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ARTICLE

Refined genotype–phenotype correlations in cases of chromosome 6p deletion syndromes

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Clinical reports of cases with deletions in chromosome 6p are relatively rare. We present a detailed study by fluorescent *in situ* hybridisation (FISH) of six new cases with distinct but overlapping 6p deletions involving the 6p24–pter chromosomal segment. Chromosomal breakpoints in individual cases were investigated using a large panel of probes previously mapped and characterised in our laboratory to cover the distal region of 6p. These cases have allowed refinement of genotype–phenotype correlations and strongly suggest a gene involved in regulating the development of hearing is localised within 6p25. There is also evidence for one or more loci involved in heart, skeletal and craniofacial development in the 6p24–p25 region. Furthermore, the Dandy–Walker malformation is associated with deletion of 6p24–pter.
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Introduction

Chromosome 6p deletions are rare events within the population. At present, there are 43 cases in the medical literature, excluding ring chromosome 6 anomalies. These include 27 reports on terminal deletions of 6p, six cases on unbalanced translocations, and the remaining 10 cases describe interstitial deletions with the entire 6p22, 6p22.2–p25.2 or 6p24–p25 segment deleted[1–11] (see references therein).

The clinical findings vary in the interstitial deletion 6p syndromes, and include orofacial clefting, short neck, clinodactyly or syndactyly and brain, heart and kidney defects. In contrast, corneal opacity, iridogoniodysgenesis anomaly, various Rieger type anomalies, hypertelorism and deafness are associated with 6p terminal deletions.

A large number of mouse model studies have provided valuable insights into the function of genes located in the syntenic segment of mouse chromosome 13, including roles in organogenesis, and in particular eye, cardiac and skeletal development.[12–17]

In this paper, we describe six new cases that enable us to delineate the 6p deletion syndrome involving the 10 Mbp long 6p24–pter chromosomal segment with further accuracy. Our findings allow us to associate developmental defects (see listing in Discussion) to deletions of specific chromosomal segments and to candidate genes therein.

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Patient 1

The youngest of two siblings (DOB 08/11/95) was born after an uneventful pregnancy and delivery: the patient was male, with a birth weight of 3.6 kg (50th centile), head

circumference was 49.8 cm (25th–50th centiles) and height was on the 25th centile.

Symptoms at birth
Hypertelorism and down-slanting palpebral fissures.

Initial cytogenetic data
G-banded preparations showed that the subtelomeric segment was absent from the derivative chromosome 6. Subsequent *in situ* hybridisation confirmed the initial karyotype: 46,XY,del(6)(p25.1). This event is *de novo* as both parents' karyotypes were normal.

Additional clinical data
Ophthalmolgical assessment revealed strabismus (which recurred after surgery) and marked posterior embryotoxon of both eyes. Anterior fibres from the iris adhered to the embryotoxon in places, but the anterior chamber was otherwise normally formed. The intra ocular pressure was normal.

The patient's ears were slightly low set and posteriorly rotated (Figure 1). A CT scan of the ears showed a poorly pneumatised mastoid on the left side. He had a broad nasal bridge, a highly arched palate, abnormal dentition.

Physical examination at 5 years noted a harsh systolic and a very soft early diastolic murmur after the aortic component of the second sound. Echocardiography demonstrated a minor structural abnormality of his aortic valve, though it was functioning normally.

He also had a left-sided cervical skin tag, slightly broad toes and his audiometry revealed bilateral mild to moderate low-frequency sensorineural hearing loss. He had moderate learning difficulties, language delay and motor milestones were delayed, as he sat at 11 months and walked at around approximately 16 months. A Griffith's assessment (14-01-99) showed him to be a year behind his chronological age of 4 years (see Table 1 for summary of phenotypes).

Patient 2

Female patient (DOB 3/05/00) was born to a 29-year-old woman after a full-term delivery, complicated by *abruptio*

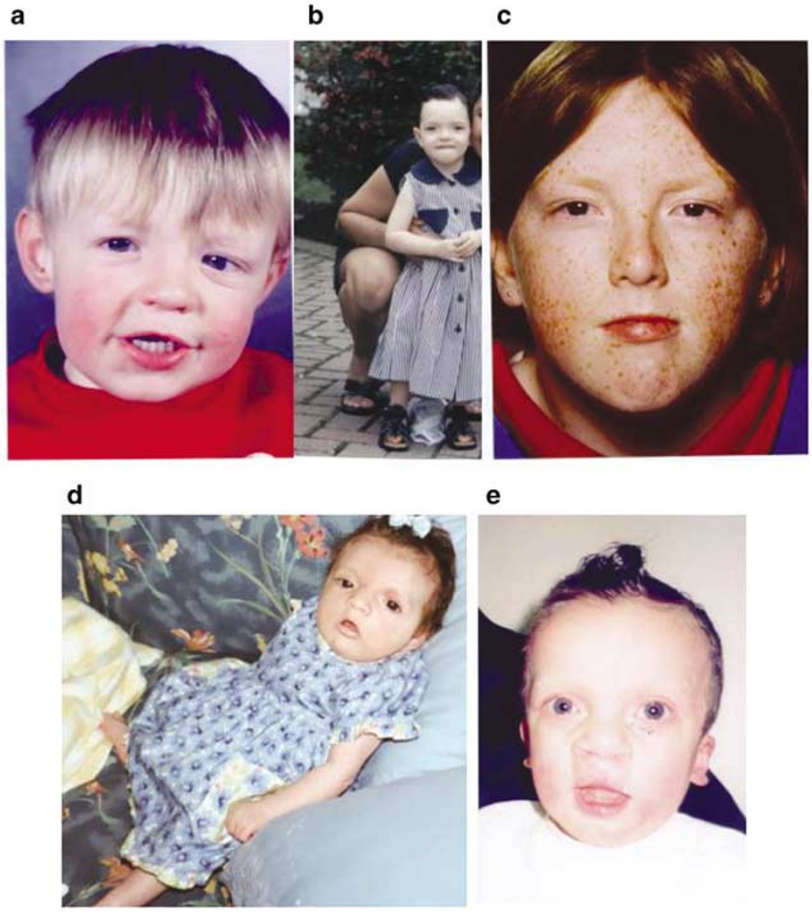

Figure 1 Photographs of five of the 6p deletion cases; (**a**) Patient 1; (**b**) Patient 2; (**c**) Patient 3; (**d**) Patient 5; (**e**) Patient 6.

Table 1 Main characteristics of chromosome 6p deletion cases presented in this study

Patients Clinical Features	*1*	*2*	*3*	*4*	*5*	*6*
DOB	8-11-95	03-05-00	13-01-84	14-12-00	02-04-02	12-3-00
Sex	Male	Female	Female	Female	Female	Male
Develomental delay/hypotonia	+	+	+	+	+	
Abnormal skull				+		
shape/hydrocephalus			+			
Hypertelorism	+	+	+		+	+
Palpebral fissures defects	+		+			
Structural eye defects	+	+	+		+	+
Anterior eye chamber anomalies	+		+	+		
Palatal abnormalities	+				+	+
Dental abnormalites	+	+	+			
Structural ear anomalies	+					+
Structural nose defects	+					+
Skin anomalies						+
Skin tags	+					
Heart defect	+	+		+	+	+
Kidney defect						+
Genital anomaly						+
Pectus excavatum					+	+
Sensorineural/conductive hearing loss	+		+	+		
Imperforate anus						+
Neuronal defect	+	+	+	+		
Motor delay	+	+	+		+	
Extremities anomalies	+		+			+
Language delay	+	+				

placenta requiring emergency caesarean. Birth weight was 3.2 kg (45th centile).

Symptoms at birth

Hypotonia, facial dysmorphism, abnormal creases in the hand and feet and hyperflexion of both feet.

Initial cytogenetic data

Cytogenetic analysis using G-banded chromosomes revealed the karyotype: 46,XX, del(6)(p24). This event is *de novo* as both parents' karyotypes were normal.

Additional clinical data

At 20 months, she was treated for pneumonia and had been diagnosed with asthma.

Examination at 2 years revealed a prominence in the occipital area, hypertelorism, hypermetropia and a strabismus (Figure 1). The teeth were abnormal and low in enamel. She had a carped shaped mouth, a small patent ductus arteriosus (PDA) and patent foramen ovale (PFO). Both feet had a rocker bottom appearance with bilateral ankle pronation. Hypotonia and joint hyperlaxity conditions were still present.

Gross motor skills were judged to be at 15 months level as she was not able to walk unassisted at 2 years. Adaptive skills were judged to be at 16–19 months level, cognitive skills were normal at 2 years. Skeletal development was showing delay and the anterior fontanel had not closed. She displayed hypersensitivity to temperature, auditory hypersensitivity and the mouth showed poor response to stimulation. A CT scan revealed a prominence in the fourth ventricle with questionable changes in the cerebella vermis and cerebella white matter.

In contrast to the parents, who are Hispanic, the baby was very fair. Thyroid stimulating hormone (TSH) levels were high, a blood test indicated high immunoglobulin E (IgE) levels and diagnosed anaemia. She was undergoing occupational and speech therapy in addition to physiotherapy.

Her proprioceptor input showed hyperactive irregularity and she displayed seizures that occur with a frequency of around 60 per day lasting for up to 1 min per seizure. It is thought these seizures were non specific and can occur in children with a developmental delay (see Table 1 for summary of phenotypes).

Patient 3

A girl, the eldest of two siblings (DOB 13-01-84), was born after an uneventful pregnancy and delivery, with a birth weight of 4.0 kg (>90th centile). The parents are non consanguineous and show no apparent family history of malformations. She presented at the age of 1 year with developmental delay and concerns about her hearing and vision.

Initial cytogenetic data

At 5 years, her chromosomes were analysed and a *de novo* del (6) p25→pter karyotype was identified.

Additional clinical data
Examination at 13 years revealed macrocephaly (head circumference 56.5 cm), upslanting short palpebral fissures, prominent epicanthic folds, mild dysmorphic features and a short philtrum. Eye examination revealed telecanthus, anterior segment dysgenesis, a divergent squint, hypermetropia, and mild pigmentary retinopathy (Figure 1). The teeth were prominent and overcrowded. Her toes were clawed, with a short second toe and bilateral fifth finger clinodactyly. She also had hip dysplasia, diagnosed as bilateral Perthes disease. She had undergone tonsillectomy, adenoidectomy, insertion of grommets and had previously suffered from faecal soiling secondary to severe constipation and overflow diarrhoea. In addition, she suffered from conductive hearing loss, with impaired motor skills and slow speech.

Patient 4
A second child, female was born to healthy nonconsanguineous parents (DOB 14-12-00), with a birth weight of 3.7 kg (60th centile).

Symptoms at birth
Examination at birth showed her to have hydrocephalus. She had been diagnosed with Dandy–Walker Syndrome with possible agenesis of the corpus callosum. A ventriculoperitoneal shunt had been inserted at 6 weeks of age.

Initial cytogenetic data
G-banded chromosome analysis revealed her to be: 46,XX, del (6)(p24.3→pter): *in situ* hybridisation also confirmed this karyotype. The parents' metaphase chromosomes were also analysed, both were normal for the respective region, indicating a *de novo* event.

Additional clinical data
Corneal opacities consistent with Peter's anomaly: she received bilateral cornea transplants at 2 weeks of age. A ventricular septal defect (VSD) was diagnosed and subsequently repaired at age 4 weeks. A gastrointestinal tube was inserted for feeding at approximately 6 weeks of age (see Table 1 for summary of phenotypes).

Mild sensorineural hearing loss in the right ear (35 db) and moderate to severe hearing loss in the left ear (60 db). Assessment at around 2 months indicated neurodevelopmental delay.

Patient 5
A female (DOB 02/04/02) was born to a 33- and 36-year-old mother and father, respectively. This was their first child and there was no clinical history of genetic abnormalities. Patient 5 was born at full term via a Caesarean section due to a breech presentation, with a birth weight of 3.54 kg (50th centile).

Symptoms at birth
Features present at birth included hypertelorism, a short neck, and a severely displaced hip: the latter condition possibly attributable to the breech presentation. Cerebral magnetic resonance imaging (MRI) revealed no significant abnormalities. Eye examinations, hearing tests and sonograms revealed no other abnormalities. The palate was highly arched. MRI detected some communication in the anterior flax. This was felt to have no clinical significance.

Initial cytogenetic data
Cytogenetic examination using G-banded chromosomes revealed a 46,XX,del(6)(p24.3) karyotype.

Additional clinical data
By 1 month of age, she had extensive hemangiomas and telangiectasias on the right arm and right shoulder girdle. A check up at 13 weeks showed occipital flattening, and mildly low set ears, mild pectus excavatum and mild hypotonia were present; radiographic examination showed the hips had healed. Patient 5 had suffered from numerous episodes of bronchiolitis and ear infections. A check up at 5 months revealed blue sclerae, hypotonia and loose joints (Figure 1). There was moderate motor delay, but otherwise, her growth, social and visual skills were good.

Examination of Patient 5 at 6 months showed relative brachycephaly and plagiocephaly appearance with right occipital bones somewhat more prominent than left, the anterior fontanel had not closed. The ears are posteriorly rotated and the nose was broad based. The abdomen was mildly protuberant with small fingertip-sized defect in the abdominal wall and umbilical hernia. The fingers were long and with ulnar deviation, the thumbs were bilaterally low set. The shoulder was particularly hyperflexible. The deep tendon reflexes were normal and symmetric throughout (see Table 1 for summary of phenotypes).

Patient 6
A first-born male (DOB 13-3-00) to a nonconsanguineous Hispanic mother and father of 23 and 28 years, respectively. The boy was born at 35 weeks gestation following treatment for preterm labour from 29 weeks.

Symptoms at birth
At birth, he had asymmetric flattening of the occiput, bilateral cleft lip, PDA, atrial septal defect (ASD); mild pelviectasis was noted on renal ultrasound and he had an imperforate anus. A brain stem auditory evoked response was normal. The bilateral cleft lip and imperforate anus were repaired in the first year.

Initial cytogenetic data

Cytogenetic analysis using G-banded chromosomes revealed a *de novo* interstitial deletion that was initially reported as: 46XY,del (6)(p23p24.3) *de novo*.ish 6pter (TelVysion probe 6p x 2).

Additional clinical data

At $7\frac{1}{2}$ months, physical examination showed hypertelorism with prominent eyes, flat supraorbital ridges and grey-blue sclerae (Figure 1), small posteriorly rotated, mildly dysplastic ears with bilateral preauricular pits. The left ear was overfolded and the right ear lacked the superior ramus of the antihelix. He had frontal upsweep of hair, a broad nose with a bulbous tip, but the palate was intact. There was mild pectus excavatum, a small umbilical hernia and an imperforate anus. The penis was small and testes were in the scrotum. Mild eczema was present. The middle toes were overlapping and thumbs were often adducted. The digits were otherwise normal except for spoon-shaped toenails. Neurological examination was normal except for mild drooling. Growth and development were essentially normal. At 13 months, he was at the 90th percentile for height and the 25th percentile for weight. Head circumference was also normal at the 10–25th percentile. At 13 months, the patient was taking one or two steps independently and he could say at least four words. His developmental assessment was age appropriate (see Table 1 for summary of phenotypes).

Family history included heart defects present in maternal second-degree relatives.

Methods

Tissue culture and fluorescent *in situ* hybridisation (FISH) were performed as described previously.[1] In total, 13 YAC, 44 PAC, two BAC clones and one cosmid, that had been previously FISH mapped to defined locations on 6p or contained identifiable genetic markers and genes, were used. The PAC clones were chosen from the Sanger Institute's FPC database (http://www.sanger.ac.uk/cgi-bin/humace/fpcwebmap, accessed May 2003); clones were ordered according to fingerprinting and marker content results. Clones present in Ensembl are a selection of the fingerprinted clones, and it is therefore possible to estimate the position of the clones used in this study in the Ensembl database (http://www.ensembl.org/, accessed May 2003). The YAC clones were ordered according to reported marker content by cross-referencing in both databases and confirmed by our own FISH-based clone ordering experiments. Additional data were compiled from the Ensembl database (May 2003) to describe genes identified so far on distal 6p with respect to their markers, known function, distance from the telomere and chromosomal localisation (see Table 2). Breakpoint positions in the cases described above are shown in Table 2 and reflect our best estimates.

Results

Deletions were confirmed by FISH as terminal or interstitial using probes mapping to 6p23–pter (Table 3). A dense collection of probes was used around regions where breakpoints occurred (Table 3). Clones were denoted as positive when a hybridisation signal was detected on the derivative chromosome 6 [del (6)] and negative when no signal was present.

Patient 1 has the smallest deleted 6p segment. YAC 952h4, which localises in 6p25.3, was positive on the respective del (6). However, PAC dJ223B1, which lies distal to the YAC, did not produce a hybridisation signal on del (6). The breakpoint lies between these probes: based on this result, we have refined the cytogenetic diagnosis and positioned the breakpoint in 6p25.2 (Table 3).

Patient 2 has the deletion breakpoint positioned in PAC DJ287K15, just distal of YAC 878B10, which produces a positive signal on del (6) and lies proximal in 6p25 (Table 3).

Patient 3 has the breakpoint positioned within the proximal segment of 6p25 with all probes distal to PAC dJ23O21 proving negative on del (6) (Table 3).

Patient 4 has the largest deleted segment with the breakpoint occurring within 6p24.3. PAC dJ436h5 did not hybridise on del (6), the probe lies just distal to BAC 648N19, which gave a positive signal on del (6) (Table 3). We were able to confirm the original cytogenetic diagnosis as 46XX,del (6)(p24.3).

PAC dJ118B18, which contains the FOXC1 gene and lies in the subtelomeric region of 6p, was used for FISH on chromosomes of patient 5. The results showed this distal region was indeed not deleted on del (6). The most proximal probe that did not hybridise on del (6) was PAC dJ133h11, this probe lies in 6p24.3, immediately distal to dJ167k8, that gave a positive signal on del (6) (Table 3). Thus, we were able to confirm the cytogenetic finding as 46XX,del (6)(p24.3p25.3).

The distal segment of 6p25 in Patient 6 was not deleted: instead, we have shown that the deletion is interstitial with BAC B18 marking the distal breakpoint and PAC 524G21 the proximal breakpoint (Table 3). Thus, we were able to refine the initial karyotype finding to 46XY,del (6)(p24p25.2). The extent of the deletion is less than previously thought from conventional cytogenetic methods.

Examples of FISH results of all cases are represented in Figure 2. Based on the FISH results and mapping data available from the Ensemble and the Sanger Centre's physical mapping database, the total amount deleted from chromosome 6 is: over 3.2 Mb from Patient 1, 5.8 Mb from Patient 2, 6 Mb from Patient 3, 9 Mb from Patient 4, 6.9 Mb from Patient 5 and 7 Mb from Patient 6. This corresponds to 0.09, 0.18, 0.17, 0.23, 0.17 and 0.14 % of the total human genome deleted, respectively. The phenotypes of the collective cases and corresponding photographs are shown in Table 1 and Figure 1, respectively.

Table 2 Data compiled from the Ensembl database (http://www.ensembl.org/), and the Sanger Institute's FPC database (see Methods for description)

	Genes	*Marker/clone information*	*Additional information*	*Distance from 6p Telomere (Mb)*	*Chromosome location 6p*
1	NOVEL	AL353654	Similar to Septin	0.117	25.2
2	Q9NRW4		Protein kinase phosphatase	0.277	25.2
3	IRF4		Interferon Regulatory Factor 4	0.377	25.2
4	Q96KP1		Ambiguous	0.471	25.2
5	QUJM7		Exocyst Complex component		25.2
6	NOVEL	AL031770	Unknown	0.587	25.2
7	Q9C009	AL499606	Winged Helix Forkhead	1.3	25.2
8	FOXF2	RP11-13J16	Forkhead Box Protein F2		25.2
9	FOXC1	AL034344	Forkhead Domain Transcription Factor	1.56	25.2
10	GMDS	AL033517	GDP Mannose,4,6 Dehydratase	1.71	25.2
11	NOVEL		Unknown	1.89	25.2
Patient 5	Distal	Breakpoint			
12	AL035693	RP1-33B19		2.07	25.2
13	000366	RP1-136B1	Belongs to LINE1	2.4	25.2
14	NOVEL	RP1-136B1	Reverse Transcriptase	2.4	25.2
15	Q96MT4	RP11-145H9	Unknown	2.6	25.2
16	NOVEL	RP11-145H9	Myosin Light Chain Kinase,Smooth Muscle		25.2
17	Q8WV26	AL139092	A Werner Helicase Interacting Protein isoform 1,	2.7	25.2
18	SERPIN B1	RP11420G6	Monocyte/Neutrophil Elastase Inhibitor	2.8	25.2
19	SERPIN B9	AL133351(RP1-90J20)	Cytoplasmic Antiproteinase 3	2.8	25.2
20	SERPIN B0	RP1-90J20	Placental Thrombin Inhibitor	2.9	25.2
21	Q96NM8	RP1-90J20	Unknown	2.97	25.2
22	NQ02	RP1-90J20	Quinone Reductase 2(Dehydrogenase)	3	25.2
23	NOVEL	RP1-40E16	Unknown	3	25.2
24	RIPK1	RP1-40E16(D6S1338)	Receptor-interacting Serine/Threonine Protein Kinase 2	3.06	25.2
25	BPHL	RP1-40E16(D6S1890)	Biphenylhydrolase-like	3.1	25.2
26	TUBB	RP1-40E16	Tubulin	3.13	25.2
27	NOVEL		Tubulin protein Family	3.14	25.2
Patient 6	Distal	Breakpoint			
28	NOVEL		Ion Transporter	3.25	25.2
29	Q9UFY2	RP11-15N12	Ion Transporter Family	3.3	25.2
30	NOVEL	RP1-72E17(D6S1196E)	Unknown	3.7	25.2
31	Q9Y247		Belongs to XAP5 Protein HXC26 family	3.8	25.2
32	Q9HD87	AL138831	Unknown	3.96	25.2
33	Q8TDP2,Q9NQH:	RP5-1013A10(D6S1932)	Serine/Threonine protein kinase		25.2
34	NOVEL	D6S1142E	Unknown	4.05	25.2
35	PECI	D6S1142E	Peroxisomal 3,2-trans-enoyl-coa isomerase		25.2
Patient 1	Breakpoint				
36	NOVEL		Unknown	4.1	
37	CDYL	RP5-1013A10	Chromodomain protein, Y-chromosome like	4.7	25.1
40	Q9BW22		Overlaps with CDYL		25.1
41	Q9UI73	RP3-430A16	Unknown	4.9	25.1
42	NOVEL	RP11-428J1		4.9	25.1
43	NOVEL	AL359643	Unknown		25.1
44	RP40	RP11-428J1	A ribonuclease P protein subunit P40	4.98	25.1
45	Q8WY88	D6S1789	Unknown		25.1

Table 2 Continued

	Genes	*Marker/clone information*	*Additional information*	*Distance from 6p Telomere (Mb)*	*Chromosome location 6p*
46	Q96DD8	RP1-67E13	CG1-203 protein	5.1	25.1
47	Q95363	RP11-105L5	Phenylalanine-tRNA, Synthetase	5.35	25.1
48	Q9NPY7		Unknown	5.35	25.1
49	095363	D6S1466	Phenyalanine-tRNA synthetase	5.4	25.1
50	Q9NTM1	D6S1685		5.7	25.1
51	Q9NPD7	D6S477		6	25.1
52	NRN-1	AL136307	Neuritin		25.1
Patient 2	Breakpoint				
53	F13A	D6S142	Coagulation Factor X111 Achain		25.1
54	Q9BX20	RP11-232H4		6.13	25.1
55	Q9NQP5	RP398D13		6.2	25.1
56	MD1-HUMAN	D6S1442		6.57	25.1
Patient 3	Breakpoint				
57	AL031123.14.1	RP11-320C15		6.57	25.1
58	RREB1		Ras responsive element binding protein	7.1	24.3
59	SSR1	RP11-69L16	Tranlocon-associated protein	7.27	24.3
60	Q8TC20	RP11-69L16	Unknown	7.3	24.3
61	Q9BRS2	D6S1640	Unknown	7.4	24.3
62	NOVEL		Belongs to nuclear ribonucleoprotein	7.5	24.3
63	Q9H302		NADP & specific isocitrate dehydrogenase	7.5	24.3
64	DSP	RP3-512B11	Desmoplakin	7.5	24.3
65	Q96MK1	512B11	Novel	7.57	24.3
66	Q9H4S5	AL390026			24.3
67	Q9H4S4	DJ336K20	Bipartite nuclear localisation signal, unknown protein family	7.6	24.3
68	BMP6	D6S1842	Bone morphogenetic protein 6 precursor	7.7	24.3
69	Q8TCT2	D6S1931	Thioredoxin related protein	7.9	24.3
70	Q8TBY6	RP1-303A1	Similar to phosphatidylinositol-4-phosphate5 kinase, type1-alpha	7.97	24.3
71	075662	RP1-303A1	Muted protein	8	24.3
72	Q9NU16			8	24.3
73	075662	D6S1674	Overlaps with muted protein	8.01	24.3
74	EEF1E1	D6S1855	Multi synthetase complex auxiliary component	8.1	24.3
75	CAA19223.7	D6S309	Overlaps with EEF1E1		24.3
Patient 5	Proximal	Breakpoint			
76	Q9H1N7		CG1-19 protein	8.4	24.3
Patient 4	Breakpoint				
77	NM153003	DJ503N11	MRDS1, orofacial clefting chromosomal breakpoint region 1	10.02	24.3
Patient 6	Proximal	Breakpoint			
78	NOVEL	D6S1955	Ribosomal	10.2	24.3
79	TFAP2A	RP1-290I10	Transcription factor	10.46	24.3/24.2
80	NOVEL		Unknown	10.5	24.2
81	CAC38372.1	RP11-360019			24.2

Table 3 List of probes used for *in situ* hybridisation on chromosomes of the 6p deletion cases

Location on 6p	*Clones 6p Tel*	*Markers & details*	*Patient 1*	*Patient 2*	*Patient 3*	*Patient 4*	*Patient 5*	*Patient 6*
25.3	6k23		−		−			
25.3	DJ15h23	(D6S1600)B76465GSS		−	−		+	+
25	DJ116 B8 }	(D6S344)AL589989						+
25	DJ118 B18 }	(870D6)AL034344(FOXC1)	−	−		−	+	+
25	905F3	AFMBO34YA5	−					
25	870D6	AFMBO34YA5	−					+
25	DJ33 B19	GMDS		−	−		+	
25	DJ90 J20 }	AL133351		−	−			
25	DJ40 E16 }	P1 REGION AL031963	−	−	−			
25	B20	P19	−	−	−		−	+
25	DJ283 K9 }	(AL1603980)	−		−		−	+
25	DJ262 P19 }		−	−	−		−	
25	DJ223 B1	AL0319433	−			−		−
25	DJ72 E17	AL38881	−				−	−
25	952H4 }	AFMA339YD9	+					
25	DJ52 F12 }	AQ081478GSS						
25	DJ21 P18	AL1333939	+	−				−
25	DJ36 I2	PROX TO CDYL	+	−			−	−
25	DJ67 E13	AL035653	+	−		−		
25	DJ232 P20	D6S1466		−	−			
25	DJ182 O16	D6S1685		−	−			
25	DJ103H18	D6S1677	+					
25	DJ287 K15	D6S1574 &(D6S477)(F13A)		−/+	−	−		−
25	878B10	D6S1574	+	+	−			
25	663A12	F13A	+		−			
25	853C3	D6S1574	+	+				
25	DJ23 O21	D6S1677	+	+	−/+			
25	DJ80 N2	PRP1		+	−/+	−	−	
25	938B10	D6S1598	+					
25/24.3	DJ129 N15 }	BMP 6			+	−	−	−
25/24.3	DJ155 I9 }	D6S1640			+	−		−
25/24.3	DJ29 L9	DSP	+	+	+		−	−
24.3	DJ164F5	WI-672			+			−
24.3	DJ133H11	D6S410			+		−	
24.3	DJ167K8	STS34084		+			+	−
24.3	DJ110P13	SGC33794			+	−		−
24.3	DJ135K15	SGC33794						
24.3	DJ103M22	AL031904					+	
24	Dj90 O12	AL031906		+		−		
24.3	DJ62A14	STSG11171		+	+	−	+	−
24	DJ436h5	STSG11216		+		−		
24	ba648N19	AL159986		+	+	+		−
24	DJ398a12	AL021332				+		
24	DJ359L13	AL1359316	+			+		+
24	DJ524G21	STSG24768		+	+	+		+
24	DJ380L24	AL139332				+		+
24.1–24.3	DJ503N11	AL031122						+
24	DJ242F16	STSG1197						+
24.1–24.3	DJ93h4	AL031122						+
24	DJ151c13	STSG26237				+		+
24	DJ172h1	STS23597					+	+
24	DJ290I10	TFAP2a				+		+
24.2	938D8	AP2,D6S470						+
24.1	886A2	D6S429	+					
23	772B2	D6S289				+		+
23	912G9	D6S1667						+
23	683H6	CD83,D6S259		+				+
23	930D2	JMJ,AFMA139WE5				+		+
23	DJ149A2	SCA1	+				+	

The cytogenetic position of the probes is indicated (left column), the name of the clones and information about marker content (middle column) and hybridisation results on patient's derivative 6 chromosomes (columns 1–6 to the right). Hybridisation (+) or failure of hybridisation (−) of probes to the derivative chromosome 6 is shown. A weak signal on the derivative 6 is described as +/−, indicating the breakpoint is close. The clones have been positioned in order using FISH and according to the Sanger centre database (http://www.sanger.ac.uk/cgi-bin/humace/fpcwebmap).

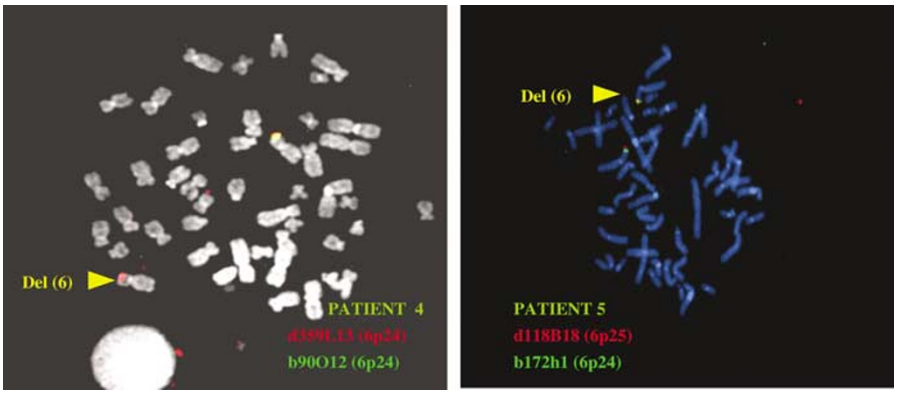

Figure 2 Examples of results showing metaphase chromosomes prepared from a selection of cases under investigation. Probes used for *in situ* hybridisation were labelled with biotin or digoxygenin and detected using fluorescein isothiocyanate or tetramethylrhodamine isothiocyanate systems. Hybridisation signals are indicated by *arrowheads*. (Left) Mapping the terminal deletion of Patient 4; PAC 359L13 (mapping in 6p24, red) hybridised to both chromosome 6 and del (6), whereas the more distal Pac 90O12 (also mapping to 6p24, green) hybridised only to the normal chromosome 6. Therefore the breakpoint lies between these two clones within band 6p24. (Right) Mapping the interstitial deletion in Patient 5; PAC118B18(red), mapping in 6p25, hybridised to both chromosome 6 and del (6), and PAC 172H1 (green) hybridised proximal to PAC118B18 within 6p24 on normal chromosome 6p. A green signal derived from PAC 172H1 is also present on del (6), and overlaps with the red signal (PAC118B18) in 6p25, indicating that a significant proportion of the region distal to 6p24 and proximal to 6p25 has been deleted.

Discussion

A number of genes have now been localised to the short arm of human chromosome 6, and current available data for this region have been analysed in an effort to gain direct relevance to our cases (Table 2). In addition, progress has been made in creating knockout mice for specific genes[12,17] or mice carrying defined chromosomal deletions.[14] Phenotypic characterisation of these mice and comparison to human patients with relevant chromosomal deletions enables us to extract information about gene function in this region. The main phenotypes observed in the cases described here are discussed below:

Ocular defects

Our four 6p terminal deletion cases exhibit ocular abnormalities, sensorineuronal hearing loss and developmental delay (clinical findings that constitute the description of 6p terminal deletion syndrome).[1] Although the extent of the deletion is different in each case, all are haploinsufficient for the region on 6p25 containing FOXC1 and associated with disorders of the eye, namely iridogoniodysgenesis, Axenfeld–Rieger anomaly/syndrome, primary congenital glaucoma and iris hypoplasia.[18,19] The variable penetrance of the eye anomalies observed in our 6p terminal deletion cases may be explained by different genetic backgrounds in agreement with the mouse model,[14] but parental origin effects, stochastic developmental events or mutations in other genes of related function may contribute. In addition, the ocular finding of grey-blue sclerae (Patients 5 and 6) can be associated with deletion of the 6p24–p25 segment distal to AP-2 and proximal to FOXC1 (Tables 1 and 2).

Hydrocephalus

The mouse Mf1 (FOXC1) knockout model and the allelic *ch* mouse show pleiotropic skeletal and ocular phenotypes.[12,14] These mice on various genetic backgrounds present craniofacial defects, lethal congenital hydrocephalus, eye defects and abnormalities of the central nervous, urogenital and skeletal systems. Parts of these phenotypes, like the ocular defects, can occur in mice heterozygous for the Foxc1 mutation.[15] Patient 4 presents with congenital hydrocephalus, heart and anterior eye developmental defects, showing considerable similarity to the congenital hydrocephalus (*ch*) mouse model. It would appear that by comparing our four terminal deletion cases (Table 3), hemizygosity for both Bone morphogenetic protein 6 (BMP6) and the FOXC1 gene produces such a phenotype as hypothesised by Kume *et al*.[12] However, it is worth noting that the case reported by Navarro *et al*[3] (and GK Mirza, AM Perle and J Ragoussis, unpublished results) and an unbalanced translocation case (CA in Davies *et al*[1]), present with hydrocephalus associated with the deletion of FOXC1, while BMP 6 is retained. Therefore, we can conclude that the factors determining the occurrence of hydrocephalus could be more complex than the contiguous deletion of the FOXC1-BMP6 chromosomal segment.

Heart defects
Patient 1 and one of our published 6p terminal deletion cases,[1] carry deletions of FOXC1 and present with aortic valve abnormalities. Furthermore, aortic valve abnormalities have been diagnosed in the FOXC1 mouse model.[13] Thus we conclude that FOXC1 hemizygosity appears to be an important determinant of aortic valve development. Suggestions that evolve from analysis of cases in the literature with molecular characterisations are that regions deleted proximal of 6p25 attribute to other heart defects, in particular ASD, VSD and PDA. In combination with Patients 5 and 6, we suggest the involvement of other loci positioned between 6p24.3 and 6p25 in heart development. Of the genes identified within the relevant chromosomal segment, BMP6 is a possible candidate since it is expressed in the heart.[17]

Bone formation
(a) Joint formation Three cases presented with joint hyperlaxity; Patients 2, 5 and 3. The latter case presented with *osteochondritis juvenilis deformans* (Perthes disease). Additional reports, including the case described by Guillen-Navarro[3] and an unbalanced translocation case (BD in Davies *et al*[1]), all with deletion of 6p25, present with delayed bone maturation and hip dislocations. In the case of Patients 2 and 3, these findings agree with the Mf1 knockout model; however, Patient 5 presents evidence for other genes further centromeric that contribute to chondrogenesis.

(b) Pectus excavatum A further interesting aspect of Patients 5 and 6 is that the segment containing the BMP6 gene is deleted. Both cases exhibit pectus excavatum, consistent with the phenotypic finding in one of our previously reported cases carrying a deletion of BMP6.[9] Mice lacking BMP6 (the heterozygotes are reported normal) show defects in the development of the sternum[17] indicating that the human protein may have a similar function to the mouse homologue.

Hearing defects Our 6p terminal deletion cases 1,3 and 4 present with hearing loss, and case 2 demonstrates auditory hypersensitivity. The interstitial deletion cases lack such symptoms and therefore we suggest that a gene involved in hearing development can be positioned distal to the most telomeric interstitial breakpoint observed (case 5), that is, distal to the region containing the GMDS gene (Tables 2 and 3).

Craniofacial anomalies Our interstitial deletion cases, 5 and 6, are reported present with a highly arched palate and a cleft lip, respectively: the proximal breakpoints of both deletions lie in 6p24.3. A number of studies have shown linkage to this region for orofacial clefting.[20–22] Patient 2 is the first case to have both cleft lip and palate and this could result from an interaction between loci in 6p24 and 6p25. In summary these cases support the evidence that genes contributing to orofacial clefting lie distal to AP2 and proximal to FOXC1 gene.

Skin defects Haploinsufficiency for the desmoplakin (DSP) gene has been implicated in causing an inherited skin disorder, palmoplanter keratoderma. Patients 4, 5 and 6 are hemizygous for this gene. Only the latter case showed defects to the skin, namely eczema. Other related cases have reported excessively dry skin but none so far have reported palmoplantar keratoderma. We conclude that hemizygosity for DSP on its own is not sufficient to cause this disease.

Cognitive/neuromotor delay With the exception of Patient 1, our cases are hemizygous for the gene neuritin (Table 2) and except for Patient 6, our cases present with either neuronal or motor development delay. Patient 2 presents with epilepsy. Neuritin is induced by neuronal activity and promotes neuritogenesis;[23] therefore, haploinsufficiency for this gene may contribute to developmental delay in our cases.

Tooth development defects Our terminal deletion patients, 1, 2 and 3, all show tooth abnormalities; Patients 5 and 6 have normal dentition. We can conclude that hemizygosity for the region between the most telomeric breakpoint in the vicinity of marker D6S1617 and the telomeric breakpoint of patient 6 results in such defects.

An important point that requires highlighting is that all cases have deletions that arose *de novo*. Patient 2 and our previous cases BD, GD, HH and SG have deletion breakpoints within the F13A gene.[1] This part of the chromosome contains the fra (6) 6p25.1 common, aphidicolin-type fragile site, and it is of interest to fine map and characterise this site and to determine whether it is involved in the deletions presented here. The extent of deletion in our cases does not correlate with the severity of the phenotype (Tables 2 and 3b). Patient 1 has the most severe phenotype, but the least amount of deleted genome compared to the other five cases. Genetic background differences, parent-of-origin effects or undetected mosaicism may be responsible for the lack of correlation between phenotype severity and size of the deletions in our cases.

It is clear that the generation and detailed study of mouse models and human deletion cases have contributed significantly to understanding the molecular basis of chromosome 6p deletion syndromes. Further work involving generation of mutations in genes located within the segment syntenic to human chromosome 6p in mice will increase our understanding of the function and nature of developmental defects associated with these deletions (http://www.mgc.har.mrc.ac.uk/physical/del36h.html).

Further details and links on 6p deletion cases are available at our website (http://www.well.ox.ac.uk/genomics).

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