



Understanding Chromosome & Gene Disorders

Single Nucleotide Polymorphism array (SNP array)

rarechromo.org

Chromosomes, genes and development

Every cell in our body contains two complete copies of our genome. Our genome contains all the instructions for making and maintaining every part of our body. Our genome is made from DNA, which contains a genetic code made from four letters A (adenosine), C (cytosine), T (thymine) and G (guanine). Our genetic code is made from over 3.2 billion letters.

Our DNA is packaged into 23 pairs of chromosomes; we inherit one of each chromosome from each of our parents. The shuffling of these pairs of chromosomes is why we are more similar to our siblings than to strangers, but not identical to them (unless we have an identical twin). Our chromosomes are numbered from 1 to 22 in approximate order of their length (these are called autosomes); the 23rd pair are the **SEX CHROMOSOMES** called the X chromosome and the Y chromosome. Girls usually have two X chromosomes (XX), whereas boys usually have an X and a Y chromosome (XY). Each chromosome has a short arm (called the p-arm) and a long arm (called the q-arm).

You can think of the genome as like a recipe book containing 23 chapters. Each chapter (chromosome) contains recipes (genes) which explain the method (genetic code) to make a dish (usually a protein).

Genetic variation

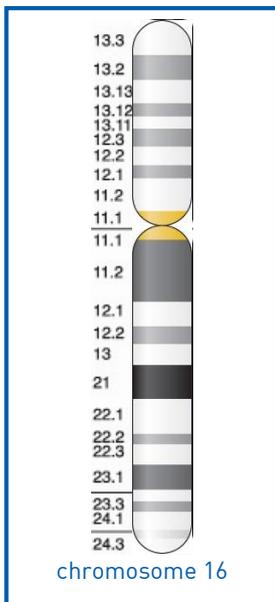
A **COPY NUMBER VARIANT** (called a CNV for short) is a change in the genetic code which results in some of it being deleted (having one less copy, or occasionally two less (this is called a deletion) or being duplicated (having more copies than expected (this is called a duplication))). This could be viewed a bit like if a sentence or paragraph was missing or repeated in a recipe book. Consider the instructions of a recipe to make a birthday cake which requires the cake mix to be stirred for ten minutes. If the instruction was changed to stir the cake mixture for five minutes (half the time) or twenty minutes (double the time), the cake would probably not be affected much. However, if the cake recipe had an important instruction missing, such as to add an egg, or if it repeated an instruction and so two eggs were added instead of one, the cake might have a more noticeable change. This could be viewed as similar to what happens with CNVs, if we have a piece of DNA deleted or duplicated, our development and functioning can be affected. When a CNV is thought to affect us in such a way, it's called a **PATHOGENIC VARIANT** or a causative variant. When a CNV is not thought to have any effect on our development and functioning, it is known as a **BENIGN VARIANT**.

CNVs can range in size from very large (a whole chromosome) to very small (just a few letters). They can include part of a gene, a whole gene, or many genes. To detect CNVs in the genetic code, a test is needed which quantifies the number of copies of the genetic code. This is called **CHROMOSOME ANALYSIS** or **MICROARRAY**.

A **SINGLE NUCLEOTIDE VARIANT** (or SNV for short) is a change in a single letter of the genetic code; a bit like a word that is spelt differently in a sentence. Consider the spelling of ingredients in a recipe. If one recipe included instructions to serve a dish with frozen yoghurt and another to serve with frozen yogurt, you would still know how to serve the dish, because these are just common variants of a spelling. In terms of DNA, this would be described as a **BENIGN VARIANT**, a variant that has no meaningful affect. However, if a recipe had a single letter change and instructions to add custard were changed to add mustard – the dish would not be prepared as expected. In terms of DNA, this would be called a **PATHOGENIC VARIANT** because it is an unexpected change to an important instruction that affects health and/or development.

SNVs change just one or a small number of letters, affecting one gene only. To detect SNVs in the genetic code, a test which reads the actual genetic code is needed. This is called **SEQUENCING**.

Both CNVs and SNVs often occur by chance in an individual and can also be inherited. The majority of CNVs and SNVs are completely benign. Usually, a CNV or an SNV will be present in every cell within the body; occasionally, a CNV or an SNV is present in only some cells and the proportion of cells with the CNV or SNV can vary depending on the tissue that is tested. This is called **MOSAICISM**.



Studying 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 opposite. They are numbered outwards starting from where the short and long arms meet (the centromere). By looking at your child's (or your) chromosomes in this way, often referred to as **KARYOTYPING**, it is possible to see a chromosome imbalance (loss or gain of chromosome material - CNV) if the change or rearrangement is large enough

Clinical Scientists who do the analysis are very skilled at detecting small and often very subtle changes. However, some CNVs are too small to see on a routine chromosome test even by the most skilled of scientists. A test which is capable of detecting smaller changes is needed, this test is called a microarray.

What is a microarray?

Chromosomal microarrays are a significant advance in technology that allow detection of chromosome imbalances that are too small to be detected by looking down the microscope. These smaller alterations, often called submicroscopic alterations because they cannot be seen down the microscope, can still disrupt growth and development. These very small changes are often called microdeletions and microduplications.

What are the different types of microarray?

As technology has improved, the ability to detect smaller and smaller CNVs has also improved. This is called improved **RESOLUTION**. If your child has been seeking a diagnosis for some time, they may have already been tested using some of the earlier microarray types – for example **BAC ARRAY** or oligonucleotide array comparative genomic hybridisation (**ACGH**). A single nucleotide polymorphism, or **SNP ARRAY**, is the most up-to-date type of microarray and usually has increased resolution as well as the ability to detect some rare variants which do not change the copy number.

Why has SNP array testing been offered for our child?

Your doctor or geneticist may consider SNP array testing if your child is born with physical or medical concerns, or delays with learning, development, or behaviour. Recent studies have shown that around 25 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 SNP array. Your child may be offered a SNP array even if they have had a previous normal microarray result because it can detect smaller imbalances and some variants which do not affect the copy number. Your child could also be offered a SNP array if they have had a CNV detected by a previous microarray if the improved resolution can help to clarify the significance of the previous result.

“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.”

What samples are needed for microarray testing?

SNP array can be performed using DNA extracted from a variety of tissues, but usually blood or saliva is used. DNA can be extracted from a fresh sample, or, if previous genetic testing has already been undertaken, stored DNA may be available.

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 (and possibly your) 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 a geneticist for a more detailed explanation (if appropriate) once family studies (if needed) are completed.

How long do the results take?

Results can be available in 6-8 weeks but might take up to six months for busy clinics. Testing during pregnancy or of a newborn baby with multiple concerns is considered a priority and therefore results should be available sooner.

Understanding a microarray result

The microarray results that you will be given will look like a long string of numbers and letters that are specific to the genetic change(s) that have been identified in your child's, or your DNA sample. A few examples are explained below.

■ **arr(X,1-22)x2**

This indicates a standard result for a female without any unusual genetic changes. **arr** signifies an array test was carried out; **(X,1-22)x2** signifies that two X chromosomes and 2 of each chromosome numbered 1-22 were identified in the sample.

■ **arr(X,Y)x1(1-22)x2**

This indicates a standard result for a male without any unusual genetic changes. **arr** signifies an array test was carried out; **(X,Y)x1** signifies that a single X and a single Y chromosome were identified (as opposed to the two X chromosomes found in females), **(1-22)x2** signifies that 2 of each chromosome numbered 1-22 were identified in the sample.

Both of the results shown above indicate that no CNVs were detected that are considered to be contributing to the symptoms and features identified in the person who provided the sample.

Depending on where your child, and possibly you, were tested, if a CNV is detected, the description may vary slightly. However, the genetics report is likely to comprise the elements below:

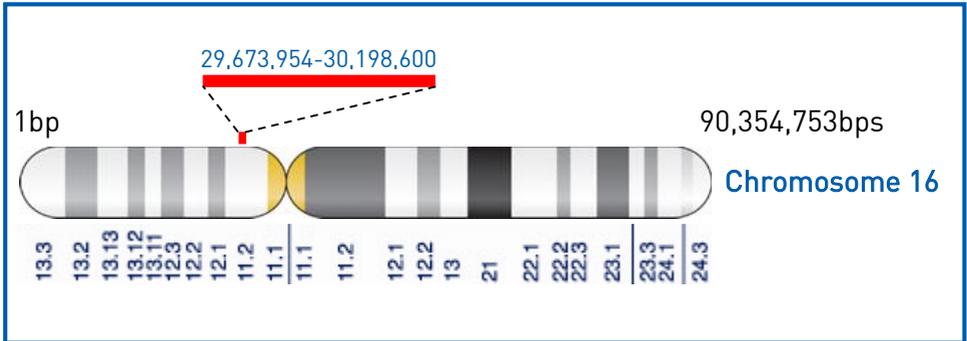
■ **arr[GRCh38] 16p11.2(29,673,954-30,198,600)x1 [0.8] dn**

The description of a CNV can look very confusing but each part includes a useful piece of information and should be described in words in the report that are easier to understand. A breakdown of the CNV shown above can be described as follows:

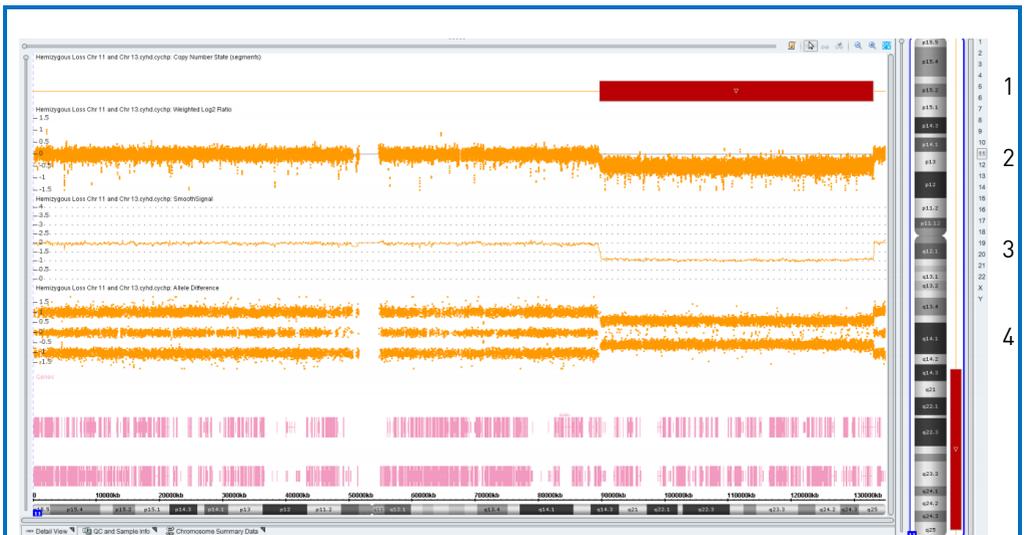
- **arr** - is short for microarray and describes the test undertaken
- **[GRCh38]** - denotes the version of the reference genome, in square brackets, that the result refers to. Reference genomes, also known as genome builds, are like a dictionary of the genome and are updated from time to time, so positions of the same CNV can vary between different genome builds.
- **16** - indicates which chromosome has been identified as having a CNV
- **p** - indicates which 'arm' of the chromosome is affected (each chromosome has a short 'p' arm and a long 'q' arm).
- **11.2** - indicates the chromosomal band (or bands, if more than one is listed) that is affected (each chromosome arm can be further divided into different numbered bands).
- **(29,673,954-30,198,600)** - indicates the first and last letters of the genetic code that is found to be affected by the CNV, in round brackets. The difference between these numbers is the size of the CNV.
- **x1** - indicates the copy number of the CNV. Since we usually have two copies of each numbered chromosome, x1 indicates a loss of one copy (a deletion) and x3 indicates a gain of one copy (a duplication). The situation is slightly different for X and Y chromosomes depending on whether the person being tested is male or female.
- **[0.8]** if present, a figure at the end of the result in square brackets indicates whether the genetic change is thought to be mosaic, meaning it is not found in all cells of the body. In this case 0.8 signifies that not all cells are affected and the genetic change is mosaic.
- **dn** - is an abbreviation of 'de novo' and indicates that this genetic change has occurred for the first time in the person being tested and is not thought to be inherited from a parent. If **mat** is noted in place of dn it indicates that the CNV was inherited from the mother (mat for maternal), **pat** would indicate a CNV that's been inherited from the father (pat for paternal).

Although this detail is very important, you may find the results are abbreviated slightly during discussions, in the case of the test result above, in conversation it would just be referred to as a 16p11.2 deletion (or microdeletion, meaning a very small deletion).

These results can be represented diagrammatically as shown in the image below. (This is not however commonly seen in a genetics report).



The results provided by the computer software used to analyse the array data look quite different. The following image is an example of what the scientists see.



The above image is a screen shot of SNP array software showing a deletion of part of the long arm of chromosome 11, indicated by a red bar (row 1). The evidence for the deletion is shown in rows 2 (raw data) and 3 (smoothed data), where the copy number number is reduced from two copies to just one copy. This is similar to previous versions of a microarray. However, the SNP array also shows information about the polymorphic variants (row 4) which increase the ability to detect some rarer variants which do not affect copy number.

“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.”

Understanding what the CNV means for your child

SNP array reports should have a summary of CNVs detected and explain the significance of them.

A **PATHOGENIC** result means a CNV has been detected which affects a stretch of DNA, gene, or number of genes, in a way which is well established and understood and therefore explains the clinical features of the person being tested. This confirms a diagnosis of a chromosome disorder.

A **LIKELY PATHOGENIC** result means a CNV has been detected which affects a stretch of DNA, gene, or number of genes, in a way that is reasonably well understood and is likely to explain the clinical features of the person being tested, but for which some uncertainty remains. For example, if a few people have been identified with a similar CNV but do not have similar clinical features or if the CNV is rare and there is insufficient data to be sure of the clinical features associated with it.

A **VARIANT OF UNKNOWN SIGNIFICANCE** (sometimes called a **VUS**) is a CNV affecting a stretch of DNA, gene, or number of genes, which are not sufficiently well understood to enable a meaningful interpretation. It may be that the CNV is very rare or even **UNIQUE** to the child so there is no information about what the effects are. Sometimes, the laboratory may request to test parents for the CNV to see if it was inherited from one of them or if the child is the first person in the family to be affected. An inherited CNV is usually less likely to be responsible for a pronounced clinical feature if a parent who has the same CNV does not have that clinical feature.

A **LIKELY BENIGN** or **BENIGN** CNV is one which is considered to be a normal variation and irrelevant to the health and development of the person being tested. These are unlikely to be mentioned in a genetics report.

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

Yes, they can. **INCIDENTAL FINDINGS** and **SECONDARY FINDINGS** are rare types of results but they are important. An incidental finding describes a CNV which might be of medical relevance that is discovered unexpectedly during the course of analysis but is not thought to be related to the reason why the SNP array was requested. Secondary findings are similar but they are actively sought by the

laboratory; they are of medical relevance but are not related to the person's symptoms and features. For example, a five-year-old girl has a SNP array because she has some unusual facial features and developmental delay. The SNP 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.

Is everyone with the same or similar CNV affected in the same way?

No, people with the same or similar CNV can be affected differently, even children within the same family with the same genetic change can have different features and symptoms, or have features with different severities.

Occasionally, a CNV is found which is said to have **INCOMPLETE PENETRANCE**. This means that the CNV is found more often in people with a particular feature than it is in people who do not have the CNV, but not all people who have the CNV will have the particular feature. It can be thought of as a **SUSCEPTIBILITY** to the feature rather than a certainty. This is often the case in CNVs associated with neurodevelopmental disorders such as autism. This suggests that there are other unknown influences which contribute to the development of the feature in addition to the CNV, such as the environment and experiences of the person as well as their own unique genetic background.

There is also another factor to consider, known as **VARIABLE EXPRESSIVITY**. While for some genetic changes, it is thought that everybody with the same or similar change will have a specific clinical feature or symptom, it is often the case that a spectrum of features and symptoms are identified in individuals with the same or similar genetic change. Also, while some people may be found to be mildly affected, others may be moderately, severely or profoundly affected. If the majority of individuals are identified as having similar features, in terms of type and severity, then expressivity may be described as low. In contrast, if there is a broad spectrum of features observed in different people, and there is a broad range of severity, then expressivity is described as high.

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

A SNP array test can identify genes which are missing or duplicated. At present, the role of only a small number of genes, and their association with particular clinical features, is understood. However, where a gene's association with a particular clinical feature is known, it can be informative for the care and monitoring of your or your child's health to know that the gene is present, absent

or duplicated. It may also be reassuring to discover that an important 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 you or your child is interesting and may help guide future studies, it does not lead directly to immediate improved treatment. At present, the function and impact of many of our genes is unknown so it is not possible to predict what the consequence is of having one copy of 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 you or 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 a microarray?

The main advantage of a microarray is the ability to study all 46 chromosomes in a single test and to detect extra or missing chromosomes and very small losses or gains of chromosome material much more precisely than conventional chromosome analyses. This 'genome-wide' quantification can detect chromosome imbalances even when there are no specific clues to what the chromosome anomaly might be. Receiving a diagnosis from a microarray may mean that your child can avoid having to undergo many other tests in order to discover a reason for their difficulties.

"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."

How is a SNP array different from other arrays?

Early microarrays could detect copy number changes only. A SNP array can detect copy number changes, just like previous microarrays and karyotyping, but has higher resolution and therefore can potentially detect smaller CNVs. In addition, it can provide additional information related to a small number of specific, common changes of single letters of the genetic code (single nucleotide polymorphisms - SNVs) which can also help to identify some other types of rare genetic anomalies.

One of these rare types of anomaly is **UNIPARENTAL DISOMY**. This is where a child has inherited two copies of a chromosome or a chromosomal region from one parent – instead of the usual one copy from each parent. This has no effect on most genes. However, some genes are only functional on the chromosome from the mother **or** from the father and not both (these are known as **imprinted genes**).

Inheriting both copies of a gene from one parent can result in two non-functional copies of that gene, or two active copies of a gene which usually only has one active copy.

Another rare type of anomaly is called **TRIPLODY**. This indicates three copies of **all** the chromosomes are present instead of two copies; this is usually only seen in early pregnancies but can be detected in **MOSAIC** form in children.

What are the benefits of SNP array testing?

SNP array testing provides the most comprehensive analysis of chromosome copy number variants currently routinely available. A diagnosis may help you and your doctor to watch for common health conditions 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 condition it may help to guide management or monitoring for your child.

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 concerns.

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 the copy number variant, or in some cases, an increased risk of late onset health conditions.

"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."

"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.”

Can I have prenatal testing using SNP arrays?

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

Will a SNP array finding change my child’s treatment?

SNP array may offer 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 their care or it may give you an indication of health concerns 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 SNP array?

A SNP array will **not** detect:

- Balanced chromosome rearrangements which do not result in loss or gain of DNA, such as translocations (when a piece of DNA has moved to a different chromosome or within a chromosome) or inversions (when a piece of DNA has been broken off, flipped around and reinserted into a chromosome ‘back-to-front’). This requires a standard chromosome analysis or karyotype (when chromosomes are stained and viewed under a microscope).

- The majority of Single Nucleotide Variants (SNVs) – single letter changes in the genetic code. This requires sequencing.
- CNVs that are smaller than the resolution of the SNP array. There are many different types of SNP array, technical information should be provided on the report.
- Mosaic results present in too small a proportion of cells to be detected.
- Some ring chromosomes, which do not result in loss of DNA at the chromosome ends.

A SNP array can detect a CNV of uncertain significance which can be difficult to understand. It is possible that the CNV can be re-interpreted more accurately in the future as more information is gathered about it. A CNV could re-interpreted as **more** likely to be pathogenic or as **less** likely to be pathogenic as evidence emerges.

A SNP array cannot define the precise length of a CNV, but rather a minimum and maximum size based on the interval between the SNPs. Occasionally, a gene which may be pathogenic may be located between the minimum and maximum size of the CNV and make it difficult to interpret without further clarification. Occasionally a CNV may not contain the coding part of a gene but it might contain DNA sequences that control the activity of a gene, these are very difficult to identify and interpret.

A SNP array can detect a CNV with secondary or incidental findings which may have unexpected implications for the future health of the child and even other family members, such as a predisposition to cancer. This may be useful information to have in the long term, since it may allow for increased cancer screening, but may be unexpected and upsetting news. In some countries it may also have health insurance implications. You should have an option to 'opt out' of being informed of some or all additional findings if that is your preference.

What do the results mean for the wider family?

Your 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.

What if a SNP array test does not detect a chromosome imbalance?

A SNP array will only detect a chromosome change in around 25 per cent of people who are tested (compared with 5 per cent who have a chromosome change that would have been detected by looking at chromosomes down a

microscope). So for every hundred children who have a SNP array 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, such as sequencing, may be appropriate.

How do I request a SNP array for my child?

SNP array tests are widely available and in most areas in the UK have replaced conventional chromosome analysis (karyotyping) and an earlier type of microarray called array Comparative Genomic Hybridisation (aCGH). If your child has already had a conventional chromosome analysis or an aCGH test that had a normal result you may like to discuss with your geneticist or paediatrician the availability and potential benefit of a SNP array test versus alternatives such as sequencing. The vast majority of Unique families in the UK who have had a SNP array test for their child had the test within the NHS. If the SNP array is not available in your local genetics service, the sample can be sent to an NHS lab where the test is available. Your geneticist or genetic counsellor will be able to advise you further.

Abbreviations

CNV = copy number variant

SNV = single nucleotide variant

SNP array = Single Nucleotide Polymorphism array

aCGH = array Comparative Genomic Hybridisation

VUS = variant of uncertain significance

Pathogenic variant = genetic variant that causes symptom and features

Benign variant = genetic variant thought not to have an effect
on development and functioning

Inform Network Support



Understanding Chromosome & Gene Disorders

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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 Dr Philippa May and Professor Caroline Ogilvie, Guy's Hospital, London together with Unique (AP).

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