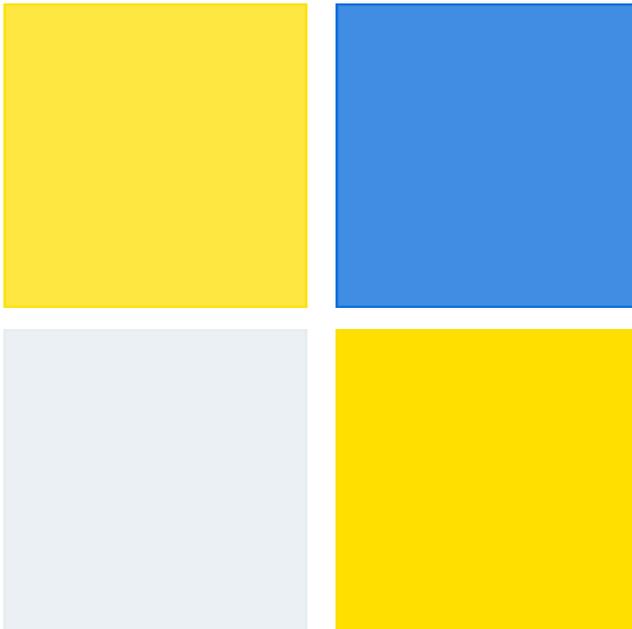


Unique[™]

**Microarray-based
comparative
genomic
hybridisation
(array CGH)**



Microarray-based comparative genomic hybridisation (array CGH)

Chromosomes

Chromosomes are the structures in each of the body's cells that carry the genetic information (DNA) that tells the body how to develop and function. They come in pairs, one from each parent, and are numbered 1 to 22 approximately from largest to smallest. Each person has another pair of chromosomes, called the sex chromosomes. Girls have two Xs (XX), whereas boys have an X and a Y chromosome (XY). Each chromosome has a short (p) arm and a long (q) arm.

Cytogenetic (chromosome) testing can detect if there is too much (gain) or too little (loss) of chromosomes or pieces of chromosomes. People with changes in their DNA or in the number or structure of their chromosomes may have an increased risk of birth defects, developmental delay, behavioural problems and intellectual disability.

Looking at chromosomes (chromosome analysis)

Chromosomes cannot be seen with the naked eye but if they are stained and magnified under a microscope it is possible to see that each one has a distinctive pattern of light and dark bands that look like horizontal stripes. You can see these bands in the diagram of chromosome 16 shown in Figure 1. They are numbered outwards starting from where the short and long arms meet (the centromere). By looking at your child's chromosomes in this way, often referred to as **karyotyping**, it is possible if the change is large enough to see if there is a chromosome imbalance (loss or gain of chromosome material) or if the chromosome is rearranged in any way.

Clinical scientists who do the analysis are very skilled at detecting very small and often very subtle changes. However, as the amount of material gained (duplicated) or lost (deleted) can often be extremely small and impossible to see on a routine chromosome test even by the most skilled of scientists, your child may have been told their chromosome analysis was normal. The clinician may have indicated that an underlying genetic basis was still likely. The newest test now available for looking at chromosomes is called a **microarray-based comparative genomic hybridisation** (also called an **array CGH**) test.

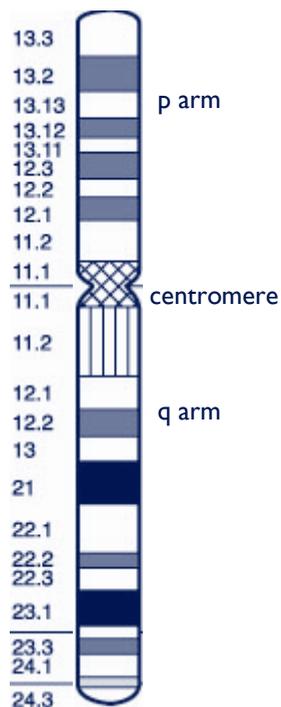


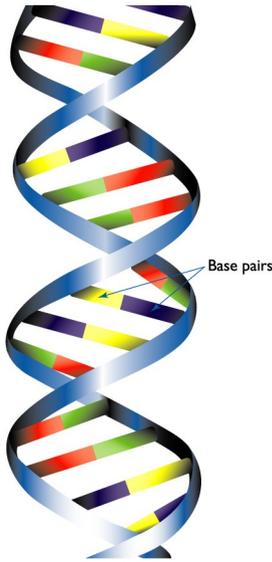
Fig 1. Chromosome 16

What is array CGH?

Array CGH is an advance in technology that allows detection of chromosome imbalances that are smaller than can be detected through looking down the microscope. Karyotyping is only as good as the resolution of the microscope and is not able to detect subtle chromosome changes. These smaller alterations, often called **submicroscopic** alterations because they cannot be seen through the microscope, can still disrupt growth and development. These very small changes are often called **microdeletions** and **microduplications**. Array CGH is also sometimes called **CGH array** or simply a **microarray**.

Array CGH compares your child’s DNA with a control DNA sample and identifies differences between the two sets of DNA. In this way, deletions or duplications (imbalances) in your child’s DNA can be identified. From this, the gene content of any such imbalance can be established.

How does array CGH work?



A microarray works by exploiting the ability of a DNA molecule (or strand) to bind specifically to, or **hybridise** to, another DNA molecule (strand).

The DNA in our cells is arranged as a double helix (see Figure 2) in which the two strands of DNA are bound (‘paired’) together by bonds between the **base pairs** (bps). A single-stranded DNA fragment is made up of four different building blocks (called **nucleotides**), adenine (A), thymine (T), guanine (G), and cytosine (C) which are linked end to end. Adenine (A) will always pair with thymine (T) and guanine (G) will always pair with cytosine (C) (see Figure 3). When two **complementary** sequences find each other they will lock together, or **hybridise**.

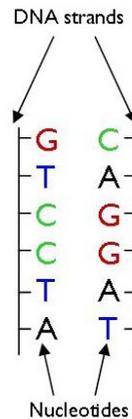
Descriptions		
bp	=	base pair
kb	=	kilobase pair or 1 000 base pairs
Mb	=	megabase pair or 1 million base pairs

Two strands of DNA are held together in the shape of a double helix by the bonds between base pairs.

Fig 2

The microarray comprises thousands of short sequences of DNA (“probes”), arranged in a precise grid on a glass slide called a **chip**. DNA from the patient is “digested” (chopped up into short lengths or fragments), then these fragments are labelled with a coloured fluorescent dye. Reference DNA, from a person, or pool of people, with no genetic abnormalities, is labelled with a different coloured fluorescent dye. The fluorescent dyes commonly used are red and green.

Reference and patient samples are mixed together and applied to the chip and **hybridisation** takes place – the fragments of DNA hybridise with their matching probes on the array (see Figure 4). The chip is then scanned in a machine called a microarray



One strand of DNA binds to another by base pairing between the nucleotides

Fig 3

scanner which measures the amount of red and green fluorescence on each probe. For example, the patient sample may be labelled with a red fluorescent dye and the reference sample may be labelled with green. The microarray scanner together with computer analytical software calculates the ratio of the red to green fluorescent dyes (see figure 5) to determine whether, for the piece of DNA represented by each probe, the patient sample has the correct amount of DNA (shown as yellow), too much DNA (a duplication) which would be shown by too much red, or too little DNA (a deletion) shown by too much green.

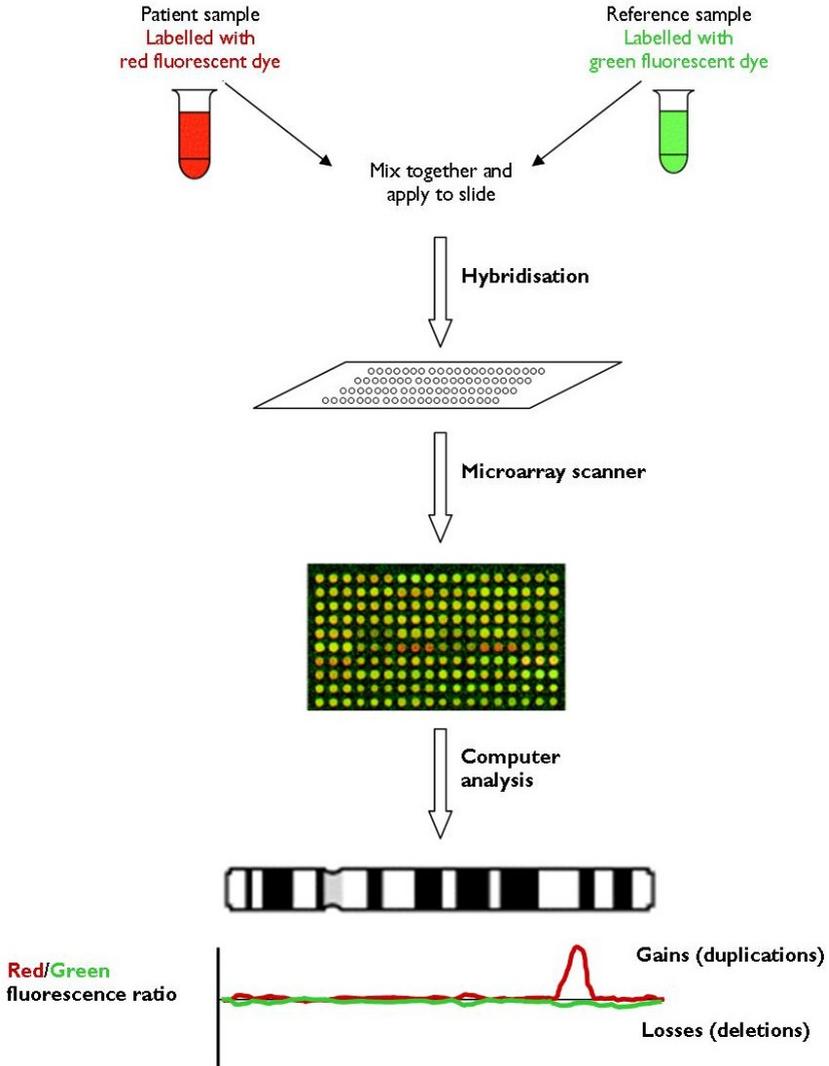


Fig 4. An overview of array CGH

This example shows that the patient has a duplication

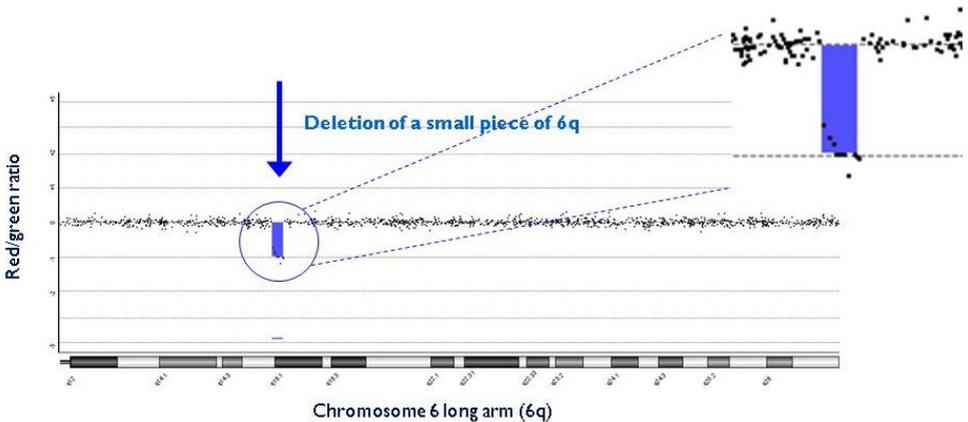


Fig 5. A deletion in chromosome 6, detected by an array CGH test. This can be described as: $\text{arr } 6q15q16.1(91,655,389-93,233,563)\times 1$. Each black dot represents an oligo probe; there are 10 probes in the deleted region (shown in blue, and enlarged on the right).

“ Our geneticist used an analogy which made things clearer for us. He said that previous test results were like an old-fashioned map of the world which showed just a wide overview (country level) and that doing an array is more like using Google earth which allows us to zoom in much more closely, even down to street level, to give a closer and clearer idea of which genes, if any, are missing or duplicated.

What are the different types of array CGH?

The two types of probe most commonly used for array CGH to detect chromosomal changes are bacterial artificial chromosome (BAC) clones (which contain short stretches human DNA) and oligonucleotide (oligo) probes (small sequences of artificial DNA). Both types of probes have advantages and disadvantages. BACs, which are usually 80-200 kbs may miss alterations smaller than the size of a clone but are less likely to detect alterations of unclear clinical significance. Oligos, which are much smaller probes, usually 60 bps, may detect small alterations that would not be seen using a BAC microarray, but oligo arrays are more likely to detect small alterations of unclear clinical significance (see [What are the limitations of array CGH](#) page 10).

What samples are needed for array CGH testing?

Array CGH can be performed on a blood sample from an adult or child, or from stored DNA from previous tests.

Why has array CGH been offered for our child?

Your doctor or geneticist may consider array CGH testing if your child has problems with learning, physical development, behaviour or birth defects or medical concerns such as seizures. Recent studies have shown that around 15 per cent of children with unexplained learning and/or developmental disability will have chromosome changes that could not be detected by conventional chromosome analysis but can be detected through array CGH.

“ We had a family history of undiagnosed children and all other tests came back negative.

“ Our son had global developmental delay and significant speech and language delay. The doctors always thought there was an underlying genetic problem for his problems but all previous tests had come back ‘normal’.

“ Although our daughter had been diagnosed with autism and severe learning difficulties, it was generally felt that there was an underlying genetic cause.

“ Our son had some unusual facial features and behavioural/learning difficulties so it was thought that there may be a chromosome anomaly.

How will we be given the results?

The results are likely to be given to you by your geneticist who will talk you through your child’s results. You will almost certainly then receive a follow-up letter summarising the consultation. Alternatively, you may receive a preliminary result from the doctor doing the test and then refer to geneticist (if appropriate) once family studies (if needed) are completed.

How long do the results take?

Results are usually available in 6-8 weeks. Testing a newborn baby with multiple problems is considered a priority and therefore results may be available slightly sooner.

Interpretations of the results

Depending on where you have been tested and which test was used, the presentation of the results may differ. However, your child’s results are likely to look like one of the following examples:

If your child had a BAC array the results may look like this:

arr cgh	arr cgh 2p15p16.1(RP11-479F13->RP11-260K8)x1
2	The analysis was by array comparative genomic hybridization (array CGH)
p15p16.1	The deletion is from chromosome 2
(RP11-479F13->RP11-260K8)x1	The chromosome has one breakpoint in band 2p15 and one breakpoint in band 2p16 and the intervening genetic material is missing
	This represents the portion of the chromosome that is only present in one copy (instead of the usual two). The region that is missing is a stretch of DNA between markers RP11-479F13 and RP11-260K8

If your child had an oligo array, the results may look like this:

arr cgh arr cgh 16p11.2(29581455-30106101)x1
16p11.2 The analysis was by array-CGH
(29581455-30106101)x1 A change was found in band 16p11.2
The first base pair shown to be missing (see Figure 6 below) is number 29,581,455 counting from the left of the chromosome. The last base pair shown to be missing is 30,106,101

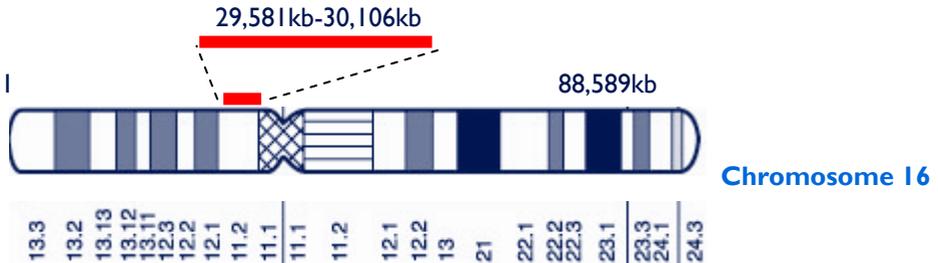


Fig 6

Some reports may also detail the individual genes that have been deleted or duplicated and/or a picture of the array CGH analysis.

“ We were pleased to have a diagnosis, even if only a series of numbers and letters. It confirmed the difficulties we knew our son was having and this was a help to us. We are now able to tell people he has a chromosome anomaly and this has helped in asking agencies for support with respite etc.

My geneticist says my child is missing x genes. How do I find out what those genes do?

With an array CGH test is often possible to determine how many and which genes are missing or duplicated. At present we only understand the role of a small minority of genes and their association with particular clinical features. However, where a gene’s association with a particular clinical feature is known, it can be informative for the care and monitoring of your child’s health to know that the gene is present, absent or duplicated. It may also be reassuring to discover that such a gene is NOT included in your child’s duplication or deletion.

However, it is important to remember that while identifying the gene(s) that are missing or duplicated in your child is interesting and may help guide future studies, it does not lead directly to immediate improved treatment. **At present, we do not know the function and impact of many of our genes so it is not possible to predict what the consequence is of having a particular gene missing or duplicated.** Additionally, even if a particular gene is thought to be responsible for a particular feature, it does not always

mean that the associated feature(s) will be present. Other genetic and environmental factors often have a role in determining the presence or absence of a particular feature. If you would like further information on the specific genes involved in your child's chromosome imbalance, you should request an appointment with your geneticist or genetic counsellor who would be able to discuss this with you more fully.

What are the advantages of array CGH?

The main advantage of array CGH is the ability to explore all 46 chromosomes in a single test and to detect any DNA imbalance including extra or missing chromosomes and loss or gain of chromosome material much more precisely than conventional chromosome analyses. Therefore, many more children will get a diagnosis from an array CGH than having a routine karyotype analysis. Additionally, array CGH can detect chromosome imbalances when there are no clues to what the chromosome anomaly might be and so would not be detected by performing specific genetic tests (such as FISH). Receiving a diagnosis from an array CGH may avoid your child having to undergo many other tests in order to discover a reason for your child's difficulties. It can reveal which specific genes are included in the deletion or duplication.

Array CGH will detect some but not all cases of mosaicism (the chromosome anomaly is not present in **all** the cells of the body; a proportion of the body's cells have a different chromosome make-up).

Array CGH may also be useful to further define breakpoints in imbalances that are already known.

“ We got a sort of closure that we at last had a diagnosis and relief that our son did not need to go through any more diagnostic testing.

What are the benefits to my child of array CGH testing?

Array CGH testing provides the most comprehensive analysis of chromosome structure available to date. A diagnosis may help you and your doctor to watch for common health problems that occur with your child's chromosome imbalance and may help predict what to expect as your child gets older. It may show which specific genes are included in your child's deletion or duplication. If the gene(s) has been associated with a particular feature or health problem it may help to guide management or treatment for your child.

Also, some parents find it helpful to give their child's diagnosis to the school system to obtain special services. Others choose to join a support group to meet other parents facing similar challenges.

Additionally, when a specific chromosome imbalance is diagnosed, the parents (and other family members) can be tested to see if they are carriers of changes in their DNA that put them at risk of having more children with a chromosome change.

“ I was pleased to get a diagnosis and now feel like we belong to a group and can get help and support.

“ Although we were initially devastated to find out something was wrong, it has made a

big difference as we now know his condition and can focus on the weaknesses in our gorgeous, special son.

“ I no longer feel so alone. We now have a leaflet on her chromosome disorder to give to specialists. It helps to be able to tell people what is wrong and some of her odd behaviours are now partly explained.

“ It is good to have a diagnosis, although there is an unclear prognosis as no-one else is known with the same deletion.

“ It helped us to get in touch with other families with children who have the same syndrome.

“ Without array CGH we wouldn't know what my son had. Now that we know, it has made me look forward and get on with our lives.

“ Sometimes I wish we hadn't found out the results so we didn't feel that our son has a medical label on him. However, the majority of the time I'm glad that we found out the source of his problems as we can be prepared for the future and be ready to deal with any problems he may have as he grows up. I also feel that due to the results we received much faster access to speech therapy.

“ It gave closure and we are very grateful for the test. We, and anyone involved in her lifelong care, will be better equipped for the future.

“ It was such a relief to get a diagnosis. I had always blamed myself for having done something wrong during pregnancy or birth. I was upset and tearful for about a week or so. I was shocked. Then I was amazed to find other children that looked just like my son when I had spent years looking for them!

Can I have prenatal testing using arrays?

Most doctors believe that we don't yet know enough about how normal development is affected by small changes in the chromosomes. Finding these changes in pregnancies could therefore cause unnecessary anxiety for the parents, and may in some cases lead to termination of a pregnancy which would have resulted in a normal baby.

Where there are severe abnormalities detected on prenatal ultrasound, the parents may be offered prenatal testing using arrays, to find out if there are chromosome changes which might explain the ultrasound findings.

Will array CGH change my child's treatment?

Array CGH offers a genetic explanation of the learning or developmental difficulties that affect your child but does not necessarily lead directly to immediate improved treatment. However, if a gene or a region of a chromosome that is associated with a specific clinical feature has been shown to be either duplicated or deleted in your child, this may have an impact on your child's care or it may give you an indication of health problems to watch out for that may occur with your child's chromosome disorder

“ Although it has made no difference to the care of our daughter, it has helped with obtaining services.

What are the limitations of array CGH?

Balanced chromosome rearrangements, such as balanced translocations and inversions (where a section of a chromosome is inverted or reversed), will not be identified using array CGH. This is because balanced chromosome rearrangements do not result in any loss or gain of chromosome material. It will also not detect some types of polyploidy (more than the usual 2 sets of chromosomes), such as triploidy (three sets of chromosomes). A standard karyotype is still available and would be undertaken when needed.

It is also important to note that genetic conditions are caused not only by chromosome imbalances, but may also be caused by point (single base pair) changes in the DNA. Array CGH cannot detect these tiny changes.

The other disadvantage of array CGH is that it may identify chromosome changes, known as copy number variants (CNVs). These changes are common in the general population and are often completely harmless. But sometimes a CNV can affect health or development. CNVs can make interpreting an array CGH difficult so the parents may need to be tested to help interpret the results. Sometimes a CNV can be unrelated to your child's problems at the time of testing, but may have implications for his/her future health or development, or the health of other family members. Please discuss this with your genetics specialist. Further information may become available about the significance of some CNVs as more children are tested by array CGH. Please contact your genetics specialist for advice on new developments

“ The array CGH discovered that our daughter had a duplication. However, after testing us [her parents] it was shown that the duplication had been inherited from me [mother] and so the duplication is not the cause of my child's problems. It is important not to pin all your hopes on the array CGH – I know a lot of children who have been diagnosed in this way, but I also know a lot that were not.

What if an array CGH test does not detect a chromosome imbalance in my child?

An array CGH test will only detect a chromosome change in 25 per cent of those children who are tested (compared with 5 per cent who have a chromosome change that would have been detected using conventional chromosome analysis). So for every hundred children who have an array CGH test, 75 children will receive a 'normal' test result - a chromosome imbalance will not have been detected. Your geneticist or genetic counsellor will be able to discuss this outcome and advise whether other tests may be appropriate for your child. In this case, the geneticist would discuss if any additional screening might be recommended for your child.

How do I request an array CGH for my child?

Array CGH tests are becoming more widely available and in some areas may have replaced conventional chromosome analysis (karyotyping). However, if your child has had a conventional chromosome analysis that is normal you may like to discuss with your geneticist or paediatrician the availability and potential benefit of an array CGH test for your child. The vast majority of *Unique* families in the UK who have had an array CGH test for their child had the test with the NHS but it is also possible to have the test done privately. Your geneticist or genetic counsellor will be able to advise you further.

Notes



Support and Information

**Rare Chromosome Disorder Support Group,
PO Box 2189,
Caterham,
Surrey CR3 5GN,
UK**

**Tel/Fax: +44(0)1883 330766
info@rarechromo.org
www.rarechromo.org**

**Unique is a charity without government funding, existing entirely on donations and grants. If you are able to support our work in any way, however small, please make a donation via our website at www.rarechromo.org/html/MakingADonation.asp
Please help us to help you!**



Produced with the support of EuroGentest
(www.eurogentest.org)

This leaflet is not a substitute for personal medical advice. Families should consult a medically qualified clinician in all matters relating to genetic diagnosis, management and health. The information is believed to be the best available at the time of publication. It was compiled by *Unique* and reviewed by Dr Shehla Mohammed and Dr Caroline Ogilvie, Guy's Hospital, London, UK and Professor Maj Hultén Professor of Reproductive Genetics, University of Warwick, UK and Chief Medical Advisor to *Unique*.

2010, 2011 (SW)

Copyright © Unique 2011