



Headlines

Craniofacial Support

No 1

What causes Craniosynostosis?

**a general discussion,
with a focus on genetic aspects (syndromes)**

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Introduction

Craniosynostosis is the medical term used to describe the premature fusion of the sutures (seams) that separate the skull bones. It is not one condition, but a group of conditions with different causes. This article is written with an emphasis on the genetic causes; however, it is important to emphasise at the outset that a *majority of craniosynostosis does not have a simple genetic cause*. At present, only about 2 in 10 (20%) of cases have a demonstrable genetic origin and although more research needs to be done, it is unlikely that more than 3 in 10 (30%) of cases have a simple genetic cause. The reasons that this article focuses on the genetically determined minority are that (i) on average, the affected individuals have more complex problems and (ii) to provide a guide through some of the technical language of genetics for those affected by a genetically determined condition. This article is organised under the following broad headings:

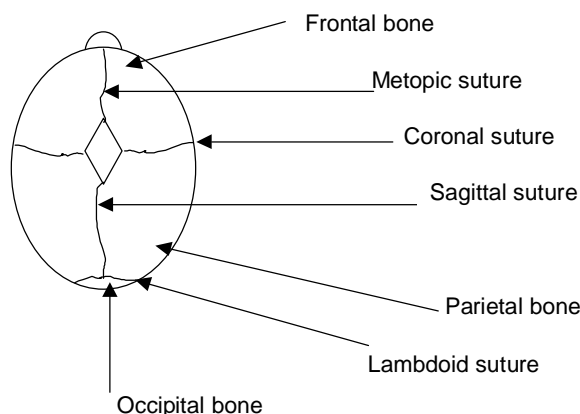
1. How craniosynostosis is classified. Classification is important when thinking about causes.
2. Common causes of craniosynostosis: mechanical constraint and alterations in the genes (mutations).
3. A focus on the genetic causes of craniosynostosis. You will see that the question "What causes craniosynostosis?" can be answered at several different levels.
4. Practical information about genetic risks and prenatal diagnosis.

1. Classification

There are two main ways to classify craniosynostosis:

- By the suture/s that is/are closed
- By additional physical features that may enable recognition of a pattern or 'syndrome'

As a baby's head gets bigger, growth occurs along the seams, or sutures, of the skull bones. The diagram on the next page identifies the names of the sutures and skull bones. The sutures commonly involved in craniosynostosis are the sagittal, coronal and metopic sutures. If one of these closes up prematurely, growth of the skull along the line of the suture is arrested. For the brain to continue to grow, it must expand in other directions, which it does by stimulating the growth at the sutures that remain open. The result is a skull with an unusual shape.



Craniosynostosis affects 1 in 2,500 children, so it isn't that uncommon. A breakdown of the pattern of suture involvement is roughly as follows:

Sagittal	45%
Coronal (unilateral or bilateral)	20%
Metopic	15%
Lambdoid	<2%
Multiple sutures	5%
Associated malformations (syndromes)	15%

It is apparent that the commonest single suture synostoses, in descending order of frequency, are sagittal, coronal and metopic. In the majority of cases the facial appearance is normal and there are no other associated problems, so the condition is recognised solely from the consequent change in head shape. In sagittal synostosis the head is narrow from side to side and long from front to back, a shape termed *scaphocephaly*.

Metopic synostosis creates a ridge down the forehead and the eyes are closer together; it is easier to see if viewing the head from above, when the triangular shaped forehead is apparent. This shape is termed *trigonocephaly*. In coronal synostosis, either both sides (bilateral) or just one side (unilateral) are affected. In bilateral cases the consequence is a symmetrically broad head that may be flat from front to back (*brachycephaly*) and /or tower-shaped (*turricephaly*). By contrast, if just one of the coronal sutures is affected, restricting growth on one side of the face, an asymmetry is created, pulling back the eye socket on the affected side with compensatory overgrowth on the unaffected side. This is termed *anterior plagiocephaly*. These different types of craniosynostosis are explained further in Headlines leaflet 3 *Non-syndromic craniosynostosis*.

Craniosynostosis syndromes

Unlike craniosynostosis that occurs just in the skull vault, in syndromic cases the little sutures of the base of the skull are affected as well, giving rise to a more complex abnormality, influencing facial as well as skull development. This makes the surgical management of these children more difficult and also means they more frequently have additional problems with mental development, vision, hearing, breathing (because the nasal passages are small) and the teeth (because the upper jaw is under-developed).

Of the many recognisable syndromes involving craniosynostosis, five are commonly encountered (although, as outlined above, together they only comprise about 15% of all cases). These are Crouzon, Pfeiffer, Apert, Saethre-Chotzen and craniofrontonasal syndromes (another syndrome that is not so readily recognised, Muenke syndrome, is mentioned later in this article). The first three syndromes are associated with a similar facial appearance. The eyes are rather prominent because the sockets are too shallow, pushing the eyeball forward; the middle part of the face is under-developed, because there is a deficiency in the growth of the underlying bones; the nose may be beak-shaped and the lower jaw prominent. However, we can't readily distinguish Crouzon, Pfeiffer and Apert syndromes by looking at the facial appearance, because it is rather similar; instead, we examine the hands and feet. In *Crouzon syndrome* the hands and feet appear normal (although on X-ray you might find that some small bones are fused together). In *Pfeiffer syndrome* the big toes and sometimes the thumbs are broad, occasionally with mild skin webbing between some of the other digits. In *Apert syndrome*, at least the central three digits are fused together and this involves the underlying bone as well as the skin.

Although the facial appearance of these three syndromes is similar, there are differences in the way that the craniosynostosis behaves. Research performed by the Paris group, in which children were followed up for several years, showed that in a typical child with Crouzon syndrome, the sutures tend to close simultaneously. This means that the skull proportions remain relatively normal, but brain growth may be restricted, so a common problem is raised intra-cranial pressure (ICP). Additionally, the brain can begin to push down through the exit at the bottom of the skull, causing pressure on the spinal column which may lead to problems with walking. By comparison, in Apert syndrome the coronal sutures usually close much earlier than the others; the expanding brain then splays the plates of bone away from the mid-line, leaving a wide central cleft through which the forehead bulges. The craniosynostosis associated with Pfeiffer syndrome tends to be most variable, including both of the above patterns as well as a severe form, caused by the early fusion of multiple sutures, termed cloverleaf skull.

Saethre-Chotzen syndrome is rather more difficult to diagnose. The eyes are slightly wider spaced, the head is broad, the hairline is low and the eyelids can be a bit droopy. The face tends to be flat and the ears small, but the features are all rather subtle. Sometimes the limbs can be affected, with webbing between the 2nd and 3rd digits of the hand and a broad big toe.

Finally, *Craniofrontonasal syndrome* is the rarest of the conditions I have mentioned. The great majority of affected individuals are female. It involves a combination of craniosynostosis (affecting the skull) with *Frontonasal dysplasia* (affecting the front of the face and nose). Here the eyes are very widely spaced and the nose is broad with a groove down the mid-line. Another characteristic feature is the longitudinal splits in the nails. In addition, the digits may have disproportionate lengths, sometimes with webbing and/or extra digits, and the shoulders are sloping.

Further information on these syndromes is provided in several leaflets produced by Headlines.

2. Causes of craniosynostosis

There is a whole diversity of causes of craniosynostosis, many of which are rare. There are two common causes:

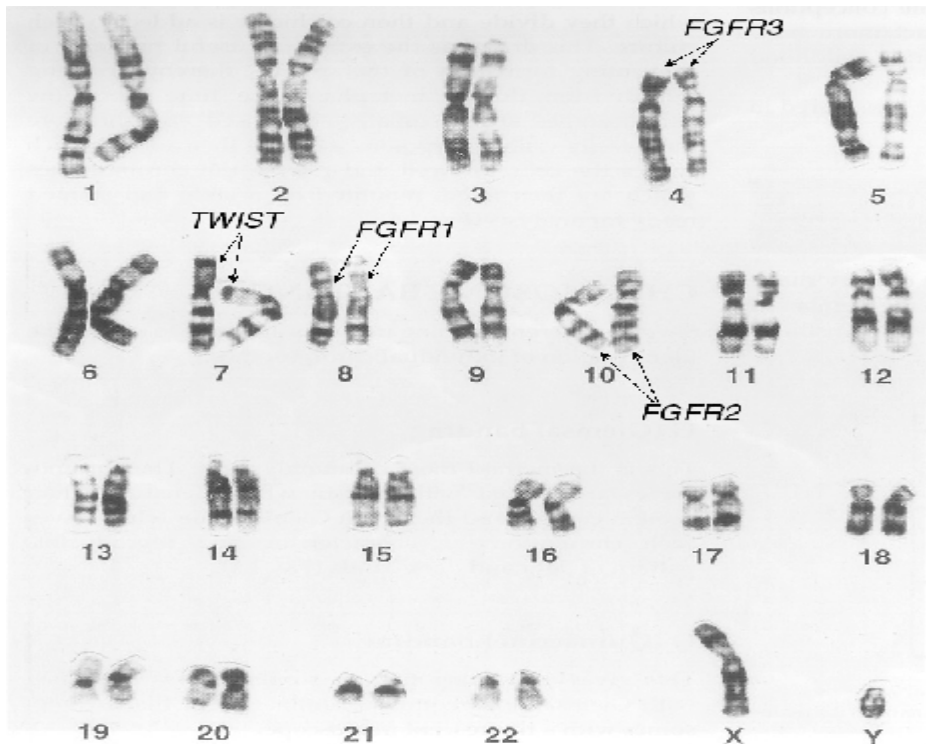
Mechanical constraint

My hunch is that this is the single most common cause of craniosynostosis, although in the individual case, the evidence for its contribution is usually at best circumstantial. In non-syndromic cases, it is common for the mother to say that during the pregnancy she had a feeling of discomfort under the rib cage, that the baby was in an unusual position inside the womb, or was one of twins. In these cases, the abnormal pressure on the developing skull may push the bones together, causing bony bridging across a suture. Once this bridging occurs, the affected suture fuses along its length rather like a zip.

If mechanical constraint is a contributing factor, the genetic predisposition is usually low and the chance that craniosynostosis will recur in another pregnancy is relatively small (between 1 in 50 and 1 in 20, or 2-5%). This is why your doctor may ask questions about the pregnancy and delivery to elicit these details. Incidentally, parents often worry that their baby's craniosynostosis has been caused by a difficult delivery. In fact, it is more likely that the child already had craniosynostosis and the unusual head shape hampered its progress down the birth canal. Babies born extremely prematurely also more frequently have craniosynostosis; because they cannot lift their head, it tends to stay in the same position for long periods, causing abnormal pressure on the skull bones.

Genetics

People get rather scared about genes, but I assure you it's really very simple! Basically, genes are the chemical instructions (code) for making the proteins (building blocks) of our bodies. Each cell in the body has the same combination of genes, which are packaged into chromosomes. If you look down a microscope you can actually see the chromosomes. We all have twenty-two pairs of chromosomes plus the sex chromosomes (either XX for a girl, or XY for a boy). The picture shows the chromosomes from a boy, numbered in pairs from 1-22, with a single X and Y. In the cell they are all jumbled up together, but for ease of visualisation they have been lined up side by side with their partners. Note that each chromosome is present as a pair, except for the unpaired X and Y. In the eggs and sperm, only one chromosome from each pair is present, so when an egg and sperm come together at fertilisation, you get back to a pair again.



Chromosomes are like a string of chemical information. Although you can see the chromosomes, the individual bits of information are much too small to see down a microscope. There are six billion bits of chemical information in each full set of paired chromosomes, present in every cell in the body. If each was a tennis ball, enough to lay in a line to reach to the moon.

In the case of craniosynostosis, if you look down the microscope at the chromosomes they usually look normal. However occasionally there is a change, where a piece of material has been swapped from one chromosome to 'land on' another (called a translocation). There is no point looking for this change in a classic case of Crouzon, Pfeiffer or Apert syndrome but it can be quite important in the investigation of Saethre-Chotzen syndrome. This is because occasionally translocations or deletions of chromosome 7 can disrupt the *TWIST* gene, the gene that is altered in Saethre-Chotzen syndrome (see below). Each gene has a specific "address" in the chromosome makeup; the positions of the most important genes in craniosynostosis are shown in the picture.

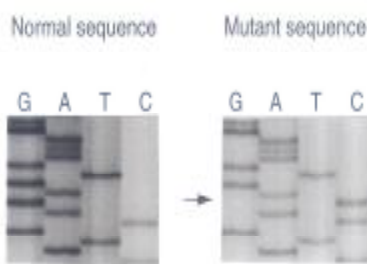
3. Focus on genetics

So what is a gene?

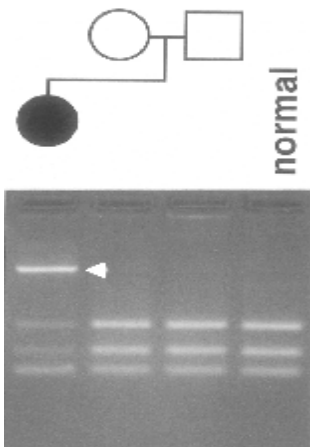
A gene is an instruction for making a specific protein. Different proteins have different functions – some provide structural building blocks, others serve as chemical signals and yet others are components of chemical factories. It's been estimated there are about 35,000 genes in total, but only four of these are currently known to be significant in craniosynostosis (the gene involved in craniofrontonasal syndrome has not yet been identified). Three genes belong to the same family, the fibroblast growth factor receptors: these are *FGFR1*, *FGFR2* and *FGFR3*. The fourth gene is unrelated to the others and is called *TWIST*. The *TWIST* gene was originally identified in the fruit fly and is so-called that because the fly's developing embryo has a twisted appearance if this gene isn't working properly. Surprisingly, our genes are remarkably similar to those of flies, so you can identify in the human the exact equivalent of many fly genes. Flies don't have skulls of course, so in humans the *TWIST* gene has been adopted to have an additional function in skull development.

Each gene has a specific position on a specific chromosome. The *FGFR2* gene, involved in Crouzon, Pfeiffer and Apert syndromes, is on chromosome 10; the related genes *FGFR1* and *FGFR3* are on chromosomes 8 and 4, respectively; *TWIST*, as already mentioned, is on chromosome 7. Because the chromosomes occur in pairs, so do the genes; one copy of every gene pair is inherited from our mother, the other from our father (see picture of the chromosomes on previous page).

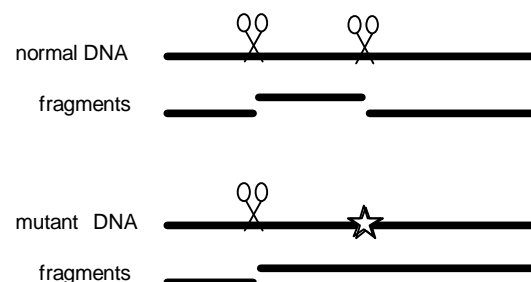
Genes themselves comprise of a string of chemicals, called DNA, that make a code. We can decipher this code and are now close to being able to read the entire DNA sequence of the human body. DNA is made up of four different chemicals called A, C, G, and T. Imagine that these are four different coloured beads that can be threaded on a string. The sequence of colours (chemicals) at any particular point on the gene occurs in a very specific order. This sequence is critical for the body to decipher the code and make the correct protein. Most of the genetic changes (termed *mutations*) that cause Crouzon, Pfeiffer, Apert and Saethre-Chotzen syndromes are changes to the identity of a single chemical in one of these genes, which cause an altered protein to be made. That's literally a single change in the six billion bits of DNA present in every cell.



To give you more of a grasp of how we detect these tiny changes, the pictures show illustrative examples of mutations of the *FGFR2* genes in children with Crouzon and Pfeiffer syndromes. The figure (above) shows the DNA sequence, which can be read off like a barcode from the bottom to the top; for clarity, the sequences of the normal and mutant copies of the gene have been separated. Note that the sequences exactly coincide, except at the single position where a G in the normal sequence is replaced by a C in the mutant sequence (arrows). The G to C change in the DNA causes the encoded *FGFR2* protein to substitute a component called "cysteine" for the usual "serine" at one particular position. Unfortunately, the cysteine is damaging to the protein, causing the individual component proteins to stick together abnormally.



The example, on the left, shows the analysis of a short segment of DNA from the *FGFR2* gene, using an electric current to separate fragments in a jelly-like support (gel). The DNA starts at the top of the gel and large fragments move more slowly than small ones. The fragments have been mixed with a special enzyme that cuts DNA (just like a pair of scissors) only when the sequence G-A-T-C is present. In the piece of *FGFR2* examined, two GATC sequences are normally present so that the fragment is cut into three smaller pieces, as indicated in the diagram.



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These three fragments show up as three parallel bands in the gel. However, in a girl affected with Pfeiffer syndrome (filled circle) a mutation in *FGFR2* has occurred so that one of her genes has the sequence T-A-T-C, which is no longer cut by the enzyme. The position of the mutation is shown as a star in the diagram. This generates a larger fragment that moves more slowly through the gel (white arrowhead). We can use this method to compare the DNA between the child and her parents (who are shown as the unfilled circle and square). In the example shown, the parents' *FGFR2* genes cut normally at this point: this means that there has been a *new mutation in the child's gene*. Why such mutations occur is discussed in the later section "Why us?"

Inheritance

Most craniosynostosis syndromes show *dominant* inheritance. This occurs when you only need an alteration in one of the pair of genes to cause the condition. When someone affected by the condition has children, each sperm or egg that they make contains only one of the two genes. Therefore, it's 50:50 whether each offspring will be affected or unaffected by the condition. If the child is affected, they are then in the same situation as the parent – they have a 50:50 chance of passing it on to their child. If the child has inherited the normal gene copy, then not only are they unaffected, they cannot transmit the condition to their own children either.

In contrast, the other type of inheritance is *recessive*, where the condition only occurs when both genes copies are malfunctioning. People with one gene copy working and the other not working are perfectly healthy and are termed "carriers". When two people who happen to carry the same alteration have children, 1 in 4 of their children will inherit the mutant gene from both parents. These individuals have no normal copies of the gene and will have the condition. However, recessive inheritance seems to be fairly unusual in the context of craniosynostosis.

Specific mutations occurring in craniosynostosis

The three FGFR genes are closely related to each other, and very specific mutations in these genes give rise to very specific syndromes. For example, 99 out of 100 children with Apert syndrome have one or other of two particular changes in the *FGFR2* gene. Crouzon and Pfeiffer syndrome tend to have a slightly wider range of possibilities, but the mutations are still mostly concentrated within a single region of the *FGFR2* gene.

There is also a specific change (termed P250R) in the *FGFR3* gene. This was only recognised in 1996 and is associated with a condition that is now called Muenke syndrome. This syndrome cannot be confidently recognised by looking at the facial features, but we now know from genetic testing that it is a rather common cause of coronal synostosis.

The situation for the *TWIST* gene is different because instead of generating an altered protein like the FGFRs, the *TWIST* mutations stop the protein working altogether. Hence a much greater diversity of changes in this gene cause Saethre-Chotzen syndrome than is the case for conditions caused by FGFR mutations.

To give you an idea of the relative frequency of the different mutations, in a four-year period at the Oxford Craniofacial Unit, 32 patients had a clear genetic basis for their condition. *FGFR2* mutations were most common (35%), followed by mutations in *FGFR3* (28%) and *TWIST* (22%). Less common were craniofrontonasal syndrome (9%) and miscellaneous chromosome abnormalities (6%), whilst no *FGFR1* mutations were identified in this group.

When molecular diagnosis matters

Generally speaking you don't need a genetic test to make a diagnosis of (for example) Crouzon or Apert syndrome - an experienced doctor can usually do this simply by carefully examining a child. But there are three situations where it is very helpful to have a genetic test.

The discovery of the P250R mutation in the *FGFR3* gene, described by Dr Muenke and colleagues in 1996, identified a new syndrome defined by the specific gene change rather than based on a pattern of recognisable clinical features (see previous section). If your child has non-syndromic coronal synostosis, it is very important for him/her to be tested for this mutation, because about 3 in 10 children test positive. However, this test is not necessary in isolated sagittal or metopic synostosis. Muenke syndrome is described in Headlines leaflet 15.

Another important observation has emerged from testing of the *TWIST* gene. Either there may be a very specific change within the gene itself, or the entire gene is deleted out with other genes either side of it. These latter, deletional cases of Saethre-Chotzen syndrome are more likely to be associated with learning difficulties, owing to the additional missing genes.

Finally, molecular diagnosis is important if you wish to have further children and want to have an accurate assessment of the risks of the recurrence of the condition.

FGFRs – what are they and what do they do?

A fibroblast growth factor receptor (FGFR) is a protein that binds to a fibroblast growth factor (FGF), another protein which specifically 'fits' the receptor structure. This binding sends a chemical signal to the cell. The two common mutations in Apert syndrome occur in the linker between the two FGF-binding regions of the receptor. When the FGF protein binds to the FGFR2 Apert mutant, it sticks harder and so signals to the cell for a longer period. So the FGFR2 protein in Apert syndrome is 'over-active'.

Another way to pose the question "What causes craniosynostosis" is to ask what is happening in the cranial sutures. We cannot investigate this in humans, because craniosynostosis arises in the fetus during the pregnancy. To find the answer, we have to study the process in animals. Research on mouse embryos shows that the FGFRs are very important within the suture itself and act at a stage before the bone is made. In the case of FGFR2, the receptor seems to be important in maintaining the cells in a growing state. If the protein is over-active because of a mutation, the cells stop multiplying, and instead start making bone. That's how you end up with craniosynostosis.

Why us?

The final level of looking at the question posed in the title is to ask "What causes these mutations to occur in the first place?" Earlier I illustrated a case where a child with Pfeiffer syndrome has the mutation and the parents clearly don't have it – it started in the child. We know that making each egg and sperm involves copying three billion pieces of chemical information (which combine to make the DNA in the cell). Whenever we undertake a complex copying process, things go wrong. Imagine sitting down with a large encyclopaedia and, starting with the letter A in the first volume, copying out each entry, line by line, and moving volume by volume to Z. Inevitably some mis-copies would occur, however careful you were. The same is true for the egg and particularly (in this context), the sperm. In fact, every time we have a child, it has been estimated that the child has about 100 mis-copies that weren't present in the parents. In other words, the process of miscopying (mutation) is an entirely normal and inevitable part of having children. Usually, these miscopies have no consequence, so we cannot tell that they have occurred in our children. Occasionally, a miscopy occurs at a critical position in a critical gene, and the baby is born with a specific genetic disorder.

When do mutations happen?

The explanation in the previous section presented the situation where the mutation arose at conception, but this is not the only time that it could have occurred. Asking the question "*when* did the mutation happen?" is important, because it will determine whether the condition could recur in another child, or whether it was probably just a one-off.

Copying of the complete genetic information occurs every time a cell divides. If the mutation was present at conception, it will be passed down to every cell in the growing person. Alternatively, the embryo could start off genetically normal with the mutation arising later during embryonic development. This would have different consequences. For example, if the mutation occurred in a cell destined to make eggs and sperm, the change would be present in multiple eggs or sperm but wouldn't show up in the rest of the body (including the blood). When they grew up, the person would appear physically normal and a genetic test of the blood would also be normal (because the mutation was only present in the egg or sperm); yet they could be carrying multiple eggs or sperm with the same mutation and therefore, could have another child with the same condition. This process is termed *germline mosaicism*.

On the other hand, imagine that the mutation occurred at a later stage when the person was already an adult and in the final process of making an individual egg or sperm, then that mutation will only be present in that particular egg or sperm. In that case, the chances of another egg or sperm being affected would be extremely small, so it would be very unlikely that another child would have the condition.

The paternal factor

As explained above, to give parents accurate risks for having another affected child, we have to work out when the mutations are arising. One thing we have learnt about FGFR mutations is that if you look at the ages of fathers of children with Crouzon, Pfeiffer and Apert syndromes, they tend to be somewhat older than average. Many children with these conditions are born to relatively young parents, because most parents have children when they are in their 20s or 30s. Nevertheless, if the population age distribution of parents is taken into account, it can be shown that between the ages of 20 to 50, the risk of fathering a child with Crouzon, Pfeiffer or Apert syndromes goes up about twenty-fold. It is possible to extend this analysis a bit further and find out from which parent the *FGFR2* mutation originated. In the case of Apert syndrome, out of 55 families studied, in all 55 the mutation originated from the father. A similar conclusion has been reached in Crouzon and Pfeiffer syndromes, based on rather smaller numbers.

Given that the mutation probably came from the father and that the risk increases with age, this suggests it is not something that occurred when the father himself was an embryo. Rather, it looks as if the miscopy is occurring in connection with the ageing process. So for a healthy couple who have had a child with craniosynostosis due to an *FGFR* mutation, the chances of having another affected child are very small, under 1 in 100 (1%).

4. Some practical considerations

Risk assessment

This section summarises the risks of having another child with craniosynostosis. We first consider the situation where a causative mutation has been identified in the family.

We have to separate those cases where one parent is themselves affected and carries the mutation, and those where both parents are unaffected and have been tested negative for the mutation. In the case where a parent is affected, the risk of transmitting the condition to each child is 1 in 2 (50%). In the case where both parents are unaffected and the mutation identified is in one of the *FGFR* genes, then, as explained in the previous section, the risk of another child with the same condition is small, less than 1 in 100 (1%). If the mutation is in the *TWIST* gene, however, we don't know at the moment how common germline mosaicism is, so the risk might be higher (a few percent).

If the condition is non-syndromic sagittal or metopic synostosis, a major causative factor may be head constraint during the pregnancy, and it is unusual (but not unheard of) to have another affected child. The risks are a little bit higher in coronal synostosis - about 1 in 20 (5%) and that's if the specific condition of Muenke syndrome has been excluded.

Prenatal testing – its uses and limitations

Finally, a few words about a prenatal diagnosis (diagnosis of craniosynostosis in the unborn baby). There are two general approaches that can be taken:

1. *Ultrasound scanning.* The advantage is that it is non-invasive (that is, it exposes the baby to no extra risk of miscarriage). The disadvantage is the cranial sutures only develop in the baby at 16 weeks of pregnancy, so any scan performed before this time to look for craniosynostosis will inevitably be normal. Routine scans are usually done at 16-20 weeks, when there has been very little time for any distortion in the skull shape, as a result of craniosynostosis, to have occurred. Routine ultrasound scanning of the skull is therefore not reliable, although it may detect some very severe cases. What can be detected at this stage is the bony syndactyly of the hand in Apert syndrome – but you have to be specifically looking for this and it requires good equipment, an experienced operator and lots of patience to obtain good views of the tiny fetal limbs.
2. *Genetic testing.* It is only possible to do prenatal genetic testing for craniosynostosis if a specific mutation has already been identified in the family. If a mutation has been found, testing of the pregnancy is relatively simple technically. The question is how best to obtain genetic material from the growing baby, and whether the risk associated with the procedure (in terms of causing a miscarriage of a potentially healthy baby) is worth taking. This is very much a personal decision on the part of the mother or couple. Chorionic villus sampling (CVS) can be done early in pregnancy, at 11 weeks, but carries a higher chance of causing miscarriage of 1-2 in 100 (1-2%). At 15 weeks, amniocentesis carries a lower risk, about 1 in 200 (0.5%).

Finally I should mention that *all the above information is for guidance only*. If you have any questions concerning the diagnosis or genetic implications with regard to your child, I would strongly recommend that you seek expert advice from a specialist in Clinical Genetics. This can be arranged either by your GP or by the craniofacial team caring for your child.

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It is a revised version of the talk he gave at the Headlines Family Conference in April 2001.

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