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Original articles

Alagille syndrome and deletion of 20p

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Abstract

We add five cases of 20p deletion to the 10 cases already published. Four had craniofacial, vertebral, ocular, and cardiovascular features of Alagille syndrome, which adds weight to the assignment of this disorder to the short arm of chromosome 20. Included in our series is the first report of familial transmission of a 20p deletion.

In 1969, Alagille *et al*[1] described a syndrome characterised by intrahepatic biliary ductal hypoplasia, peripheral pulmonary artery stenosis, a typical facies with deep set eyes and a bossed forehead, posterior embryotoxon of the eye, and vertebral defects. A variety of less common features included renal defects, short stature, and developmental delay. Several reports of affected subjects in more than one generation favoured an autosomal dominant trait with variable expression.[2–4] In 1986, Byrne *et al*[5] reported 20p deletion in an infant with features of Alagille syndrome and drew attention to similar clinical features in two other reported cases with this deletion. In 1989, Schnittger *et al*[6] reported a 19 year old girl with Alagille syndrome associated with interstitial deletion of 20p11.23–p12.1. More recently, Zhang *et al*[7] and Legius *et al*[8] reported a re-examination of the chromosomes of two boys with Alagille syndrome which showed a deletion of 20p with the breakpoints at p11.13 and p11.2, respectively.

We report five further cases of 20p− to add to the total of 10 already published. Details of four published cases[5–8] taken together with our cases confirm the assignment of Alagille syndrome to 20p and expand the phenotype associated with deletion of this region.

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Case reports

CASE 1

The proband was born at 39 weeks' gestation after a normal pregnancy. She was the first and only child of a young, unrelated couple of white European origin. Her birthweight was 2350 g (less than the 10th centile) and she was noted to be dysmorphic from birth with a prominent forehead and deep set eyes. Difficulty in feeding and frequent vomiting led to initial failure to thrive. She developed jaundice on her third day of life and required phototherapy for four days with a maximum serum bilirubin level of 222 mmol/l. Apart from this short lived neonatal jaundice, she had never developed liver problems and review at 13 years showed normal liver function test with a bilirubin of 6 mmol/l. Serum cholesterol was normal at 5·5 mmol/l but triglyceride levels were raised at 3·4 mmol/l (normal range 0·1 to 2·1). Renal glycosuria, mild to moderate uraemia (blood urea of 16·9 mmol/l), and a generalised aminoaciduria were noted in infancy; these resolved spontaneously. At the age of 5 months she was found to be hypercalcaemic. This responded to a low calcium diet for some time but she continued to fail to thrive. Intravenous pyelography showed a duplex right kidney with bilateral mild to moderate vesicoureteric reflux. Renal biopsy performed at the age of 10 months showed sclerosed glomeruli, tubular atrophy, and calcification. Long term prophylactic antibiotics were introduced. Evidence of impaired renal function persisted. At the age of 13 years her creatinine was 150 μmol/l and urea was 8·1 mmol/l. It is anticipated that she will require dialysis or a transplant during adolescence.

Cardiac catheterisation was performed at 18 months to investigate a heart murmur. This showed a mild bilateral pulmonary branch stenosis.

Growth and development were impaired. She was of short stature throughout childhood. Thelarche began at 9 years and menarche at 11 years. By 13 years she had reached a height of 136 cm (below 3rd centile). A moderate degree of mental handicap resulted in attendance at a school for children with special educational needs and only partial independence in day to day activities at 13 years. A partial left sensorineural hearing loss was detected.

A convergent strabismus was corrected at 11 years. Ophthalmological review at 13 years confirmed the presence of bilateral posterior embryotoxon.

Her dysmorphic features were compatible with those seen in Alagille syndrome (fig 1A and B). Other physical features were a short neck, small ears, crowded teeth, small hands and feet, and mild hypoplasia of all finger and toenails.

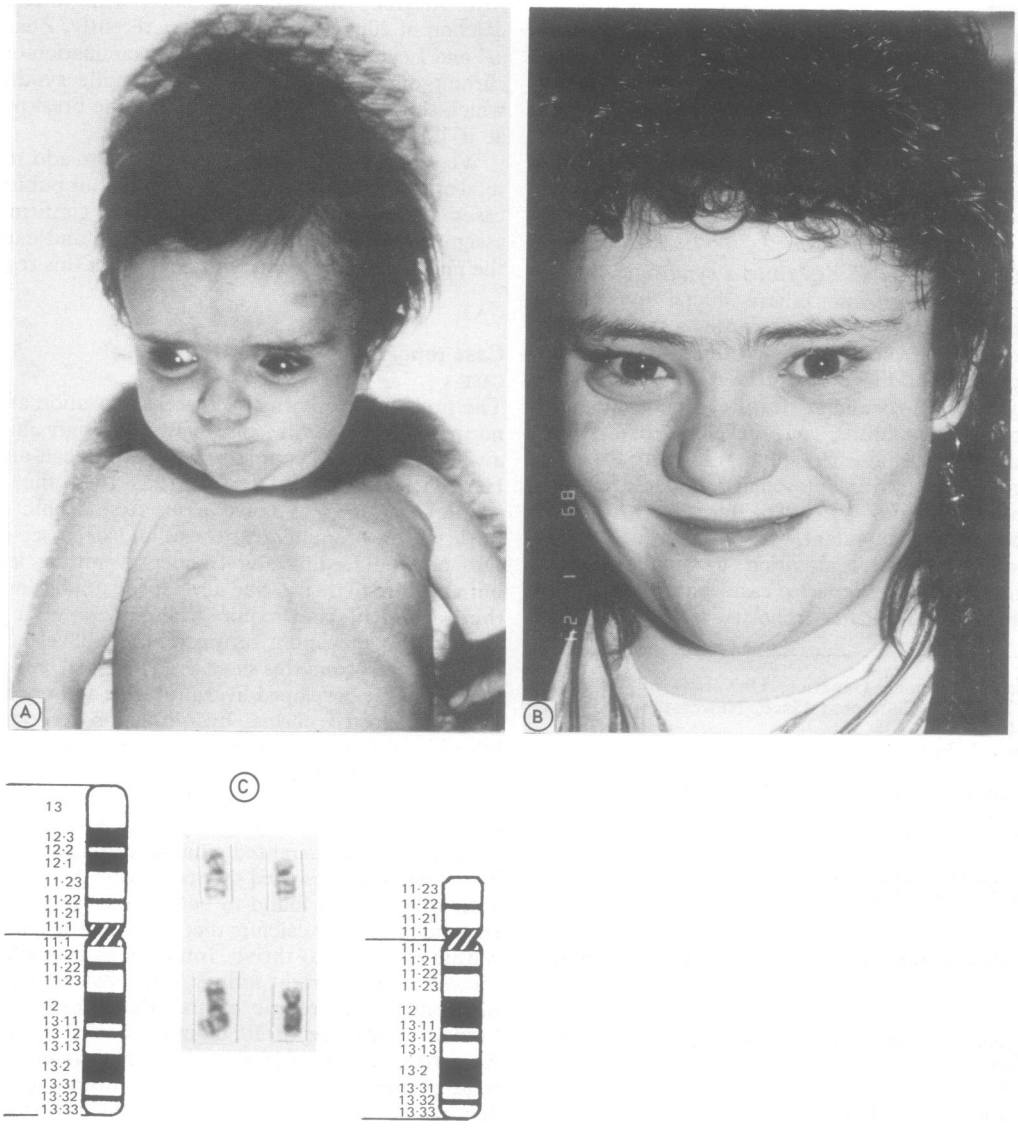

Figure 1 (A) Facial appearance of case 1 in infancy and (B) at 13 years. Note the frontal bossing with a concave nasal bridge, upward slanting palpebral fissures, and a short philtrum. (C) The de novo deletion (20) (p11.23–p13) in case 1.

Radiological examination showed butterfly-like deformity affecting multiple thoracic vertebrae with a complete sagittal cleft of the body of T4.

Cytogenetic studies

Cytogenetic studies showed deletion of the short arm of chromosome 20 with a breakpoint in band 20p11.23 (46,XX,del(20)(p11.23–p13) (fig 1C). Parental chromosomes were normal.

CASE 2

This female infant was born at term by normal vaginal delivery after a normal pregnancy weighing 3310 g. The mother at that time was 29 years of age and father 32 years of age and they were unrelated and of white European origin. A male sib born in 1983 was normal.

At birth the proband was noted to have a left sided cleft lip, a high arched palate, small, low set ears, overfolded helices, a flat nasal bridge, hypertelorism, epicanthic folds, widely separated skull sutures with a large anterior fontanelle, a short neck, and generalised hypotonia. During the first week of her life an ejection systolic murmur was noted in the pulmonary area radiating to the back. ECG showed right ventricular hypertrophy and features of the Wolff-Parkinson-White syndrome. Echocardiogram showed a left superior vena cava joining into the coronary sinus and a normal pulmonary valve and main pulmonary artery. These findings were felt to be consistent with a mild to moderate peripheral pulmonary artery stenosis. Skeletal survey showed spina bifida occulta of the first cervical vertebra and hypoplastic acetabula.

From birth she exhibited poor sucking with uncoordinated swallowing and had several episodes of aspiration with poor weight gain. This initial failure to thrive persisted throughout life and despite several hospital admissions and attempts to increase calorie intake she remained below the 3rd centile.

At the age of 3 years 6 months she developed mild jaundice. Investigations showed slightly raised liver enzyme levels but there was no evidence of chronic hepatitis or autoimmune disease. The jaundice gradually resolved and on review at the age of 5 years her total serum bilirubin was 6 mmol/l. However, both serum alkaline phosphatase and gamma-glutamyl transpeptidase were raised (666 IU/l and 87 IU/l, respectively). The rest of the liver enzymes and serum lipids were within normal limits.

Examination at 18 months and 5 years of age showed a short, thin child wearing hearing aids who had the following facial features: successfully repaired left cleft lip, long philtrum, moderate hypertelorism, a depressed nasal bridge, frontal bossing, and downward slanting palpebral fissures. She had a short chest with pectus carinatum. Cardiovascular examination indicated persistence of the pulmonary systolic murmur. Ophthalmological examination showed no evidence of eye defects. The facial appearance was not typical of Alagille syndrome (permission to publish photograph withheld).

Developmental history indicated moderate global delay. Her milestones had been delayed to sitting unsupported at 18 months and walking independently at 3 years. She began to vocalise at 3 years 7 months. Significant hearing impairment had been noted and bilateral hearing aids provided. She has been placed in a school for the educationally subnormal. At the age of 5 years she could say several words and was able to join two or three words together in sentences, though her speech was difficult to understand. She was hypotonic but not weak.

Cytogenetic studies

Studies in the neonatal period showed a deletion of the short arm of chromosome 20. Subsequent analysis showed the breakpoint to be at p12 (46,XX,del(20)(p12–p13) (fig 2). The maternal karyotype was normal, but the father and brother were not available for testing.

CASE 3

This female child was the first offspring of unrelated parents of white European origin, aged 28 and 27 years. She was born on 10.5.88 after a normal delivery at 39 weeks' gestation, birthweight 3090 g. The pregnancy was complicated by an episode of gastroenteritis but was otherwise normal. She was admitted to special care because of failure to suck adequately and mild hypoglycaemia, but was discharged at 3 days. She was readmitted at 4 days of age with irritability, jaundice, and feeding problems. On day 6 abnormal liver function tests but a normal liver ultrasound were reported. She was discharged at 17 days and readmitted at 6 weeks with hepatomegaly

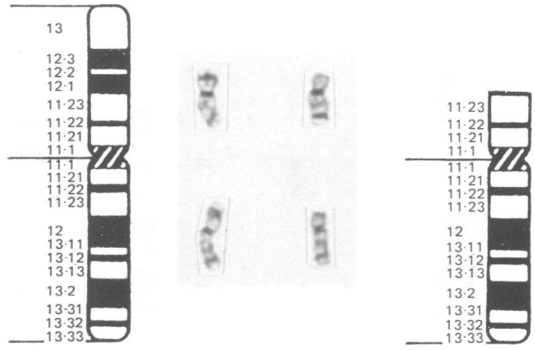

Figure 2 Cytogenetic abnormality in case 2: 46,XX,del(20)(p12–p13).

and splenomegaly, possibly owing to neonatal hepatitis. Persistent jaundice was noted and a liver biopsy showed chronic persistent hepatitis. An echocardiogram showed patent ductus arteriosus with possible peripheral pulmonary artery stenosis. Eye examination indicated anterior segment changes of posterior embryotoxon. *X* ray investigation showed a normal spine and normal ribs.

Examination at 6 months of age showed a small infant with both height and weight on the 3rd centile. There was no evidence of jaundice. Frontal bossing, antimongoloid slant, and a prominent nasal root were evident (fig 3). The head circumference was 40·8 cm with an inner canthal distance of 2·6 cm, interpupillary distance of 4·5 cm, and an outer canthal distance of 7·1 cm. These measurements represent mild hypertelorism in a child otherwise on the 3rd centile for physical growth. Repeat examination at 14 months showed a head circumference of 42·5 cm and a weight and height still on the 3rd centile. Development was grossly normal.

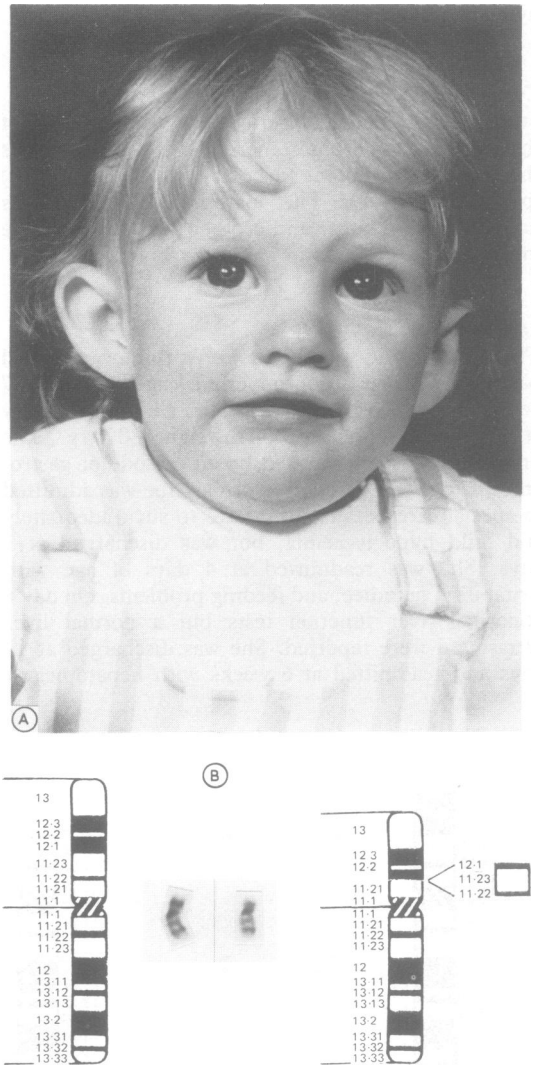

Figure 3 (A) Facial appearance of case 3 showing deep set eyes and a short philtrum. (B) The de novo interstitial deletion (20)(p11.2-12) in case 3.

Cytogenetic studies

The karyotype showed an interstitial deletion in the short arm of chromosome 20 with breakpoints within bands p11.2 and p12, 46,XX,del(20)(p11.2p12). The abnormal chromosome is shown in fig 3B. Parental chromosomes were normal.

CASE 4

This 3 year old boy (fig 4A) presented at six weeks for further investigation of prolonged conjugated hyperbilirubinaemia, renal dysplasia, congenital heart disease, abnormal facies, and poor weight gain. He was born at 37 weeks' gestation by forceps delivery after an uneventful pregnancy complicated only by the finding of intrauterine growth retardation and a dilated right renal tract on ultrasound scan. The infant's immediate neonatal period was complicated by a septicaemic illness and acute renal failure. He responded to antibiotics and conservative management of his renal failure. The infant was first noted to be jaundiced on day 2. The jaundice persisted and, on further investigation, was found to be obstructive in nature.

On examination, he was a small, jaundiced infant weighing 2·65 kg. He had abnormal facies with a high forehead, small, pointed chin, and an iris coloboma on the right. He had a long systolic murmur at the pulmonary area radiating to all parts of the lung fields with a soft pulmonary component to the second sound. He had a 2 cm palpable, smooth, non-tender liver. There were no other abnormalities.

Investigations confirmed a conjugated hyperbilirubinaemia with abnormal liver function tests, and a raised creatinine of 70 μmol/l. Abdominal ultrasound scan showed a normal liver and gallbladder and bilateral small dysplastic kidneys with dilatation of the right collecting system. A liver biopsy showed biliary duct hypoplasia. An electrocardiogram showed right ventricular strain and echocardiography indicated pulmonary stenosis and pulmonary artery stenosis. Ophthalmological assessment confirmed the presence of unilateral anterior segment dysmorphism but no other abnormalities. All other investigations were normal.

A diagnosis of Alagille syndrome was made on the basis of biliary duct hypoplasia, abnormal facies,

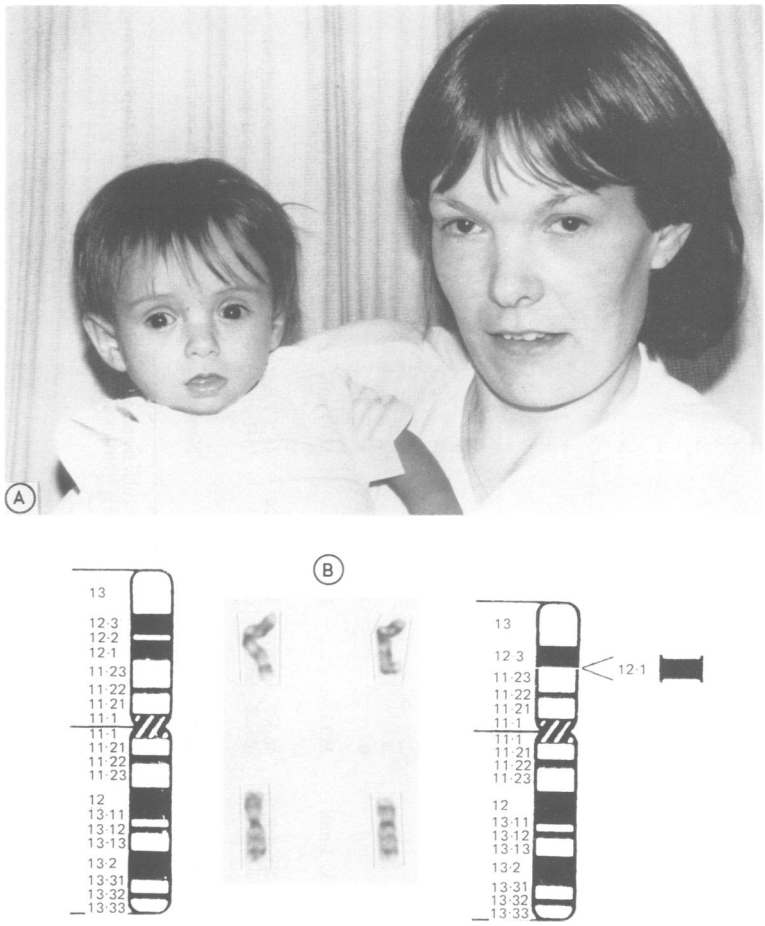

Figure 4 (A) Facial appearance of cases 4 and 5. (B) Cytogenetic abnormality showing an interstitial deletion present in mother and son: del(20)(p11.2–p12.2).

congenital heart disease, and growth retardation. There was no evidence of vertebral arch defects or hypogonadism.

The infant was treated initially with modified feeds, vitamin supplements, cholestyramine, and phenobarbitone. He remained well although his growth was poor, persistently well below the 3rd centile but parallel to it. He showed evidence of developmental delay.

The child was readmitted at 17 months of age with poor weight gain and diarrhoea, for which no explanation could be found. It has been noted that some patients with Alagille syndrome have pancreatic insufficiency and the child was therefore started empirically on pancreatic enzymes. He responded well with good weight gain and the diarrhoea ceased. Despite continual surveillance for signs of rickets, the child presented at 2 years 3 months with clinical rickets confirmed biochemically and radiologically. Rapid healing occurred with treatment with oral calcium supplements and intramuscular injections of cholecalciferol.

At the age of 2 years 10 months the boy presented with a rash on his hands which had been worsening over the past two weeks. Examination by a derma-

Table 1 Principal features of Alagille syndrome.

	%
Major features	
Chronic cholestasis	91
Characteristic facies	95
Cardiac abnormalities (68/80)	85
(Peripheral pulmonary artery stenosis 56/80)	70
Vertebral abnormality (butterfly-like)	87
Posterior embryotoxon*	88
Minor features	
Mental retardation	16
Growth retardation	50
Other skeletal abnormality (vertebral) 23/43	53
Glomerular renal involvement (mesangiolipidosis) 17/23	74

*Embryotoxon: a congenital condition of the eye in which the margin of the cornea is opaque. The condition resembles that of arcus senilis and is sometimes referred to as arcus juvenilis.

Table 2 Summary of the clinical features in the 15 cases of 20p deletion.

Clinical features	Kalousek and Therien[10]	Kogame *et al*[11]	Garcia-Cruz *et al*[12]	Byrne *et al*[5]	Vianna-Morgante *et al*[13]	Silengo *et al*[14]	Kiss and Osztovics[15]
Retarded growth: Prenatal (SFD/BW)	+/2570 g	+/2150 g	+/2250 g	+/2112 g	+/2900 g	+/2150 g	+/2000 g
Postnatal (FTT/SS)	+	+	+	+	+	+	+
Developmental/mental retardation	+	+	+	+	+	+	+
Characteristic facies							
Frontal bossing	+	0	+	+	+	+	+
Saddle/prominent straight nose	+	+	+	+	+	+	+
Flat midface	+	+	+	+	+	+	+
Mild/moderate hypertelorism	+	+	+	+	+	+	+
Deep set eyes	±	0	+	+	−	−	+
Epicanthic folds	+	+	+	+	−	0	−
Small/low set/deformed ears	+	+	+	+	−	+	−
Peripheral pulmonary arterial stenosis	+	+	−	+	−	−	−
Other cardiac defects	Complex	Lt SVC	TF	−	−	VSD	PDA
Skeletal abnormalities	+	+	+	+	+	+	+
Vertebral defects	+	+	+	+	+	+	−
Thoracic vertebral defects	+	+	+	+	+	+	−
Butterfly-like thoracic vertebral defects	+	−	+	−	−	+	−
Other features							
Hearing loss	0	+	+	0	0	0	0
Chronic cholestasis/abn liver function	−	−	−	+	−	−	−
Structural renal abnormalities	−	−	−	−	−	+	−
Atretic/stenotic bowel abnormalities	+	−	−	+	−	−	−
Posterior embryotoxon	−	−	−	−	−	−	−
Finger/toes/nail abnormalities	0	+	0	+	+	0	0
Sex/age at time of reporting	F/11 mth	M/5y 3 mth	M/16 y	F/15 mth	M/14 mth	F/10 mth	F/12 mth
Karyotype							
Patient	46,XX,del(20) (p11–pter)	46,XY,del(20) (p11–pter)	46,XY,del(20) (p12.2–pter)	46,XX,del(20) (p11.2–pter)	46,XY,del(20) (p11–?pter)	46,XX,del(20) (p11)/46,XX mosaicism	46,XX,del(20) (p11–pter)
Parents	Normal	Normal	Normal	Normal	Normal	Normal	Normal

+=present. ±=borderline or difficult to judge from illustrations. −=absent. 0=information not available. VSD=ventricular septal defect. TF=tetralogy of Fallot. PDA=persistent ductus arteriosus. Lt SVC=left superior vena cava. FTT=failure to thrive. SS=short stature. SFD=small for dates. BW=birth weight.

Table 2 (cont).

Schnittger *et al*[6]	Zhang *et al*[7]	Legius *et al*[8]	Case 1	Case 2	Case 3	Case 4	Case 5	No of affected patients
+/3000 g	0	+/2460 g	+/2359 g	−/3310 g	−/3090 g	+/2650 g	0	10/13
+	+	+	+	+	+	+	0	13/14
+	0	+	+	+	−	+	+	13/14
+	0	+	+	+	+	+	+	13/13
+	0	+	+	+	+	+	+	14/14
0	0	+	+	+	+	+	+	13/13
+	0	+	+	+	+	−	+	13/14
+	0	+	+	+	+	+	+	10/13
0	0	−	−	+	−	−	−	5/12
0	0	−	+	+	+	−	−	8/13
+	+	+	+	+	?+	+	−	9/15
−	−	PVS	−	Lt SVC	PDA	PVS	PVS	10/15
+	0	0	+	+	−	−	0	11/13
+	0	0	+	+	−	−	0	10/13
+	0	0	+	−	−	−	0	9/13
+	+	0	+	−	−	−	0	6/13
+	0	0	+	+	−	−	−	5/8
+	+	+	−	+	+	+	−	7/15
−	0	−	+	−	−	+	0	3/13
−	0	−	−	−	−	−	−	2/14
+	+	−	+	−	+	−	+	5/15
0	0	−	+	+	−	−	−	5/9
F/20 y	M/8 y	M/6 mth	F/13 y	F/4 y	F/14 mth	M/3 y	F/30 y	M/F 6:9
46,XX,del(20) (p11.22–p12.2) interstitial del	46,XY,del(20) (p11.23–p12.3)	46,XY,del(20) (p11.2–pter)	46,XX,del(20) (p?11.23–p13)	46,XX,del(20) (p12–p13)	46,XX,del(20) (p11.2–p12)	46,XY,del(20) (p11.23–p12.2)	46,XX,del(20)	
Normal	Normal	Normal	Normal	Normal mother, father not tested	Normal	Father normal, mother case 5	Not tested	

tologist showed areas of erythema, vesicles, crusted lesions with blood staining, and considerable scarring. The lesions were thought to be compatible with porphyria cutanea tarda or variegate porphyria. Porphyria screening was therefore carried out which showed normal urine and stool porphyrins, but massively raised blood protoporphyrins making a diagnosis of erythropoietic protoporphyria.

Cytogenetic studies

Chromosome analysis showed an interstitial deletion of chromosome 20 (p11.23–p12.2) (fig 4B).

CASE 5

A review of the parents of case 4 showed that his mother also had the typical facies of Alagille syndrome (fig 4A) with a high forehead and pointed chin. In addition, like her son, she had iris colobomata bilaterally. Further enquiries showed that she had never been jaundiced as an infant but was being followed up because of right ventricular outflow tract obstruction. She had been reviewed by an ophthalmologist as a child and found to have both bilateral anterior segment dysmorphism and posterior embryotoxon. She was also of limited intelligence. A diagnosis of Alagille syndrome without hepatic involvement was therefore made. The child's father was entirely normal. Both parents had normal porphyrin levels. The mother's chromosomes showed the same pattern as that described in her son (fig 4B). The father had normal chromosomes.

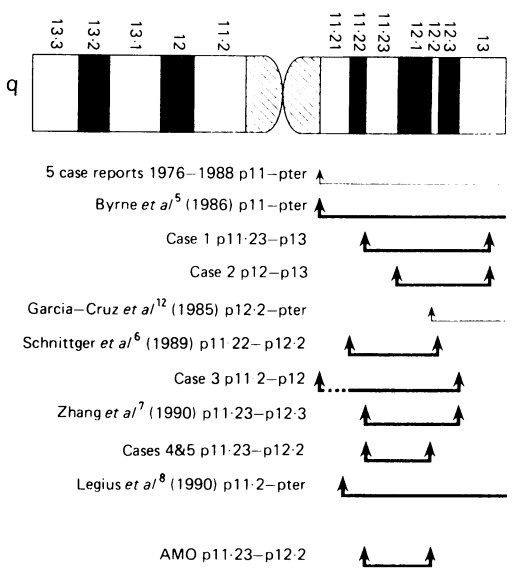

Figure 5 Diagrammatic representation of 15 cases of 20p deletion. Note that cases 1 and 2 from the present report are not shown as extending to the telomere. This is based on preliminary molecular genetic studies which have shown the presence of two copies of probes from the telomeric region (I Hansmann, personal communication). The area of minimal overlap (AMO) on cytogenetic grounds is p11.23–p12.2.

Discussion

Table 1 contains a recent review of the clinical features of 80 cases of Alagille syndrome[9] and table 2 those of all cases of deletion of chromosome 20p including our five cases. Cases 1, 3, 4, and 5 in the present report show clinical features which would be compatible with a diagnosis of Alagille syndrome. Case 2 has evidence of the typical heart defect, growth and mental retardation, vertebral abnormalities, and some liver function derangement though the craniofacial features shared less in common with Alagille syndrome. This may be the result of a somewhat more distal breakpoint involving 20p12 and a more extensive loss of material from the short arm. The significance of the erythropoietic protoporphyria in case 4 remains to be elucidated.

These cases together with the reports by Schnittger *et al*,[6] Zhang *et al*,[7] and Legius *et al*[8] confirm the proposal by Byrne *et al*[5] that Alagille syndrome results from a genetic defect on the short arm of chromosome 20 and we concur with the proposal by Schnittger *et al*[6] that the characteristic features result from a deletion involving the region p11.23–p12.2 (fig 5). Molecular genetic studies are in progress to refine the area of minimum overlap further and to establish whether this syndrome constitutes a contiguous gene defect. Linkage studies in familial cases of Alagille syndrome are now indicated.

The following clinicians have been involved in the care and investigation of the reported cases: Case 1 Dr M Oo, Dr M Coulthard; case 2 Dr F N Porter, Dr Kuzemko, Dr H L Smith; case 3 Dr R Beach, Dr C Bennett, Dr C Ball, Dr G Mieli-Vergani. Linda Burn prepared the manuscript.

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