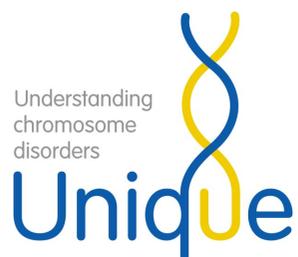


Inform Network Support



Rare Chromosome Disorder Support Group

G1, The Stables, Station Rd West, Oxted, Surrey. RH8 9EE

Tel: +44(0)1883 723356

info@rarechromo.org | www.rarechromo.org

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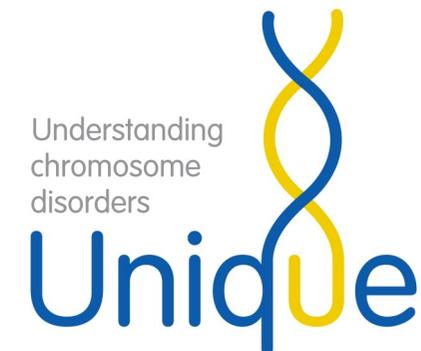
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Please help us to help you!

The publication of this guide was made possible through the generous support of The Worshipful Company of Grocers

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 Caroline Wright, DDD project manager, Wellcome Trust Institute, Cambridge, UK and Dr Helen Firth, Consultant Clinical Geneticist, Cambridge, UK.

2013 Version 1.0 (SW)
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DNA sequencing: whole genome and exome sequencing

What are chromosomes and DNA?

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. Chromosomes (and genes) usually come in pairs with one half of each chromosome pair being inherited from each parent. Humans have 23 pairs of chromosomes giving a total of 46 individual chromosomes. Of these 46 chromosomes, two are the sex chromosomes that determine gender. Females have two X chromosomes and males have one X chromosome and one Y chromosome. The remaining 44 chromosomes are grouped in 22 pairs, numbered 1 to 22 approximately from the largest to the smallest. Each chromosome has a short (p) arm and a long (q) arm. The full set of genetic information, packaged on the chromosomes, is called a [genome](#).

DNA is a code written in only four 'letters' (known as nucleotides), called A, C, G and T. There are around six billion letters in every human genome, and the largest chromosome (chromosome 1) contains around 250 million letters. The meaning of this code lies in the sequence of the letters A, C, G and T in the same way that the meaning of a word lies in the sequence of alphabet letters.

Every chromosome contains thousands of [genes](#) which may be thought of as individual instruction booklets (or recipes) that contain all the genetic information telling the body how to develop, grow and function. The human genome contains around 20,000 genes. A gene is a functional region of DNA that provides the instructions to make ('code' for) a protein. Proteins play many important roles in the body. They do most of the work in cells and are required for the structure, function, and regulation of the body's tissues and organs.

Looking at chromosomes (chromosome analysis)

Chromosomes cannot be seen with the naked eye, but if you stain them and magnify them many hundreds of times under a microscope, you can see that each one has a distinctive pattern of light and dark bands. By looking at your 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. A recent enhanced test is called a microarray-based comparative genomic hybridisation ([array CGH](#)) test. Unique has a separate guide on Array CGH. Array CGH allows detection of DNA imbalances that are smaller than can be detected by looking down a microscope. These smaller alterations are often called microdeletions and microduplications. However, a more recent advance in technology that can detect even smaller changes is [DNA sequencing](#). Sequencing can detect alterations as small as a change in a single nucleotide ('letter').

but they may have implications for your child's future health or development, or the health of other family members. Currently these results are not usually shared with patients because it is hard to know what they mean.

What if the sequencing does not give a diagnosis for my child?

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. There is currently a lot of research being done to understand genomes, so it is possible that a diagnosis will be found in future.

How do I request sequencing for my child?

Presently genome sequencing is usually only available as part of research projects such as the DDD project. However, in the future it is likely that sequencing will become more widely available.

Notes

What are the limitations of sequencing?

Unlike single gene diagnostic sequencing, interpreting the result from whole genome or exome sequencing is very complicated. Every person has a unique DNA sequence and there are lots of tiny genetic (DNA) differences between all of us, some common and some rare. This makes finding the genetic differences that cause a particular developmental disorder especially challenging. These DNA changes are known as **copy number variants (CNVs)** or **single nucleotide variants (SNVs)**. CNVs and SNVs are common in the general population and are often completely harmless. These are known as **benign** CNVs and SNVs. But sometimes a CNV or SNV can affect health or development. These are called **pathogenic** CNVs and SNVs. However, your child's sequencing results may include a DNA change whose significance is not yet known (known as CNVs and SNVs with **uncertain significance**). These variations (CNVs and SNVs) can make interpreting the test results very difficult. In addition, some DNA changes, such as highly repetitive changes (for example those found in Huntington's disease), are hard to detect using current genome sequencing technologies. For disorders caused by these sorts of changes, a different technique is used.

Can I have prenatal testing using sequencing?

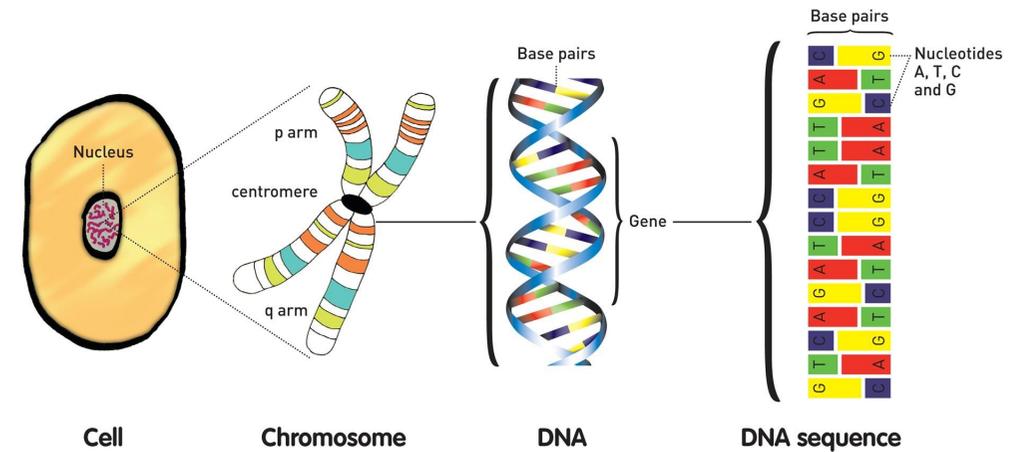
As sequencing is such a new and emerging technology it is not yet used in prenatal testing and further research is needed. However, if a genetic variant identified through sequencing and confirmed to be causal for a serious condition has been found, then prenatal testing may be a possibility using CVS at 11 weeks gestation, or even perhaps in future by PGD (pre-implantation genetic diagnosis).

Will sequencing change my child's treatment?

Detecting a chromosome or DNA change by sequencing might offer a genetic explanation for any 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 duplicated, deleted or changed 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 or DNA change.

What else can sequencing reveal about me?

A human genome contains many changes, some of which are relevant to current or future diseases and other personal characteristics. Like array CGH, a sequencing result may raise unexpected genetic risks unrelated to your child's original reason for referral. These findings are often called 'incidental' or 'unexpected' findings as they do not relate to the reason your child was tested



What is sequencing?

Sequencing involves reading the exact order of letters - As, Cs, Gs and Ts - along a piece of DNA. This is the most detailed genetic test possible. It allows us to read a person's genome from start to finish, or to dip in and out and read selected regions of particular importance. Your child's sequencing result will then be compared to standards and references of what is common in the population and the result interpreted by laboratory and clinical genetics specialists. Using sequencing, it is possible to detect spelling mistakes, as well as extra or missing words. This technology means that almost any changes to the DNA that might have caused the child's developmental disorder can potentially be found. However, interpreting the sequence data is a highly complex task and it will be some years before the full diagnostic potential of this technology is realised.

What are the different types of sequencing?

There are several possible ways to undertake sequencing for clinical purposes:

■ Whole genome sequencing

This is when a person's entire ('whole') genome is sequenced. It is known that less than two percent of the genome codes for protein. Genes consist partly of coding DNA (known as **exons**) and partly of non-coding DNA. The entire coding DNA that is contained in all the exons is called the **exome**. The rest of the DNA is made up of the same four letters as coding DNA but it doesn't have the same meaning. We are not sure what all of this non-coding DNA is for. We know that some of it controls when genes are switched on or off and other bits are important for the structure of chromosomes.

■ Exome sequencing

It is known that most of the changes that occur in DNA sequences that lead to

genetic disorders are located in the coding part of the genes (exons). Therefore, sequencing the exome ([exome sequencing](#)), rather than whole genome sequencing, is currently a more efficient and economical way to sequence a person's DNA to discover most of the genetic causes of diseases, disabilities or developmental delay.

■ Panel sequencing

It is also possible to sequence a subset of specific genes, such as all those known to be involved in retinal (eye) disease or cancer. This is even cheaper than exome sequencing, but limits the possibility of finding new diagnoses.

■ Single gene sequencing

This is the traditional approach but requires the clinician to select which one of 20,000 genes to select for testing. This is possible for some disorders, for example cystic fibrosis, but not for the majority where a similar clinical condition can be caused by changes in a variety of genes, making it difficult to select which gene to sequence.

What samples are needed for sequencing?

Sequencing can be performed on any DNA sample from an adult or child, including blood or saliva.

Why has sequencing been offered for our child?

Genome sequencing is particularly valuable for serious undiagnosed disorders where a small genetic change(s) somewhere in the genome is likely to be the cause of the disorder. In such cases, older technologies such as those available routinely in clinic may be unable to find the DNA change. Genome sequencing is not yet routinely available in a clinical setting. You may have been offered sequencing as part of a research project, such as the Deciphering Developmental Disorders (DDD) project (<http://www.ddduk.org>). It is likely that sequencing will become more routinely available in the future.

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 referral to a geneticist (if appropriate) once family studies (if needed) are completed.

How long do the results take?

As whole genome or exome sequencing is a new and emerging technology, results may take some time to be reported back to parents. Sequencing results obtained in the DDD project are currently taking at least a year, often more.

Interpretation of the results

Sequencing is a way to find changes in a person's DNA including small missing (losses) or extra (gains) of parts of the chromosomes and/or genes, or changes in a single nucleotide within a gene. Many of these changes are very common and seem to have little or no medical effect. Some are relatively frequently seen and have a well understood pattern of medical problems. However, some sequencing results are rare and may never have been seen before. In most cases, it is not yet possible to work out if a particular DNA change will cause a problem or not, or what medical problems may be expected. Parental testing can sometimes help to clarify this type of uncertain result.

My geneticist says my child has a change in a specific gene. How do I find out what this gene does?

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 management of your child's health to know which gene is affected. If you would like further information on the specific gene(s) involved in your child's disorder, 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 sequencing?

Sequencing provides the most comprehensive analysis of chromosomes and DNA available to date. Therefore, many more children are likely get a diagnosis from sequencing than from other genetic tests. Additionally, sequencing can detect changes in DNA and chromosomes when there are no clues to what a child's diagnosis might be and so would not be detected by performing specific genetic tests. Receiving a diagnosis from sequencing may avoid your child having to undergo many other tests in order to discover a reason for your child's difficulties.

A diagnosis may help you and your doctor to watch for common health problems that occur with your child's DNA change and may help predict what to expect as your child gets older. 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. Some parents choose to join a support group to meet other parents facing similar challenges.

Additionally, when a specific DNA change is detected, 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 or DNA change.