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16p subtelomeric duplication: a clinically recognizable syndrome

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We report on two patients with duplication of the subterminal region of chromosome 16p (dup16p) recognized by fluorescent *in situ* hybridization (FISH) telomere analysis, presenting with closely overlapping facial features and neurological impairment. Distinct facial anomalies included high forehead, sparse eyebrows, blepharophimosis, short nose, everted upper lip, high-arched palate, wide-spaced teeth, and cupped anteverted ears. Susceptibility to vascular anomalies, in particular pulmonary hypertension and portal cavernoma, was found in one patient. Subtelomeric analysis by FISH demonstrated a *de novo* duplication of the subtelomeric region of chromosome 16p and a deletion of the subtelomeric region of chromosome 4q in case 1, and duplication of the subtelomeric region of 16p and a deletion of the subtelomeric region of 21q, resulting from malsegregation of a balanced maternal translocation t(16pter;21qter) in case 2. The extension of duplicated regions measured by array-comparative genome hybridization was about 12 Mb on 16p13.3p13.13 in case 1, and about 8.5 Mb on 16p13.3p13.2 in case 2. In conclusion, we reported a clinically recognizable disorder in two patients with dup16p. Pulmonary hypertension, vascular ring, and manifestations of vascular disruption, as terminal hypoplasia of hands and aplasia cutis, have been previously described in association with dup16p. Thus, susceptibility to pulmonary vascular disease and other vascular anomalies can be a feature of dup16p, suggesting that this subtelomeric region in some respect could be related to vascular anomalies.

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Introduction

Partial trisomy 16p is a rare chromosome imbalance characterized by mental retardation, prenatal and postnatal growth deficiency, facial anomalies, cleft palate, congenital heart defect, and urogenital anomalies.^{1–9} Only a few patients have been reported, the majority of which manifest chromosome duplications resolved by standard cytogenetic techniques. Previous studies have

established that the phenotypic severity could not be correlated with the size of the duplicated segment, and region 16p13.1–p13.3 is 'critical' in determining this disorder. Telomere analysis has also identified cryptic duplications of the subterminal region of chromosome 16p (dup16p), which was considered less rare than originally thought.^{8,10,11}

We report on two patients with dup16p recognized by fluorescent *in situ* hybridization (FISH) telomere analysis, presenting with closely overlapping facial features, neurological impairment, and urinary malformations. Susceptibility to vascular anomalies, in particular pulmonary hypertension and portal cavernoma, was found in one case.

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Subjects Patients

Patient 1 This patient, a female, was the first child of healthy nonconsanguineous parents. At birth, the mother was 28-year old and the father 23. Family history was unremarkable. The baby was born by vaginal delivery at the 35th week of an uneventful gestation. Birth weight was 2500 g, length 48 cm, and head circumference 33.5 cm. Apgar scores were 8 and 9 at 1' and 5', respectively. The patient was first seen by us at 6 months. Weight was 5300 g (<3rd centile), length 58.5 cm (<3rd centile), and head circumference 38 cm (<3rd centile). Clinical evaluation disclosed microcephaly, bilateral blepharophimosis, everted upper lip, anteverted ears, and mild fingers' camptodactyly. The baby was hypotonic and developmental milestones were delayed. Feeding difficulties required nasogastric tubes in the first 4 months of life. The patient underwent surgical correction of bilateral inguinal hernia at 3 months.

Portal hypertension was diagnosed in the first month of postnatal life. Magnetic resonance angiography demonstrated a portal vein cavernoma with esophageal varices. Liver and spleen were enlarged. A corrective meso-portal bypass surgery was performed.

Two-dimensional color Doppler echocardiography showed patent ductus arteriosus and a small atrial septal defect ostium secundum type. Patent ductus arteriosus was operated at 2 months. A routine color Doppler echocardiography at 2 years disclosed severe pulmonary hypertension with dilatation of the right heart chambers and the pulmonary artery. The pulmonary hypertension was idiopathic and not related to congenital heart defects. Cardiac catheterization showed systemic level pulmonary artery pressure, elevated pulmonary vascular resistance, and no response to administration of oxygen and nitric oxide.

Cerebral MRI demonstrated corpus callosum hypoplasia. EEG and brain stem audiometry were normal. Ophthalmological examination revealed bilateral mild optic nerve hypoplasia. Renal ultrasonography showed an ectopic left kidney.

At 2.4 years, weight was 8.350 kg (<3rd centile), height 68 cm (<3rd centile), and head circumference 44.5 cm (<3rd centile). Clinical characteristics included blond fine hairs, sparse eyebrows, bilateral palpebral ptosis with narrow palpebral fissures, asymmetric nares, short philtrum, open mouth with everted upper lip, narrow palate, dental malposition with diastema, and cupped anteverted ears (Figure 1a and b). Moderate mental retardation was detected by neuropsychological testing, and language was absent.

Patient 2 This patient was a 3.7-year-old female. At birth, the mother was 28-year old and the father 30. Parents were nonconsanguineous, and family history was unremarkable.

The baby had a healthy dizygotic co-twin. The proband was born by vaginal delivery at the 39th week of an uneventful gestation. Birth weight was 2430 g, length 47 cm, and head circumference 34 cm. Apgar scores were 6 and 8 at 1' and 5', respectively. The patient was evaluated by us at 3.7 years. Weight was 12 Kg (<3rd centile), height 101 cm (75th–90th centile), and head circumference 50 cm (50th centile). On clinical evaluation, the patient disclosed blond thin hair (blonder than the familial background), high frontal hairline, narrow palpebral fissures with bilateral ptosis, short philtrum, everted upper lip, high arched palate, dental caries, micrognathia, cupped anteverted ears (Figure 1c and d), and hypoplastic distal phalanges with clubbed nails. A right congenital hip dysplasia was operated at 6 months. Developmental milestones were moderately retarded. The baby was sitting at 12 months, and walked alone at 2 years. Language was absent. She was hyperactive and showed self-injurious behavior. Feeding difficulties were recorded in the first 2 years of life (Figure 2).

Cerebral MRI, ophthalmologic evaluation, brainstem audiometry, and two-dimensional color Doppler echocardiography were normal. Renal ultrasonography and cystourethrography disclosed grade IV left vesicoureteral reflux with unilateral hydronephrosis and hypoplastic kidney on the left.

Results

Chromosome analyses were carried out on peripheral lymphocytes using standard techniques. Subtelomeric regions were analyzed using ToTelVysion kit (Vysis Inc., Downers Grove, IL, USA), according to manufacturer's suggestions. To characterize the extension of the subtelomeric imbalances, array-comparative genome hybridization (CGH) analysis was performed using an oligonucleotide microarray with a genomic resolution of about 100 kb (44K; Agilent Technologies, Waldbronn, Germany), according to Bernardini *et al.*¹² Slides were imaged with Feature Extraction software (v8.5) and chromosomal profiles were obtained using the CGH Analytics software (v3.4.40; Agilent Technologies).

In patient 1, standard chromosome analysis disclosed a normal 46,XX karyotype. Subtelomeric analysis by FISH demonstrated a *de novo* duplication of the subtelomeric region of chromosome 16p and a deletion of the subtelomeric region of chromosome 4q (Figure 3a and b). The extension of unbalanced regions was measured by array-CGH, which showed that the duplicated 16p13.3p13.13 region extended for about 12 Mb, from the probe A_14_P101770 to A_14_P122811 (12 088 408 bp; Figure 4), whereas the deleted 4q35.2 band included about 4 Mb of genomic DNA spanning from probe A_14_P123925 (187 372 895 bp) to probe A_14_P129637 (191 259 499 bp).



Figure 1 Frontal and lateral appearances of patient 1 (a, b) and patient 2 (c, d).

In patient 2, standard chromosome analysis was normal (46,XX), whereas subtelomeric analysis disclosed a duplication of the subtelomeric region of 16p and a deletion of the subtelomeric region of 21q (Figure 3c and d). This imbalance resulted from malsegregation of a balanced maternal translocation $t(16pter;21qter)$. Array-CGH showed that the duplicated region covered about 8.5 Mb on 16p13.3p13.2, from probe A_14_P101770 to probe A_14_P121597 (8 857 047 bp; Figure 4). Deletion of chromosome 21 spanned for about 300 kb in the subterminal region 21q22.3.

A duplication of the subtelomeric region of 16p in the parents of patients 1 and 2 was excluded by array-CGH.

Discussion

The distinguishing clinical features of dup16p syndrome, limited to the terminal 16p13.1–p13.3 'critical' region,



Figure 2 Facial appearance of the patient with dup16p reported by Sommer *et al*⁸, showing striking similarity of the phenotype with patients 1 and 2 in this report (courtesy of Dr Sommer).

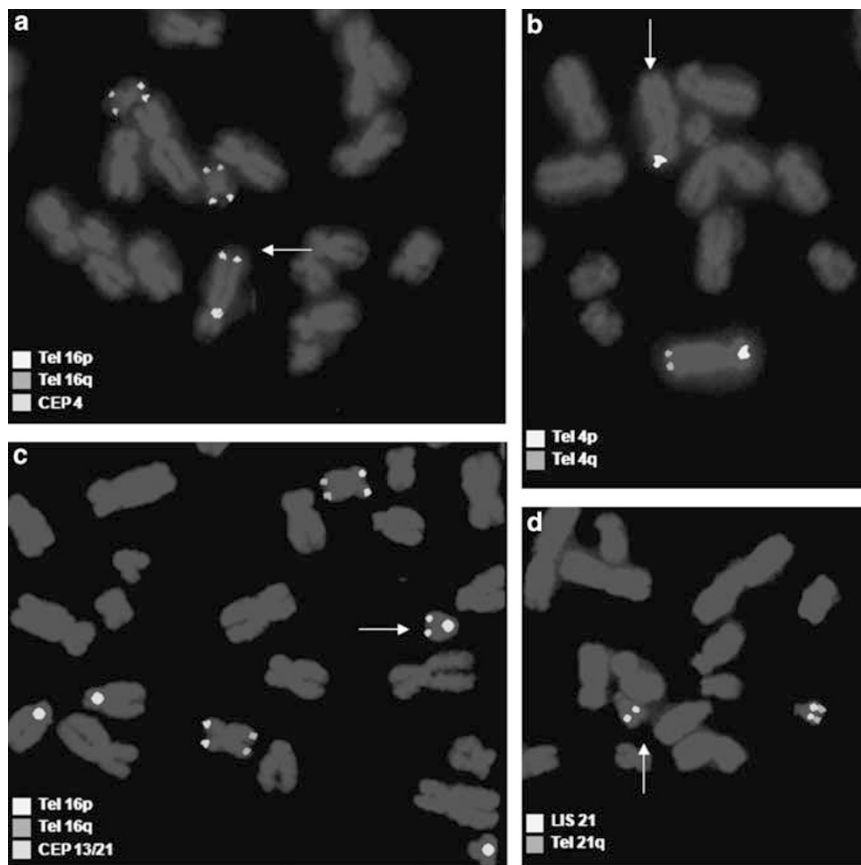


Figure 3 Patient 1: (a) Analysis of subtelomeric regions showing the duplication of the distal tip of 16p onto the subtelomeric region 4q, which was deleted (b). Patient 2: (c, d) The subtelomere 16p probe discloses an additional signal (duplication) onto the distal 21q region, which has only one signal (deletion). Arrows point to duplicated and deleted regions.

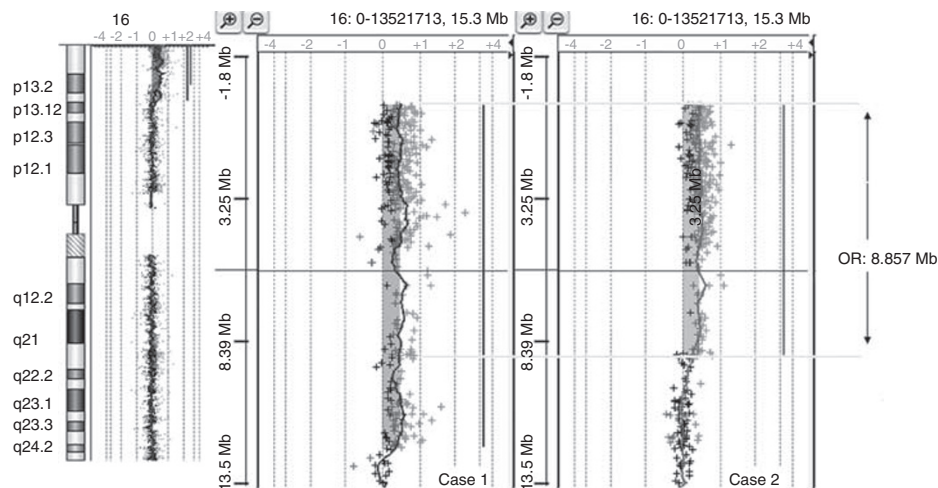


Figure 4 Oligonucleotide array-CGH analysis shows that duplication 16p in case 1 (blue vertical line) encompasses about 12 Mb, whereas in case 2 (green vertical line) is spanning for about 8.8 Mb, thus defining the overlapping duplicated region.

include specific facial anomalies, mental retardation, congenital cardiac and vascular defects, urinary malformations, and hypoplastic distal phalanges of hands (Table 1).

Craniofacial appearance is characterized by blond thin hairs, high forehead, sparse eyebrows, blepharophimosis with palpebral ptosis, short nose, open mouth with everted

Table 1 Clinical features of patients with microduplication of the subtelomeric region of chromosome 16p (dup 16p)

Features	Present reports		Martin, 2002					Tschernigg, 2002	de Ravel, 2005	Sommer, 2006	Ruiter, 2007	Total number, %
	Patient 1	Patient 2	1	2	3	4	5					
<i>Facial anomalies</i>	+	+	+	+	+	+	+	+	+	+	+	11/11, 100%
Thin hair	+	+	+	N	N	+	+	+	+	+	N	8/8, 100%
<i>Blepharophimosis/palpebral ptosis</i>	+	+	N	N	+	+	+	+	+	+	N	8/8, 100%
Short nose	+	+	-	-	-	-	-	-	+	-	N	3/10, 30%
Everted upper lip	+	+	N	N	N	N	N	-	+	+	N	4/5, 80%
High arched palate	+	+	N	N	+	-	-	+	+	+	N	6/8, 75%
Cleft lip/palate	-	-	-	+	+	+	+	+	+	-	+	7/11, 63%
Micrognathia	+	+	+	+	N	-	-	-	+	+	N	6/9, 67%
Cupped/dysmorphic ears	+	+	+	+	N	+	+	+	+	+	N	9/9, 100%
<i>Hands/feet anomalies</i>												
Hypoplastic digital phalanges	-	+	N	N	+	-	-	-	-	-	-	2/9, 22%
2-3 toe syndactyly	-	-	N	+	N	+	+	-	-	-	-	3/9, 33%
<i>Congenital heart defect</i>	+	-	-	-	+	+	-	+	-	-	-	4/11, 36%
Atrial septal defect	+	-	N	N	+	+	-	+	-	-	-	4/9, 44%
Ventricular septal defect	-	-	N	N	-	-	-	+	-	-	-	1/9, 11%
Aortic coarctation	-	-	N	N	-	-	-	+	-	-	-	1/9, 11%
Patent ductus arteriosus	+	-	N	N	-	-	-	-	-	-	-	1/9, 11%
<i>Vascular anomalies</i>	+	-	+	-	-	-	+	+	-	-	-	4/11, 36%
Pulmonary hypertension	+	-	-	-	-	-	-	+	-	-	-	2/11, 18%
Portal vein cavernoma	+	-	-	-	-	-	-	-	-	-	-	1/11, 9%
Vascular ring	-	-	N	N	-	-	+	-	-	-	-	1/9, 11%
Aplasia cutis	-	-	+	N	N	-	-	-	-	-	-	1/9, 11%
<i>Urinary abnormalities</i>	+	+	-	+	-	+	+	-	-	-	-	5/11, 45%
Vesicoureteral reflux/hydronephrosis	-	+	N	+	N	+	+	-	-	-	-	4/9, 44%
Renal hypoplasia	-	+	N	N	N	N	N	-	-	-	-	1/6, 17%
Ectopic kidney	+	-	-	-	-	-	-	-	-	-	-	1/11, 9%
Congenital hip dislocation	-	+	-	-	-	-	-	-	-	-	-	1/11, 9%
Mental retardation	+	+	+	+	+	+	+	+	+	+	+	11/11, 100%
Seizures	-	-	+	-	+	-	-	+	+	+	-	5/11, 45%
Corpus callosum hypoplasia	+	-	+	-	N	+	N	-	-	N	-	3/8, 38%
Microcephaly	+	-	+	-	+	+	-	+	-	+	-	6/11, 55%
Hearing loss	-	-	+	N	+	+	+	N	-	-	-	4/9, 44%
Approximate size of the duplication (Mb)	12	8.5	3.5	4.5	10	10	10	N	4.5	N	<0.2	

+ denotes present, - absent, and N not reported.

upper lip, high-arched palate, wide-spaced teeth, and cupped everted ears. Facial anomalies appeared strikingly similar in our two patients, and in those reported by de Ravel *et al*¹⁰ and Sommer *et al* (Figure 2).⁹

Congenital heart defect, prevalently atrial septal defects, can be a feature of dup16p syndrome. Patients carrying large 16p duplications detected using standard techniques can manifest also tetralogy of Fallot or ventricular septal defect.^{5,13} A baseline cardiological evaluation is recommended at diagnosis, and periodic monitoring is warranted for the potential onset of pulmonary hypertension. Early onset of pulmonary vascular disease unresponsive to oxygen, as illustrated in our case 1, has been reported

previously in an infant with an inverted duplication of 16p11.2-p13.3.⁵ These two cases suggest that susceptibility to pulmonary vascular disease can be a feature of dup16p syndrome. Fixed pulmonary hypertension has been described also in an additional patient with a 46,XY,add(16)(p16.3).ish dup16(pterp13) karyotype.⁷ However, in this case pulmonary hypertension could be related to congenital heart defect, consisting in a large perimembranous ventricular septal defect, an atrial septal defect, and hypoplastic aortic arch. Our case 1 had also a congenital portal cavernoma leading to portal hypertension, a feature not previously recognized in these patients. The portal cavernoma and the coexisting pulmonary

vascular disease in the same patient expand the clinical spectrum of trisomy 16p syndrome, and argue for a possible association between this imbalance and vascular anomalies. Interestingly, vascular ring and manifestations of vascular disruption, as terminal hypoplasia of hands and aplasia cutis, have also been reported in association with dup16p (present patient 2).⁸

The phenotype of patients with dup16p is not specific for a specific Mendelian syndrome, although some aspects of facial appearance including blepharophimosis and hypoplastic teeth could suggest Ohdo syndrome.¹⁴

Previous observations have stated that chromosomal region 16p13.1–p13.3 is 'critical' in determining clinical anomalies of the syndrome, and that the phenotypic severity could not be related to the size of the duplicated segment. Nevertheless, in patients with complete trisomy 16p it was not possible to identify a characteristic phenotype similar to that found in cases with duplication of the distal segment.¹⁵ Pre- and postnatal growth and mental retardation were more severe in patients with complete trisomy 16p. A distinctive phenotype different to that identified in our patients has been recently described in a girl with duplication limited to the Rubinstein–Taybi region on 16p13.3, probably representing a single gene disorder.¹⁶ Clinical manifestations in that case included moderate mental retardation, tall stature, specific facial appearance, and hand/foot anomalies.

In our two patients, dup16p overlapped for about 9 Mb, from the 16p subtelomeric region to 16p13.2 band, and was associated with distal deletion of 4q in case 1 and distal deletion of 21q in case 2. The clinical implications of the second imbalances should be also considered. However, the distinct facial dysmorphisms and vascular anomalies usually are not found in patients with these isolated cryptic deletions, suggesting that the distinguishing features of dup16p syndrome result from gene dosage of the distal 8.8 Mb region duplicated in our two patients (Figure 4).

In conclusion, we have confirmed the existence of a clinically recognizable disorder in patients with dup16p, involving the terminal 16p13.1–p13.3 region. Mental retardation, facial anomalies, and terminal hypoplasia of fingers are clinical handles for suspecting this rare syndrome, which can be confirmed by molecular-cytogenetic testing. Susceptibility to pulmonary hypertension and other vascular anomalies, as occurring in one of the present patients and in other published cases, can be a

feature of dup16p, suggesting that this subtelomeric region in some respect could be related to vascular anomalies.

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