Bristol	3.8	Planned 06/07
	Oxford	3	Yes
	Southampton	(Research laboratory)	Yes
Wales	Cardiff	3	Yes
Scotland	Edinburgh	1.8	Yes

(a) Equipment

Eleven laboratories had invested in their own equipment to undertake array CGH, whereas a further three were outsourcing the work - in two cases to the Sanger Centre and, in a further case, to another NHS laboratory.

Laboratories had a range of equipment. The most common scanners were Agilent (6) and Perkin Elmer (3). More recently, several laboratories are investing in the 1Mb Cytochip. The predicted lifespan for equipment, largely as a result of obsolescence ranged from 2-10 years though mostly around 5 years. Arrays used were mostly commercial including those from Array Genomics, Affymetrix, Spectral Genomics, Agilent Array Genomics, and Oligoarrays and one laboratory (Southampton) made their own.

The most common reasons for choice of platform included: requirement for chip type (5), recommendation (7), compatibility with other systems (3) and price (7). In other cases choice was a result of trials (1) and testing of the scanner as part of the evaluation (1).

(b) Developing experience in the use of array CGH in the context of LD

Laboratories were asked the main reasons for adopting array CGH in the context of LD. In all cases this included increased detection of abnormalities. Nine of the 14 laboratories (64%) intended to use it for prevention of recurrence and all but one (93%) for better characterisation of known abnormalities. Three laboratories also cited the opportunity of future development linked to the technology including applications for acquired abnormalities such as in cancer diagnosis.

Current applications included targeted array of known syndromic loci (6/14), targeted array of known oncogenic loci (1/14), SNP array for detection of constitutional imbalances and uniparental disomy (UPD) (1/14), SNP array for detection of acquired UPD(1/14),1 Mb array for detection of constitutional abnormalities (11/14), tiling array for detection of constitutional abnormalities (2/14).

(c) Use of platforms for applications other than array CGH and by other groups

Laboratories were asked if the platforms were used for applications other than array CGH and by other groups. Two laboratories were currently using their platform for SNP genotyping. One laboratory noted that it was being used for targeted array. Most laboratories reported that other groups had current access to the equipment (e.g. other research groups or other disciplines such as haematology or pharmacogenetics) or that shared access was planned. There is thus a general requirement that the platforms should be versatile.

(d) Estimated demand for tests

Laboratories were asked to estimate the demand for tests - both currently for learning disability and in the future. Population rates are given in Table 6.2. Both were thought difficult to quantify. For current estimates some were limited by what had been funded. Both current and future demand would depend on whether array CGH will be used as a front line test or after routine cytogenetic analysis. Laboratories that estimated use in latter context included Cambridge, GOSH, Cardiff and Leeds (estimate based on people with LD referred for sub-telomere FISH analysis) with current rates per million population being 25.0, 37.5 and 66.7 and 40. If CGH were to be introduced into clinical practice there would initially be a need to test the accumulated backlog.

Future numbers would depend on eventual clinical utility, developments of the technology in other fields, cost and willingness to fund. Most laboratories felt that there were too many variables to put a number on this at this stage. However, if all cases of LD were tested expected rates per million estimated by Manchester (36.4), Cambridge (100) or Cardiff (200) suggest that these rates would rise three to four fold. Other laboratories give much higher estimates.

Table 6.2 Estimated current and future demand for array CGH tests in learning disability

Region	Population served (million)	Current	Rate per million	Estimated future	Rate per million
Manchester	5.5	50	9.1	200	36.4
Sheffield	1.8	650	361.1		
Leeds	3.75	75	20.0	150	40.0

Nottingham	2.2				
Birmingham	5	2000	400.0	3000	600.0
Cambridge	2	50	25.0	200	100.0
North East London (GOSH)	4	150	37.5		
Guy's Hospital	4				
North West Thames (Kennedy Galton Centre)	3.6	750	208.3	1000	277.8
Bristol	3.8	500	131.6	7000	1842.1
Oxford	3				
Southampton	(Research laboratory)				
Cardiff	3	200	66.7	600	200.0
Edinburgh	1.8	500	277.8	500	277.8

(e) Costs

Laboratories were asked to estimate costs for capital equipment, consumables and staff time. Capital was difficult to estimate as laboratories had obtained equipment at discounted rates or some elements of equipment obtained for other purposes was being used. For those that had invested in the whole range of equipment (arrayer, hybridisation chamber, reader and related IT) the whole cost ranged from £123.5K to £162K.

The total cost of an array investigation was estimated by laboratories. The range was 200-1500 with an average of £775. This cost is very similar to that estimated by in the detailed economic analysis undertaken by the Oxford Genetics Knowledge Park and described in Chapter 8.

In terms of staff time, the range of total WTE spent on array CGH was from 0.4 to 2 with an average of 1.0 WTE. Staff involved were mainly clinical scientists with a small input from technologists and clinical geneticists.

(f) Local referral systems for testing

Gate-keeping systems for array CGH will be important in determining the numbers and appropriateness of referrals within any system. Currently seven laboratories accepted referrals from clinical genetics and only the Cambridge service from a wider group including community paediatricians. One service selected patients using an established checklist and one service only research patients.

(g) Use of databases

Array CGH is capable of detecting chromosomal abnormalities that had not previously been described. It is generally thought that these should be recorded along with information about phenotype allowing clinicians and families in the longer term to identify those with similar conditions in order to inform clinical management and prognosis. Laboratories were asked if they routinely used these databases. Six laboratories currently made use of the DECIPHER database, and one laboratory used an in-house database. Four laboratories intended to use a database but had not decided between the two main databases (DECIPHER and ECARUCA).

(h) Array CGH fit with other laboratory systems

Laboratories were asked how array CGH fits in with their laboratory system. In most cases it fits in with the routine cytogenetic investigation of dysmorphology, complementing and extending existing analyses (karyotype and FISH). It would be used where other chromosomal results were normal or to further define abnormalities. Two laboratories commented that it would be likely to replace karyotype or become part of routine analysis.

(i) Experience with array CGH – testing unknown cases

Four laboratories had undertaken testing of array CGH with unknown cases who fulfilled clinical criteria for a likely genetic diagnosis and had already gone through the routine cytogenetic testing including FISH and/or MLPA as appropriate. A total of 55 patients were tested and 14 (25%) were positive for a genetic abnormality although the significance of these was uncertain. (Results for Cambridge are included in the published results as part of the meta-analysis).

(j) Experience with array CGH – controls

Of the four laboratories undertaking tests of patients with unknown diagnoses three were also testing controls – including use of commercially available male and female genomic DNA (pooled sources) (Manchester), sex mismatches and known abnormals (Birmingham) 10 cases with known small chromosomal imbalances (Cardiff).

(k) Comments and opinions

Centralised testing

The development of array CGH as a more specialised technique in a small number of laboratories nationwide was considered as an option by laboratories who were asked to comment on its feasibility and possible advantages and disadvantages. It was generally thought that centralisation might be an option whilst array CGH is a second line test but that once it was robust enough to be a first line test most services would want to offer it locally. This would mean all medium and large sized laboratories. It was thought that many disciplines would want the technology as it had wide applications across genetics and pathology. Centralisation was thought to be practical and feasible in terms of sample transport and processing though it would be difficult to enforce a system nationally.

Advantages suggested by laboratories included pooling of expertise in techniques (1) and interpretation of results (2), streamlining processes (1), standardised testing (3), financial savings (4), turnaround time (3) and focusing of experience and equipment (1).

Disadvantages included: destabilisation of current services (3), deskilling of workforce (4), loss of local control and potential difficulties in coordinating and undertaking follow-up investigations (1), limited opportunity for intellectual and technical discussion (1), loss of opportunity to develop arrays across pathology in general (1) and overall diminished opportunity for research in this area.

Impact on funding of other group involvement

Only two laboratories commented on the potential impact on funding of other groups involvement in array CGH. There was currently lack of experience from prospective studies although, in the longer term there was thought to be potential to "revolutionise diagnostic cytogenetics for a large sector of the work load". It would probably be necessary to have increased funding for a period of time in order to investigate the savings from better first line

tests. As most referrals for the high risk group are made from clinical genetics departments, no major additional costs, such as counselling related costs were expected as a result of higher detection rate in the LD group.

Advantages and disadvantages of array CGH

Laboratories were asked to summarise the overall advantages and disadvantages of array CGH and barriers to use in practice.

Main advantages cited were:

- Higher resolution
- Increased detection
- Speed
- Higher objectivity and reproducibility
- Accurate reporting
- Less labour intensive, can be automated and no cell culture required
- Provides new knowledge of new syndromes, including more accurate definition and information on genotype phenotype correlation
- May help to avoid other costly investigations

Disadvantages included:

- Detecting polymorphisms of no clinical significance
- Expense in time and money
- Hard to interpret
- Complexity and reliability
- Unable to detect balanced translocations
- Hard to standardise software
- Subsequent work-up needed

Barriers included:

- Complex to implement
- Robustness of technique
- Cost
- Time
- Training
- Capital requirement
- Lack of reliable commercial kits
- Questions over clinical utility

Chapter 7 Evaluation of array CGH

7.1 The ACCE Framework

Decisions to introduce genetics tests into laboratory and clinical practice should be based on a systematic and validated process. The UK has initiated a process of genetic test evaluation for molecular genetic tests for rare diseases that are provided by the NHS. This is the UK Genetic Testing Network (UKGTN) *Gene Dossier* evaluation framework based on the ACCE framework.

The ACCE framework for the evaluation of new genetic tests was pioneered by the Centers for Disease Control (CDC), USA(1). ACCE is an acronym derived from the four components of the evaluation namely:

1. **A**nalytic validity: the ability of the test to accurately and reliably measure the genotype of interest.
2. **C**linical validity: the ability of the test to detect or predict the associated disorder (phenotype).
3. **C**linical utility: consideration of the risks and benefits associated with introduction of the test into routine practice.
4. **E**thical, legal, and social implications (ELSI): these may arise in relation to all the components described above, and include economic considerations.

7.2 Methodological issues in array CGH

The evaluation of array CGH as a genetic test, however, presents methodological issues. As well as being available as a substitute technology to identify known syndromes, the greater resolution of the technology allows us to identify hitherto unknown syndromes with genetic abnormalities that cannot be identified by other means. In other words, it takes us into an arena where there is no 'gold standard' or independent means of knowing the truth. In this latter situation some elements of the formal ACCE evaluation cannot be undertaken.

Thus, we conceptually divided our evaluation into two main areas:

1. Evaluation of the use of array CGH in diagnosing known genetic abnormalities
2. Evaluation of the technology as an "investigative technology used for discovery" to diagnose hitherto unknown syndromes

In the latter case the objective is to identify genotypic subsets of learning disability and the evaluation seeks to determine the effectiveness and cost-effectiveness of this. Appropriate measures are:

- Diagnostic yield ('true positives')
- Proportion of non-causal variants ('false positives')

7.3 Evaluation of test performance in known syndromes

Methods

We addressed the question of analytic sensitivity by seeking studies that used microarray CGH to confirm known genetic abnormalities. We included case series or cohort studies in which genetic abnormalities were known to be present based on positive cytogenetic tests. The cases included in the study had to be patients with learning disabilities (mental retardation), developmental delay or dysmorphism. Studies using microarray CGH in the diagnosis and characterisation of cancer were excluded. Methods for the systematic review are provided in Box 7.1.

Box 7.1 Systematic review methods

Search Strategy

Electronic searches were conducted using the MEDLINE, EMBASE and BIOSIS databases. The search strategy combined a component for the clinical condition and a component for the test and is provided below. Searches were not to be limited by language or publication type.

MEDLINE: ((learning disability) OR (mental retardation) OR (abnormalities) OR (learning disorders) OR (developmental disabilities) OR (syndromes) OR (multiple congenital anomalies)) AND (array CGH OR microarray)

EMBASE:

1 array CGH OR microarray
2 DNA-MICROARRAY#.DE. OR GENE-EXPRESSION-PROFILING#.DE.
3 mental ADJ retardation OR learning ADJ disorders OR learning ADJ disability OR developmental ADJ disorders OR abnormalities
4 LEARNING-DISORDER.DE. OR EDUCATION.W..DE. OR MENTAL-DEFICIENCY.DE. OR FRAGILE-X-SYNDROME.DE. OR AUTISM.W..DE.
5 1 OR 2
6 3 OR 4
7 5 AND 6

BIOSIS: al: (array CGH OR microarray) and ts: "mental retardation" or "learning disability" or "learning disorder" or "developmental disorder" or "abnormalities" and su: (Human)

Data Extraction

A data extraction form was designed for the project. This was piloted on three studies and modified as appropriate. Two reviewers (SI & GS) independently assessed the studies and extracted data. Where there were differences or difficulties in the interpretation of data, this was resolved in discussion with two other members of the team (SS & CSS).

Meta-analysis (unknown syndromes only)

Meta-analysis is a statistical technique for combining the results of studies addressing the same question. Meta-analytic methods use weighted averages to combine effects, so that larger studies have more influence over the results than smaller ones. Diagnostic yields (proportion of tested

individuals in whom a variant was found and concluded to be causal) were analysed on the logit scale (log(p/(1-p)) and transformed to the original scale for presentation. Prior to meta-analysis, consistency of findings was assessed using a standard χ^2 test for variation across studies (often called heterogeneity) and using the I^2 statistic, which describes the proportion of total variation in estimates due to heterogeneity rather than random error. A 'fixed-effect' meta-analysis model assumes that there is a common effect underlying all of the studies. A 'random-effects' meta-analysis model assumes that heterogeneity can be represented by a distribution of underlying effects, conventionally a normal distribution. In the absence of heterogeneity, the models yield the same result. This was the case in our analyses for this project.

Findings

From among 449 citations retrieved by the search (January 2006), we identified seven studies in which the authors performed array CGH testing in groups of patients with previously identified chromosomal anomalies. The initial identification in these cases, which were mostly defined chromosomal syndromes, had been achieved by conventional cytogenetic tests such as karyotyping and FISH testing. The objective of these studies was to validate the use of array CGH in the diagnosis of chromosomal abnormalities. Three studies were syndrome-specific and their study population included patients with conditions such as 1p36 deletion (2), Wolf Hirschhorn syndrome (4p deletion) (3), and X-linked mental retardation with chromosomal imbalance (4). The other three studies were case series that included patients with different genomic abnormalities. In four of the studies, normal controls were also tested. The details of these studies are provided in Table 7.1.

Table 7.1 Study description

Author & Year	Country	Patients	Setting	Controls	Methods
Yu W et al. 2003(2)	USA	Patients with 1 p36 deletion	-	Nil	Array of 97 clones from 1p36, 41 subtelomeric and 3 each from X & Y
Cheung S W et al. 2005(5)	USA	Various genomic disorders (25)	Genetics Service	Normal male & female (1 each)	Array of 362 clones for known genetic conditions and 41 subtelomeric clones
Harada N 2005(3) Validation	Japan	Wolf Hirschhorn syndrome (4p deletion)	Genetics Service	Nil	Array of 43 subtelomeric clones
Rickman L et al. 2005, 1 Mb array(6)	UK, Spain & Italy	Patient samples for prenatal diagnosis / postnatal blood samples with known cytogenetic abnormalities	Prenatal diagnosis service	30 normal healthy blood donors	1 Mb array
Rickman L Custom made array(6)	UK, Spain & Italy				Custom designed array of 600 large insert clones which are concentrated on areas of clinical significance

Bauters M et al. 2005(4)	Belgium	XL MR, X- linked ichthyosis	Genetics Service	Cross validation of 3 sets of clones and in 4 female & male samples	Complete tiling path array of X chromosome
Bejjani BA et al. 2005(7)	USA	Cell lines from patients with known chromosomal abnormalities	-	50 normal controls (25 M & 25 F; including multiple ethnicities)	Array generated by assessing 906 clones (589 selected)

The results of these validation studies indicate that, in four of these, array CGH could detect all of the known chromosomal abnormalities, thus providing 100% sensitivity. In two of the studies (6,7), however, array CGH did not detect some of the chromosomal abnormalities previously diagnosed by cytogenetics. Details are given in Table 7.2.

Table 7.2 Results

Author & Year	No of Patients	Resolution	No. with causal abnormalities*	Sensitivity (%)
Yu W et al. 2003(2)	25	100-300 Kb	25	100 (25/25)
Cheung S W et al. 2005(5)	25	NS	25 (25)	100 (25/25)
Harada N 2005(3) (Validation)	5	NS	5	100 (5/5)
Rickman L et al. 2005 1 Mb array(6)	30	1 Mb	22	73 (22/30)
Rickman L Custom made array(6)	30	NS	29	97 (29/30)
Bauters M et al. 2005(4)	7	82 Kb	7	100 (7/7)
Bejjani BA et al. 2005(7)	36	1 Mb	35	97 (35/36)

* Numbers in brackets indicate those confirmed by FISH.
NS: Not stated.

Discussion

A review of seven studies has demonstrated that array CGH testing is able to identify existing genetic abnormalities, previously detected by karyotyping, with a high sensitivity (100% in four studies). These studies demonstrate the sensitivity of array CGH in the diagnosis of genetic defects, relative to karyotyping and FISH testing. However some of the results also indicate the limitations of CGH arrays. These studies, although observational in nature, cover a range of genomic conditions, and have been performed with rigorous attention to technical detail. Four of the seven studies have also tested control samples from individuals or pooled samples.

The major outlier among these studies is the report from Rickman et al. (6), where the 1 Mb array has provided false negative results in seven cases, owing to the low clone density in this

array for the following critical regions: 7q, 22q, 17p and 4p. These findings point to a potential shortcoming with array design, which may be remedied by designing appropriate custom made arrays, as the authors have demonstrated in the study.

Another case in point is the single false negative result (from a cohort of 36) in the study by Bejjani and colleagues(7). However, more importantly, in this study, only 65% of the commercially available clones were used in the test array, as the remaining clones (35%) were found to have been mapped to the wrong location. The authors raise a cautionary note regarding the validity of clone specification.

Hence it would be reasonable to conclude that array CGH testing using the appropriate set of clones is capable of achieving a high level of analytic validity in the detection of genetic abnormalities, although modifications in array design are necessary to ensure consistently high specificity.

The need to design appropriate arrays needs to be borne in mind, if array CGH testing is to be extended to the wider population of learning disabled individuals. In the NHS context this would require that the arrays that are selected for clinical use must contain the appropriate clones to detect the genetic abnormalities related to the common phenotypes that can be clinically identified. These may otherwise be missed, leading to an unacceptably high proportion of false negatives on array CGH testing.

7.4 Diagnostic yield in unknown syndromes

Methods

We evaluated the performance (diagnostic yield and false positive proportion) of array CGH in patients with learning disability in whom a genetic abnormality is suspected and in whom conventional cytogenetic analysis has proven negative. Studies were included in the systematic review if they used microarray CGH to identify genetic abnormalities, and confirmed their results using FISH test. We included both case series and cohort studies. The cases included in the study had to be patients with learning disabilities (mental retardation), developmental delay or dysmorphism. Studies using microarray CGH in the diagnosis and characterisation of cancer were excluded. Methods of the systematic review are as provided in Box 7.1 in the previous section.

We also sought studies that reported genomic variation detectable by array CGH in phenotypically normal people.

Diagnostic yield

From among 449 citations identified by the search (January 2006), we found seven studies that selected a cohort of patients who were negative for conventional cytogenetic testing and conducted the array CGH test for these patients. These studies selected the patients to be included in the cohort to be tested using certain clinical criteria, an example of which is shown in Box 7.2

Box 7.2 Clinical Criteria for offering array CGH testing in a research context

An example from Shaw-Smith et al. 2005 (9)

Learning disability mild moderate or severe plus one of:
- family history of LD
- overgrowth or growth failure
- behavioural problems
- seizures
- facial dysmorphism or major structural malformation

and normal G-banded karyotype at 400 to 500 resolution

However most studies have used a modification or subset of the clinical checklist developed by de Vries (10) and are summarised in Table 7.3.

Table 7.3 Checklist for selecting patients for array testing (10)

Items	Score
Family history of mental retardation	1
Compatible with Mendelian inheritance	2
Incompatible with Mendelian inheritance (including discordant phenotypes)	1
Prenatal onset growth retardation	2
Postnatal growth abnormalities For each of the following 1 point (max 2):microcephaly (1), short stature (1) macrocephaly (1), tall stature (1)	2
>2 facial dysmorphic features Notably hypertelorism, nasal abnormalities, ear anomalies	2
Non-facial dysmorphism and congenital abnormalities For each anomaly 1 point (max 2) Notably hand anomaly (1), heart anomaly (1), hypospadias +/- undescended testis (1)	2

These studies include cohorts reported from a number of European countries, as well as Japan, Brazil and the USA. All studies have included sampling of control DNA as part of their protocol. All but two have used a 1 Mb resolution array for investigating the whole genome. One group has used an array with a resolution of 50 Kb (11), and another has used a set of 2173 clones, resulting in an average resolution of 1.4 Mb (8).

The control samples included in the studies varies from 2 persons (1 male & 1 female) up to 40 people. Some studies have used pooled DNA sample tests as control and one group has also included a positive control. Menten and colleagues (12) have used the samples from other patients in the cohort as controls. The characteristics of the studies are described in detail in Table 7.4.

Table 7.4 Study description

Author & Year	Country	Patients (negative to cytogenetic testing)	Setting	Controls	Methods
Menten B et al. 2006 (12)	Belgium	Idiopathic MR with multiple congenital anomalies	Genetics services	Other patients in the cohort	1 Mb array
Miyake et al. 2006(8)	Japan	Idiopathic MR with some dysmorphic features	Various	2 (1 M & 1F) negative & 1 positive control	For whole genome: 2173 FISH – confirmed clones
de Vries BB et al. 2005(11)	Netherlands	Mental retardation, negative for karyotyping & MLPA. Scored by a checklist of clinical criteria (0-10)	Genetics Service	Samples from 72 parents of the cases.	Tiling resolution whole genome array
Shaw-Smith et al. 2005(9)	U.K., France	Moderate to severe MR, non-consanguineous, with at least 1 clinical criteria (out of 4)	Genetics Services	Pooled DNA from normal people: 20 M & 20 F	1 Mb array
Schoumans J et al. 2005(13)	Sweden	Mild to severe MR, with phenotype suggestive of chromosomal origin- i.e. dysmorphism, malformations and/or family history, scoring at least 3 points on the de Vries checklist	Molecular Medicine	Reference DNA of a pool of 10 normal individuals	1 Mb array
Rosenberg C et al. 2005(14)	Nether-lands, Brazil & U.K.	Mild to severe MR, with cranial/ facial dysmorphisms and at least one additional congenital abnormality. Family history and consanguinity were not considered	Genetics Service	100 control observations for each chromosome pair.	1 Mb array
Vissers LE et al. 2003(15)	Nether-lands & USA	Patients with mental retardation and additional dysmorphisms, scoring 3 points or more on de Vries' checklist		Four normal healthy blood donors (2 M, 2 F)	1 Mb array

In the group of patients with learning disability who were selected for array CGH testing, detection rates of causal chromosomal abnormalities varied from 10 to 16.7%. The anomalies detected took the form of deletions, duplications, and unbalanced translocations. The results of the studies are summarised in Table 7.5.

Table 7.5 Causal genetic abnormalities in idiopathic learning disability.

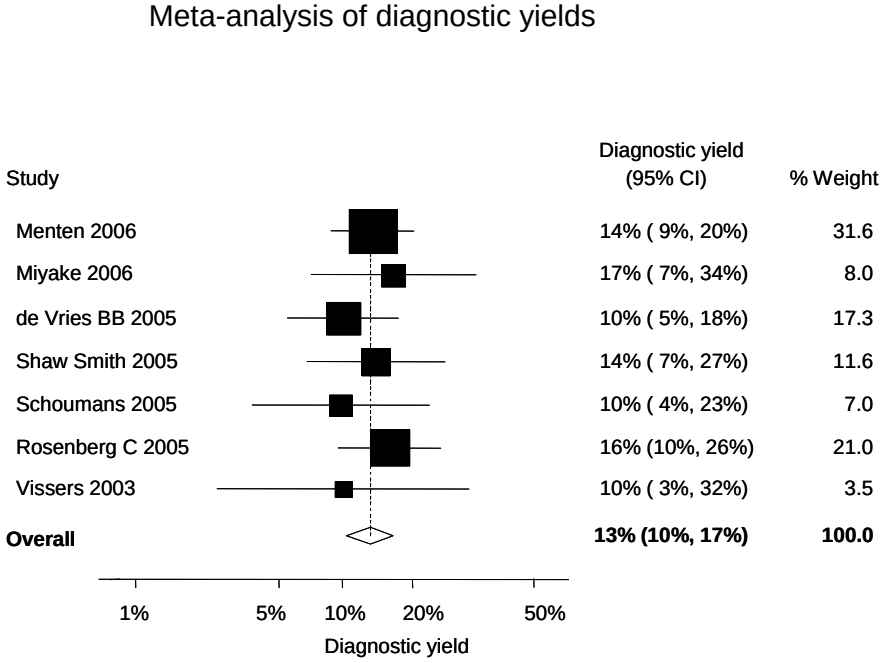
Author & Year	No. of Patients	Resolution	No. with causal abnormalities*	No. with non-casual abnormalities (%)	Diagnostic Yield (%)
Menten B et al. 2006(12)	140	1 Mb	19 (11)	9(6.4)	13.6 (19/140)
Miyake et al. 2006(8)	30	1.4 Mb	5 (5)	20 (66)	16.7 (5/30)
de Vries BB et al. 2005(11)	100	50 Kb	10 (10)	5 (5)	10 (10/100)
Shaw-Smith et al. 2005(9)	50	1 Mb	7 (7)	5 (10)	14 (7/50)
Schoumans J et al. 2005(13)	41	1 Mb	4 (4)	NS**	10 (4/41)
Rosenberg C et al. 2005(14)	81	1 Mb	13 (13)	7 (8.6)	16 (13/81)
Vissers LE et al. 2003(15)	20	1 Mb	2 (2)	1 (5)	10 (2/20)

* Numbers in brackets indicate those confirmed by FISH.
**A total of 151 copy number polymorphisms (CNP) detected in the cohort; number of patients with CNPs not stated.

Figure 7.1 provides a forest plot, illustrating the results of the seven studies and the result of a meta-analysis. The numbers to the right show each study's diagnostic yield, along with a 95% confidence interval and its weight in the meta-analysis (closely related to sample size). The areas of the black squares are proportional to these weights and the horizontal lines depict the 95% confidence intervals. The overall estimate and confidence interval is shown in the diamond at the bottom of the figure, with the vertical dotted line showing this combined estimate in relation to each of the contributing studies.

The overall diagnostic yield from the seven studies was 13% (95% confidence interval: 10-17%). There was no evidence of variation between studies (heterogeneity statistic I^2= 0%, p=0.8, not statistically significant). This result represents the overall diagnostic yield of array CGH testing in patients with learning disabilities in whom conventional cytogenetic analysis (+/- additional FISH and/or MLPA) was negative and who have fulfilled the clinical criteria for an array CGH test.

Figure 7.1 Array CGH meta-analysis (random effects) of diagnostic yields



Numbers needed to test

The diagnostic yield can be expressed in another, more intuitive way, as the numbers needed to test (NNT) to achieve one additional positive diagnosis.

NNT = 100 X 1/ (absolute increase % in diagnostic yield)

Given a diagnostic yield of 13%, about 8 patients (100/13) will need to be tested with array CGH to obtain one positive result.

Non-causal abnormalities (false positives)

A careful interpretation of genomic copy number variations detected by array CGH tests must also consider the background variation in the human genome, which we shall review briefly in the next section. The existence of these variations mean that the abnormalities detected by array CGH need to be carefully evaluated to rule out the possibility that they may be part of the normal, common variation in the human genetic makeup. This requires an understanding of the ways in which geneticists can compare variations found in the patient with the parental genome and understanding the background variation in the human genome.

Table 7.5 showed that the use of array CGH in the process of diagnosing learning disability gave rise to a not insignificant number of patients with abnormalities that were ultimately thought not to be causal. Box 7.3 gives an outline of the investigative routine that is undertaken with each positive test to reach a clinical interpretation of whether the abnormality is probably causal or not. This includes interrogation of online international databases of known polymorphic

variations, databases such as DECIPHER that provide information on genotype, phenotype correlations, and clinical examination and testing of parent. The proportion of variants eventually thought to be non-causal ranged from 5% to 67% of the total sample.

Box 7.3 Clinical interpretation of an array CGH result

Researchers who test cohorts or large case series of patients with learning disabilities using array CGH, compare the genomic abnormalities that they detect with previously reported normal variations in the human genome.

Variations in the human genome identified by researchers around the world are recorded in the human genome database http://www.gdb.org/.
Similarly, the Single Nucleotide Polymorphism database (dbSNP) is a public-domain archive for a broad collection of simple genetic polymorphisms http://www.ncbi.nlm.nih.gov/projects/SNP/.

Other online databases record and update details of phenotypes correlated to genetic abnormalities.
Examples include DECIPHER http://www.sanger.ac.uk/PostGenomics/decipher and ECARUCA http://www.ecaruca.net. These collections contribute to the repository of knowledge on genetic variants and their association with an abnormal phenotype. It is hoped that, as the collections enlarge, they will prove valuable in the interpretation of results from array CGH studies, by providing researchers with a tool for the rapid assessment of the significance of their observations.

Clinicians also endeavour to determine whether the genetic abnormality is inherited, or one arising *de novo* by testing the parents of the patients, if available. If abnormalities are detected in the parent(s), there are also attempts to review the parent(s) clinically to ascertain if they have any phenotypic features similar to those in the patient.

Information from all these sources is considered and the results are reported in three categories:

1. genomic abnormalities that are probably causally related to the learning disability.
This group includes:
    i. *de novo* genomic abnormalities in a patient that can be demonstrated to be absent in both parents, and have not been reported previously in phenotypically normal people and
    ii. inherited abnormalities in patients whose parent(s) test positive for the same abnormality and also display similar (usually milder) phenotypic features as the patient.

2. genomic polymorphisms of no pathological significance.
This group includes:
    i. abnormalities that are demonstrated to be inherited from phenotypically normal parents, i.e. detected in patients as well as their normal parent(s), and
    ii. polymorphisms previously reported in phenotypically normal people.

3. genomic variations of uncertain significance.

This group includes those abnormalities that have insufficient information about them to conclusively prove pathogenicity. For example, parental samples may not be available for testing for a patient with a demonstrated abnormality, and hence the possibility of it being an inherited normal variation cannot be excluded.

Clinicians typically follow a conservative approach in reaching a decision about the causative role of a genetic abnormality. This is also true of the studies included in this review.

Common variations in the human genome

Some indication of the possibility of incorrectly diagnosing a patient with a causal genomic variant can be gleaned from studies of phenotypically normal people. Knowledge of normal genomic variation that might be identified by array CGH allows exclusion of these variants from consideration.

Gains and losses of larger amounts of genetic material (>200 bp) result in variations in the human genome, which are collectively called copy number polymorphisms (CNP). The characterisation of CNPs has recently been an area of keen research interest. Some of the recent studies, which attempt to identify such variation, are listed in Table 7.6, along with their main findings.

Table 7.6 Copy number polymorphisms in the human genome

First author & year	Sample details	No. of variations detected	Type of variation	Average No. of CNP per person	Gene involvement
Iafrate JA 2004(16)	55 people- 39 normal 16 with chromosomal imbalances	255	-	124	142 (56%)
Sebat J 2004(17)	20	221 (76 unique)	CNP > 100 kb	11	70 (32%)
Tuzun E 2005(19)	Human genome reference sequence vs. another (fosmid paired end sequences)	297	Insertions: 139 Deletions: 102 Inversion breakpoints: 36	297	-
Sharp AJ 2005(18)	47 (from 4 continents) focus on 130 rearrangement hotspots in the genome	160 variations (119 CNP regions)	-	-	Overlaps 141 genes partly or completely
Altshuler D 2005(22)	269 (4 populations: Intn'l hapmap project)	> 1.3 Million	SNPs	-	-

Conrad DF 2006(20)	60 (from the Intn'l hapmap project)	586	Deletions	30- 50 deletions >5kb size	Span 267 known and predicted genes
McCarroll SA 2006(21)	269 (Intn'l hapmap project)	541	Deletions 1-745 Kb size		287

Experimental approaches are able to detect the presence of variation in the large scale, from 100 KB and more (16-18) whereas a computational approach using fosmid end sequences has demonstrated variation from >8kb (19). CNPs are widely distributed throughout the genome (17), may affect genes, and may be involved with pathogenesis of certain diseases. The more recent reports of deletion polymorphisms (20, 21) indicate that they may be in linkage disequilibrium with SNPs as well as being associated with segmental duplications.

7.5 Discussion

Meta analysis of the results from seven papers using array CGH testing shows that the test has a greater clinical sensitivity relative to karyotyping in selected patient groups. The array CGH test is able to detect causal genetic abnormalities in 13% (95% CI 10% - 17%) of patients in whom karyotyping did not demonstrate any abnormalities, and who fulfilled the clinical criteria, a number needed to test of eight (95% confidence interval 6 – 10). The findings are remarkably consistent especially as each study has used different selection criteria, array platforms, resolutions, scanners and software.

Array CGH also identifies false positive abnormalities. If the study by Miyake is excluded (false positive yield 67%), the false positive yield ranges from 5%-10%. The reasons for Miyake's extreme result are unclear; it does not appear to be related to the array's resolution because they used the lowest resolution array (1.4 Mb) and the study with the highest resolution array (de Vries) had one of the lowest false positive yields (5%). The spectrum of patients tested also appears to be similar to the other studies. One possible explanation is that there are important differences in the design, calibration, and use of their array and especially their choice of clones. A less likely explanation is that ethnicity may be influencing the results as this was the only study reporting data from patients in the Eastern hemisphere. It will be interesting to see whether future studies conducted in Asian patients report similar results.

The assessment of specificity requires that there is a reference method that can identify those with, and without, the target disorder so that true positives and true negatives can be reliably determined. Whilst it is possible to determine with some degree of certainty whether a patient with a positive array CGH result really does have a causal genetic abnormality (see Box 7.3), it is not possible to determine whether patients with a negative array CGH test result really do not have a causal genetic abnormality. Even after making judicious use of all available testing modalities, the majority of patients do not have a genetic diagnosis, although the data suggest that array CGH detects more abnormalities than conventional cytogenetic analysis, due to the increased resolution.

Additional diagnostic yield of array CGH

The systematic review process has identified the studies that examine the diagnostic yield of microarrays, and a meta-analysis has quantified the effect as detection of genetic abnormalities in an additional 13% of patients in whom karyotype +/- FISH/MLPA was negative. This needs to be

placed in the overall context of the diagnostic processes through which diagnosis of a chromosomal abnormality is reached for patients with learning disability. The routine laboratory analysis usually involves karyotyping on all patients, followed by FISH/MLPA for those patients who are negative, but still suspected on clinical grounds to have a chromosomal diagnosis. Array CGH has then been used on patients who are found negative on FISH/MLPA and further clinical criteria are sometimes applied at this stage.

The de Vries study follows this process through. This cohort excluded Down syndrome patients and the overall yield for the cohort was:

Karyotype	(Diagnostic Yield 4.8%)	34/710 = 4.8%
MLPA	(Diagnostic Yield 4.7% of those tested)	14/710 = 2.0%
Array CGH	(Diagnostic Yield 10% of those tested)	10/710 = 1.4%

This provides an order of magnitude for the extra diagnoses achieved by using array CGH as an adjunct to current routine analysis. Our meta-analysis suggests that the yield for array CGH might be very slightly higher than this. It can be noted that, for diagnosis made by MLPA a recent Cambridge technical report of 150 consecutive cases referred for FISH with normal karyotypes in 7(4.7%) abnormalities were discovered by FISH and in 9 (6%) by MLPA. http://webgroups.phgu.org.uk/file_admin/secure_file.php?file_ID=646

From our literature review, there are no studies that follow through a full cohort of patients in order – doing the next stage analysis on all patients who were negative or which use array CGH as a first line test without prior karyotyping. We have therefore attempted to produce a model for this in our discussion in Chapter 9.

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Chapter 8 Cost analysis

8.1 Introduction

As the number of tests using genomic information increases, health service providers require a framework for decision making. Economic evaluation contributes to this framework and compares the costs and effects (outcomes) of health care interventions and identifies efficient ways of using limited NHS resources. The Oxford Genetics Knowledge Park (OGKP) is currently performing an economic evaluation of array CGH compared with karyotyping for detecting chromosomal abnormalities in learning disability. This chapter presents the methods employed and the results of the cost-analysis component of the evaluation. This cost-analysis is important because very little is known about the true costs of array CGH in the NHS. Whilst some prices are available, it is important to remember that true costs and tariffs (or prices) are not the same and also that we should be reflecting the opportunity costs and not costs in the financial sense.

8.2 Methods

Costing perspective

The aim of our cost-analysis was to provide a cost for a single sample (child/patient) being tested for learning disabilities. The perspective taken was that of the NHS, rather than society. As such, certain costs were excluded from the analysis, for example, the time taken from paid work for patients travelling for appointments for blood samples to be taken.

Data collection

Primary data collection for the unit costs of defined resource categories (staff, consumables, capital, and overheads) can be derived using two main approaches, top-down and micro (bottom-up costing). With the former the intervention is broken down into large components, which need to be identified, such as the average cost for a hospital day for a surgical intervention. Alternatively, micro costing requires the identification of all underlying activities, such as the consumables and staff time which form this hospital day (1). In practice most economic evaluations use a combination of the two approaches and the decision of when to use each one is often based on the ease of data collection (2). However, in some situations one approach could perform better than the other. A recent costing methodology study by Wordsworth and Ludbrook (2005) provides empirical evidence that, when directly comparing these two costing approaches, micro costing is more likely to reflect the true costs of health care interventions and is therefore a preferable approach in many situations (3).

Four main resource categories were examined: staff, equipment, consumables and overheads (Trust on costs). Using a micro-costing approach, emphasis was placed on collecting detailed information at the laboratory level. This micro-costing enabled the identification of very specific items of resource use, such as the time spent by staff on various activities and these individual items were built up to create a total cost for a sample being tested.

The basis for information on resource use for the arrays was a laboratory within the Wellcome Trust Centre for Human Genetics (University of Oxford). Data were collected within an academic setting as the scientists helping with the costing were part of the OGKP array study, exploring the translation of array technology from a research setting into the NHS. Any areas that were likely to be different within an NHS setting were highlighted by staff in the

Oxford Regional Cytogenetics Laboratory (Churchill Hospital, Oxford), where the arrays were being translated into clinical practice.

In order to make the cost results as generalisable as possible to other UK laboratories, besides data from Oxford, cost data from the Birmingham Regional Genetics Laboratory and the South East Scotland Cytogenetics Laboratory in Edinburgh were also collected. This was undertaken by producing an array costing questionnaire which was completed by the Birmingham and Edinburgh laboratory directors (available on request from S. Wordsworth). Finally, the laboratory array questionnaires produced as part of this array working group provided additional information for the costing as the questionnaire had some basic questions on laboratory resource use for the arrays.

The costing for the arrays was based on the use of genome-wide oligonucleotide arrays, specifically the Agilent 44K array. However, there is considerable variation in the cost of arrays and a range of costs, including those of targeted and genome-wide BAC chips is therefore incorporated into the sensitivity analysis.

For the karyotyping, information on resource use was collected within the Oxford Cytogenetics Laboratory and the Birmingham Regional Genetics Laboratory, again through the use of a specially designed costing questionnaire (available on request from S. Wordsworth).

Testing Process

The first step for the costing was to identify the testing pathway from a sample arriving at the laboratories, through to the reporting of results. This highlighted seven stages in the array and karyotyping testing processes as detailed in Figures 8.1 and 8.2 respectively. The next step was then to identify what resources would be required in each of these stages.

Figure 8.1 Array CGH testing process

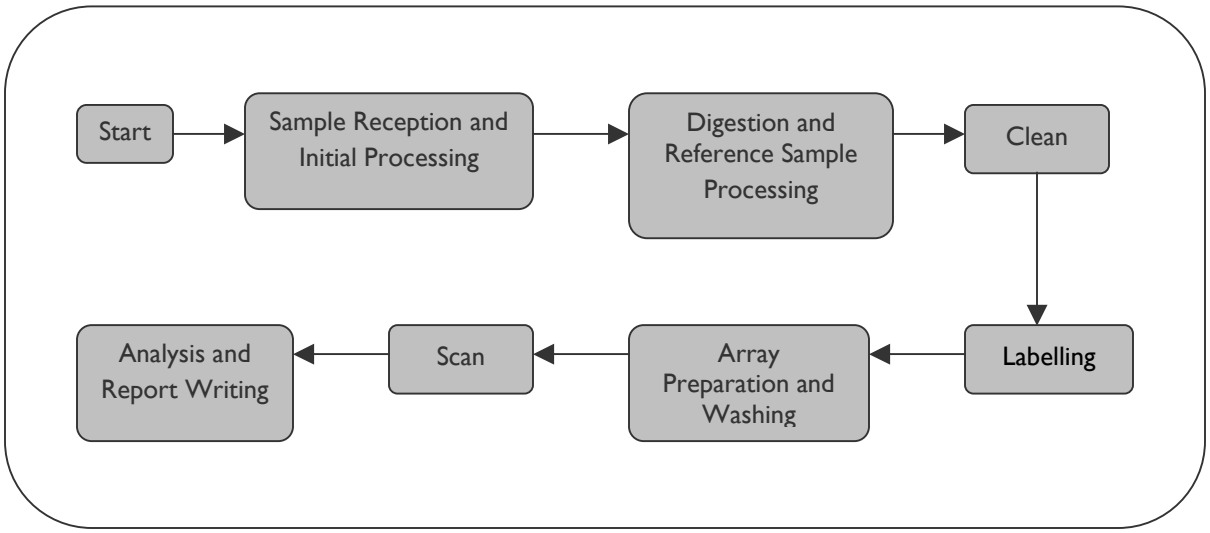
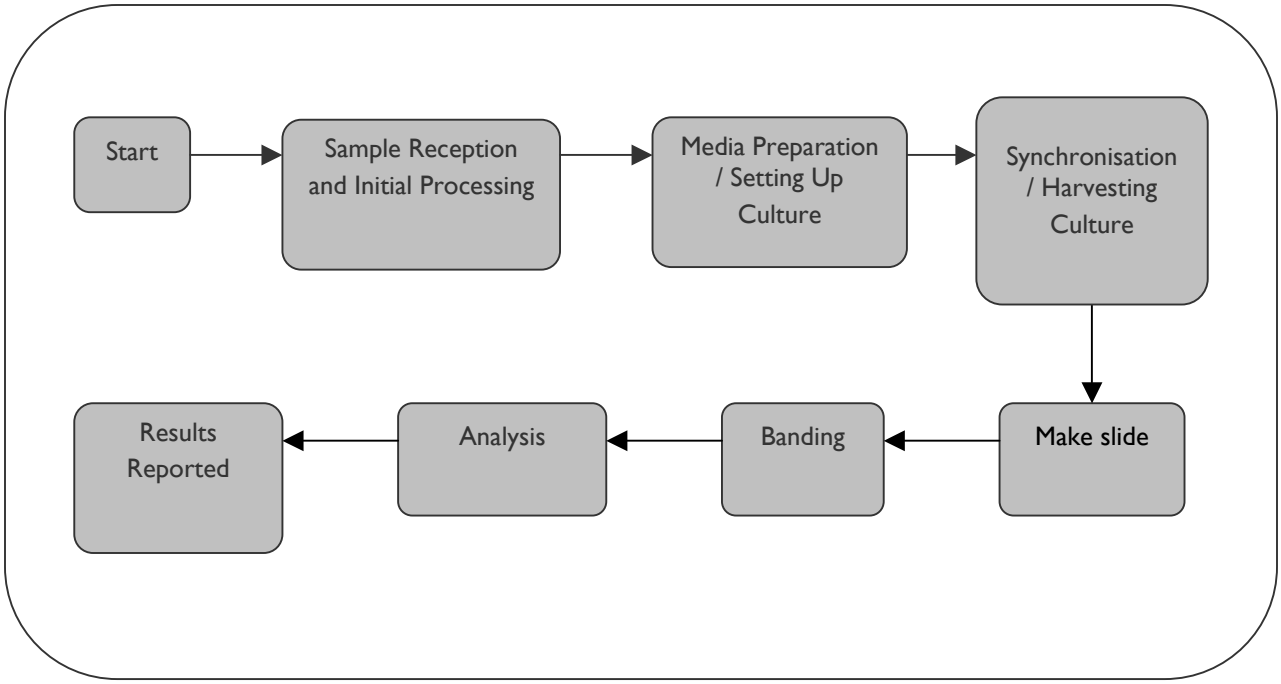


Figure 8.2 Karyotyping testing process



Staffing

Data on staff resource use were obtained from scientists estimating how long all activities took in the testing processes. For the arrays this was scientists in the Oxford (Wellcome Centre), Edinburgh and Birmingham laboratories and for the karyotyping, the Oxford Cytogenetics Laboratory and the Birmingham laboratory. Unit costs were then attached to these time estimates taken from the NHS Reference Costs Database (4). Staff were assumed to work 37.5 hour weeks and 46 weeks per annum. Mid-points were taken of the national salary range, which adds to the generalisability even further. Finally, national insurance and superannuation were added at 20%.

Equipment

Data on major equipment items such as array CGH scanners were identified from the two Oxford laboratories (Wellcome Trust and Cytogenetics laboratory) and from the laboratory survey outlined in this report. Data on smaller equipment items such as computers were identified from the two Oxford laboratories. All equipment items included VAT at 17.5%, which is arguably a transfer payment for the government (simply goes from NHS to treasury and back in another form). However, we include VAT here as it is a real cost faced by the laboratories. For the equipment items we annuitised the initial capital outlay over the predicted lifetime of the item, that is, we calculated an equivalent annual cost (EAC). The EAC calculation produces an annual cost payment that would be equivalent to renting or leasing the item of equipment. This adjusts for the depreciation of the capital item over time and the fact that money spent purchasing the item cannot be spent on other services, the opportunity cost (5). For the EAC various life spans were used for the equipment items based on estimates from the laboratories providing the costing data, with a discount rate of 3.5% being applied.

Consumables

Consumables were largely the disposable items such as reagents. Once the quantities of consumables were identified, information on unit costs was derived from laboratory price lists and VAT added.

Overheads

This category of cost included resources required for the provision of the service, which are shared with other departments, such as heating, power and lighting (sometimes referred to as Trust 'On Costs'). They also include those which form part of the general running costs of the hospital, but are not directly used, such as medical records. The capital element of the building space is also covered in this category, as it is assumed to be included in the estimates of Trust 'On Costs' (ranging from 15% to over 25%), which were used and difficult to separate from the total cost figure.

Reporting Scenarios

The testing processes for both arrays and karyotyping from the reporting stage onwards can take a number of different paths, depending on what the preliminary findings are. Given that array CGH is newer than karyotyping, we focused on the various potential scenarios for the arrays including tests on both parental samples, using CGH arrays, FISH and MLPA.

Laboratory Throughput

For the arrays a baseline level of 25 tests per week was chosen (1,150 tests over a 46 week year), which reflected the current typical availability of equipment and staff in Oxford. For karyotyping, the baseline level was 2,800 per annum, again based on Oxford throughput. Due to potential variability in these throughput levels across different UK laboratories, these throughput levels were examined in the sensitivity analysis (see below).

Sensitivity Analysis

Due to the degree of uncertainty surrounding certain cost estimates and assumptions, the impact of changes in these variables was examined using both one-way and multi-way sensitivity analysis. We chose cost items which were likely to have the greatest impact on the total costs of the tests, such as staffing times and grades, cost of the arrays and scanner, the percentage used to calculate overheads, cost of obtaining a chromosomal sample; batch sizes; impact of using different grades of staff at various stages of the process; probes for karyotyping, test throughput, and the discount rate employed in the EAC for equipment.

Finally, all costs are reported in British pounds (£) in 2005-2006 prices. Any costs used prior to 2005, were inflated to 2006 using the hospital and community health services (HCHS) pay and price index. Data analyses were performed using Microsoft Excel 2003.

8.3 Results

Array CGH

Table 8.1 provides a breakdown of the staff resources required for the array CGH testing. The total laboratory staff hands on time is estimated to be just under 2.5 hours, from the time taken from the sample being handled at reception and initial processing to report writing. Obviously these time estimates do not reflect how long the array processing actual takes because there

will be various steps where the staff start a process (such as restriction enzyme digestion), then perform other activities whilst waiting for this to be completed. In terms of costs, Table 8.1 shows an average cost of £42 per sample for staffing. A point to note is that this is a pure hands on array cost so does not include costs such as staff meetings, any training and conferences that are assumed to be similar for the arrays and karyotyping so would therefore cancel each other out.

Table 8.1 Array CGH staff resource use

Staff Type	Cost Per Hour[a]	Hands-on time (minutes)	Cost Per Test
Medical Technical Officer	£14.69	61	£10.92 [b]
Clinical Research Scientist	£21.65	76	£27.55
Consultant Grade Scientist	£44.09	5	£3.67
Total		**2 hours and 22 minutes**	**£42**

[a] Includes Superannuation and National Insurance; [b] MTO Cost per test does not equal cost per hour multiplied by time spent on one test due to samples being batched together.

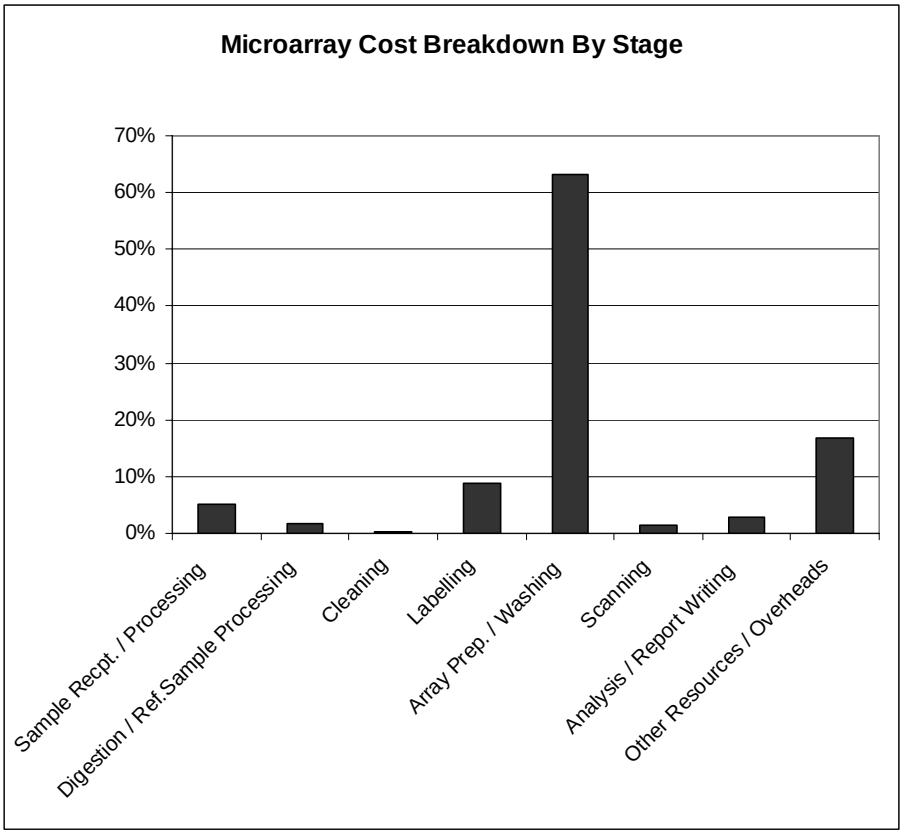
In terms of the overall costs for the arrays, Table 8.2 provides a breakdown of the summary costs associated with each stage of the testing for using array CGH, again on a per sample basis. These summary costs include staff, equipment and consumables for the 7 testing stages, plus Trust on costs. The table shows that the total average cost for the array testing is £892 per sample, with a range between £412 and £1,141. Unsurprisingly, the main cost driver for this total is the array component at £563, which includes an average of £500 per array, plus £63 for array preparation. The second largest cost is Trust on costs at £148, followed by labelling at £78. These costs are also presented graphically in Figure 8.3.

Table 8.2 Array CGH cost breakdown

Stage [a]	Cost	Range
Sample Reception and Initial Processing	£45	-
Digestion / Reference Sample Processing	£15	-
Cleaning	£4	-
Labelling	£78	-
Arrays, Plus Preparation and Washing	£563	£163 - £763 [b]
Scanning	£14	£10 - £19 [c]
Analysis and Report Writing	£24	-
General resources (e.g. pc computer)	£1	-
Trust on costs	£148	£37 - £186 [d]
Total	**£892**	**£377 - £1,135**

[a] Cost of getting blood sample not included; [b] Array range - £100 - £700; [c] Scanner range - £25,000 - £75,000; [d] Overheads range 15% to 25%.

Figure 8.3



Because different pathways could be followed after initial report writing, Table 8.3 provides a breakdown of potential alternative scenarios. Scenario 1 is considered the basic scenario where no putative mutation is found, at a cost of £895 (slightly different to the £892 figure in Table 8.2, which was the patient test cost for the most commonly anticipated scenario - scenario 2, where a putative mutation is found and one or both of the parents shows the same mutation. £892 is a proportion of the total cost of £2582 for the stage).

This is followed by a scenario where a decision is made to also test both parents using FISH (£1,031) and then arrays (£2,582). Further scenarios include either a FISH test (£2,650) or MLPA being performed (£2,798).

Table 8.3 Costs for alternative scenarios for the arrays

Scenario [a]	Cost
1 No putative mutation found in patient sample.	£895
2 Putative mutation found in patient, **FISH** test parents	£1,031
3 Putative mutation found in patient sample. One or both of the parents shows the same mutation.	£2,582
4 Putative mutation found in patient. Neither parent shows this mutation. **FISH** test performed on patient sample. Mutation confirmed.	£2,650
5 Putative mutation found in patient. Neither parent shows this mutation. **FISH** test performed on patient sample. Mutation not confirmed.	£2,650
6 Putative mutation found in patient. Neither parent shows this mutation. FISH test not possible – **MLPA** test used instead. Mutation confirmed.	£2,798
7 Putative mutation found in patient. Neither parent shows this mutation. FISH test not possible – **MLPA** test used instead. Mutation not confirmed.	£2,798

[a] Scenario 1 is a patient (child) sample only; remaining scenarios include child and both parents being tested.

With respect to sensitivity analysis, clearly the major cost driver of total cost results for the basic array testing is the initial cost of the arrays. Because the arrays are an emerging and fast growing technology, extensive sensitivity analyses were performed on the costs. Table 8.4 therefore presents the sensitivity analysis performed on the arrays themselves. We used a range of £100 to £700, to ensure that we included the prospect of the arrays decreasing in cost, which is probably the most likely, but also costs higher than our average of £500, in case there are laboratories who are currently paying more than the average figure if they have been unable to secure any discounts with companies. If a cost of £100 is imputed in the analysis, the total cost figure could be as low as £412. In contrast changing the costs for staff and scanners had little impact upon the total costs of the arrays, in comparison with the array costs. For instance replacing a clinical scientist with a consultant grade had the impact of increasing the costs by only £34 per sample.

Table 8.4 Sensitivity analysis on array costs

Array Cost	Total Test Cost	Difference	% Difference
£100	£412	-£480	-53.8%
£200	£532	-£360	-40.4%
£300	£652	-£240	-26.9%
£400	£772	-£120	-13.5%
£500	£892	£0	0%
£600	£1,012	+£120	-13.5%
£700	£1,132	+£240	-26.9%

Karyotyping

Table 8.5 provides a breakdown of the staff resource use required for karyotyping. These figures are based on an average of the staff used in the Oxford Cytogenetics and the Birmingham Regional Genetics Laboratories. The table highlights that clinical scientist time accounts for the largest single component of staff costs. The average staff cost per sample is £85, with a range of £73 to £96.

Table 8.5 Karyotyping staff resource use

Staff Type	Cost Per Hour [a]	Hands-on time (minutes) [b]	Cost Per Sample [b]	Hands-on Time Range (minutes)	Cost Per Sample Range
Medical Technical Officer	£15.46 - £19.35	76.5	£22.41	40 – 113	£10.31 - £34.51
Clinical Scientist	£23.00 - £24.56	98.5	£40.32	5 - 192	£1.92 - £78.71
Consultant Grade	£43.78 - £44.40	27.5	£20.18	10 – 45	£7.05 - £33.30
Secretarial Staff	£12.98	7.5	£1.62	0 - 15	£0 - £3.24
Total		**210**	**£85**	**178 - 242**	**£73 - £96**

[a] Includes Superannuation and National Insurance; [b] Mid-points of range

Table 8.6 then provides information on the cost of each stage of the testing process from sample reception to any clinical liaison after report writing. The average costs for the two laboratories is £117 per sample (range £103 - £131). The table shows that the analysis and reporting results stages are the main cost drivers for karyotyping.

Table 8.6 Karyotyping cost breakdown

Stage	Cost	Range
Sample Reception and Initial Processing	£4.53	£4.48 - £4.58
Media Preparation / Setting Up Culture	£2.39	£1.62 - £3.16
Synchronisation / Harvesting Culture	£3.81	£2.93 - £4.69
Slide-making	£1.93	£1.05 - £2.81
Banding	£3.36	£2.28 - £4.45
Analysis (including checking)	£47.11	£39.15 - £55.08
Reporting Results and Authorisation	£29.39	£27.94 - £30.84
Clinical Liaison	£1.27	£0.37 - £2.18
Other Costs – general resources (e.g. PC)	£2.00	£2.00 - £2.00
Overheads	£21.29	£21.28 - £21.30
Total	**£117**	**£103 - £131**

With respect to sensitivity analysis for karyotyping, the area which is likely to have the greatest impact upon total costs is the amount of staff time devoted to the analysis stage. Using more automated analysis could save up to £27 for this stage of testing, bring the total cost below £100.

In Table 8.7 a comparison of the costs by resource category are provided for the arrays and karyotyping. This highlights a total cost difference of almost £800 per sample.

Table 8.7 Comparison of array CGH and Karyotyping Costs (1)

Cost category	Array CGH (cost per sample)	Karyotyping (cost per sample)	Cost Difference
Staff	£42.14	£84.53	-£42.39
Equipment	£15.25	£9.27	+£655.73
Consumables	£650		
Overheads	£148.44	£21.29	+£127.15
Other costs	£36.46 [a]	£2	+£34.46
Total	**£892**	**£117**	**£775**

[a] Includes other general resource costs, plus the cost of chromosomal extraction

8.4 Discussion

This Chapter has reported the methods employed and results obtained from a cost-analysis of array CGH compared with karyotyping for diagnosis of learning disability. A micro-costing approach was used to try and reflect the true underlying costs of the two technologies rather than simply using prices. The Oxford Genetics Knowledge Park was used as the basis for the study. However, in order to make the results more generalisable to the rest of the UK, other laboratories performing array CGH and karyotyping also provided cost data for the analysis, as well as the survey conducted as part of this working group report.

With respect to the array costs, the average total cost for a single sample (child) being tested is £892, based on the cost of the arrays themselves being £500. However, the array sensitivity analysis showed that the total cost could be as low as £412, if the arrays were priced at £100.

In terms of the follow on tests from the initial test, if a MLPA is performed, this, as well as both parents being tested could increase the total cost of testing to just under £3000. Although the frequency with which this would be required will vary across laboratories.

With respect to the karyotyping costs, the average cost per patient is £117. The major cost driver is the analysis and checking results stage at £47, which is the most labour intensive part of the process.

In comparing the costs for the two interventions, we can see that staff costs for karyotyping are twice as much as those for the arrays at £84 and £42 respectively. The largest difference between the two forms of testing however is the cost of consumables, with an average cost for a single array at £500, overshadowing all other costs. The relatively high cost of the arrays creates an average cost difference of £775 for the two technologies.

In terms of interpreting these results, it is important to note that in future it is anticipated that the array costs will be reduced by developments in array technology including increased probe density, multi-sample array formats and introduction of cheaper methods of array production (such as spotting of oligos as opposed to their *in situ* synthesis on the array). For instance, as this report is being finalised, the biotech company Agilent have announced that they are launching new CGH format arrays 4x44k (G4426A). These would be priced at under £500 per slide and each slide would hold data for 4 patients. This development would obviously make a large difference to the future costs of arrays. In addition, anticipated improvements in array software are likely to reduce the analysis time, reducing costs even further.

This study provides a detailed costing of arrays and karyotyping based on empirical data. However, in order to provide a more comprehensive picture of the potential for using array CGH in the NHS, a full cost-effectiveness analysis is required. The collection of effectiveness data is an ongoing piece of work in the Oxford Genetics Knowledge Park. The collection of information on the effects of the two technologies will hopefully be completed by Spring 2007 and combined with the costs to produce an estimate of cost-effectiveness for the technologies.

Compared with other health care interventions the effectiveness of array CGH versus karyotyping is actually quite complex. Common effectiveness measures in economic evaluation are life years saved or quality-adjusted life years (where a quality of life dimension is added to survival). The use of arrays is unlikely to have an impact on hard outcome measures such as survival, but may improve the chance of reaching a diagnosis, thereby reducing costs of clinical follow-up and additional investigations. As such, the Oxford research is gathering data on the range of clinical pathways which result from karyotyping, particularly in those cases where there is an obvious clinical phenotype but the karyotype is normal and exploring the impact of using arrays instead.

8.5 References

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Contributors

The cost-analysis reported in this chapter was undertaken by Dr Sarah Wordsworth and James Buchanan both funded by OGKP and based in the Health Economics Research Centre, University of Oxford. Costing was carried out with the assistance of Dr Regina Regan and Dr Sam Knight at the Wellcome Trust Centre for Human Genetics, University of Oxford, Dr Val Davison (Director) and Sara Dyer of the Birmingham Regional Genetics Laboratory, Dr Eddy Maher (Edinburgh) and Kim Smith (Director) and Carolyn Campbell, of the Oxford Regional Cytogenetics Laboratory and Dr Jenny Taylor, Director of the Oxford Genetics Knowledge Park.

Chapter 9 Discussion

Our discussion centres on three main areas:

1. A model for estimating the yield from cytogenetic diagnosis that utilise array CGH at different stages in the routine work-up and considerations of cost and cost effectiveness.
2. Main issues for laboratories and associated clinical services.
3. A consideration of the next steps that need to be undertaken to develop the technology for most effective routine use.

9.1 Diagnostic model

We have assessed the diagnostic yield from array CGH testing in the diagnosis of learning disability, when used in combination with other genetic testing methods. This chapter makes proposals for three different models and considers the availability of data for generating them, and their interpretation. Some estimates for the different diagnostic yield from the use of array CGH in these different models are made using evidence from the literature.

When samples from patients with learning disability plus or minus associated features are sent for cytogenetic analysis the usual routine involves karyotype for all patients followed by further investigation for those with negative results, (usually FISH or MLPA) on subgroups selected on clinical criteria similar to those described in Table 7.3 (page 53). In some services, such as those described in Chapter 6, array CGH can still be offered for those with negative results after FISH or MLPA, usually on a research basis. Clinical selection is not an exact science and involves the potential of losing positives in the group that is not selected. This is important because, although the diagnostic yield (proportion) in groups that do not fulfil clinical criteria is smaller, the groups are numerically larger and so can contribute a majority of the overall positive findings. Our proposed models include different proportions of cases clinically selected to proceed to further investigation following a negative karyotype.

As mentioned in Chapter 7, there are no reports in the literature from research groups that have followed through entire cohorts with the full range of tests. We used available literature and findings from work undertaken at the Regional Cytogenetics Laboratory in Cambridge to generate some estimates of the proportions of the cohort with clinical features for which further investigation might be undertaken and the diagnostic yield for the various components of the testing regime.

Background data

Proportions of the cohort with important clinical features for likely chromosomal abnormality and possible yield from these groups

Tables 9.1 and 9.2 below provide data from the Cambridge Regional Cytogenetics Laboratory SHIRE database (database of patient samples and records). This work included the analysis of referrals to cytogenetic testing for learning disability patients (excluding Down syndrome) between July 1995 and August 2004 (Joanne Staines personal communication). Clinical category of referral, and abnormality rates after routine laboratory testing (karyotype +/- subsequent investigations such as FISH). During this time a total of 1631 referrals were made.

Table 9.1 Analysis of cohort according to presence of absence of dysmorphic features and presence or absence of abnormalities after cytogenetic investigation

No. of cases	Abnormal	Variant –non causal	Normal	Total analysed	Yield (%)	Proportion of referrals
Dysmorphic+	13	1	151	165	7.9	10.2 %
Non-dysmorphic+	63	18	1361	1442	4.4	89.8 %
TOTAL	76	19	1512	1607	4.7	100 %
Proportion of abnormalities that are in the dysmorphic group = 17.1%						

Table 9.2 Analysis of cohort according to presence or absence of severe learning disability and presence or absence of abnormalities after cytogenetic investigation

No. of cases	Abnormal	Variant	Normal	Total Analysed	Yield	Proportion of referrals
Severe developmental delay+	9	1	154	164	5.5	10.2 %
Non-severe delay + (and other)	67	18	1358	1443	4.6	89.8 %
TOTAL	76	19	1512	1607	4.7	100 %
Proportion of abnormalities that are in the severe delay group 11.8%						

Thus, it can be seen that, although the yield is greater in the groups of patients who are dysmorphic than in those who are not dysmorphic (7.9% compared with 4.4%) and in the severe developmental delay compared with the mild or moderate delay (5.5% versus 4.6%), overall the majority of patients in whom abnormalities are found come from the non-dysmorphic and/or mild/moderate delay groups. This is because these groups are numerically larger.

Diagnostic yield of the component steps

Karyotyping: An estimate from the De Vries paper (Chapter 6 reference 10) from a cohort of patients with learning disability excluding Down syndrome is 4.8%

FISH/MLPA: The proportion of moderate to severe learning disability caused by subtelomeric abnormalities is estimated to be 3% in an editorial by Flint et al. (1) who also conducted one of the original studies in this area. When selection of patients is based on clinical criteria, the yield is 7.4%. van Karnebeek et al. in a systematic review of the yield of diagnostic studies in patients with mental retardation (2) estimated that the median yield of sub-telomeric studies was 4.4%, varying from 6.7% in moderate or profoundly learning disabled patients to 0.5% in mild learning disability. More recently work by Koolen et al. (3) on MLPA testing in patients with unexplained mental retardation showed that 'abnormalities with clinical relevance occurred in 6.3%, 5.1%, and 1.7% of severely, moderately, and mildly retarded patients, respectively'.

The finding for clinically selected patients is reinforced by Cambridge data. In a recent technical report from the Cambridge laboratory (Howard Martin, personal communication) of 150 consecutive cases referred for FISH with normal karyotypes 7 (4.7%) abnormalities were discovered by FISH and 9 (6%) by MLPA.

Array CGH: The meta-analysis that we conducted indicates that the mean diagnostic yield of array CGH test is 13%. However this relates to patients selected using a clinical scoring system.

It is assumed that array CGH can identify all the abnormalities that would have been detected by karyotype or FISH.

Clinical selection

An important step in estimating the diagnostic yield in the different models is to assess how this varies with the use of clinical criteria to select cases for further investigation following the initial finding of a negative karyotype. There is very little data in the literature to assist in making these estimates and none for array CGH. Table 9.3 shows the assumptions and figures that have been made for modelling. Steps 2 and 3 assume negative findings at previous steps.

Table 9.3 Yield estimates used in modelling

Test method	Overall 'unselected' cohort yield	Yield for cohort selected for further investigation on clinical criteria	Notes
Step 1			
Karyotyping	4.8%		Estimated from de Vries et al.
Step 2			
FISH	3%	7.4% (1)	Assumes the unselected group includes patients with mild and moderate learning disability and other problems such as autism
Step 3			
Array CGH	6.5%	13% (our meta-analysis)	We have estimated a 50% reduction in yield for unselected cohort. The actual yield might be less than this.
For FISH and Array CGH we assumed that the yield from the 'clinically selected' group would apply where 40% of cases were selected for further investigation following a negative karyotype and thereafter the yield was reduced in equal gradations to the level for the unselected initial cohort of referrals.			

Models for testing for patients with learning disability

Two models were compared with respect to the estimated overall yield and the number of abnormalities missed.

Model 1 (clinical selection)

Karyotyping ⇒ FISH/ MLPA (criterion based) ⇒ array CGH
Model assumptions:

a) All patients with learning disability referred for cytogenetic testing are given a karyotyping test. This is the recommended standard at present.

b) Clinical criteria are used on patients who test negative after karyotype to select a group who will be offered further investigation.

Model 2

Karyotyping ⇒ FISH/ MLPA ⇒ array CGH
Model assumptions:

a) All patients with learning disability referred for cytogenetic testing are given a karyotyping test. This is the recommended standard at present.

b) All patients testing negative at each step proceed to the next test in the sequence.

A *third potential model* exists where patients are investigated by array CGH alone.

Diagnostic yield

We undertook modelling of the overall diagnostic yield for sequential testing for an **initial cohort of 100 patients with learning disability with or without other clinical features who are referred for cytogenetic investigation**. **(This excluded patients with Down syndrome).** All patients have an initial karyotype and for those who are negative a proportion of cases is selected on the basis of clinical criteria for FISH or MLPA. For these 'selected' patients array CGH is undertaken on all negatives. The 'clinical selection' models have been based on 10-90% selection proportions. Model 2 corresponds to 100% selection, or all patients negative after karyotype proceeding to further investigation. For full table of modelling see Appendix 3.

Figure 9.1. shows the overall yields for an initial cohort of 100 patients in diagrammatic form for programmes with three scenarios for selection proportion: 10%, 50% and 100%. The number of diagnoses made varies from 7, where 10% of patients are selected for further analysis to 14 where all patients have further analysis, implying that in the first scenario seven diagnoses would be missed.

Figure 9.1 Diagram showing yield based on various models of clinical selection

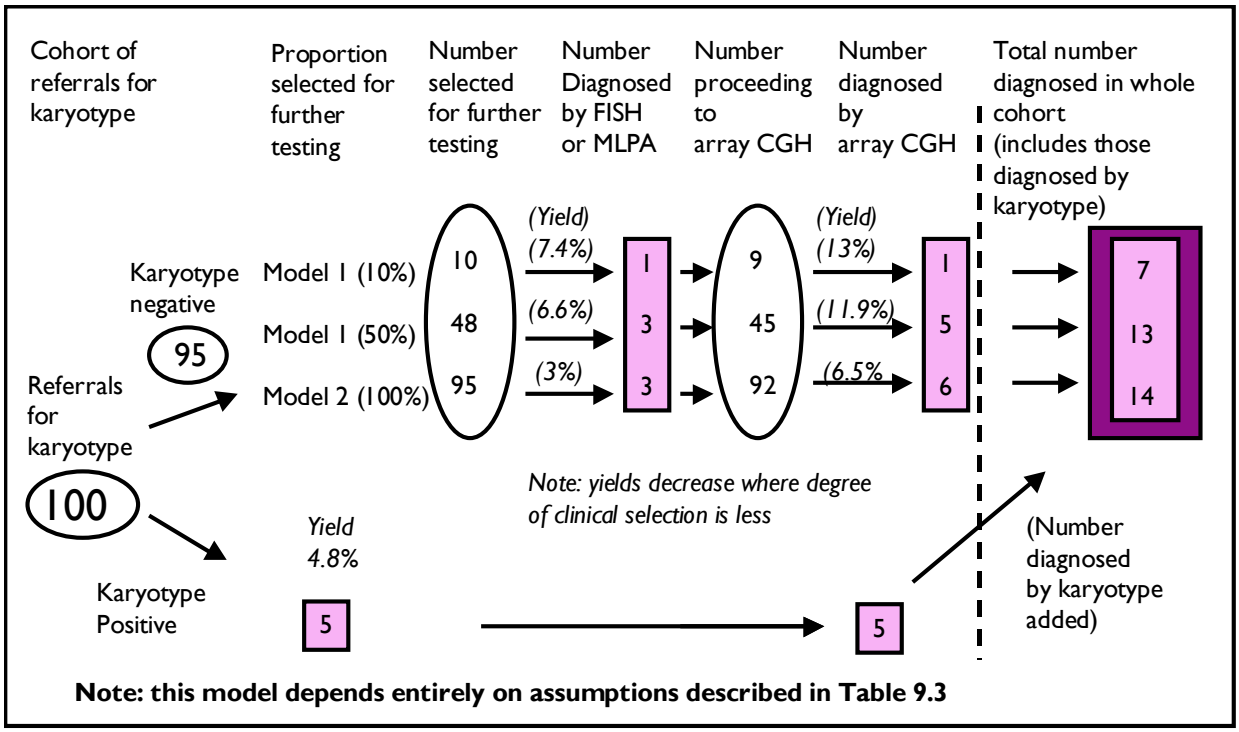

Table 9.4 (following page) shows the full table of modelling for programmes with selection proportions from 0 to 100% including numbers of missed cases.

Table 9.4 Modelling of numbers of diagnoses made and total number of tests undertaken. Numbers have not been rounded in order to maintain clarity of calculations. However, it is self-evident that numbers of diagnoses would need to be integers.

A	B	C	D	E	F	G	H	I	J	K
Initial cohort	**Number diagnosed by karyotype**	**Number available for FISH**	**Proportion proceeding to further analysis**	**Number proceeding to further analysis**	**Yield**	**Number diagnosed**	**Number proceeding to further analysis**	**Diagnostic yield**	**Number diagnosed**	**Total diagnosed**
Karyotype only										
100.00	4.80	95.20	0.00	0.00		0.00	0.00		0.00	4.80
Model 1										
100.00	4.80	95.20	0.10	9.52	0.074	0.70	8.82	0.130	1.15	6.65
100.00	4.80	95.20	0.20	19.04	0.074	1.41	17.63	0.130	2.29	8.50
100.00	4.80	95.20	0.30	28.56	0.074	2.11	26.45	0.130	3.44	10.35
100.00	4.80	95.20	0.40	38.08	0.074	2.82	35.26	0.130	4.58	12.20
100.00	4.80	95.20	0.50	47.60	0.074	3.17	44.43	0.130	5.29	13.27
100.00	4.80	95.20	0.60	57.12	0.067	3.39	53.73	0.119	5.82	14.01
100.00	4.80	95.20	0.70	66.64	0.059	3.47	63.17	0.108	6.16	14.42
100.00	4.80	95.20	0.80	76.16	0.052	3.40	72.76	0.098	6.31	14.51
100.00	4.80	95.20	0.90	85.68	0.045	3.20	82.48	0.087	6.25	14.25*
Model 2 (Equivalent to array CGH for all patients as first line)										
100.00	4.80	95.20	1.00	95.20	0.03	2.86	92.34		6.00	13.66*

* It should be noted that the number of diagnoses made levels out as higher proportions go for testing. Fluctuations are due to assumptions about the way in which diagnostic yield decreases with decreasing patient selection.

Array CGH as first line testing: a third model

A third model could be suggested in which all patients have an initial array CGH. Here it would be assumed that array CGH could pick up all the abnormalities identified by karyotype, FISH, and MLPA (i.e. the same as for Model 2 with 100% clinical selection). Our best estimates of the yield here on an unselected cohort would be 14%.

(For patients selected initially on strict clinical criteria allowing higher average yield levels to be applied, we estimate that the maximum yield applying clinically selected rates at all stages could be as high as 23%).

Economic considerations and cost effectiveness

The costs of karyotype (£117), FISH (£52) and array CGH (£892) obtained in the economic costing were then applied in each of the selection scenarios. Because karyotype is the cheapest investigation and itself has a yield of almost 5%, a programme which only uses this investigation is the most cost effective at about £2,500 for each diagnosis made. However, a number of diagnoses are missed with such a programme and it is generally deemed appropriate clinical practice for further investigations (FISH/MLPA +/- array CGH) to be undertaken on negative cases. The key questions are – what is affordable in terms of cost per patient and what is the most cost effective additional programme? Table 9.5 sets out the cost per patient for each scenario and the cost per diagnosis made. The full modelling table is given in Appendix 3.

Table 9.5 Estimates of patient costs and cost per diagnoses under different selection scenarios

Scenario	Proportion proceeding to further analysis	Patient cost for each scenario (£)	Cost per diagnosis made (£)
Karyotype only			
	0.00	117	2,438
Model 1			
	0.10	201	3,016
	0.20	284	3,343
	0.30	368	3,553
	0.40	451	3,699
	0.50	538	4,055
	0.60	626	4,468
	0.70	715	4,958
	0.80	806	5,553
	0.90	897	6,295
Model 2			
	1.00	990	7,250

The modelling was then extended to estimate the marginal cost of achieving an extra diagnosis as testing programmes include a higher proportion of cases with negative karyotype for further investigation. This is very dependent on the assumptions made about how the yield will decrease as the cohort receiving further investigation becomes less clinically selected. The

model below uses the assumptions about yield given in Table 9.3. Results are given in Table 9.6. It can be seen that, at the current cost of array CGH (£892), the marginal cost per extra diagnosis achieved of increasing the programme of further testing from 10 to 20% is £4,516 per diagnosis, whereas, an increase from 60 to 70% entails a much higher marginal cost (£21,500 per diagnosis). This results from the fact that the yield to be obtained by investigating patients who do not fulfil clinical criteria is smaller.

The lower section of the table includes the same modelling with reduced costs of array CGH.

Table 9.6 Estimates for marginal cost per case diagnosed with increasing 'further testing' proportions

Array cost	Increment in proportion tested Between	Marginal cost per case diagnosed
£892	10 and 20%	£4,516
	40 and 50%	£8,136
	60 and 70%	£21,500
Sensitivity of marginal cost for each new diagnosis with lower cost of array CGH		
£500	10 and 20%	£2,649
	40 and 50%	£4,767
	60 and 70%	£12,575
£300	10 and 20%	£1,697
	40 and 50%	£3.045
	60 and 70%	£8,023

Array CGH as a first line test

The cost of using array CGH as a first line test in all patients would be £892 per patient and £6,530 per diagnosis made- equivalent to the cost of a programme in which further investigation is done on nearly all (90%) of patients. This reflects the fact that array CGH is markedly more expensive than the other tests at present. However, if the cost of array CGH were to come down significantly, or, if it could be shown that utilising array CGH is cost effective in making an immediate diagnosis and saving time in further laboratory and clinical consideration and investigation, the possibility of using it as a first line test should be considered. Modelling using the same estimates shows that, at a cost of around £250 it may be as cost effective to do array CGH as the first step as to undertake the current stepwise programmes with up to 40% 'selection' for further analysis. However, this does not take into account any extra clinical and laboratory work-up arising from a possible increased number of false positives with array CGH.

9.2 Main issues for laboratories and associated clinical services

The use of array CGH in genetics service laboratories offers a wide range of benefits, both for the diagnosis of chromosomal causes of learning disability and also for wider applications, such as characterisation of cancers. In particular, the technique offers an unprecedented level of accuracy and resolution, and this is likely to continue to improve as technological innovation and development proceeds. Many UK laboratories are already using array CGH.

However, there is a range of issues associated with the potential introduction of array CGH on a national basis. Of note, the performance of array CGH is affected by multiple factors including

the choice of array platform and type currently available from several different suppliers. The optimal choice may depend not only on personal experience with a particular system or systems, but also the nature of abnormalities to be investigated (for example, large or small scale copy number changes), the nature of the patient specimens to be analysed, and the intended application of results. For example, the optimum resolution selected for research purposes may not fully satisfy criteria for clinical applications; quality control of platforms, equipment and procedures is an important factor for consideration.

Other issues of possible concern include:

Equitable access to superior technology – the introduction of technology to facilitate improved levels of genetic diagnosis requires that all patients should have access to equivalent services in this respect.

Systematic and coordinated implementation - different laboratories would need to work in a coordinated fashion to efficiently and appropriately evaluate the use of different platforms, scanners and methodologies. Laboratory quality control measures are likely to be adequate but would again need to be agreed across different laboratories.

Expense - initial set-up costs for laboratories to switch to array CGH diagnosis would be expensive; centres that already use the technology but have systems other than those agreed to be optimal might also have to reinvest in new equipment, although staff training costs would not be as high as for centres where the technique was wholly new. In the longer term, higher costs might not be a factor especially in comparison with labour intensive techniques such as FISH. Costs have already fallen substantially and are likely to continue to fall; however, there would be a need for continuing investment in equipment, which will rapidly become obsolete as new and improved systems emerge.

Resource allocation – although implementation of array CGH could significantly improve diagnostic capability for genetics centres, the technique could not wholly replace older techniques such as karyotyping and FISH. It would be important that these remained available for specific clinical situations in which the use of array CGH alone would be inappropriate or inadequate for diagnosis.

Movement towards centralised high-throughput systems to handle testing from various centres and for different purposes may be a realistic requirement to optimise the use of resources.

Wider range of applications - the expanding use of array CGH for other areas such as cancer diagnosis and the possible use of targeted arrays for prenatal diagnosis is highly probable, and application of new facilities to these different ends would help to offset the investment cost.

Potential increased levels of testing – as array CGH analysis could be applicable to a large proportion of overall referrals to a genetics centre, there would be a need to define selected patient groups for initial clinical introduction and full validation, with future extension to wider groups, ideally linking in with related EU initiatives.

False positives - polymorphisms - a high rate of false positive results arising from detection of polymorphisms (abnormalities identified by array CGH that are in fact associated with normal or nearly normal phenotypes) might be expected when using the technique to investigate possible causes of learning disability, and there would be potential dangers associated with producing incorrect genetic diagnoses for patients. This could be avoided provided that experts were honest about uncertain results, although such results are problematic for patients.

Linking clinical and cytogenetic information via a database – databases of linked clinical and cytogenetic information such as DECIPHER, for increasing knowledge of which chromosomal abnormalities are linked with what phenotypic traits (including those present in 'normal' populations), would be very important. Phased introduction of array CGH to clinical

practice (including robust quality control procedures) should have a concomitant commitment to information deposition in databases.

Confirmation of reproductive implications – the use of array CGH could significantly increase the number of families for whom an accurate estimation of the risk of recurrence of a chromosomal abnormality in future pregnancies could be made.

9.3 Next Steps

Although applying sequential tests to a high proportion of cases does increase the diagnostic yield, there is a diminishing return as the number of tests needed to achieve this increases. If, as is found, array CGH can pick up abnormalities with an almost 100% sensitivity then, when the cost of array CGH has reduced enough, it might be more effective and efficient to undertake this as the first line analysis with karyotype, FISH, MLPA or other tests reserved for those with specific indications (e.g. clinical features suggestive of a particular diagnosis). However, this requires further understanding of the possible disadvantages of this strategy, including, particularly, the numbers of false positives (identification of non-causal variants) and the amount and nature of further clinical and laboratory work needed to interpret these correctly. In general, this involves interrogation of databases, and genetic testing and clinical review of parents to determine whether the abnormality was inherited or arose *de novo*.

A full prospective assessment of the use of array CGH as a replacement for initial standard karyotype will be necessary and this should include not only the technical measures of the test but also the overall laboratory and clinical management of the referral and the wider context of clinical utility and overall economics.

9.4 References

1. Flint J, Knight SJL. Screening chromosome ends for learning disability. BMJ 2000; 321: 1240.

2. van Karnebeek CDM et al., Jansweijer MCE. Diagnostic investigations in individuals with mental retardation: a systematic literature review of their usefulness. European Journal of Human Genetics 2005; 13:6-25.

3. Koolen DA, Nillesen WM et al. Screening for subtelomeric rearrangements in 210 patients with unexplained mental retardation using multiplex ligation dependent probe amplification (MLPA). J Med Genet. 2004; 41(12):892-9.

Chapter 10 Conclusions and recommendations

Achieving a diagnosis in learning disability is greatly valued by parents and has clinical utility in aiding management decisions and genetic counselling. Our review shows that array CGH is an effective adjunct to the investigation of learning disability. At present it is used, almost exclusively, in cases where karyotype is normal and second stage testing by FISH or MLPA in clinically selected cases is also normal. Array CGH does not require prior knowledge of specific areas of chromosomes and so can be used for further investigation looking for unknown chromosomal rearrangements. Our systematic review and meta-analysis showed that, for unknown syndromes the overall diagnostic yield in patients in whom conventional cytogenetic analysis was negative and who fulfilled clinical criteria was about 13%. The use of clinical criteria to select cases for further testing after karyotype approximately doubles the diagnostic yield. However, these criteria are by no means 100% sensitive or specific and undoubtedly positive cases are lost in the group not further investigated. Additionally, the large size of the group who do not fulfil criteria (often mild to moderate learning disability without dysmorphism) may mean that, in absolute terms current laboratory regimes risk missing many cases.

The overall additional yield from array CGH depends on the proportion of cases selected for further investigation. In the one cohort study available (de Vries et al.) where 44% of cases were selected for MLPA the respective yield from the various stages (excluding Down syndrome) was: karyotype 4.8%, MLPA 2.0% and array CGH 1.8%, giving a total yield of 8.2%. We modelled the effect of using array CGH on all patients, making best estimates of the diagnostic yield in unselected cases, and estimate that the maximum yield that could be achieved using karyotype and a sequence of MLPA or FISH and then array CGH on all patients with negative results would be around 14% (excluding Down syndrome). However, this represents an inefficient use of resources as, under this model, at least 86% of patients would have karyotype, FISH or MLPA and array CGH.

Our cost analysis shows that array CGH is still moderately expensive with average costs being £892 per sample (£412 to £1,141) and the main cost driver being the array component. There is anecdotal evidence that the cost of arrays has already fallen since the cost analysis was undertaken and there would be further price reductions with large volume orders.

Array CGH is a powerful new technology with such potential that experts believe it will inevitably become a prime tool in pathological diagnosis across a range of clinical areas. Our review has shown that it is already being tested and used in this clinical area in 13 laboratories across the UK. However, services face a number of barriers before it can be used more widely. The high cost of arrays is the main limiting factor but of importance also are the complexity of development of arrays and software to optimize the technology, integration into the service, standardisation and quality assurance of the systems.

In the longer term, the advantages of array CGH in providing higher resolution and the ability to diagnose hitherto unrecognised syndromes may best be achieved by using the technique on all patients instead of the current regime of karyotype +/- FISH or MLPA +/- array CGH. Elimination of a large number of these current routine investigations will offset the high cost to some extent, whilst higher volume contracts should provide an incentive to price reduction. However, this requires further understanding of the possible disadvantages of this strategy, including, particularly, the numbers of false positives (identification of non-causal variants) and the amount and nature of further clinical and laboratory work needed to interpret these correctly. In general, this involves interrogation of databases, and genetic testing and clinical

review of parents to determine whether the abnormality was inherited or arose *de novo*. The transition of this technology from research to mainstream practice has thus not yet been achieved and we believe that these elements should be investigated further in a prospective study.

We make the following recommendations:

1) Array CGH should continue to be available as an adjunct to routine laboratory cytogenetic analysis for investigation of cases of children with learning disability. Cases will usually be referred for array CGH following assessment by a clinical geneticist and application of appropriate selection criteria (modified from Shaw-Smith C. *et al.*[i]). As the cost of array CGH decreases consideration should be given to the affordability of increasing the proportion of patients having further investigation beyond karyotype in order to minimise missed diagnosis.

2) Means should be explored that will allow the revenue costs of array CGH testing already being performed in genetics laboratories to be met.

3) Work should continue to optimise the technology to ensure maximum sensitivity for known syndromes and genome wide screening (in proportion to gene density) and to minimise the incidence of false positives.

4) A multi-centre prospective cohort trial of array CGH should be undertaken to compare a cohort of patients managed by the current cytogenetic routine analysis, with one in which all patients receive an initial array CGH investigation. The trial should cover investigation of different platforms, potential selection criteria, clinical and social impact, different centres, economic aspects, laboratory and clinical follow-up for positives and negatives, implications for education and training of laboratory staff, and implications for information and education for parents.

5) A quality control system for array CGH should be devised and incorporated into the NEQAS system.

6) Geneticists should record findings from array CGH (in terms of genotype and phenotype for novel abnormalities) in a suitable database such as DECIPHER, to facilitate information sharing.

7) Cost-effectiveness analysis to explore the real cost of current LD investigations (i.e. frequency with which karyotyping, multiple FISH, telomere and MLPA tests are performed and cost of these) should be undertaken.

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Appendix 3 Modelling of total cost per patient and per diagnosis made based on different array CGH cost

The table below shows details of the modelling to estimate the way in which total costs per patient and the cost per diagnosis (a measure of cost effectiveness), vary as the cost of array CGH reduces from its current level of £892 to £200. Figures in bold show 'break even' points at which it may be cost effective to undertake array CGH as a first level investigation.

NOTE: The figures are derived using estimates given in Table 9.3 (page 76) and should be regarded as giving 'order of magnitude' only

Proportion proceeding to further analysis	**Patient cost for each scenario (£)**	**Cost per diagnosis made (£)**	**Patient cost for each scenario (£)**	**Cost per diagnosis made (£)**	**Patient cost for each scenario (£)**	**Cost per diagnosis made (£)**	**Patient cost for each scenario (£)**	**Cost per diagnosis made (£)**	**Patient cost for each scenario (£)**	**Cost per diagnosis made (£)**
Array cost (£)	**892**		**500**		**400**		**300**		**200**	
Current routine analysis with various degrees of clinical selection										
0.00	117	2,438	117	2,438	117	2,438	117	2,438	117	2,438
0.10	201	3,016	166	2,496	157	2,364	148	2,231	140	2,099
0.20	284	3,343	215	2,530	197	2,322	180	2,115	162	1,908
0.30	368	3,553	264	2,551	238	2,296	211	2,040	185	1,785
0.40	451	3,699	313	2,566	278	2,277	243	1,988	**207**	**1,699**
0.50	538	4,055	364	2,743	319	2,408	275	2,073	231	1,738
0.60	626	4,468	415	2,965	362	2,581	**308**	**2,198**	254	1,814
0.70	715	4,958	468	3,241	**404**	**2,803**	341	2,365	278	1,927
0.80	806	5,553	**520**	**3,587**	448	3,086	375	2,584	302	2,083
0.90	**897**	**6,295**	574	4,027	491	3,448	409	2,869	327	2,291
1.00	990	7,250	628	4,600	536	3,923	444	3,247	351	2,571
Array CGH only										
	892	**6,531**	**500**	**3,661**	**400**	**2,929**	**300**	**2,196**	**200**	**1,464**