Understanding Chromosome & Gene Disorders

Microarray-based comparative genomic hybridisation (array CGH)

rarechromo.org

Microarray-based comparative genomic hybridization is a laboratory technique that is used on a DNA sample to see if any genetic material has been duplicated or lost.https://rarechromo.org/array-cgh/

Chromosomes

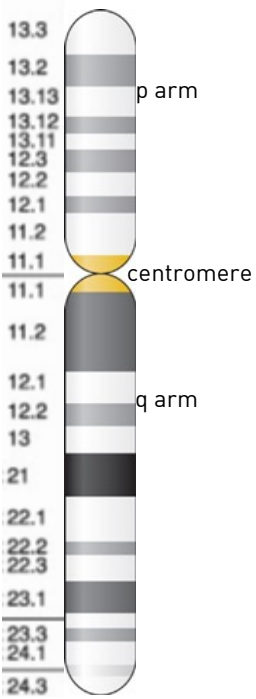
Chromosomes are the structures in our body's cells that carry our genetic information (our DNA) that tells our bodies how to grow, develop and function. Chromosomes come in pairs, one from each parent, and are numbered 1 to 22 approximately from largest to smallest. Each person has another pair of chromosomes,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

DNA testing can detect if there is too much (gain/duplication) or too little (loss/deletion) chromosomal material (either whole chromosomes or pieces of chromosomes). People with changes in their DNA or in the number or structure of their chromosomes may have an increased chance of being born with physical or medical symptoms and features, developmental delay, behavioural difficulties or 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 the image opposite. They are numbered outwards starting from where the short and long arms meet (the centromere). By looking at chromosomes in this way, often referred to as karyotyping, it is possible to see relatively large chromosomal imbalances (losses or gains of chromosome material) or if a chromosome is rearranged in some way.

Clinical scientists who do this type of analysis are very skilled at detecting very subtle changes. However, as the amount of material gained (duplicated) or lost (deleted) may be too small to see on a routine chromosome test, many people with a chromosomal imbalance will have been given a 'normal' result simply because the genetic change was to small to be visualised by this type of test.

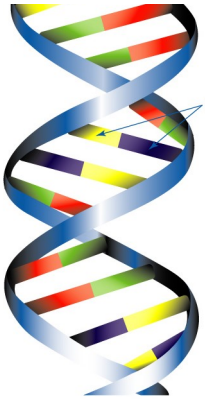


Chromosome 16

Array CGH compares a test DNA sample with a control DNA sample and identifies differences between the two sets of DNA. In this way, deletions or duplications (imbalances) in a child or adult'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).



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

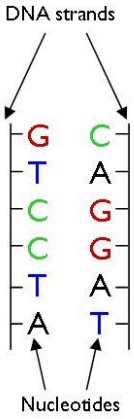
The DNA in our cells is arranged as a double helix, as seen in the image opposite, in which the two strands of DNA are bound ('paired') together by bonds between the base pairs (bps).

Descriptions:
bp = base pair
kb = kilobase pair = 1000 base pairs
Mb = megabase pair = 1 million base pairs

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) always pairs with thymine (T) and guanine (G) always pairs with cytosine (C) as shown in the image below.

When two complementary sequences find each other they will lock together, or hybridise.

A microarray comprises tens of thousands of short sequences of DNA (called "probes"), arranged in a precise grid on a glass slide called a chip. DNA from a 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 condition, 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.



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

The fragments of DNA hybridise with their matching probes on the array as shown in the image below. The chip is then scanned in a machine called a microarray scanner which measures the amount of red and green fluorescence on each probe.

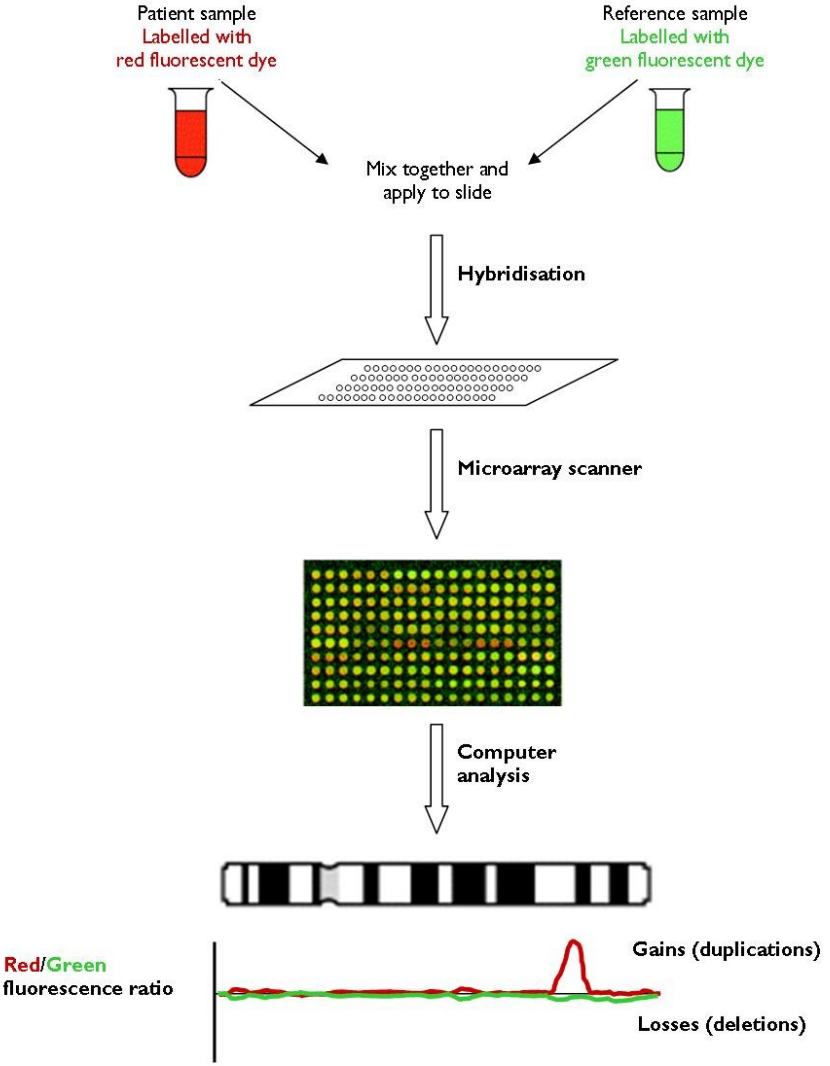
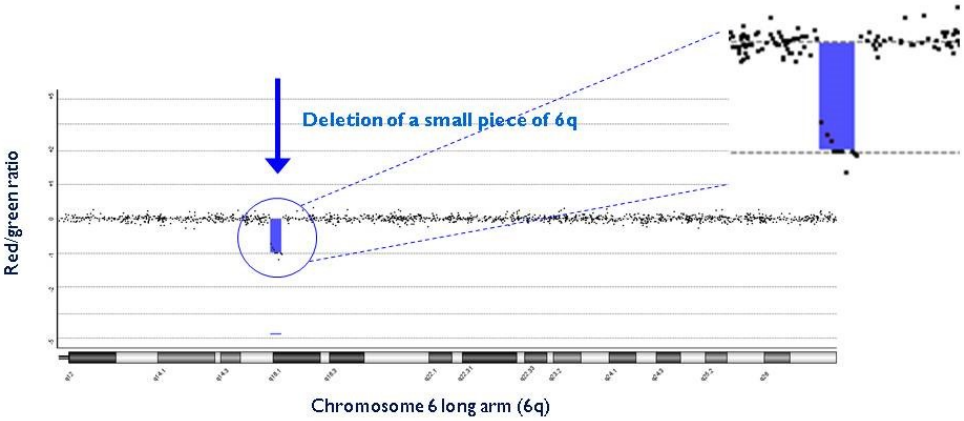


Fig 4. An overview of array CGH
This example shows that the patient has a duplication

If the patient sample is labelled with a red fluorescent dye and the reference sample is labelled with green, the microarray scanner and analytical computer software calculates the ratio of the red to green fluorescent dyes to determine whether, for the piece of DNA represented by each probe, the patient sample has the expectred 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.



A deletion in chromosome 6, detected by an array CGH test. This can be described as: arr 6q15q16.1(91,655,389-93,233,563)x1. 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 samples are needed for array CGH testing?

Array CGH can be performed on a blood or saliva 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 20 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 be referred to geneticist for a more detailed explanation (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, the presentation of the results may differ slightly. However, your child's results are likely to look like the following example:

arr[hg19] 16p11.2(29,673,954-30,198,600)x1

arr The analysis was by array-CGH

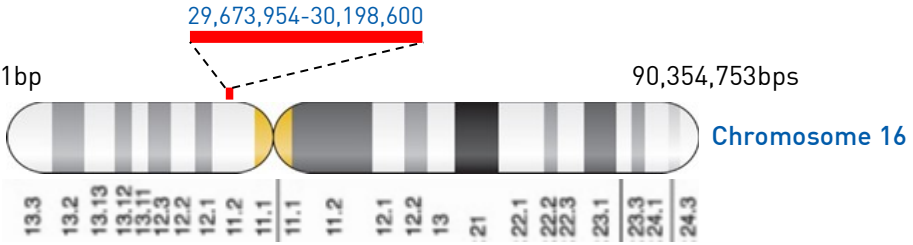
hg19 Human Genome build 19. This is the reference DNA sequence that the base pair numbers refer to. As more information about the human genome is found, new "builds" of the genome are made and the base pair numbers may be adjusted

16p11.2 A change was found in band 16p11.2

(29,673,954-30,198,600) The first base pair shown to be missing (see Figure 6 below) is number 29,673,954 counting from the left of the chromosome. The last base pair shown to be missing is 30,198,600. Take the first long number from the second and you get 524,646 (0.525Mb or 525kb). This is the number of base pairs that are deleted

x1 means there is one copy of these base pairs, not two – one on each chromosome 16 – as you would normally expect

These results are shown diagrammatically below (this sort of image is not shown on a genetics report).



Some reports also detail the individual genes that have been deleted or duplicated and/or a picture of the array CGH analysis (such as the image shown on page 5).

❝ 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 currently routinely available. 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 monitoring 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?

Where there are severe abnormalities detected on prenatal ultrasound, prenatal testing using arrays can now be offered at most centres, to find out if there are chromosome changes which might explain the ultrasound findings. However, a less detailed analysis is likely to be carried out, to reduce the chance of finding changes which are of unknown significance, and therefore likely to cause undue parental anxiety.

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 (known as variants) in the DNA. Array CGH cannot detect these tiny changes.

The other disadvantage of array CGH is that it may identify chromosome changes (CNVs) that are 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. Some CNVs are difficult to interpret without knowing whether or not a parent carries the same imbalance, which is why blood from both parents, if possible, may need to be tested to help interpret the results. A genetics specialist will be able to explain each family's situation in more detail. Further information may become available about the significance of some CNVs as more children are tested by arrays. Genetics departments can be contacted for advice on new developments. Chidren and adults who had an arrayCGH test before 2015 may be eligible to have a more recent array or sequencing test since technology has improved considerably during the last few years.

❝ 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 do the results mean for the wider family?

The geneticist will be able to give specific advice about implications of some results to the wider family, for example siblings or other relatives. If it is advisable for other relatives to be made aware of the results, a general "to whom it may concern letter" can be passed to relatives with relevant information, suggesting a referral to their local genetics centre via their own GP.

Can other genetic changes be found that are not related to my child's symptoms and features?

Yes, they can but they are quite rare. Professionals call these incidental or secondary findings, they are also referred to as unexpected or additional findings. These types of genetic changes are thought to be of medical relevance, but not thought to be related to the persons symptoms and features that lead to an array CGH test being requested.

For example, a five-year-old girl has an array CGH because she has developmental delay and some unusual facial features. The array detects a pathogenic CNV affecting a gene which can result in an increased risk of breast cancer. This would not explain the child's features but does mean that when she reaches adulthood she can seek early and frequent mammograms or take preventative measures.

Secondary and incidental findings may have unexpected implications for the future health of a child as well as other family members, for example, a predisposition to cancer. This may be useful information to have in the long term, since it may allow for increased screening, but may be unexpected and upsetting news. In some countries it may also have health insurance implications. There may be an option to 'opt out' of being informed of some or all additional findings if that is your preference.

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 now widely available and have replaced the previous karyotyping test. If your child had a karyotyping test carried out that did not pick up anything unusual, you may like to discuss with your geneticist or paediatrician the availability and potential benefit of an array CGH test. 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. More recently, arrayCGH tests are being replaced by SNParrays, which are able to provide more detailed information. Unique publishes a separate guide for SNParrays. Your geneticist or genetic counsellor will be able to advise you further.

Inform Network Support

Rare Chromosome Disorder Support Group,
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info@rarechromo.org | www.rarechromo.org

Join Unique for family links, information and support.

Unique is a charity without government funding, existing entirely on donations and grants. If you can please make a donation via our website at www.rarechromo.org/donate
Please help us to help you!

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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 Professor 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.
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