4q deletions

***Unique*, the worldwide rare chromosome disorder support group, hosted the first-ever international meeting for families with a child with a deletion from the long arm of chromosome 4 in Coventry, UK in April 2010. Families came from round the world to meet researchers, interested doctors and therapists – and each other. This report contains the presentations that were specific to 4q-. Presentations from the day are also available on *Unique's* website.**

What is 4q- syndrome?

Dr Eugen Strehle, a paediatrician in Newcastle, UK with an interest in 4q, explained that the term '4q syndrome' was first used by Dr Charles Ockey over 40 years ago in 1967.

Twelve years later, Dr Phillip Townes applied the term to a syndrome where the breakpoint is found in band 4q31 and the end of the chromosome is lost. Then in 2001, Dr Strehle suggested that the term should be used to cover all microscopically visible deletions of the long arm of chromosome 4 – that is, any deletion that you can see under a light microscope. Broadly, there are two categories of 4q deletion: interstitial deletions with two breaks and a part missing along the length of the chromosome, and terminal deletions, where the end of the chromosome is lost.

Review of people with 4q- syndrome

Dr Strehle reviewed 100 patients with 4q deletion syndrome and found an incidence of perhaps one in 100,000 with more recent data suggesting perhaps a higher incidence. The male to female ratio was roughly equal at 48:53. Some deletions occurred relatively frequently, for instance 4q12q21 interstitial deletions and 4q31 and 4q33 terminal deletions. The chromosomes of the parents were normal in about 86 per cent of cases. Among those affected, the most typical characteristics were: unusual facial features (craniofacial dysmorphism) in 99 per cent, developmental delay in 94 per cent and unusual fingers and/or toes (digital dysmorphism) in 88 per cent. Growth failure occurred in 60 per cent, skeletal and cardiac anomalies in about half. The mortality rate was almost one third, a reassuring finding compared with earlier reports of 70 per cent, which most likely relates to improved medical care. Other systems - muscles, eyes, skin and hair and the gastrointestinal tract – were involved to a lesser extent. Hearing was commonly affected, with sensorineural or conductive hearing impairment, making it important to test children's hearing. One third of children had a cleft lip or palate or a condition called Pierre Robin sequence characterised by a receding chin, a posteriorly placed tongue and also a cleft palate. There was central nervous system involvement in about 30 per cent.

Dr Strehle suggested this programme of investigations: imaging of the brain; X-rays if the child has any significant abnormality of the arms or legs; an abdominal ultrasound scan; pH studies to test for gastro-oesophageal reflux; an electrocardiogram or echocardiogram because of the common heart anomalies; an electroencephalogram (EEG) for seizures, which are relatively common; and hearing and vision tests. Children with 4q deletion syndrome should be cared for by a multidisciplinary team including a paediatrician, geneticist, a surgeon where needed, a dietician to support growth, a speech and language therapist for any swallowing difficulties, a physiotherapist, occupational therapist, a psychologist because there can be behavioural difficulties, and a special needs teacher.

Array-CGH

Dr Strehle described array-CGH, a way of testing DNA that shows extra or missing chromosomal material throughout the genome. Using this technique, his colleague Dr Taosheng Huang was able to find a potential new gene for cleft lip and palate.

> *"With this technique you can determine to the individual gene where the breakpoint is."*

Pinpointing the gene: a child with a 4q33 deletion

Dr Strehle presented a child with a terminal deletion from 4q33. She was born by caesarean section, with a birth weight of 2.9 kilograms and Apgar scores of 8 and 9 meaning that she was in good condition at birth. She had an occipital encephalocele, which is like a tumour, and this was successfully removed when she was two days old. A brain scan and MRI when she was three months old showed she had a neuronal migration defect, which means that the grey matter of the brain is inserted into the white matter. She also had an Arnold Chiari malformation, where the brain stem is pushed through a hole in the skull and which can lead to hydrocephalus – an increase in the fluid within the brain. She was the first child with an encephalocele reported with a terminal deletion of chromosome 4q. One other child was reported but with a different interstitial deletion at 4q13q23 and unfortunately she died when she was seven months old. There is a condition called Knobloch syndrome that is characterised by eye abnormalities and an occipital encephalocele, caused by a mutation of the *COL18A1* gene

on chromosome 21. It's possible that other genes on chromosome 4q are also involved in the development of encephaloceles and one way of finding out is to study patients like this little girl.

The girl also had severe, complex heart disease but urgent surgery was successful. It is known from Dr Taosheng Huang's work that when some genes are present in only one copy instead of the expected number of two (haploinsufficiency), abnormalities can occur. When the *HAND2* gene on 4q34.1 is missing, cardiovascular malformations can occur.

What were the unusual facial features? She had a large fontanelle (soft spot on top of the head), widely spaced eyes, overfolded ears, a small mouth and chin, overlapping fingers, unusually placed toes and a sacral haemangioma, like a birthmark near the base of her spine.

Normal chromosome tests showed a terminal deletion, not inherited from her parents, at 4q33. A CGH test showed a very large deletion of 25.7 megabases with a breakpoint at 4q32.3. (Bases are the bonds between the two strands of DNA that look like the rungs of a ladder; megabases are one million bases.).

In follow-up she had growth deficiency and feeding difficulties. Her developmental milestones were delayed, walking by 2½, and at 5 saying single words and following a simple instruction. At 5 her unusual facial features were still visible and her short stature was very striking.

In the wider context, a lot of children with abnormalities on any chromosome have learning difficulties and much is not yet understood. Research in animals and humans has shown that the nerve cells in the brain (neurons) have fewer connexions. So something led to decreased networking between the neurons and this may account for the learning difficulties.

Summing up

4q syndrome is a valid disease entity. It is characterised by overlapping features in which other factors may play a causative role. As for the encephalocele, the genes responsible for the development of the basement membrane may reside on chromosome 4 and if abnormal may lead to encephaloceles.

Unique survey

Dr Strehle described a survey conducted through *Unique*. Using an 8-page questionnaire, parents' views at diagnosis and subsequently were elicited. 55 questionnaires were sent out, as well as a short form to five adults, all relatives identified after their child was diagnosed. These people went through life without knowing they had 4q- until their child was born. This suggests there is quite a large number of undetected 4q deletions.

Among the adults was a 28-year-old woman with an interstitial deletion of 4q25q27 who was well and studying for a psychology degree. A man of 47 with a terminal deletion from 4q34 said he had aggressive behaviour and went to a special school but was well. Another man with a similar deletion had a cleft palate and hypermobile joints and was working as an accounting technician; another with a small terminal deletion from 4q35 also reported aggressive behaviour and dyspraxia.

Of 32 returned questionnaires (a 58 per cent response rate), there were 16 interstitial and 20 terminal deletions. The mean age of the children was 11 years, ranging from 10 months to 47 years. The chromosome abnormality was inherited in 16 per cent. All children had some learning difficulty but in almost half it was mild; in one third it was moderate and it was severe in seven. Thirty-eight per cent had growth deficiency; 65 per cent had small hands and feet. Two out of three children with a terminal deletion showed an anomaly of the fifth finger and finger nail in which the finger is small and the nail is not well developed. More than two thirds of children had feeding difficulties, mainly when young, needing nasogastric tube feeding, a gastrostomy and sometimes a fundoplication to prevent reflux.

What were parents' feelings at diagnosis? Parents said that it was the worst experience ever but didn't change the way they loved their child. Others said: 'It knocked the wind out of me' and 'The baby I brought home 'died' that day'. More positively, one family said: 'It gave our son's disability a title'.

Parents felt the diagnosis should be given by someone knowledgeable, in a personal, sincere and sympathetic manner. Some parents criticised the clinician giving the diagnosis for having insufficient knowledge or time to research the disorder thoroughly.

Some professionals were felt to be insensitive, giving an unnecessarily poor prognosis and referring to older literature. As children grew older, parents became more positive and confident and searched for more information themselves. They became more accepting, starting to take each day at a time and not looking too much into the future. There was some criticism of doctors. Many health professionals involved with the child were perceived to either not have the time or to focus on one part of the child, leading to fragmented care.

> *"My personal opinion is that the first two years of life are the most crucial. If congenital abnormalities are present but surgery goes well and is a full correction, then as the child grows older and stronger it's less likely that something severe will happen."*

> *"Often parents become specialists in their child's condition. It's not uncommon that parents will know more after a few years than the people they meet."*

Parents' own knowledge of their child's chromosome disorder was good. The medical advice given to parents was deemed to be cautious.

Some families felt abandoned by the geneticist because they did not offer follow up appointments; it may be more appropriate for a paediatrician to follow families up.

Positive experiences

What were families' positive experiences? They said that bringing up a child with 4q- had enriched their lives and provided new perspectives. Parents referred to their child as 'our gift', 'the best thing that ever happened' and 'a constant reminder of what is important'. Many children surpassed doctors' predictions. Some parents were impressed by the level of care and acquired detailed medical knowledge.

'He made us laugh, worry and cry just like any other child would.'

In conclusion

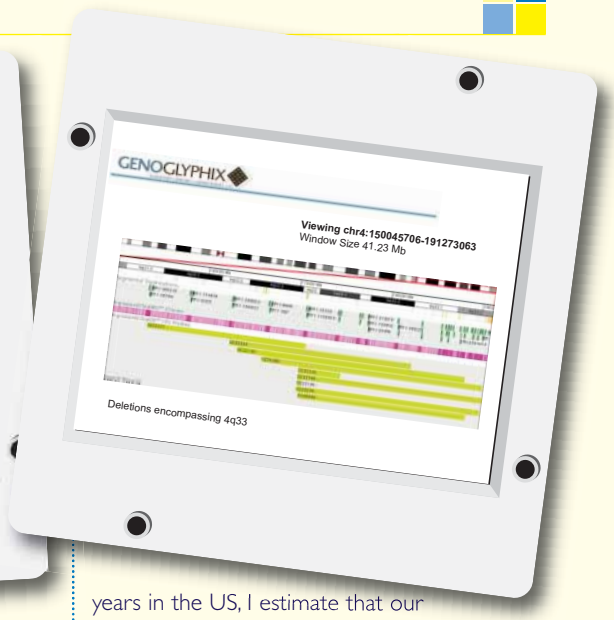
Of the 20–25,000 genes in the human genome, most display no obvious phenotype when one copy of the gene is missing because of a chromosome deletion. That means that the other copy present on the intact chromosome 4 is enough to enable the child's enzymes and proteins to work properly. But alterations in the dose of some genes can lead to disease or even death. In summary, 4q- has a fairly distinctive clinical and behavioural phenotype. Gene dosage effects may contribute to the effects of 4q-. Early diagnosis and multidisciplinary management can improve life expectancy.

Genotype-Phenotype Study

Dr Taosheng Huang is an associate professor with tenure in paediatrics, developmental biology and pathology at University of California, Irvine, USA.

He is also the director for the Cardiovascular Genetics Clinic and the co-director for the MitoMed Molecular Diagnostic laboratory. Clinically, he is interested in genetic syndromes with congenital cardiac defects, metabolic disease and genetic screening.

4q deletions



He talked about his research in creating what is called a genotype: phenotype correlation.

'A phenotype is something you can see or measure, like a picture that you can see. For instance, you can do an echocardiogram for congenital heart disease or you can measure IQ. A genotype is your genetic make-up. In the case of 4q-, if you are missing a gene, what kind of phenotype will you see? My final goal is to try to pinpoint the phenotype to particular genes and then see if we can intervene and help children with 4q- and come up with treatment. Maybe that's a big goal but that's what we aim for.

'The human genome is very complicated with a three-billion-character code within the 46 chromosomes. Chromosomes make a pair, half from the mother and half from the father. Using previous technology, looking at chromosomes under a microscope, even with the best technician and the highest resolution, you can probably see 2000 bands. Since there are 3 billion codes, each of these bands represents one to two million codes, so still a huge number. So the previous technology was used for diagnosis but it is not good enough for the questions I want to answer.

4q- or 4q- syndrome?

'Some people say 4q-, some say 4q- syndrome. The clinical phenotype is very well known in the 4q- group. For some genes on 4q, one copy is not sufficient – haploinsufficiency. That's why you have disease, for example congenital heart disease.

'My research relies on two advances in science – one is the complete sequencing of human genome in 2003, and then advances in array-CGH. People argue: should we say 4q- or 4q- syndrome? 4q- syndrome means there is a constellation, a recognisable pattern. In 4q-, it's very diverse. Each child depends on the deletion, they can be very different from each other. However, many individuals with terminal deletions do look very similar. Typically, they have a very thin upper lip and may have congenital heart disease and limb anomalies. But others look completely different. So we can argue: Should we call it 4q- syndrome or 4q-? As far as terminal deletions are concerned, it's appropriate to talk about a 4q- syndrome.

'How does the first human genome project help us? The genome is like a book but with 23 volumes and two copies of each volume. Before the human genome project finished, you knew you could take one volume or one chapter out of the genome (a deletion), but you didn't know what that chapter said. The human genome project then spelled out each word in the whole volume.

'The first version of the human genome project cost three billion dollars and took 13 years to finish. Now technology has advanced so much that I can do a genome in one week, and it would cost 50,000 dollars. Being able to read what each chapter says is critical and is a critical component of my research.

Array-CGH

'We are now using third generation array-CGH with a chip that is even smaller than a matchbox and has increased the sensitivity of the test 100–1000-fold compared with karyotyping. In the past I could only see when a whole book or a chapter was missing. Now I can see the detail in the book down to each paragraph. To use a cookbook analogy, once I know what a paragraph means, I can cook macaroni cheese!

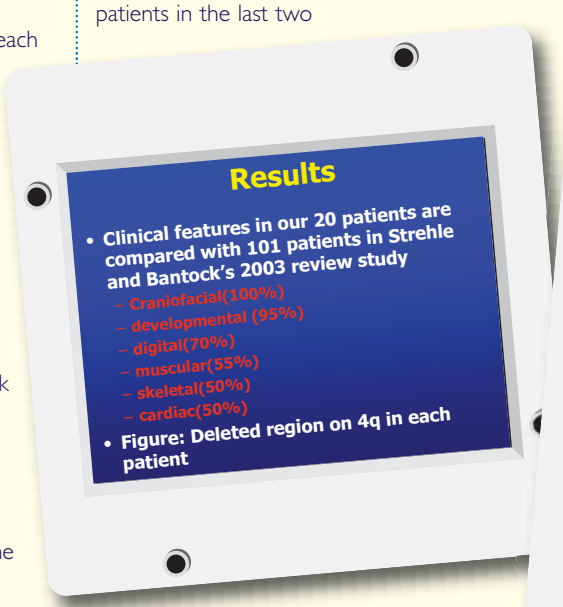
'We now realise that 4q-/4q- syndrome is way more common than we thought. We missed a lot of people with tiny deletions when we only looked under a microscope. In just one laboratory in a two-year period in the US we have 150 patients. Since we have probably made the diagnosis of 4q- in around 500 patients in the last two years in the US, I estimate that our cases represent less than one-third of the diagnoses.

Common break points

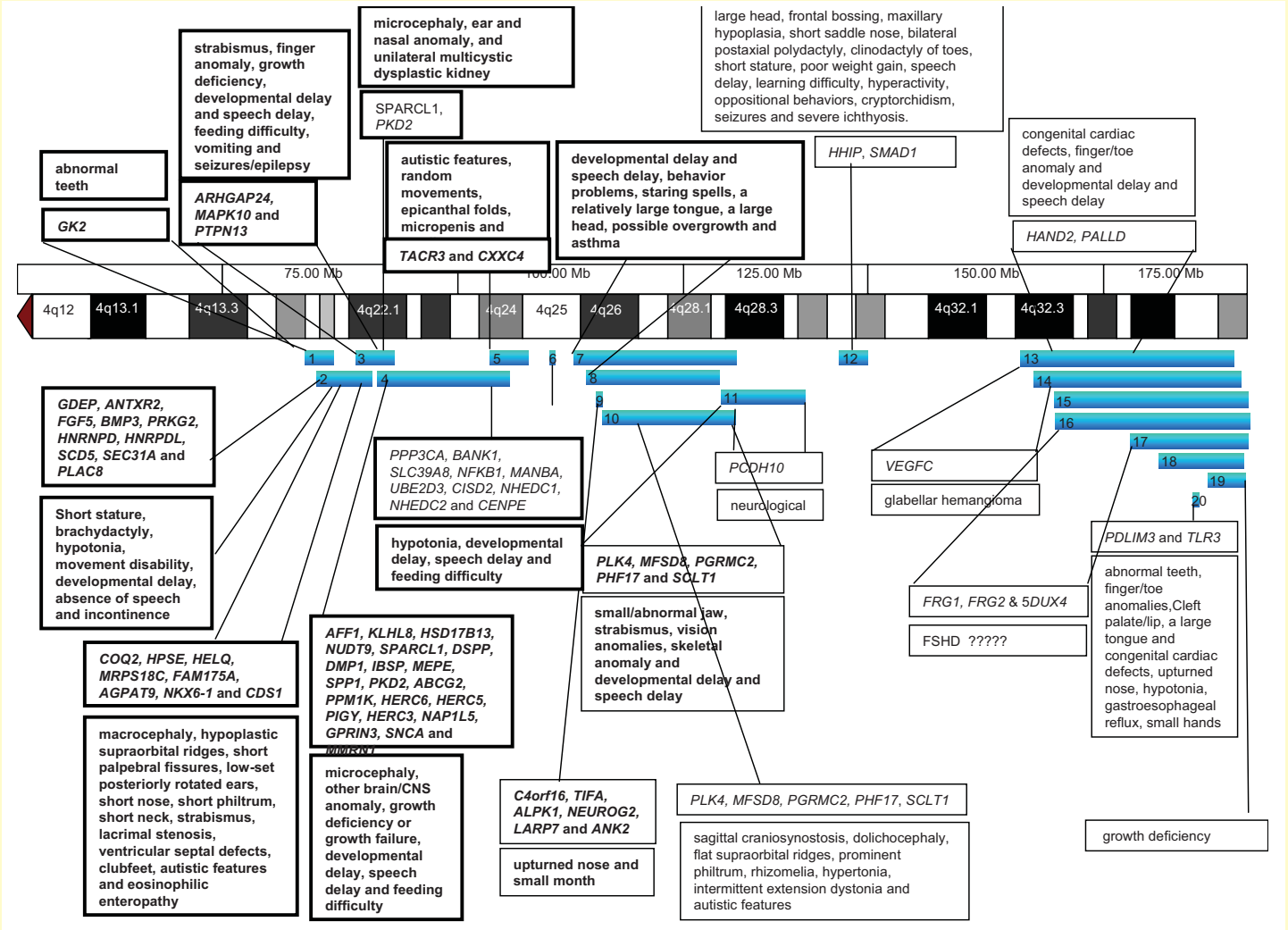
'We want to see if there are common deletions, common break points, if a lot of people's chromosomes break at the same spot (slide above, left). And we do see some hotspots where deletions are more common. If we then look in more detail (slide above, right), we can see that a lot of deletions are very similar. That means that the structure of the chromosome in that region is vulnerable to be broken.

'But research is very limited. Now we have the genome, we know how many genes and which ones have been deleted. Now we need to relate the deletions to what we see clinically. We have compiled a long questionnaire for physicians and we have data from 20 patients. What we have found so far is that if you lump them together you find the clinical phenotype is very similar to what Dr Strehle published many years ago (slide below, left).

'How many genes are there in this region? Perhaps 1000. Some deletions encompass a lot of genes; some deletions are very small. Patients with very small deletions are very valuable for us to understand which genes cause disease because they have only lost one or two genes (slide below, right).



4q deletions



Features and lost genes in 20 individuals with a 4q deletion.

'Recently we have found some people who instead of having lost one copy of a part of 4q, have an extra copy (known as a duplication), and yet the clinical phenotype can be very similar to people with a deletion. That is strange. It means that not only not having enough is the problem; sometimes too much is not good either.

'What can we do for all these patients? We can tentatively assign genes to phenotypes, but only maybe because the number of patients is still not big enough. We also search databases and we look at animal models because we can do actual haploinsufficiency studies in animals, taking out one copy of a gene and then two and watching the effects.

Genes and disease

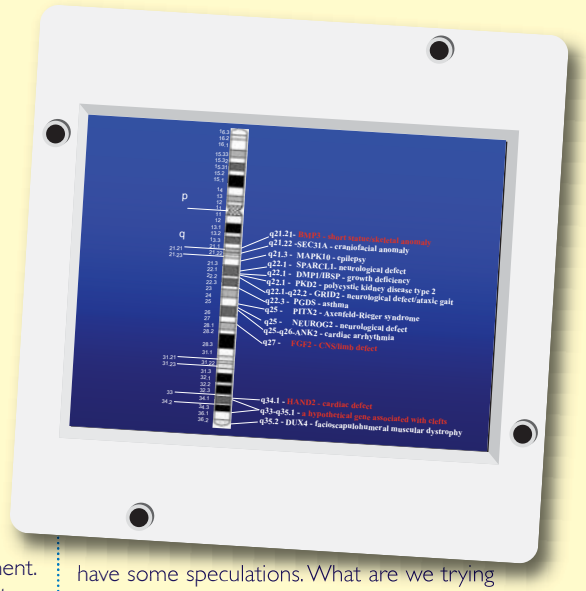
'Taking congenital heart disease, we know *HAND2* (in 4q34.1) is one gene expressed in the heart, but exclusively in the right side of the heart. So if this gene is deleted most likely you will have congenital heart disease. Also we have a cleft palate gene. We are pretty specific as to where it is because we had one patient with only three known genes deleted, no learning difficulties so far but with very complicated congenital heart disease, a limb anomaly, unusual craniofacial features and a cleft palate and cleft lip. But there are only two or three genes in the deleted region. That means that those genes are responsible for most phenotypes in 4q-. So there must be a very critical gene or genes in this region.

'Based on the database we think there may also be other genes that can be assigned to some of the phenotype such as eye anomalies or neurological defects. To pick out a few, *BMP3* (in 4q21.21) is a gene that is associated with bone development. A lot of kids we see with 4q- are very short, so we thought that may be important.

> *"Treatment at the moment is totally speculative."*

Patient care

'So how will this research apply to patient care? The purpose of research is to benefit our children. This is an ongoing project but I do have some speculations. What are we trying to do? For example, if my hypothesis is right, and *BMP3* that we know is important for bone development, is causing the short stature so kids are small, we can intervene in this pathway. Gene pathways are very complicated but many drugs are already used for other purposes in clinical trials for something like osteoporosis. If



> *One problem in the US is that 4q- is a rare disease. For research funding from the National Institutes of Health, the first thing they want to know is what the impact will be. And because 4q- is rare, the impact will be relatively small. But while it's true that 4q- is a rare disease, some of the discoveries made by studying 4q- could be applied to more common conditions. So if I can identify a cleft palate gene, cleft palate is quite common. Congenital heart disease is even more common: about one per cent of babies are born with defects of the heart. So hopefully in future we can go in that direction and say my research is using 4q- as the basis of the study but the research can apply to other areas.*

we can assign *BMP3* as responsible, maybe we can borrow a drug for this purpose.

'Recently we studied a patient who is very short and wondered: Do we want to start growth hormone treatment? Some children respond to treatment, some don't. At least one of my patients has been willing to try and so far is doing very well, fingers crossed.

'Again, the gene *FGF2* (in 4q27) is associated with the central nervous system, limb development and heart problems. The pathway is complicated but we already know many drugs that are already being used to intervene in this pathway so again we can maybe use drugs that have already been tested.

'What is our future direction? If we can pinpoint genes for some clinical signs, hopefully we can find some treatment for patients. Treatment at the moment is totally speculative because sample sizes are very small, but it is feasible.'

Questions

Growth deficiency and growth hormone treatment

Q *Growth deficiency is an obvious problem. In your professional opinions do growth hormones help or are they irrelevant?*

TH *Growth hormone has been used in many genetic disorders like Russell Silver syndrome where children are very tiny and Turner syndrome where they are short. Some respond well, some don't. I just started one of my patients and so far they are doing very well from the growth point of view. But we need a lot of studies. We don't know if children with 4q- have a growth hormone deficiency. That would be very helpful to know. The good thing is that generally growth hormones are pretty safe.*

ES *The practice of treatment with growth hormone varies from country to country. In the UK growth hormone treatment is not generally recommended for chromosome disorders apart from Turner syndrome.*

Parent *Our daughter is 14, and was diagnosed six months ago with 4q deletion. She has been on growth hormone for three years and now that we've found out that she has 4q deletion we are wondering whether it's beneficial to keep her on it. It seems to have helped her but because she has been on it for so long, we wonder how it can be good for anybody to be on hormones for so long. Now she seems to have stopped growing again and if she shows no more signs of growth they are probably going to take her off it. The growth hormone also seems to have made her stronger in that she can fight other diseases a bit better. Whether that's because of or in spite of the growth hormone we don't know.*

A show of hands revealed that out of 13 families, eight children with 4q- were short for their age and five were not. One child was tall for her age. Two children were taking growth hormone.

Parent 2 *My daughter aged 9 will have been on growth hormone for two years in July. In the first year we saw a big difference, then it tailed off but because it increased her appetite, they are keeping her on it. In two years she grew 19 centimetres and put on four pounds (about 2 kilograms) in weight.*

Q ***Those on growth hormone, did it make a difference to their strength and has that in turn helped their development?***

Parent *Our daughter suffered from really bad chest infections 15 to 20 times a year. Growth hormone treatment cut it down noticeably fast to three or four times a year.*

Parent 2 *It was seasonal. As soon as the season changes, she'd be coughing and chesty. Now I forget to give her inhalers, she's*

> *There are limitations to our work. One is the sample size. If I could have one third of the patients diagnosed in the US in the past year I would have great confidence in assigning the genes responsible for the clinical phenotype much better. So we definitely need increased sample size. A lot of things that are currently speculation would then be more real.*

As a clinical geneticist I want to tell you we are still trying to understand why 4q- happened. You should know that this is not anything you did right or wrong. Without families like you participating in studies it's impossible to get any conclusions. So I really appreciate all the families participating in the study and the physicians with their detailed clinical phenotype. Hopefully we can find treatment for at least some aspects of the condition.

got so much stronger and healthy in herself.

ES *I'm not certain that it's possible for growth hormone to have an impact on other aspects of a child's health – supporting growth is the main reason for giving growth hormone.*

Q ***Is it age-related?***

Parent *We thought that just growing makes you stronger and that was the effect. She coughs less. Apart from when she gets chest infections, she's relatively healthy.*

TH *Some children with 4q- have low muscle tone. For those children, respiratory function may be affected. Growth hormone also increases muscle strength – that's one possibility.*

One of the two children on growth hormone had a low growth hormone level before treatment; the other child did not.

TH *Most endocrinologists test a child for growth hormone deficiency and that's a definite indication for treatment. But some people will put a child on growth hormone when the level is not low.*

Neurons branching

Parent ***In relation to the branching of the neurons, when does that show up and how can it be tested for or seen?***

ES *These are research studies. In children you can do an MRI scan of the brain which shows myelination, the myelin sheath around the nerve axons and that gives you some indication about maturation of the brain. At this point we can not infer from a brain scan how a child will perform in the future from an intellectual point of view. There's an interesting book by Moyra Smith about learning difficulties and various chromosome abnormalities, called Mental Retardation and Developmental Delay published by Oxford University Press in 2005.*

Q ***Are there any interventions or diet that can help to promote learning in those children with a learning difficulty?***

ES *There are supplements like omega-3 fatty acids that may be helpful in autism as well. The yahoo group website has other suggestions such as coenzyme Q10. We are looking into this but more from a parental observational experience at present. It's not based on hard evidence but there are positive opinions about some food supplements from some parents. It can't be proven at this point that they are beneficial.*

Q ***Could they be harmful?***

ES *These are over-the-counter products which anyone can buy. I don't think there is any suggestion that they are harmful.*

TH *In my clinic, early intervention is the most effective way to help these children. They intervene early with speech and physical therapy, and for example physical therapy does seem to improve muscle strength significantly. Speech therapy also makes significant improvements.*

Q ***Is your database US children only?***

TH *It's international, with children from the UK and South America as well.*

Diagnostic test

Q ***When we got our diagnosis three years ago, how do we know what sort of test was done and how do we get a more up-to-date one?***

TH *If it was done three years ago, most likely an older technique was still being used. You have to ask the doctor what kind of arrays they used. The latest third generation uses oligoarray CGH. Getting a new array done may be done as a research resource or there may be an indication if it's important to know the exact break point. If a big chunk has been deleted, it may not be necessary to repeat the test because 95 per cent of genes are already known in that region. But if you have a small deletion and the test shows greater precision over the break points, it can maybe make a lot of difference.*

Gastro oesophageal reflux

Q ***What are the implications of reflux?***

ES *Gastro oesophageal reflux is common in children whether they have a chromosomal deletion or not. It has to do with the fact that the sphincter (ring of muscle) at the entry to the stomach is not working properly. That usually corrects itself in the first year of life but it can lead to aspiration as one of the most serious side effects. If food is flowing back into the food pipe and the child gets it into the mouth and into the lungs and cannot cough well and cough it up, then that leads to aspiration pneumonia and is serious. It needs to be taken seriously and treated appropriately with medicine in the first instance.*

Q ***How do you know if a child is aspirating?***

ES *A pH study or a barium meal or ultrasound scan can give the diagnosis. A speech and language therapist can do a videofluoroscopy to see if there is aspiration or not. The last resort treatment is a fundoplication operation and possibly a gastrostomy and that can prevent aspiration but you would want to try medicine first.*

Q ***In this group, about 69 per cent have reflux. In how many is it still a problem?***

A show of hands showed reflux was still a significant problem.

Parent *In my son it is a problem at 18, but he didn't get it until he was 10. He has had a gastrostomy since he was two or three.*

ES *One reason it improves naturally is because of the upright position and also the valve mechanism improves naturally in quite a number of children. The angle between the food pipe and the stomach changes and that improves reflux.*

TH *In the US we often use a pH probe because stomachs are very acidic and I can see the level of acidity. If it's higher, that's a risk for aspiration. In some cases thickening the formula by adding rice cereal can help.*