# Proposal form for the evaluation of a genetic test for NHS Service

**Gene Dossier**

## Test – Disease – Population Triad

<table>
<thead>
<tr>
<th>Disease – name</th>
<th>No specific disease name</th>
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<tr>
<td></td>
<td>Learning disability (mental retardation)</td>
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<td>Developmental delay</td>
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<td>Congenital anomalies</td>
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<tr>
<th>OMIM number for disease</th>
<th>No specific disease name or OMIM number</th>
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<tr>
<th>Disease – alternative names</th>
<th>please provide any alternative names you wish listed</th>
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<tr>
<td></td>
<td>• Learning disability (LD) or mental retardation is a significant impairment (IQ ≤ 70) of the cognitive and adaptive function with onset before the age of 18 years</td>
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<td>• Other common terms are learning difficulty, intellectual disability, developmental delay, impaired cognition and mental handicap.</td>
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<td>• LD is a common condition affecting 1-3% individuals worldwide</td>
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<td>• Genetic factors are estimated to be the main cause in around half of all cases with severe LD and around 15% of patients with mild LD.</td>
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<td>• Congenital anomalies generally refer to a structural variant that is present at birth. They include malformations and dysmorphic syndromes. <strong>Around 3% newborns have a major physical anomaly</strong></td>
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<td>• <strong>Around 12-25% congenital anomalies may be genetic.</strong></td>
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<td>• LD and congenital anomalies may occur together and specific combinations may be characteristic of recognised syndromes.</td>
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<th>Disease – please provide a brief description of the disease characteristics</th>
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<td><strong>The recurrence risk for developmental disabilities is often quoted as between 3 -10% for cases where the aetiology is unknown. Where genetic factors are implicated, the defect may be inherited or <em>de novo</em>. <strong>A more reliable evaluation of the mode of inheritance or recurrence risk is dependent on improved diagnosis using aCGH.</strong></strong></td>
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<td><strong>The predicted recurrence risk is dependent on the nature and origin of the imbalance.</strong> For example, the identification of an unbalanced cryptic translocation may uncover a balanced translocation in a carrier parent with a recurrence risk of ~50% for unbalanced gametes and variable rate of miscarriage dependent on the specific translocation.</td>
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<td>The recurrence risk given for a <em>de novo</em> copy number change detected by aCGH will depend on whether a parental balanced rearrangement has been excluded. If so, the risk is the germline mosaicism risk (&lt;5%); if not, then the recurrence risk may be as high as 50%.</td>
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<table>
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<tr>
<th>Disease - mode of inheritance</th>
<th>The proposed test involves a targeted screen of the whole genome for copy number variations.</th>
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<td><strong>To date, our analysis has detected imbalances that together include some 12,972 i.e. 39% known genes.</strong></td>
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### Approval Date: Sept 2010

### Submitting laboratory: Guy's & St Thomas' Cytogenetics

### Copyright UKGTN © 2010
<table>
<thead>
<tr>
<th>OMIM number for gene(s)</th>
<th>N/A</th>
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<tr>
<td>Gene – alternative names please provide any alternative names you wish listed</td>
<td>N/A</td>
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| Gene – description(s) (including number of amplicons) | • The aCGH test involves use of a custom oligonucleotide array (Agilent 44K platform) of probes designed by NGRL Wessex to target 135 established microdeletion and microduplication syndromes, with additional coverage of the whole genome, essential because of the heterogeneity of the imbalances giving rise to MR/DD + MCA and MCA (see e.g. Menten et al, J Med Genet 2006;43:625-633).  
  • The average interval across the genome between the 60mer oligos is 68kb and the maximum interval between adjacent oligos in the intergenic areas has been designed to be no greater than 250kb. |
| Mutational spectrum for which you test including details of known common mutations | • The test will detect imbalance (deletions, duplications and triplications) at a resolution that will depend upon the genomic region and the probe density therein.  
  • This approach does not detect balanced rearrangements, ploidy abnormalities such as XXX triploidy nor establish the phase of the copy number change. Neither will it detect imbalance between probes nor imbalance outside the regions analysed.  
  • Some rearrangements that appear balanced by conventional karyotype analysis have now been shown by aCGH analysis to be unbalanced. This reflects the ~100 fold higher resolution of aCGH.  
  • In addition, contrary to early concerns, aCGH can also detect mosaicism, in some circumstances at a lower level than standard cytogenetic analysis (Ballif et al 2006, Cheung et al 2007, in-house investigations, manuscript in preparation). |
| Technical Method(s) | Customised Agilent oligonucleotide array comparative genomic hybridisation (aCGH) for detection of copy number change in patients with MR/DD/MCA (NGRL Wessex constitutional array CGH V1 design # 015543 as described by Barber et al, Eur J Hum Genet 2008;16:18-27).  
  The average interval, between the 60mer oligos is 68kb and the maximum interval between adjacent oligos in the intergenic areas has been designed to be no greater than 250kb.  
  For further details, please see [http://www.ngrl.org.uk/wessex/arraycgh.htm](http://www.ngrl.org.uk/wessex/arraycgh.htm).  
  • To reduce costs we are using a 4x44K format and hybridising patients against other patients rather than a reference normal control. The patient selection is non-random to ensure that patients with a suspected similar genotype e.g. 22q11.2 duplication are not co-hybridised. We have calculated the likelihood of two random patients having exactly the same imbalance as <1/25,000 (without phenotype mismatching). |
- We have recently published the full details and validation of our method in a peer reviewed paper. **Validation and implementation of array comparative genomic hybridisation as a first line test in place of postnatal karyotyping for genome imbalance.** Ahn J, Mann K, Walsh S, Shehab M, Hoang S, Docherty Z, Mohammed S, Mackie Ogilvie C. Mol Cytogenet. 2010 Apr 15;3(1):9.

The technique of hybridising phenotypically mismatched patients to improve cost effectiveness has been widely presented at scientific and professional meetings including those held and attended by members of the Association of Clinical Cytogeneticists (ACC). The Laboratory has been successfully re-inspected by CPA(UK), the official accrediting body for NHS Pathology Laboratories since introducing the test. As indicated in the above publication, the observed detection rate as compared with other protocols using oligoarrays show that the patient/patient hybridisation strategy did not compromise the diagnostic yield. We have estimated that there is a less than 1 in 25,000 chance of failing to detect 16p11.2 deletion syndrome (the most common finding, present in ~0.62% patients) even **without** phenotype mismatching.

- All abnormal results have a repeat analysis undertaken using a second aliquot of the extracted DNA. This confirmation is by MLPA, second array, or FISH test depending on the specific anomaly. Once this is confirmed, the result is reported and parental samples are requested for inheritance studies. Parental samples may be tested using MLPA, array CGH or FISH again depending on the position and number of imbalances found in the proband. Phenotypic information is also required on the parents.

- Data is analysed using the Agilent Analytics Software that provides an independent measure of hybridisation quality (called the DLRS score) which we require to be less than 0.3. We require an abnormal ratio with at least 3 adjacent probes in order to call a copy number variant (CNV).

- Interpretation is made with reference to the available databases of genomic variants in healthy individuals and individuals with a described phenotype (see below). We have developed a custom database (MOKA) to efficiently link our aCGH analytical data to online resources and to our own patient and sample databases to facilitate the interpretation of findings.

**Distinguishing between benign and pathogenic CNVs**

Reference is made to:
1. Whether the change is **de novo** or inherited by analysis of parental samples
2. Available databases of genomic variants identified as benign and unlikely to be pathogenic and to those associated with overlapping phenotypic and genotypic features including
   a) Toronto DGV ([http://projects.tcag.ca/variation/project.html](http://projects.tcag.ca/variation/project.html)),
**Validation Process**

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<td>3. Segregation with phenotype within family</td>
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<td>4. Genetic content and size of the CNV</td>
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**Self-self hybridizations – no imbalances**

**389 cases:**
14 known abnormal karyotypes tested during validation – all concordant on arrays.
Known trisomy samples tested with no regions “called” as normal.
104 new abnormals confirmed by either FISH, MLPA or inheritance studies.

**Are you providing this test already? If yes, how many reports have you produced?**

Please give the number of mutation positive/negative samples you have reported.

The working group queried the value of 22% and Mark Kroese will discuss further.

As indicated in our publication (Ahn J. et al. 2010), following introduction into routine service for patients with suspected genomic imbalance, we have as of December 2009, undertaken **1169 first-line tests** and **1245 follow-on tests** for patients with normal karyotypes. The latter representing the cohort (back-log) of patients that need re-testing on further clinical review. Thus overall in total we have undertaken 2414 index tests. The number of actual tests is higher than this figure as this above excludes the inheritance studies.

- The respective detection of imbalance was **22% in the first-line tests** and 26% in the follow-on tests. This excludes the clinically well documented common, benign variants described on the established reference Toronto Database of Genomic Variants (DGV).
- At least 89% of the abnormalities detected by first-line testing would not have been detected by standard karyotype analysis.
- The imbalances comprised 374 deletions (range 2kb to 19.215Mb), 300 duplications (range 12kb to 34.086Mb) and 27 triplications (range 26kb to 1.786Mb).
- Parental samples were requested in all cases with imbalance not represented in the DGV but during our study period were received only for 40% cases overall. These inheritance studies showed that de novo imbalances accounted for 13% of findings from first-line tests and 35% of findings after a normal karyotype.

**For how long have you been providing this service?**

A diagnostic service has been delivered since May 2008. From May 2009, aCGH has been provided as a first line test to replace conventional postnatal karyotype analysis for referrals with learning disability, developmental delay and/or congenital anomalies (MR/DD/MCA).
### Is there specialised local clinical/research expertise for this disease?

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<th>Yes</th>
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<td>Yes</td>
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<td>The Clinical Genetics Service at Guy's has over 30 years experience of the diagnosis and management of individuals with chromosomal syndromes. Dr Joo Wook Ahn, Research Cytogeneticist at Guy’s, Cytogenetics Laboratory has an extensive research background in microarrays. The Laboratory now has some two years of research experience comparing and validating different array platforms, one year of offering array CGH as a clinical service, and 12 months experience in delivering the service as a first-line postnatal test.</td>
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### Are you testing for other genes/diseases closely allied to this one? Please give details

- Conventional karyotype analysis is still available and used for detection of structural rearrangements.
- MLPA, FISH and QfPCR are alternative techniques that may be used in association with aCGH testing or karyotyping for detection of genetic changes.

### Your Activity

#### If applicable - How many tests do you currently provide annually in your laboratory?

- **First-line index testing:**
  - ~1754 per annum (predicted on 8 months data May'09-Dec'09)
- **Inheritance studies on first-line index cases:**
  - ~22% of index cases had an imbalance for which parental samples were requested. *To date* parental samples have been received for 83 of these cases (32%) that represents 249 samples pa. Most inheritance studies are now being undertaken by MLPA analysis.
- **Follow-on test after normal karyotype test (re-test/backlog):**
  - ~1868 per annum (predicted on 8 months data May'09-Dec'09). ~24% of these cases with a previous normal karyotype had an imbalance for which parental samples were requested. *To date* these have been received in 149 cases (46%) that represents 447 samples pa.

  The total number of first-line and follow-on test index cases is thus predicted from 8 months data as **3622 pa** and the total number of inheritance tests *is predicted likewise as at least* **696 pa**. We expect the number of follow-on test cases may decrease long term as the first-line test becomes established but that number of inheritance tests will increase given the above estimate is based on a relatively short response time frame. An 80% response rate for both parental samples would yield **936 samples pa for inheritance studies**

### Your Activity

#### How many tests will you be able to provide annually in your laboratory if this gene dossier is approved and recommended for NHS funding?

- **Index cases**
  - We are currently introducing robotic processing and once complete, we would be capable of processing around 140 samples per week.
  - We are able to offer array CGH testing to other centres, either as a first test, post-normal karyotype test, or to characterise imbalances detected by other methods.
### Based on experience how many tests will be required nationally (UK wide)?

**Index cases**
If other laboratories introduce this as a first line test, the demand will equal the referrals for karyotyping for chromosome imbalance at those centres.

**Inheritance tests**
This depends on the referral & analytical criteria but is estimated at around 24% index cases based on our data. The actual number of samples received should reflect the fact that a sample will be requested from each parent but should allow for the fact one or both parental samples may not be available for all cases. The response rate in our study over an 8 month time period was 40%.

### National Activity (England, Scotland, Wales & Northern Ireland)

If your laboratory is unable to provide the full national need please could you provide information on how the national requirement may be met. For example, are you aware of any other labs (UKGTN members or otherwise) offering this test to NHS patients on a local area basis only? This question has been included in order to gauge if there could be any issues in equity of access for NHS patients. It is appreciated that some laboratories may not be able to answer this question. If this is the case please write "unknown".

It is anticipated that aCGH for this cohort of patients will be available through all regional genetic centres. The analysis may be delivered either in-house or as part of consortium arrangement with other providers.

The ACC audit reported that 23 laboratories offer an array service, 19 in-house and 3 out-source.
## Epidemiology

| **Estimated prevalence of disease in the general UK population** | Learning difficulties (LD) are estimated to affect 3 per 100 of the population, severe LD to affect 3.8 per 1,000 and mild LD a minimum of 25 per 1,000. Thus mild and severe LD affect 28.8 per 1,000.  
Data from Burton *et al.* 2006: Report of a UK GTN Working Party on array CGH.  
2-3% newborns have ≥1 major abnormality at birth. Minor abnormalities are found in 10-15%. |
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| **Estimated gene frequency**  
(Carrier frequency or allele frequency) | This can be estimated in a number of ways.  
(0.5 x 3.8/1000) + (0.15 x 25/1000) = ~6/1000  
2. As chromosome abnormalities are present in 16.1% of individuals with LD, the minimum frequency of patients with imbalances detected with the more sensitive array CGH technique can be estimated at 5 per 10,000.  
Data from Burton *et al.*, 2006, *op cit.* |
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| **Estimated penetrance**  
Please identify the information on which this is based | • The penetrance of euchromatic imbalance is close to 100% even if the degree of phenotypic expression varies greatly. This contention is supported by the rarity with which classical unbalanced chromosome abnormalities have no apparent phenotypic consequences; in a review of the literature, only 53 examples could be identified from the world literature over a period of more than 20 years (Barber, J Med Genet 2005;42:609-629).  
• Penetrance is also close to 100% in patients with the established microdeletion and microduplication syndromes identified using FISH or MLPA. In the case of e.g. the 22q11.2 deletion syndrome, as many as 10% of cases are transmitted from a parent who is often more mildly affected but rarely unaffected (see e.g. McDonald-McGinn Genet Med 2001;3:23-9; Leana-Cox J *et al.*, Am J Med Genet 1996;65:309-16).  
• The penetrance of *de novo* imbalances identified using array CGH is also likely to be close to 100%. |

| **Target Population**  
Description of the population to which this test will apply (i.e. description of the population as defined by the minimum criteria listed in the testing criteria) | Patients with learning disability (mental retardation) or developmental delay or those with (multiple) congenital abnormalities alone.  
**Indicative referral criteria include:**  
• Family history of learning difficulties (including discordant phenotypes)  
• Prenatal onset growth retardation  
• Postnatal growth abnormalities (microcephaly short stature, macrocephaly, tall stature) |
| --- | --- |
- 2 or more facial dysmorphic features notably hypertelorism, nasal anomalies, ear anomalies
- Non-facial dysmorphism and congenital abnormalities notably hand anomaly, heart anomaly, hypospadias ± undescended testes
- Significant speech and learning difficulties ± autism

**Exclusive referral criteria**
- Prenatal samples
- Recurrent miscarriages or fertility problems
- Expectation of balanced rearrangements

### Estimated prevalence of disease in the target population
- Our data to date of 1169 first-line tests supports a detection rate for imbalance of 22%. Of these cases of imbalance, 13% (11/83) were confirmed to be *de novo* that indicates an overall detection rate for clinically significant imbalance of 3% (30/1169).

- The corresponding detection rate using array CGH on referrals that are normal by conventional cytogenetics is ~26%. Of these 35% (52/149) were confirmed to be *de novo* that indicates an overall detection rate for clinically significant imbalance of 8% (101/1245).

The lower detection rate for first-line testing is likely to be due to the more stringent selection by expert dysmorphologists and clinical geneticists for post-karyotype array CGH.

- A systematic review and meta analysis of 19 studies and around 14000 subjects with learning disability and congenital anomalies in whom conventional cytogenetic analysis had proven negative, showed an overall diagnostic yield of causal abnormalities of 10% (95% confidence interval 8-12%). The overall false-positive yield of non-causal abnormalities was 7% (95% CI 5-10%). The main variables to detection are the volume of samples analysed and resolution of the arrays used.

Intended Use (Please use the questions in Annex A to inform your answers)

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<tr>
<th>Please tick the relevant clinical purpose of testing</th>
<th>YES</th>
<th>NO</th>
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<tbody>
<tr>
<td>Diagnosis</td>
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<tr>
<td>Treatment</td>
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<td>Prognosis &amp; Management</td>
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<td>Presymptomatic testing</td>
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<td>Risk Assessment for family members</td>
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<td>Risk Assessment – prenatal testing</td>
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### Test Characteristics

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<th>Analytical sensitivity and specificity</th>
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<tr>
<td>This should be based on your own laboratory data for the specific test being applied for or the analytical sensitivity and specificity of the method/technique to be used in the case of a test yet to be set up.</td>
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If more than one gene will be tested, please include your testing strategy and data on the expected proportions of positive results for each part of the process. Please illustrate this with a flow diagram.

| 2414 patients | have been tested using the Agilent 44K array CGH V1 design # 015543 that is designed to detect 135 established micro deletion and micro duplication syndromes with an average interval between probes of 68 kb and a maximum interval between adjacent probes in the intergenic areas of no greater than 250 kb. The estimated sensitivity is ~100 greater than for conventional karyotype analysis. As described, we use a threshold hybridisation score of 0.3 and require 3 adjacent abnormal ratio shifts to score a CNV. |

#### Sensitivity

- All known abnormal cases tested during validation were confirmed; all new abnormalities were followed-up and confirmed.

#### Detection rate in First-line Tests

We have a 22% detection rate for imbalance excluding well-documented, clinically benign variants.

This included a total of 311 imbalances (some patients had more than one region of imbalance) of which 151 were deletions, 145 duplications, 9 triplications, 1 marker and 5 whole chromosome (incl. 4 mosaic)

Of the 1169 total patients, 260 (22%) had a significant imbalance. Of these, 72 were inherited, 11 were de novo and 177 were of unknown inheritance. Please see flow charts below indicating overall detection rates for clinically significant imbalance in first-line, follow-on and the combined first-line and follow-on data as 3%, 8% and 5% respectively.

#### Testing Strategy (see flow chart below)

1. Select patients for array CGH according to test criteria
2. Test using array CGH.
3. If no significant copy number changes are found, report as normal (78%).
4. If copy number change is detected (22%), repeat analysis on a further aliquot of DNA extract by second array, MLPA or FISH
5. Issue preliminary report requesting parents.
6. Carry out inheritance studies as appropriate
   - If a de novo copy number change is found, report as abnormal with relevant published or DECIPHER phenotypic information. Offer genetic counselling.
   - If copy number change is found in a phenotypically normal parent, report as likely to be a benign CNV, but any unusual features shared by proband and carrier parent may be associated with the imbalance. Offer genetic counselling.
   - If 1 one or more of the parents are not available, a combination of approaches is used (see under technical details) to predict whether the CNV likely to be causal or not.
Clinical sensitivity and specificity of test in target population

The **clinical sensitivity** of a test is the probability of a positive test result when disease is known to be present; the **clinical specificity** is the probability of a negative test result when disease is known to be absent. The denominator in this case is the number with the disease (for sensitivity) or the number without disease (for specificity).

The customised 4x44K format is a pragmatic design to maximize the number of clinically significant imbalances and minimise the number of non-pathogenic copy number variations that need to be followed up.

**Clinical sensitivity for first-line testing:**
22% of individuals tested have a copy number variation and 78% are therefore normal.

A growing body of literature is available to help with the interpretation of array CGH results as are a number of databases including:
- DatabasE of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources (DECIPHER) ([http://www.sanger.ac.uk/PostGenomics/decipher/](http://www.sanger.ac.uk/PostGenomics/decipher/));
- the Database of Genomic Variants ([http://projects.tcag.ca/variation/](http://projects.tcag.ca/variation/));
- European Cytogenetic Association Register of Unbalanced Chromosome Abnormalities ([www.ecaruca.net](http://www.ecaruca.net));
- The Transmitted Sub-Telomeric Imbalance Collection ([http://www.ngrli.org.uk/Wessex/subtel_collection.htm](http://www.ngrli.org.uk/Wessex/subtel_collection.htm)).

**Clinical specificity**
CNV have been described in phenotypically normal individuals and in some cases occur at a high frequency in the general population. They range in size from 1 kb to several Mb, show variable copy number when compared to a reference genome and include both deletions and duplications of genomic loci. These structural variants are usually inherited and have been reported to encompass as much as 12% of the genome. Detection rate depends on the resolution of array but it has been estimated that there are thousands of them in the genome, some of which are very common, including deletion polymorphisms in specific genes involved in sex steroid and drug metabolism and olfaction as well as duplication and others may be rare or ethnic specific. It is not yet clear how CNVs contribute to genetic variability or their associations with disease susceptibility (Edelman and Hirschhorn 2009. Ann NY Acad Sci 1151:157-166).

Note that some patients have imbalances in genes not known to be associated with their symptoms and that may be associated with other symptoms (e.g. cancer genes). The frequency of this has yet to be established.

**Clinical validity (positive and negative predictive value in the target population)**

The **clinical validity** of a genetic test is a measure of how well the test predicts.

Please see flow charts illustrating the detected number of de novo and inherited imbalances.

In our published study, to date inheritance was only ascertained in 32% cases with a first-line imbalance. We expect this rate to increase given some requests for parental samples are...
the presence or absence of the phenotype, clinical disease or predisposition. It is measured by its positive predictive value (the probability of getting the disease given a positive test) and negative predictive value (the probability of not getting the disease given a negative test).

Clinical validity for array CGH can be broken down into three distinct classes:

1. **de novo copy number change**:
   - **Positive predictive value** is likely to be high (~99%).
   - Notes:
     - This is for *de novo* copy number changes after polymorphic variation has been excluded.
     - There are very few reported examples of *de novo* copy number changes with no apparent adverse phenotype.
   - **Negative predictive value is likely to be high (~98%).**
   - Note: this is estimated on prevalence of cases without mild and severe LD less those that have a significant copy number change.

2. **co-segregating transmitted copy number changes**:
   - **Positive predictive value** ~99%.
   - Note: this is for transmitted copy number changes co-segregating with disease after transmitted imbalances from phenotypically normal parents have been excluded.
   - **Negative predictive value ~1%.**

3. **transmitted copy number changes**:
   - **Positive predictive value** is likely to be low.
   - Note: Information is still merging as to the clinical significance of imbalance for many genomic regions in different backgrounds.
   - **Negative predictive value** is likely to be low (~2%).
   - Note: this is based on the prevalence of LD in the normal population less those with a significant array CGH result.

| Clinical utility of test in target population | The Clinical Care Pathway being followed for array CGH is the same established pathway as was used for routine karyotyping. Major referring specialties include Clinical Genetics, Paediatrics, Cardiology, Neurology and Ophthalmology. |
| (Please refer to Appendix A) | The following samples would be excluded from testing |
| Please provide a description of the clinical care pathway. | • Prenatal samples |
| | • Recurrent miscarriages or fertility problems |
| | • Expectation of balanced rearrangements |

How will the test add to the management of the patient or alter clinical outcome?

1. **Diagnosis**: arrays provide a unique opportunity of increasing the proportion of clinically significant positive test results by around 10% to 15% (Knight et al. 2006 Cytogenet Genome Res. 2006;115(3-4):215-24). No other technology since FISH has provided such a step change. This increased sensitivity has cost savings in terms of multiple test requests and avoidance of often invasive investigations in children to establish a diagnosis( Wordsworth et al. 2007. |
2. **Treatment**: there are few specific treatments for "chromosomal syndromes" but e.g. melatonin has been found to help restore normal sleep patterns in children with Smith-Magenis Syndrome and growth hormone treatment has unexpectedly been found to improve cognitive function in patients with 18q deletions.

3. **Prognosis and Management**: a chromosomal diagnosis can transform the chance of getting children statemented, of obtaining additional help in mainstream schools, of gaining entry to SEN schools or receiving respite care in the home. The growing knowledge of the phenotypes associated with array CGH imbalances also provides prognostic information for parents and social care teams. At least 89% of the abnormalities detected in our cohort would not have been detected by standard karyotyping analysis (Ahn et al. 2010).

4. **Prevention**: It has been estimated that around 1,200 diagnoses in learning disabilities per year would be missed in the UK as a result of not implementing array CGH testing (UKGTN Commissioning workshop 2009).

5. **Genetic Risk assessment**:  
   - Firstly, many of the simple deletions and duplications detected are compatible with fertility and have 50% recurrence risks in the same way as dominant conditions.
   - Secondly, we estimate that 5% of the imbalances detected using array CGH will be derived from balanced parental rearrangements. In both cases, **prenatal diagnosis can be offered** to prevent a recurrence.
   - **A pathogenic de novo abnormality** would have a low risk of recurrence and may **obviate the need for an invasive prenatal test in a subsequent pregnancy**

What impact will this test have on the NHS i.e. by removing the need for alternative management and/or investigations for this clinical population?

- Testing for imbalance using aCGH is likely to be cost effective because long term savings can be made regardless of a positive (diagnosis) or negative result. Earlier diagnoses save costs of additional diagnostic tests. Negative results are cost effective in minimising follow up test choice.
- More reliable recurrence risk offers family more informed choice for future intervention

Please provide example from own service (see new form example)

**Array CGH Case Studies**

**Patient 1:**

- A 12 year old boy with multiple congenital abnormalities, severe developmental delay and a complex clinical picture after extensive investigations was found to have an array abnormality which finally provided clear answers for the family.
- He was first seen in the neonatal period due to his small size,
distinctive facial features, severe congenital heart disease, small genitalia, inverted nipples and unusual fat distribution. He failed to thrive and had global developmental delay with bilateral sensorineural hearing loss. Over time he developed seizures and a severe scoliosis.

Facially he was always felt to have a "chromosomal gestalt" and accordingly a karyotype was repeated several times and he underwent a detailed subtelomeric analysis (standard and deletion/duplication telomeres) with normal results. Wider discussion at national genetics meetings garnered the same suggestion. The collective eye of the genetics community agreed that an underlying chromosomal aetiology was most likely. As comprehensive analysis was normal, further relevant investigations including brain MRI under general anaesthetic, an EMG, muscle biopsy, lumbar puncture and skin biopsy for detailed metabolic causes were undertaken. All yielded a normal result.

The parents were given an empirical risk of recurrence as there was no all embracing syndromic diagnosis. Array CGH after 12 years of searching for a cause finally showed him to have a de novo 19q abnormality.

The parents have expressed their enormous relief at finally have a clear answer for their son's profound difficulties. The results have enabled more accurate genetic advice to be given for the extended family.

The total cost of extensive and invasive investigations in the patient 1 is estimated at £10,800 in contrast to the cost of arrays at £250. Undoubtedly, whilst the cost savings would involve specialities other than genetics, the distress and co-morbidity of repeated invasive investigations in a small child is not insignificant. In a child presenting with a similar features now, the advent of array CGH which could provide an early answer means that alternative investigations could be obviated or minimised greatly.

Patient 2:

A 5 year old boy was referred for developmental delay, short stature and non specific dysmorphic features. There was a history of alcohol/substance misuse and the possibility of fetal alcohol syndrome was mooted. On examination however he did not have features consistent with this diagnosis and was noted to have one cafe au lait patch. Standard chromosome analysis was normal. Subsequently subtelomeric analysis showed a possible chromosome abnormality on chromosome 7p and chromosome 7q but full family studies were not feasible at the time. Four years later he returned for review as the number of cafe au lait patches had increased but were still insufficient to fulfil the clinical criteria of a diagnosis of Neurofibromatosis (NF1) and he also had inexplicable anaemia.

Array CGH showed him to have a complex rearrangement
with three regions of imbalance: a duplication of chromosome 2 and 12 (maternally inherited which was interpreted to be a CNV). Significantly he had a 1.3 Mb deletion of 17q (encompassing the NF1 locus) and mosaic loss of the entire length of chromosome 7. The NF1 deletion suggested that he was at risk of developing neurofibromatosis and is now being monitored for this.

The mosaic monosomy of chromosome 7 is associated with a significant risk of developing myelodysplastic syndrome and he is under regular review with a haematologist.

1995
NO ABNORMALITY DETECTED
46,XY
Male chromosome complement observed

2009 Array CGH

<table>
<thead>
<tr>
<th>CHROMOSOME IMBALANCE DETECTED</th>
</tr>
</thead>
<tbody>
<tr>
<td>arr 2q36.3(228,127,461-229,250,891)x3 mat,</td>
</tr>
<tr>
<td>7p22.3p11.1 (149,268-57,498,383,498,383) x1~2,</td>
</tr>
<tr>
<td>7q11.21q36.3 (62,153,588-158,602,499) x1~2,</td>
</tr>
<tr>
<td>12q24.31 (121,936,584-122,408,947)x3 mat,</td>
</tr>
<tr>
<td>17q11.2(26,085,852-27,391,327)x1.mlpa (NF1)x1</td>
</tr>
</tbody>
</table>

In patient 2 the detection of a complex alteration by array CGH means that appropriate screening and intervention for associated complications can be instituted at an early stage.

Costs
As detailed in our recent publication, we have minimised the consumable and labour costs associated with aCGH by;

- Patient /patient hybridisation
- Use of robotics
- Customised in-house application for data processing, interpretation and reporting. Note there are no software license fees.
- Delivering an average reporting time of 25 days to attract external referrals.
- Processing of appropriate volume of activity (current capacity 96 cases per week) to negotiate favourable consumable costs

Because of our strategy, we are able to offer aCGH testing at the same overall cost to that of G-banded chromosome analysis and within the same overall service budget. In terms of the total service costs, including benefits achieved through the substantially higher diagnostic yield, array CGH is considerably cheaper than karyotype analysis.

Our NHS-commissioned aCGH costs (processing, analysis and interpretation) have been negotiated and presented as a block cost for array analysis inclusive of any inheritance studies.
We do not have separate charges for positive and normal results. The DNA extraction is itemised separately as historically many index samples have already had prior screening for Fragile X syndrome and therefore an additional DNA extraction was not necessary.

The separation of the extraction activity from the analytical work is in-line with the agreed approach being adopted by UKGTN/CMGS/ACC for the common units/currency for genetic tests.

Our cost for aCGH including any necessary inheritance studies is £250.

The cost of DNA extraction, if required, is an additional £38 per sample.

Under the present practice for Fragile X testing, most index cases for LDD also have a fragile X molecular test and thus one extract can usually be used for both tests. We have reviewed our data for the time period used in our publication study and found that ~17% of index cases required a DNA extract for the aCGH test. Clearly a position needs to be taken as to how to allocate this cost component to avoid any double counting but also to ensure that the full cost is appreciated. We have taken the pragmatic approach in the financial analysis below to assign the observed 17% index cases requiring a DNA extract to the aCGH costs. However we note that should the way in which fragile X testing is currently being provided be changed, then this 17% is likely to change. The total costs allowing for all samples to have a DNA extraction are therefore also presented for completeness.

Karyotype analysis is also currently commissioned through a block contract arrangement to which marginal rates have been applied over many years. In evaluating the comparison between karyotyping and aCGH under current funding mechanisms we have assessed the impact on the overall service budget and found this to be essentially cost neutral at this time. With future developments as highlighted in the recent UKGTN array CGH workshop report, we anticipate there may be further efficiencies and savings.

Please provide consequences for not testing – as per example on new gene dossier form

It is estimated that around 1,200 diagnoses in learning disabilities per year would be missed in the UK as a result of not implementing arrayCGH testing (UKGTN workshop report). This has consequences for:

- Potential litigation for mis- or non-diagnosis
- Increased burden on families
- Missed opportunity for intervention
- Increased number of often invasive investigations in children at a higher overall cost to the health service. e.g. muscle biopsy, EMG, brain MRI necessitating a general anaesthetic
- Ethical and social consequences of discrimination
Please provide utility of testing in a couple of sentences – please see examples on new gene dossier form

The use of aCGH for LDD/MCA yields a significant (~17%) increase in diagnostic yield over karyotyping for a vulnerable, sizeable and frequently overlooked section of the population. This cannot be overlooked where it can be achieved at similar overall service cost to karyotyping.

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Is there an alternative means of diagnosis or prediction that does not involve molecular diagnosis? If so (and in particular if there is a biochemical test) please state the added advantage of the molecular test</td>
<td>No</td>
</tr>
<tr>
<td>Please describe any specific ethical, legal or social issues with this particular test?</td>
<td>None that do not already apply to conventional cytogenetic analysis.</td>
</tr>
</tbody>
</table>

Please complete the testing criteria form.
UKGTN Testing criteria

**Name of Disease(s):** Learning disability, developmental delay, congenital anomalies (first line)

**Name of gene(s):**

<table>
<thead>
<tr>
<th>Patient name:</th>
<th>Date of birth:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient postcode:</td>
<td>NHS number:</td>
</tr>
</tbody>
</table>

**Name of referrer:**

**Title/Position:**

**Lab ID:**

Referrals will only be accepted from one of the following:

<table>
<thead>
<tr>
<th>Referrer</th>
<th>Tick if this refers to you.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical Geneticists</td>
<td></td>
</tr>
<tr>
<td>Consultants in Paediatrics</td>
<td></td>
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<tr>
<td>Consultants in Neurology</td>
<td></td>
</tr>
<tr>
<td>Consultants in Ophthalmology</td>
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</table>

Minimum criteria required for testing to be appropriate as stated in the Gene Dossier:

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Tick if this patient meets criteria</th>
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<tbody>
<tr>
<td>Clinical symptoms consistent with a “chromosomal disorder” indicating one of the following:</td>
<td></td>
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<tr>
<td>Mental retardation or developmental delay</td>
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<tr>
<td>Syndromic mental retardation or developmental delay with dysmorphism</td>
<td></td>
</tr>
<tr>
<td>Congenital anomalies</td>
<td></td>
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<tr>
<td>Prenatal onset growth retardation with multiple congenital abnormalities</td>
<td></td>
</tr>
<tr>
<td>Postnatal growth abnormalities (microcephaly short stature, macrocephaly, tall stature)</td>
<td></td>
</tr>
<tr>
<td>Significant speech and learning difficulties ± autism</td>
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</table>

**Exclusion Criteria**

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<tbody>
<tr>
<td>Prenatal samples</td>
<td></td>
</tr>
<tr>
<td>Recurrent miscarriages or fertility problems</td>
<td></td>
</tr>
<tr>
<td>Expectation of balanced rearrangements</td>
<td></td>
</tr>
</tbody>
</table>

If the sample does not fulfil the clinical criteria or you are not one of the specified types of referrer and you still feel that testing should be performed please contact the laboratory to discuss testing of the sample.
Flow charts indicating detection rates for the a) first line alone, b) follow-on to normal karyotyping alone and combined first-line and follow-on testing.

(a) Flow Chart for First-line aCGH as a Replacement for Karyotyping

Patient referred for aCGH test meets defined test criteria & referred through defined pathway 1754 pa (1169 in 8 months)

Imbalance detected (excl.common benign variants) on initial & confirmatory test 22% (220)

No Imbalance 78% (904)

Parental samples & phenotype data requested for array / FISH or MLPA test (according to position & number of imbalances) (32% response to date)

De novo 1% (11)

Unknown 15% (177)

Inherited from phenotypically normal parent 5% (57)

Anticipated de novo (80% response, 13% de novo) 2% (19)

Unresolvable Parental sample not available 3% (35)

Anticipated inherited (80% response, 87% inherited 11% (123)

Inherited from phenotypically normal parent 8% (87)

Uncertain 3% (35)

Unlikely to be clinically significant 17% (195)

(b) Array CGH Flow Chart for Follow-on to Normal G-banding Karyotype

Patient referred for first-line test meets defined test criteria & referred through defined pathway 1868 pa (1245 in 8 months)

Imbalance detected (excl.common benign variants) on initial & confirmatory test 26% (325)

No Imbalance 74% (920)

Parental samples & phenotype data requested for array / FISH or MLPA test (according to position & number of imbalances) (46% response to date)

De novo 4% (52)

Unknown 14% (178)

Inherited from phenotypically normal parent 8% (97)

Anticipated de novo (80% response, 35% de novo) 4% (46)

Unresolvable Parental sample not available 3% (35)

Anticipated inherited (80% response, 65% inherited 7% (82)

Clinically Significant 8% (101)

Uncertain 3% (35)

Unlikely to be clinically significant 15% (189)
(c) Array CGH Flow Chart for First-line & Follow-on to Normal Karyotype

Patient referred for first-line test
meets defined test criteria & referred through defined pathway
3621 pa (2414 in 8 months)

Imbalance detected
(excl. common benign variants) on initial & confirmatory test
24% (585)

No Imbalance
76% (1829)

Parental samples & phenotype data requested for array / FISH or MLPA test
(according to position & number of imbalances)
(40% response to date)

De novo
2% (63)

Unknown
15% (353)

Inherited
from phenotypically normal parent
7% (169)

Anticipated de novo
(80% response, 27% de novo)
3% (68)

Unresolved
Parental sample not available
3% (70)

Anticipated inherited
(80% response, 65% inherited
9% (215)

Clinically Significant
5% (131)

Uncertain
3% (71)

Unlikely to be clinically significant
16% (375)