A summary of 7q interstitial deletions and exclusion mapping of the gene for β -glucuronidase

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SUMMARY Three patients are described with different phenotypes and differing de novo interstitial deletions of the long arm of a chromosome 7. The first patient has a deletion with loss of the proximal 7q11.23 band. Only three other cases have been reported with this particular deletion. Our second case shows mild dysmorphism similar to the other four patients reported with deletion of bands 7q21.12→21.3. Our third patient has a deletion of the 7q22.1→32.2 segment and has many of the phenotypic features of the other reported cases of del 7q22→32.

GUSB, the gene for β -glucuronidase, has been localised to the 7cen→q22 region. Analysis of β -glucuronidase levels in blood leucocytes of our patients has helped more precisely to assign this gene locus to 7q21.11 or 7q22.1.

Interstitial deletions of the long arm of chromosome 7 are still rare and 26 cases have been published to date. These case reports have been classified roughly according to the deleted segment into three groups, that is, deletion of 7q11→q21, del 7q21→q32, and del 7q32→q34.^{1,2} A further group of terminal deletions, del 7q32→qter, are said to present a consistent phenotype and will not be considered further here. The general consensus is that no clear picture emerges from the interstitial deletion reports, as they are presently grouped, to be able to establish a definite clinical phenotype. However, we suggest that high resolution banding studies would clarify many of the current discrepancies and enable a more accurate prognosis to be given for such patients.

We present details of three patients with deletions of different segments of 7q and attempt to define further the phenotypic characteristics associated with this region of the genome.

The gene coding for β -glucuronidase (GUSB) has been assigned to chromosome 7cen→q22 and, more recently, to 7q21.1→q22.³ Normal levels of β -glucuronidase activity in our three patients further narrows the location of this gene to either band 7q21.11 or 7q22.1.

Case reports

CASE 1

This male patient was born at 36 weeks' gestation by

normal vaginal delivery after an uneventful pregnancy. The parents were unrelated and the G1P0 mother was aged 22 years. Birth weight was 2450 g (<3rd centile) and head circumference was 32 cm (<3rd centile). Apgar scores were 6, 8, and 9 at one, five, and 10 minutes, respectively. At birth he was noted to have respiratory distress, a very weak cry, a small penis, hypogonadism, abnormal ears, left choanal narrowing, and bilateral hernias, which were repaired at the age of two weeks.

Episodes of apnoea preceded presentation at five months of age with myoclonic seizures. There was an overall increase in muscle tone and deep tendon reflexes. An EEG showed a hypsarhythmic pattern. CT scan of the head showed slight generalised atrophy with no evidence of any calcification. Seizures were kept under control with clonazepam.

At the age of 21 months, his head circumference was 32 cm (4 cm below the 3rd centile), and weight (7.7 kg) and height (75 cm) were below the 3rd centile. He had persistent increase in muscle tone with scissoring of his lower extremities and some plantar flexion of his ankles. Additional features included frontal bossing, brachycephaly, muscle wasting in the pelvic region, and rockerbottom feet with overriding second toes (fig 1).

There were neither abnormal dermatoglyphics nor neurocutaneous markings, no apparent visceromegaly, and no evidence of any cardiac lesion. Fundal examination was normal but there was only limited visual fixing and following. Brain stem

FIG 1 Full view and lateral face of case 1 aged 20 months.

evoked responses were also normal. Developmentally at 21 months he was functioning at no better than 11 months of age and appeared to be severely retarded.

CASE 2

This female infant was born to unrelated 26 year old parents, the product of the third pregnancy after two healthy children. The pregnancy was complicated by poor weight gain during the third trimester. Labour occurred spontaneously at 41 weeks' gestation and Apgar scores were 8 and 9 at one and five minutes. Birth weight was 3160 g (10th centile), length 49 cm (10th centile), and head circumference 32.5 cm (>10th centile).

Clinical examination showed facial dysmorphism including prominent, oedematous upper tarsal plates with eversion of the upper eyelid (fig 2) and upward slanting palpebral fissures. The nose was pointed and the philtrum elongated. There was a mild degree of micrognathia and laxity of the temporomandibular joints. The ears were alobular and showed excessive helical curving. A third fontanelle was present. A full physical examination indicated no other obvious abnormalities and ultrasound showed normal head, heart, and abdomen. The eye abnormalities resolved considerably over the first week of life and ophthalmological investigation was unremarkable.

At the age of seven months the patient's height and weight were between the 10th and 50th centiles;

the head circumference remained on the third centile. Brachycephaly was now evident. The eyes appeared normal. Nails were absent on the second and third toes bilaterally. Neurodevelopmentally, she was achieving a five to six month old level.

CASE 3

This male infant was born at 34 weeks' gestation after a pregnancy complicated by polyhydramnios to a G7P3A2 woman aged 28 years. Birth weight was 1820 g (10th to 50th centile) and head circumference 31.5 cm (50th centile). Owing to prematurity, length was not measured. Apgar scores were 9 and 9 at one and five minutes.

At birth the following features were noted: an elfin-like face, a long philtrum, a large mouth and relative micrognathia (fig 3), a prominent occiput, and large fontanelles. He was very hypotonic. CT scan showed evidence of marked generalised cerebral atrophy. There was stenosis of the left main pulmonary artery and an aberrant right subclavian artery. There was tracheomalacia and left main bronchomalacia and an annular pancreas.

He had severe gastro-oesophageal reflux and uncoordinated swallowing and had recurrent episodes of pulmonary aspiration. This settled after a Nissen fundoplication and gastrostomy feeding and his height, weight, and head circumference returned to the 50th centile levels.

Fundal examination showed a refractive error and astigmatism; fundoscopy showed a small, crowded,

FIG 2 Full face and lateral face of case 2 at 12 days.

right optic disc and a large, left, pale disc with a small inferior coloboma.

Epilepsy was diagnosed at seven months and both febrile and afebrile seizures occurred in spite of anticonvulsant therapy. At 22 months of age he had developmental delay, was hypotonic with poor head control, and was unable to sit unsupported or crawl.

Cytogenetic studies

Chromosome studies on our three patients were performed on blood lymphocyte cultures stimulated with phytohaemagglutinin. The cells were cultured for 65 hours at 37°C. The medium (Ham's F10) was supplemented with 20% fetal calf serum. The cells

FIG 3 Full face and lateral face of case 3 at two weeks.

FIG 4 Partial GTG banded karyotypes of (a) case 1, (b) case 2, (c) case 3. (i) shows the chromosomes at a resolution of approximately 500 bands. (ii) RBG bands and ideogram. The normal chromosome is on the right in the first pair and on the left in the second pair.

were harvested by the Ibraimov method⁴ and trypsin G banded. RBG bands were obtained by the method of Eichenbaum *et al*⁵ after BrdU incorporation.

CYTOGENETIC RESULTS

Patient 1 had a deletion of q11.23, a small, proximal, G band negative region of the long arm of one chromosome 7 (fig 4a). His karyotype is 46,XY,del(7)(pter→q11.22::q21.11→qter). Both parents had normal chromosomes.

Patient 2 had a deletion of the G band positive bands q21.2 and q21.3 of one chromosome 7 (fig 4b). This was visible only at a resolution of or above 850 bands (fig 4b(i)). The karyotype is 46,XX,del(7)(pter→q21.12::q22.1→qter). The parents had normal chromosomes.

Patient 3 had a deletion of band q31 of the long arm of one chromosome 7 (fig 4c). His karyotype is 46,XY,del(7)(pter→q22.1::q32.2→qter). Both parents and all three normal sibs had normal chromosomes.

Biochemical studies

β -glucuronidase activity was measured in the Department of Chemical Pathology, Adelaide Children's Hospital, which acts as an Australian National Referral Centre for the diagnosis of lysosomal storage disorders including the mucopolysaccharidoses. Control values are derived from

TABLE Results of enzyme studies.

	Patient 1	Patient 2	Patient 3	Normal range
β -glucuronidase (nmol/min/mg protein)	1.1	1.8	1.2	0.5-3.4

several thousand referrals to this centre. The fluorimetric assay is based on that described by Glaser and Sly⁶ and measures the release of 4-methylumbelliferone (4MU) from 4MU- β -glucuronide (Koch-Light) in 0.1 mol/l sodium acetate buffer, pH 4.8. Fluorescence was measured in a Perkin-Elmer LS-5 spectrophotofluorimeter equipped with a flow through cell. Leucocytes were prepared by sedimentation in dextran as described by Kampine *et al*⁷ and were disrupted by three cycles of freeze-thawing to -70°C in 0.1% (v/v) Triton X-70. A 750 g supernatant was used for assay of β -glucuronidase and protein.⁸ Plasma was used undiluted.

Biochemical results

The results of enzyme activity levels in all three patients are presented in the table.

Discussion

While there have been 26 different cases of interstitial deletion of the long arm of chromosome 7 published so far, no apparently distinct syndromes are said to have emerged.^{1 2} This may be the result, in part, of the many different breakpoints involved in these reports, of the different sizes of many of the deleted segments, and of some possible inaccuracies in several reports owing to poor resolution of chromosomes.

To date, reports involving deletions of segments q11 and q21 have been considered together for review purposes and a wide variety of features have been described. However, when one considers these two bands separately a more distinct pattern emerges (fig 5).

Those patients who have deletions of band 7q11 only, that is, our case 1,^{2(patient 3)3 9} or who have a larger deleted segment including the apparently critical band q11.23 with part of q21.1^{11(patient 2)13 28} have the following clinical features in common: profound retardation and severe growth delay, myoclonic spasms or seizures or both, microcephaly, flat occiput, abnormal muscle tone, spastic quadriplegia, abnormal ears, inguinal hernias, and most were non-ambulatory.

The two patients showing loss of the largest segment in this group²⁸ also had the most severe

FIG 5 Diagram of 7q interstitial deletions. a=Young *et al*.² b=Frydman *et al*.⁹ c=Fryns *et al*.¹⁰ d=Seabright and Lewis.¹¹ e=Crawford *et al*.¹² f=Valentine and Sergovich.¹³ g=Gibson *et al*.¹ h=Johnson *et al*.¹⁴ i=Klep-de Pater *et al*.¹⁵ j=Pfeiffer.¹⁶ k=Serup.¹⁷ l=Abuelo *et al*.¹⁸ m=Higginson *et al*.¹⁹ n=Franceschini *et al*.²⁰ o=Dennis *et al*.²¹ p=Hull *et al*.²² q=Ayraud *et al*.²³ r=Martin-Pont *et al*.²⁴ s=Stallard and Juberg.²⁵ t=Nielsen *et al*.²⁶ u=Bateman *et al*.²⁷ v=Allanson *et al*.³ w=Fryns *et al*.²⁸ x=Chitayat *et al*.²⁹

abnormalities; the sibs died at birth with severe cardiorespiratory problems owing to coarctation of the aorta. They had general oedema, flat face, webbing of the neck, and genital hypoplasia.

Four cases have been described which showed deletion of bands 7q21.2 and q21.3 (fig 5).¹ 2(patient 4)10 11(patient 1) Two of these papers¹ give another description of their karyotype and more general breakpoints, for example, del q11→q21. However, the photographs of their chromosomes clearly show that the G band negative segment q11 is unaffected by the deletion and that loss has occurred of the dark q21.2 and q21.3 bands. The clinical descriptions also indicate that they share the deleted segment and many of the features present in our case 2 and in the other two patients in this group, namely: moderate developmental delay, mild dysmorphism, facial asymmetry, hypertelorism, epicanthus, folded helices of ears, broad nasal bridge, and small, downturned corners of the mouth.

These features, although based on a small number of cases, would seem to indicate that milder phenotypic abnormalities are caused by loss of the q21.2 and 21.3 bands than by deletion of the similar sized band q11.23. Each of these bands constitutes 7% of the total length of chromosome 7.

One patient is described with loss of the whole large G band positive segment comprising band q21¹⁴ (fig 5). This report is of an abstract presentation only, so the breakpoints cannot be confirmed. The patient presented with small birth size and abnormal infolding of the helices; she had bilateral palmar creases and displayed some developmental delay.

Two further cases had a deletion of this same q21 band and part of q22.^{15 16} (fig 5). Both these patients were more severely growth and developmentally retarded than the preceding case and both were brachycephalic and had micrognathia and cleft palate. The patient of Pfeiffer¹⁶ had split hand/split foot malformation; the patient of Klep-de Pater *et al*¹⁵ (case 2) had kyphoscoliosis and at the age of four years had no speech and could neither stand nor walk unaided. Quite possibly, it is deletion of the G band negative band q22.1 which is responsible for the more severe abnormalities in these two patients.

The single most common interstitial deletion of 7q shows a loss of the bands q22.1→q32. The 11 reported cases^{2(patient 5)15(case 1)17-25} and our case 3 share many phenotypic features. All patients had 'marked' to severe developmental retardation and two died in infancy. Most commonly, the patients had severe failure to thrive with feeding difficulties owing to either poor sucking or gastro-oesophageal reflux. Most also had an unusual or high pitched cry

in infancy. Microcephaly was common as was a large mouth, micrognathia, hypertonia of the lower limbs, seizures, and low set ears. This somewhat unusual combination of features are consistently present in most reported cases so that one could possibly speak of a 'deletion 7q31 syndrome'.

Other 7q interstitial deletions include three members of one family with a deletion of bands q32→34 (fig 5) resulting from malsegregation of an inversion carrier.²⁶ No unrelated cases have been described to date with these exact breakpoints. These patients have similar phenotypic features: mental and developmental retardation, external ear malformation, hypertelorism, flat nasal bridge, a bulbous nose tip, and wide mouth.

Bateman *et al*²⁷ reported a single case with an interstitial deletion involving loss of bands q33 and q34 (patient 3), that is, his patient had a smaller deletion than the family of Nielsen *et al*²⁶ and, apart from severe growth and developmental retardation, had microcephaly, prominent forehead, upward slanting palpebral fissures, bilateral simian creases, and clitoromegaly.

Conclusions

Deletions of interstitial segments of the long arm of chromosome 7 are still relatively rare. The single most common deletion (del q31) has been found in 12 cases (11 published cases and our case 3) and a characteristic clinical picture is now emerging: severe developmental retardation, an unusual cry in infancy, a large mouth, seizures, and feeding or gastro-oesophageal difficulties.

A deletion which includes the G band negative bands q11.23 or q22.1 or both results in severe growth and developmental retardation and the prognosis is poor. Spasms and seizures are common and most patients remain non-ambulatory. By comparison, those patients whose karyotype shows loss of only G band positive bands of 7q generally display minor dysmorphism and mild to moderate developmental delay.

While current evidence supports the grouping as described in fig 5, more case reports are needed with deletion breakpoints accurately defined using high resolution banding to confirm the phenotypic pattern.

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Note added in proof

Our patient 3 died in his sleep aged two years nine months. He had suffered a grand mal seizure the previous day. We suggest naming del 7q31 'the JB syndrome' after him.

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