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ARTICLE

Detailed phenotype–genotype study in five patients with chromosome 6q16 deletion: narrowing the critical region for Prader–Willi-like phenotype

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Most patients with an interstitial deletion of 6q16 have Prader–Willi-like phenotype, featuring obesity, hypotonia, short hands and feet, and developmental delay. In all reported studies, the chromosome rearrangement was detected by karyotype analysis, which provides an overview of the entire genome but has limited resolution. Here we describe a detailed clinical presentation of five patients, two of whom were previously reported, with overlapping interstitial 6q16 deletions and Prader–Willi-like phenotype. Our patients share the following main features with previously reported cases: global developmental delay, hypotonia, obesity, hyperphagia, and eye/vision anomalies. All rearrangement breakpoints have been accurately defined through array-CGH at about 100 Kb resolution. We were able to narrow the shortest region of deletion overlap for the presumed gene(s) involved in the Prader–Willi-like syndrome to 4.1 Mb located at 6q16.1q16.2. Our results support the evidence that haploinsufficiency of the *SIM1* gene is responsible for obesity in these patients. A possible involvement of the *GRIK2* gene in autistic-like behaviour, of *POPDC3* in heart development, and of *MCHR2* in the control of feeding behaviour and energy metabolism is also hypothesized.
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Introduction

Previous studies have associated the interstitial deletion of chromosome 6q16 with a Prader–Willi-like phenotype, featuring obesity, hypotonia, short extremities, and developmental delay.[1–7] In all these studies, the chromosome rearrangement was detected by karyotype analysis, which provides an overview of the entire genome but has limited resolution. We report two previously published cases (including detailed follow-up information on one case)[2,4] further cytogenetically refined by array-CGH and three new cases with overlapping interstitial 6q deletions. One of the new cases shows the smallest deletion

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(4.1 Mb) reported until now, which led us to narrow the critical region for Prader–Willi-like phenotype at 6q16.1–6q16.2.

Our results refine the genotype-phenotype correlation for the deletion 6q16 Prader–Willi-like syndrome.

Case report

All five patients are described in more detail in the section below and their clinical features are summarized in Table 1.

Case 1

This male patient was hypotonic at birth and developed hyperphagia in the second year of his life.[2] At the age of 23 months, his weight was at the 75th centile and his stature below the 50th centile. He had almond-shaped eyes, short hands, and psychomotor delay. Although external genitalia were normal, the general phenotype suggested Prader–Willi syndrome. His karyotype was defined as 46,XY,del(6)(q16.2q21). The child was re-evaluated at the age of 16 years. He shows severe mental retardation and developmental delay. He has absent language and uses non-verbal communication.

In his behaviour and social contact, he shows some features of autism spectrum disorder (marked solitariness, poor ability to relate to others, stereotypies). He loves classical music and does not show any aggressive/self-mutilating behaviour. He has important hyperphagia and is still obese (body mass index between 29 and 30). He suffers of constipation and has no bowel control.

Case 2

This girl was first seen at the age of 4 years.[4] She was reported to be hypotonic with feeding problems in the neonatal period. She had a patent ductus arteriosus and atrial septal defects. From the age of 2 years she became obese. She was noted to have a broad forehead, scaphocephaly, full round face with almond-shaped eyes, mild ptosis, strabismus, and a broad nasal bridge. She had truncal obesity and short fingers and toes. There was moderate psychomotor retardation; she walked without support at the age of 20 months. Neurological examination revealed hypotonia with a wide-based gait and an IQ of 60 at a non-verbal Dutch intelligence test. Endocrine evaluation showed mild primary hypothyroidism and insufficient growth hormone secretion, but normal growth. A magnetic resonance imaging scan of the brain showed Arnold–Chiari malformation type 1. Her karyotype was 46,XX,del(6)(q15q21).

She was re-evaluated at the ages of 7, 10, and 13 years (Figure 1a). Her growth remained normal with heights of 128, 144.5, and 163.5 cm respectively (all 50th centile). She was mildly obese with a weight of 41.8, 58.5, and 76.8 kg (8, 10, and 7 Kg above 95th centile), respectively. She also had mild macrocephaly with a head circumference at the 98th centile for her age. She attended a school for children with learning disabilities and was able to read at low level and write her name at the age of 13 years. She had severe speech delay and communication was supported by the use of pictograms. She had a pleasant and friendly personality.

Case 3

She was the first child of healthy non-consanguineous Caucasian parents. Her mother and father were 35 and 37 years old, respectively, at the time of her birth. Her mother suffered from threatened abortion that was treated with bed rest, Isoxsuprine, and Buscopan at the 2nd and 4th months of pregnancy. She was also on regular treatment with Thyroxin (100 μg per day) for hypothyroidism. Routine ultrasound scanning at the 20th week of gestation revealed a possible heart malformation confirmed by foetal echocardiography showing tetralogy of Fallot. Karyotype analysis of amniotic fluid cells at the 23rd week of gestation showed female 46, XX chromosomes with suspicion of an interstitial deletion at the long arm of chromosome 6: 46,XX,del(6)(q16.2q22.1)dn. The child was born at the 35th week of gestation by caesarean section for preterm rupture of the membranes. Birth weight was 1.95 kg (25th centile), length was 48 cm (50th centile), head circumference was 31 cm (10th centile) and APGAR scores were 6 and 8 after 1 and 5 min, respectively. Postnatal echocardiography confirmed the tetralogy of Fallot. Transfontanellar and abdominal ultrasound scans were within normal limits. On the fourth day of life, she showed hypertelorism, low-set ears, malformed right ear (with hypoplasia of the lobe), cauliflower shaped left ear, and single palmar creases. At the age of 2 months, her weight was 2.82 Kg ($<$3rd centile). She had head control since the third week of life. Further dysmorphic features were high hairline, wide anterior fontanelle, shallow orbits, and bilateral zygomatic prominence.

Case 4

This male patient was the first child of healthy non-consanguineous parents. He was born at the 36th week of gestation. Pregnancy was referred to be regular. His birth weight was 2.75 kg (50th centile). Length, head circumference, and APGAR scores were not recorded. At birth hypotonia, feeding difficulties, and metatarsus valgus were noticed. Developmental milestones were delayed; due to a knee fracture he began to walk unsupported at the age of 6 years. His first words were pronounced at the same age.

The boy was referred at the age of 16 years (Figure 1b). His weight was 69 kg (75–90th centile); height, 164 cm (3rd–10th centile); head circumference, 55 cm (50th centile). Neurological examination showed an IQ $<$ 49 at the Griffiths Scale. The following dysmorphic features were noted: asymmetric skull, plagiocephaly, brachycephaly, round face, deep-set eyes, long posteriorly rotated ears, flat malar region, prominent columella, thin high nasal bridge, short philtrum, high palate, small mandible with irregular malpositioned teeth, tapering fingers, genus valgus, clubfoot, and truncal obesity. Cardiopulmonary

Table 1 Clinical and cytogenetic characteristic of PWS-like patients with interstitial 6q deletions

	Our cases					*Klein* et al *(2007)*			*Le Caignec* et al *(2005)*	*Verela* et al *(2006)*	*Total*
Patients no.	Case 1	Case 2	Case 3	Case 4	Case 5	Case 1	Case 2	Case 3			
6q deletion size	q16.1–q21 15.2 Mb	q16.1–q21 14.2 Mb	q16.1–21 15 Mb	q16.1–q16.3 4.1 Mb	q16.1–6q16.3 7.2 Mb	q16.2–q21 8.8–16.4 Mb	q15–q16.2 6–16.2 Mb	q15–16.2 11.3–15.7 Mb	q16.1–q21 14 Mb	q16.1–21	
Parental origin	NT	Paternal	Paternal	Maternal	Paternal	Paternal	Paternal	Paternal	NT	Paternal	
Age at last clinical evaluation	14 years	13 years	2 months	16 years	38 years	11 years	6.5 years	13 years	$2^{8/12}$ years	12 years	
Clinical features											
Developmental delay	+ Severe	+ Moderate	+	+ moderate	+ moderate	+	+ Mild	+	+ Moderate	+	10/10
Language delay	Absent	+		+		Absent			+	+	6/10
Hyperphagia	+	+	–	+	+					+	5/10
Hypotonia	+	+	–	+	–	+	+ Neonatal	+	+ Neonatal	+	8/10
Craniofacial anomalies	+	+	+	+	+	+	+	+	+	+	10/10
Obesity	+	95th	<3rd	75–90th	>95th	75–90th	10th	+	50th	>98th	7/10
Hands	Short	–		–	Large		–			Small	3/10
Tapered fingers	+		–	+	–		–				2/10
Clinodactyly				–	–	+	–	+			2/10
Small stature				–	–	+	+	–		–	2/10
Vision anomalies	–	Problems with stereotypic vision		Myopic astigmatism	–	Esotropia, visual maturation delay	Strabismus, nystagmus, retinitis pigmentosa	Myopia	Strabismus, hypermetropia	Strabismus	7/10
Behavioural problems	ASD	–		Stereotypies	Restless food, liquid seeking behaviour		Increased oral sensory sensitivity	ADHD/ OCD	ASD	Temper tantrums, stubbornness	6/10
Cardiopathy	–	+	+	–	–	–	–	–	–	–	2/10
Brain anomalies	+	+		+	+	+		–	–		5/10
EEG anomalies				+	+ Mild	+	+				4/10
Craniofacial anomalies											
Rounded face/ full cheeks	+	+		+	–					+	4/10
Skull	Brachycephaly	Scaphocephaly		Plagiocephaly Brachycephaly	Macrocephaly	Brachycephaly	Brachycephaly	Macrocephaly		Macrocephaly	7/10
Microcephaly	–	–		–	–	+	–	–			1/10
Eyes anomalies	+	+	+	+	–	+				+	5/10
Low-set ears	+	–	+	–	–	+	+	+			4/10
Malformed ears	+	+	+	+	Large ears	+	+	Low-set	+		9/10
Nose	Bulbous	Broad nasal bridge		Thin high nasal bridge	Normal	Long, narrow	Wide mid-section	High bridge	Upturned	Bulbous, anteverted nares	9/10
Philtrum	Marked			Short					Prominent		3/10
Microretrognathia	+	–	–	+	–	+	+	+	+	+	7/10
Malpositioned teeth		–		+	–				+		2/10
High arched palate	+			+	–						2/10
Perinatal											
Birth weight (percentile)	75th	10th	25th	50th	50th	5–10th	25th	95th	3rd–10th	50–75th	
Birth length (percentile)	>50th		50th	NR	50th	50th		75–90th	25th	50–75th	
OFC (percentile)			10th	NR	NR	3rd					
GA(weeks)	38	41	35	36	40				40		
Maternal age (years)	30	32	35	25	28	29	37			24	
Paternal age (years)	30	51	37	27	31	29	30			25	

ADHD, attention deficit/hyperactivity; ASD autism spectrum disorders; NR, not recorded; NT, not tested.
When a specific clinical feature was not described in the original report the item was not scored.

and abdominal physical evaluations were normal. Generalized mild hypotonia was present. Tendon reflexes could hardly be evocated, especially at the lower limbs. Fine motor incoordination was also evident. Primary nocturnal enuresis was present. Sensorineural retrocochlear left side hypoacusia was documented together with myopic astigmatism with normal fundus oculi. EEG showed slow and epileptic anomalies in the postero-temporal region, more pronounced on the right side.

Cerebral MRI revealed an enlarged third ventricle and hypertrophy of the left choroid plexus.

His behaviour was characterized by the presence of highly restricted interests and stereotypes reminiscent of autistic spectrum disorders.

Case 5

This male patient came to our attention at the age of 28 years. He is the second child of healthy, non-consanguineous Caucasian parents. His two brothers are healthy. He was born after an uncomplicated pregnancy and delivery. There is no history of neonatal hypotonia or feeding problems. He is mentally retarded, his IQ is 44. He became obese at 10 years of age. His weight at age 28 years was 130 kg (20 Kg > 95th centile), his height 186 cm (75th centile), and his head circumference 61.5 cm (98th centile). He had mild dysmorphic features, ocular hypertelorism, and large ears (> 98th centile), but no hypogonadism, and his hands and feet were not small. He has a craving for drinking and eating, with food seeking behaviour. He is restless and sometimes has an aggressive temper. Routine chromosomal analysis showed a normal male karyotype, 46,XY. On re-evaluation at the age of 38 years, his weight was 160 kg (50 kg > 95th centile) (Figure 1c). Chromosomal investigation was repeated and now an interstitial deletion of chromosome 6 was found: 46,XY,del(6)(q16.1q21).

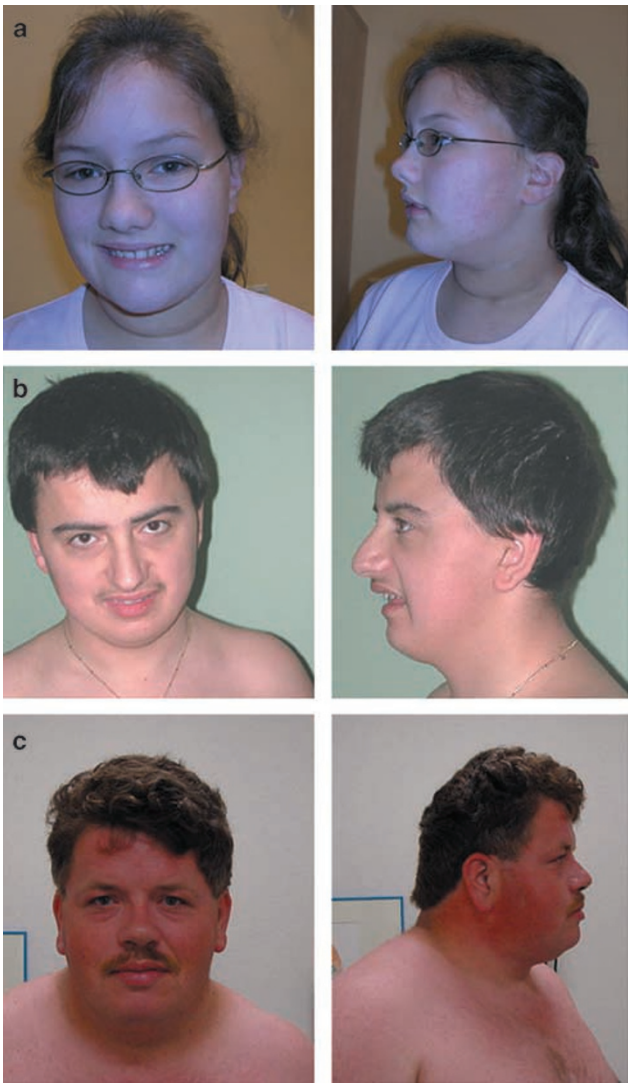

Figure 1 Front and lateral views of patients. (**a**) Patient 2 at the age of 13 years. Note the large skull, full nose, and flat midface. (**b**) Patient 4 at the age of 16 years. Note the large skull, short philtrum, and small mandible. (**c**) Patient 5 at the age of 38 years. Note the obesity, the large skull, hypertelorism, and large ears.

Materials and methods

Karyotyping was performed on metaphase spreads prepared from peripheral blood lymphocytes by conventional methods. Molecular karyotyping was performed through array-CGH with Agilent oligo-chip. The Agilent platform is a 60-mer oligonucleotide-based microarray that allows genome-wide survey and molecular profiling of genomic aberrations with a resolution of ~100 Kb (kit 44B). DNAs (7 μg) of the patient and controls of the same sex (Promega) were double-digested with *Rsa*I and *Alu*I for 2 h at 37°C. After column purification, 2 μg of each digested sample was labelled by random priming (Invitrogen) for 2 h using Cy5-dUTP for the patients/parents DNA and Cy3-dUTP for the control DNA. Labelled products were column purified. After probe denaturation and pre-annealing with 50 μg of Cot-1 DNA, hybridization was performed at 65°C with rotation for 40 h. After two washing steps, the array was analysed through the Agilent scanner with the Feature Extraction software (version 8.1). Graphical overview was obtained using the CGH analytics software (version 3.2.32).

Genotyping of polymorphic loci was performed by amplification with primers labelled with fluorescent probes (ABI 5-Fam, Hex and Tet) followed by analysis on an ABI 310 Genetic Analyzer (Applied Biosystems, Monza). Amplifications were performed with AmpliTaq Gold (Applied Biosystems) using standard protocols.

Results

The karyotypes of the patients as obtained by routine cytogenetic analysis were; Case 1, 46,XY,del(6)(q16.1q21);[2] Case 2, 46,XX,del(6)(q15q21);[4] Case 3,

46,XX,del(6)(q16.2-q21); Case 5, 46,XY,del(6)(q16.1q21). In case 4, karyotype analysis and subtelomeric FISH test revealed no abnormalities. Array-CGH analysis (Supplementary Figure 1), however, detected a *de novo* 6q interstitial deletion of 4.1 Mb between oligomers at 98.569 Mb (first deleted) and 102.689 Mb (last deleted), flanked by oligomers at 98.326 and 102.879 Mb (both present).

The size and chromosomal breakpoints redefined by array-CGH (Supplementary Figure 1) in the other four cases were:

- Case 1: deletion size, 15.2 Mb between oligomers at 94.349 Mb (first deleted) and 109.625 Mb (last deleted) flanked by oligomers at 94.176 and 109.698 Mb (both present).
- Case 2: deletion size, 14.2 Mb between oligomers at 92.102 Mb (first deleted) and 106.363 Mb (last deleted) flanked by oligomers at 91.890 and 106.650 Mb (both present).
- Case 3: deletion size, 15 Mb between oligomers at 98.569 Mb (first deleted) and 113.685 Mb (last deleted) flanked by oligomers at 98.326 and 114.075 Mb (both present).
- Case 5: deletion size, 7.2 Mb between oligomers at 95 641 Mb (first deleted) and 102 879 (last deleted) flanked by oligomers at 94 277 and 103 038 Mb (both present).

Typing of a number of polymorphic chromosome 6 markers in the deleted interval in the probands and their parents showed that the deletion involved the paternal chromosome 6 in cases 2, 3, and 5, and the maternal chromosome 6 in case 4 (Supplementary Table I). For patient 1, parental samples were not available.

Discussion

At least 20 rare syndromes that are caused by genetic defects or chromosomal abnormalities, both autosomal and X-linked, are characterized by obesity (OMIM 601665). Most of these syndromes can be distinguished by the presence of associated abnormalities, which include mental retardation and phenotypic abnormalities. Among these, PWS is the commonest form of syndromic obesity, with an incidence of approximately 1/25 000.[8–10] It is characterized by distinct physical and behavioural features including severe obesity, neonatal hypotonia, cognitive impairment of variable degree, behavioural abnormalities, hypogonadotropic hypogonadism, and short stature.

A Prader–Willi-like phenotype has been described in patients with chromosome Xq duplication,[11,12] fragile X,[13] monosomy 1p36,[14] and 6q deletion.[1–7] Here we report five patients with interstitial 6q16 deletion defined by array-CGH analysis. Common clinical features in our patients are global developmental delay, hypotonia, obesity, several craniofacial anomalies (skull, eye/vision anomalies, abnormal/low ear), hyperphagia, and behavioural problems (Table 1). The absence of obesity in case 3 is not indicative, because she is only 3 months old and hyperphagia and obesity usually start after 1 year of age.[6,7,15] The features noted in our patients are also frequently described in other reported cases with monosomy covering the 6q16 chromosome region.[6,7,15] Case 4 enabled us to narrow the shortest region of deletion overlap (SRO) in which the gene(s) involved in the Prader–Willi-like phenotype should be located to 4.1 Mb (Figure 2).

Obesity was present in four of our five patients and in three recently reported cases (Table 1) suggesting that the 4.1 Mb SRO between 6q16.1–16.3 deletions can be regarded as a region of interest for obesity. The *SIM1* gene (MIM 603128), mapping to the common 6q16.2deletion region, has been proposed as a candidate for the obesity observed in all these subjects. This hypothesis is further supported by the finding that the gene has been found disrupted by a *de novo* balanced translocation 46,XX,t(1;6)(p22.1q16.2) in a patient with severe obesity and normal development.[16] Case 4 confirms the involvement of *SIM1* in obesity.

Case 3, a female newborn, was ascertained because of heart malformations and facial dysmorphisms. Although she is too young to show obesity, hypotonia and short hands/feet were not present. A single case with a 6q deletion of $\approx$14 Mb encompassing band 6q16, showing no features of Prader–Willi-like phenotype, has been reported.[15] This case suggests that the deletion is necessary but not sufficient to develop obesity. This situation reflects incomplete penetrance, which might be due, for instance, to digenic inheritance, similar to the situation described for TAR and Bardet–Biedl syndromes.[17,18]

Other genes present in the common critical region (Figure 2) are: *MCHR2* (MIM: 606111), a G protein-coupled receptor for melanin-concentrating hormone, a neuropeptide that plays an important role in the control of feeding behaviour and energy metabolism;[19] *GRIK2* (MIM: 138244), encoding a subunit of a kainate glutamate receptor. Association and linkage studies suggested its involvement in autism.[20,21] Glutamate receptors mediate the majority of excitatory neurotransmission in the brain.[22] Haploinsufficiency of this gene might cause mental retardation.

Additional genes in the 4.1 Mb critical region (Figure 2) are: the activating signal cointegrator 1 complex (*ASCC3*) subunit of unknown function; the ubiquitin specific protease 45 R (*COQ3*); *FBXL4* (MIM: 605654); and *POU3F2* (MIM: 600494) that is predominantly expressed in the central nervous system (CNS).

It is not clear whether one or more specific genes are the cause of psychomotor delay/mental retardation in our deletion patients. Cases 2 and 4 have, in addition, an autistic spectrum disorder phenotype (poor social interaction,

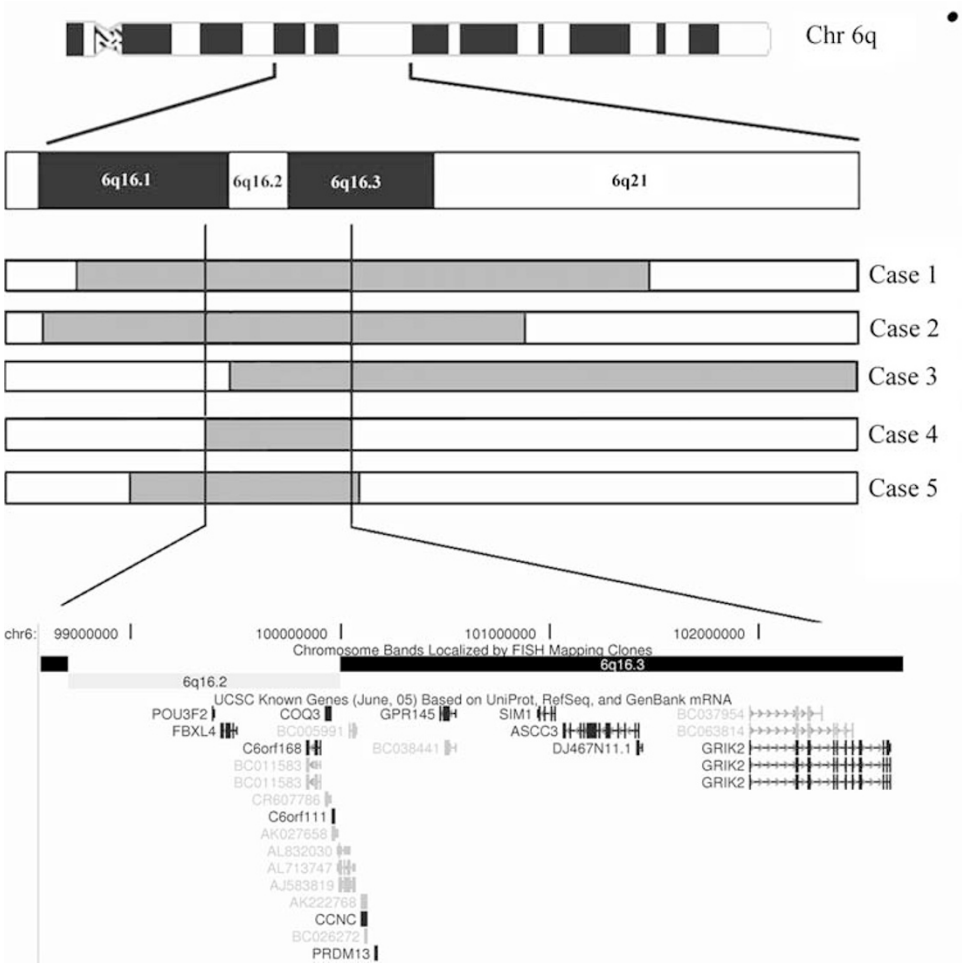

Figure 2 Top: ideogram of the long arm of chromosome 6 and enlargement of the 6q16.1–q21 region. The deleted regions of our five cases are shown by grey boxes. Bottom: further enlargement of the shortest region of deletion overlap (SRO) covering 6q16.2–q16.3 where the gene(s) involved in Prader–Willi-like syndrome are presumed to be located.

stereotypic and repetitive movements) also present in the case reported by Le Caignec *et al*,[15] suggesting a possible role for *GRIK2* haploinsufficiency. It is noteworthy that both cases 2 and 3 show heart defects (ductus arteriosus and atrial septal defects in case 2, tetralogy of Fallot in case 3) and are haploinsufficient for the *POPDC3* gene, belonging to the Popeye family (MIM 605824); being expressed in cardiac and skeletal muscle, *POPDC3* may play an important role in heart development.[23] Here again the deletion of this gene in case 4, who does not have heart defects, suggests a more complex inheritance. Of course, positional effects on the expression of genes outside the deletion interval in some patients cannot be ruled out.

In summary, we have mapped aberrations in five cases with 6q interstitial deletion associated with PWS-like phenotype in at least four cases, using array-CGH to improve the understanding of the relationship between genotype and phenotype. We have shown that an overlapping deleted region of 4.1 Mb supports the evidence that haploinsufficiency of the *SIM1* gene is responsible for obesity in these patients. A possible involvement of the *GRIK2* gene in autistic-like behaviour, *MCHR2* in the control of feeding behaviour and energy metabolism, and *POPDC3* in heart development is also hypothesized.

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References

1 Turleau C, Demay G, Cabanis MO, Lenoir G, de Grouchy J: 6q1 monosomy: a distinctive syndrome. *Clin Genet* 1988; **34**: 38–42.
2 Villa A, Urioste M, Bofarull JM, Martinez-Frias ML: *De novo* interstitial deletion q16.2q21 on chromosome 6. *Am J Med Genet* 1995; **55**: 379–383.
3 Stein CK, Stred SE, Thomson LL, Smith FC, Hoo JJ: Interstitial 6q deletion and Prader–Willi-like phenotype. *Clin Genet* 1996; **49**: 306–310.

4 Gilhuis HJ, van Ravenswaaij CM, Hamel BJ, Gabreels FJ: Interstitial 6q deletion with a Prader–Willi-like phenotype: a new case and review of the literature. *Eur J Paediatr Neurol* 2000; **4**: 39–43.
5 Faivre L, Cormier-Daire V, Lapierre JM *et al*: Deletion of the SIM1 gene (6q16.2) in a patient with a Prader–Willi-like phenotype. *J Med Genet* 2002; **39**: 594–596.
6 Varela MC, Simoes-Sato AY, Kim CA, Bertola DR, De Castro CI, Koiffmann CP: A new case of interstitial 6q16.2 deletion in a patient with Prader–Willi-like phenotype and investigation of SIM1 gene deletion in 87 patients with syndromic obesity. *Eur J Med Genet* 2006; **49**: 298–305.
7 Klein OD, Cotter PD, Moore MW *et al*: Interstitial deletions of chromosome 6q: genotype-phenotype correlation utilizing array CGH. *Clin Genet* 2007; **71**: 260–266.
8 Whittington JE, Holland AJ, Webb T, Butler J, Clarke D, Boer H: Population prevalence and estimated birth incidence and mortality rate for people with Prader–Willi syndrome in one UK health region. *J Med Genet* 2001; **38**: 792–798.
9 Smith A, Egan J, Ridley G *et al*: Birth prevalence of Prader–Willi syndrome in Australia. *Am J Clin Nutr* 2003; **88**: 263–264.
10 Vogels A, Van Den Ende J, Keymolen K *et al*: Minimum prevalence, birth incidence and cause of death for Prader–Willi syndrome in Flanders. *Eur J Hum Genet* 2004; **12**: 238–240.
11 Stalker HJ, Keller KL, Gray BA, Zori RT: Concurrence of fragile X syndrome and 47, XYY in an individual with a Prader–Willi-like phenotype. *Am J Med Genet A* 2003; **116**: 176–178.
12 Monaghan KG, Van Dyke DL, Feldman GL: Prader–Willi-like syndrome in a patient with an Xq23q25 duplication. *Am J Med Genet* 1998; **80**: 227–231.
13 De Vries BB, Niermeijer MF: The Prader–Willi-like phenotype in fragile X patients: a designation facilitating clinical (and molecular) differential diagnosis. *J Med Genet* 1994; **31**: 820.
14 D'Angelo CS, Da Paz JA, Kim CA *et al*: Prader–Willi-like phenotype: investigation of 1p36 deletion in 41 patients with delayed psychomotor development, hypotonia, obesity and/or hyperphagia, learning disabilities and behavioral problems. *Eur J Med Genet* 2006; **49**: 451–460.
15 Le Caignec C, Swillen A, Van Asche E, Fryns JP, Vermeesch JR: Interstitial 6q deletion: clinical and array CGH characterisation of a new patient. *Eur J Med Genet* 2005; **48**: 339–345.
16 Holder Jr JL, Butte NF, Zinn AR: Profound obesity associated with a balanced translocation that disrupts the SIM1 gene. *Hum Mol Genet* 2000; **9**: 101–108.
17 Klopocki E, Schulze H, Strauss G *et al*: Complex inheritance pattern resembling autosomal recessive inheritance involving a microdeletion in thrombocytopenia-absent radius syndrome. *Am J Hum Genet* 2007; **80**: 232–240.
18 Beales P: Lifting the lid on Pandora's box: the Bardet-Biedl syndrome. *Curr Opin Genet Dev* 2005; **15**: 315–353.
19 Sailer AW, Sano H, Zeng Z *et al*: Identification and characterization of a second melanin-concentrating hormone receptor, MCH-2R. *Proc Natl Acad Sci USA* 2001; **98**: 7564–7569.
20 Jamain S, Betancur C, Quach H *et al*: Linkage and association of the glutamate receptor 6 gene with autism. *Mol Psychiatry* 2002; **7**: 302–310.
21 Shuang M, Liu J, Jia MX *et al*: Family-based association study between autism and glutamate receptor 6 gene in Chinese Han trios. *Am J Med Genet B Neuropsychiatr Genet* 2004; **131**: 48–50.
22 Paschen W, Blackstone CD, Huganir RL, Ross CA: Human GluR6 kainate receptor (GRIK2): molecular cloning, expression, polymorphism, and chromosomal assignment. *Genomics* 1994; **20**: 435–440.
23 Andree B, Hillemann T, Kessler-Ieckson G *et al*: Isolation and characterization of the novel Popeye gene family expressed in skeletal muscle and heart. *Dev Biol* 2000; **223**: 371–382.

Supplementary Information accompanies the paper on European Journal of Human Genetics website (http://www.nature.com/ejhg)