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ARTICLE

Effect of the size of the deletion and clinical manifestation in Wolf-Hirschhorn syndrome: analysis of 13 patients with a *de novo* deletion

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We performed clinical, cytogenetic, and molecular analyses on 13 patients (8 females and 5 males, aged 6 months to 13 years) with Wolf-Hirschhorn syndrome due to *de novo* deletions of chromosome 4p. All patients presented with the typical facial gestalt, microcephaly, and profound mental retardation. Other clinical signs were low birth weight (10/13; 77%), postnatal short stature (8/12; 66%), muscular hypotonia (12/13; 92%), seizures (11/13; 85%), congenital heart defects (4/13; 31%), colobomata of iris (4/12; 33%), genital anomalies (4/13; 31%), deafness (3/13; 23%), and renal anomalies (3/13; 23%). The smallest deletion was a submicroscopic terminal deletion of nearly 2.5 Mb. The largest was a terminal deletion of nearly 30 Mb. Cleft lip/palate, preauricular pits/tags, and congenital heart defects were present only in patients with terminal deletions larger than 10 Mb. The deviations from mean birth weight, birth length, and postnatal head circumference correlated with the size of the deletion. Determining the parental origin of the deletion with microsatellite markers, the maternal allele was missing in three patients and the paternal allele in eight patients. Our observations support the existence of a partial genotype–phenotype correlation in Wolf-Hirschhorn syndrome. *European Journal of Human Genetics* (2000) **8**, 519–526.

Keywords: Wolf-Hirschhorn syndrome; deletion size; clinical manifestation; *de novo* deletion; parental origin of deletion

Introduction

Wolf-Hirschhorn syndrome (WHS) was first and independently published in 1965 by Wolf *et al*[1] and Hirschhorn *et al*[2] and describes a wide spectrum of clinical signs in patients with terminal 4p deletions.[3–5] The minimal critical region has been narrowed down to 165 kb in the chromosomal band 4p16.3.[6] Potential candidate genes such as *WHSC1, WHSC2,* and *LETM1* have been identified.[7–9] Although multiorganic expression corresponds well with affected organs in WHS, a direct link between these candidate genes and the broad clinical spectrum is missing.

Different mechanisms causing WHS have been observed: *de novo* deletions, familial translocations, and *de novo* translocations.[3,10] The rate of familial translocations in WHS patients has been reported to be up to 15%,[10] 66% of them maternally inherited.[11] *De novo* events are estimated to occur in 85–87% of WHS patients[10] and here the paternally derived deletions are more frequent.[12,13]

Here, we evaluate 13 WHS patients with *de novo* deletions by clinical, cytogenetic, and molecular analyses to assess the severity of the clinical phenotype in context of the size and origin of the deletion.

However, it is still an open debate as to whether the size of the deletion in WHS patients influences the clinical phenotype.[14] The aim of this study was to assess the influence of the

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size of the deletion on the clinical phenotype in the context of previously published reports.

Subjects and methods

Ascertainment

Since 1996, we performed clinical, cytogenetic, molecular, and molecular–cytogenetic investigations in 22 patients with Wolf-Hirschhorn syndrome. *De novo* deletions have been observed in 13 of the patients described here. Another nine patients had familial (3/22) or *de novo* translocations (6/22) resulting in Wolf-Hirschhorn syndrome. They are described elsewhere.[15]

Clinical data

Thirteen patients, eight female and five male, aged 6 months to 13 years, were investigated (Table 1). Birth occurred usually at term, after 35 to 42 gestational weeks (mean 39 weeks). Mean birth weight was 2241 g (2.6 SD below mean). Mean birth length was 46.7 cm (1.8 SD below mean), and mean

Table 1 Clinical findings in 13 WHS patients with *de novo* deletions

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	
Sex	m	f	m	f	m	m	f	f	f	f	f	m	f	8f/5m
Characteristic facial phenotype	+	+	+	+	+	+	+	+	+	+	+	+	+	13/13
Hypertelorism	+	+	+	+	+	+	+	+	+	+	+	+	+	13/13
Prominent glabella	–	–	+	–	+	+	+	+	+	+	+	–	–	8/13
Broad nose	+	+	+	+	+	+	+	+	+	+	+	+	+	13/13
Beaked nose	+	–	–	+	–	+	+	–	+	+	–	+	+	8/13
Short philtrum	+	+	+	+	+	+	+	+	+	+	+	+	+	13/13
Micrognathia	+	+	+	+	+	+	+	+	+	+	+	+	+	13/13
Downturned corners of the mouth	–	+	–	–	+	+	+	+	+	+	+	+	+	10/13
Cleft lip/palate	–	–	–	–	–	+	–	–	+	–	–	+	–	3/13
Dysplastic ears	+	+	+	+	+	+	+	+	–	+	+	+	+	12/13
Preauricular tag/pit	–	–	–	–	–	–	+	+	–	–	–	–	+	3/13
Gestational weeks at birth	41	36	35	41	36	40	41	42	39	42	38	40	40	Ø39
Weight in g	2640	1750	1580	2750	2650	2050	2360	2720	2250	2150	2020	2120	2090	2241
[difference from mean/SD][a]	–2.3	–2.0	–2.9	–1.3	mean	–4.9	–2.1	–1.8	–2.3	–3.0	–2.8	–4.7	–4.0	Ø–2.6
Length in cm	51	41.5	43	46	51	51	45	48	45	47	46	47	46	46.7
[difference from mean/SD][a]	–0.8	–1.6	–0.9	–2.6	+1.3	–1.2	–3.0	–2.1	–2.3	–2.5	–1.6	–2.9	–3.7	Ø–1.8
OFC in cm	33	29	n.r.	n.r.	n.r.	31	33	34	30	30.5	31	30	30	31.2
[difference from mean/SD][a]	–2.4	–2.9				–5.1	–1.3	–1.0	–3.7	–3.9	–2.0	–6.2	–3.9	Ø–3.2
Age at examination[years]	5 9/12	1 1/12	1 10/12	13	10 9/12	13	9	1 3/12	6	9/12	2	8	10	
Postnatal stature in cm[b]	108	66	72	132	110	145.7	97.7	64.5	89	n.r.	85	102	127	
[difference from mean/SD]	–1.4	–3.7	–5.1	–3.6	–5.4	–1.2	–6.2	–5.2	–5.8		–0.8	–4.8	–1.9	Ø–3.8
Postnatal weight in kg[c]	n.r.	5.5	6.5	34	19	33.5	12.5	5.1	9.5	4.2	8.0	15.5	23.5	
[difference from mean/sd]		–3.6	–4.7	–2.0	–3.6	–0.7	–4.4	–4.2	–7.1	–4.3	–3.5	–3.7	–2.1	Ø–3.7
Postnatal OFC in cm[d]	48.5	40.5	42.5	50.5	48	47.6	45.3	41.4	45.5	n.r.	42.5	44.9	44.5	
[difference from mean/SD]	–2.1	–4.1	–4.8	–2.3	–3.7	–4.2	–4.9	–3.9	–3.8		–4.3	–5.3	–5.1	Ø–4.0
Mental retardation	+	+	+	+	+	+	+	+	+	+	+	+	+	13/13
Sitting without support [years]	1 1/12	–	2	2	–	1 1/12	5	–	3.5	–	–	–	–	6/13
Walking without support [years]	5	–	4	3	–	4	–	–	–	–	–	–	–	4/13
Speech [years]	1 6/12	–	4	4	–	–	–	–	–	–	–	–	–	3/13
Muscular hypotonia	+	+	+	+	+	+	+	+	+	+	+	–	+	12/13
Seizures	+	+	+	–	+	+	+	+	+	+	+	+	–	11/13
Clinical findings														
Colobomata of iris	–	–	–	–	n.r.	–	+	–	–	+	–	+	+	4/12
Strabism	+	+	–	+	+	+	+	+	–	–	–	–	+	8/13
Deafness	–	–	+	–	–	–	–	–	–	–	+	–	+	3/13
Congenital heart defects	–	–	–	–	–	–	–	–	+	+	+	+	–	4/13
									ASD Pst	ASDI	VSD ASDII	VSD		
Clubbing of fingers/toes	–	+	–	+	–	–	+	+	–	–	+	–	+	6/13
Scoliosis	–	–	–	–	+	–	–	–	–	–	–	–	+	2/13
Club feet	–	–	–	–	+	–	–	–	–	–	–	–	+	2/13
Renal anomalies	–	–	+	–	+	–	–	–	–	+	–	–	–	3/13
Genital anomalies	–	–	+	–	–	–	+	–	–	–	–	+	+	4/13
Sacral dimple	–	–	–	–	–	–	–	–	–	–	–	n.r.	+	1/12

n.r.: not reported; f: female; m: male; Ø: average; ASD: atrial septal defect; VSD: ventricular septal defect; Pst: pulmonic stenosis; [a]according to Keen & Pearse;[49] [b]according to van Wieringen;[50] [c]according to Kunze;[51] [d]according to Nellhaus.[52]

head circumference at birth was 31.2 cm (3.2 SD below mean). Diagnosis was suggested in these patients because of the typical facial formation comprising hypertelorism, strabism, prominent glabella, broad nose with flat tip, short philtrum, downturned corners of the mouth, cleft lip/palate, micrognathia, dysplastic ears, and preauricular pits/tags. The facial phenotypes of the patients are illustrated in Figure 1. All patients were mentally retarded. Formal IQ testing has not been done. Other common clinical findings in WHS were muscular hypotonia (12/13), seizures (11/13), strabism (8/13), clubbing of fingers/toes (6/13), congenital heart defect (4/13) including ASD, VSD, and pulmonic stenosis, and genital anomalies (4/13) such as crypto-orchidism and hypertrophy of the clitoris, as well as colobomata of the iris (4/12), deafness (3/13), renal anomalies (3/13) such as ureter duplex, unilateral renal agenesis, and vesico-ureteral reflux, scoliosis (2/13), club foot (2/13), and sacral dimple (1/12) (Table 1).

Cytogenetic investigations

Chromosome studies including GTG-banding were performed on peripheral blood lymphocytes according to slightly modified standard techniques[16] in all patients and their parents. The karyotypes of patients 1–13 are listed in Table 2.

In patients 1–4 the deletion is submicroscopic and is not visible at a banding resolution of 400 bands per haploid genome. Further molecular cytogenetic investigations were necessary to confirm the diagnosis. The remainder of the deletions range from 4p16.2 to 4p15.2.[17] The chromosomes of all parents showed a normal karyotype (46,XX or 46,XY).

Fluorescence *in situ* hybridisation (FISH)

DNA from cosmids pC847.351 (D4F26),[18] CD2 (D4S90),[19] 33c6 (D4S43),[20] L21f12 (D4S180), L228a7 (D4S81),[21] L247f6 containing the *FGFR3* gene,[22] and inter-Alu PCR products[23] from YAC 877G6, 405D10, 794D12, 225D2, 435A11, 400F9, 848G12, and 796D3 (CEPH) were labelled with digoxigenin by using a BRL nick-translation kit (Gibco, Life Technologies, Gaitherburg, MD, USA). Labelled cosmid and YAC DNA was separated from unincorporated nucleotides by using 1800 ml Centricon 30 filters (Amicon, Beverly, MA, USA). FISH was performed as described by Lichter *et al*.[24] Labelled cosmid DNA (50 ng) or YAC DNA (100 ng) was mixed with human Cot-1 fraction DNA to suppress repetitive sequences or block

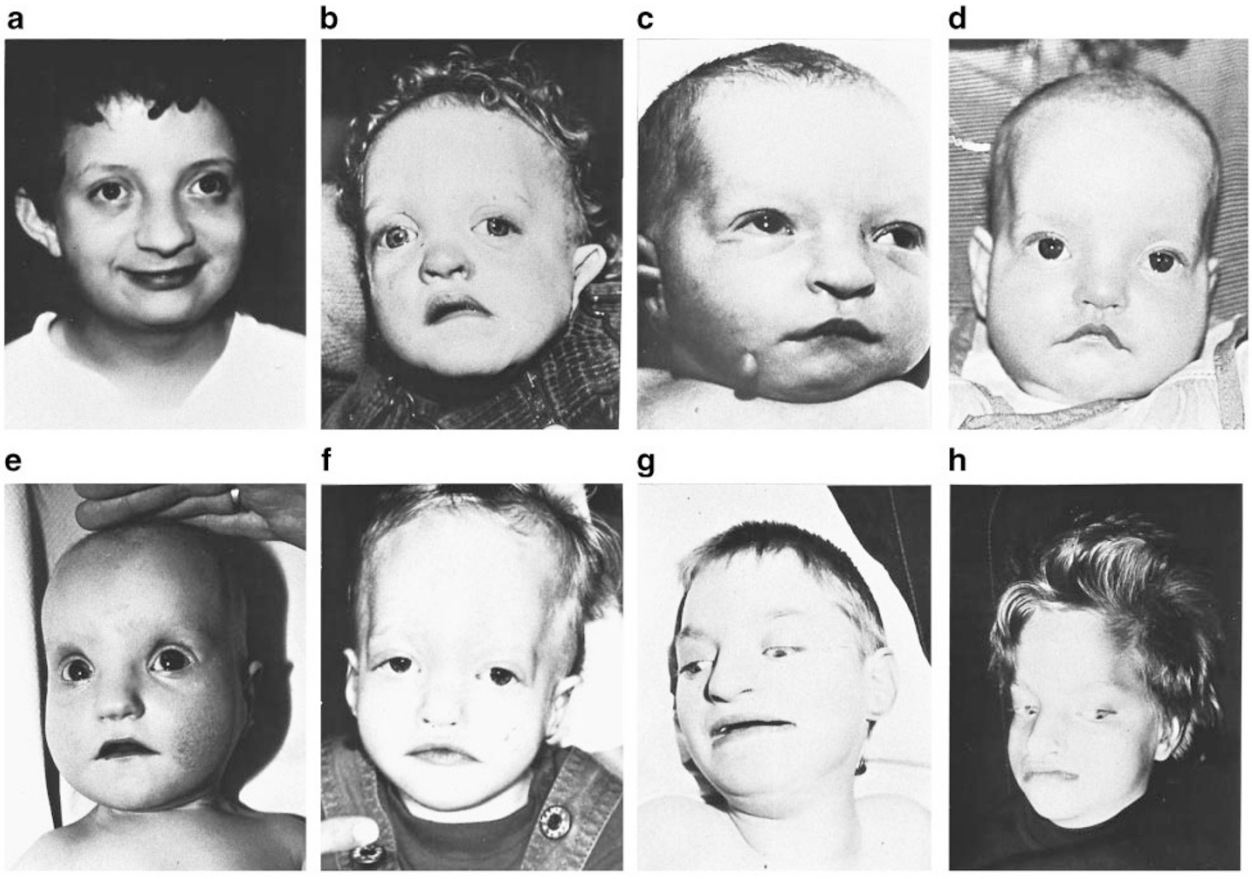

Figure 1 Facial features of 8 patients with WHS. **a** Patient 4, **b** Patient 6, **c** Patient 7, **d** Patient 9, **e** Patient 10, **f** Patient 11, **g** Patient 12, **h** Patient 13.

Table 2 Cytogenetic and molecular results in 13 WHS patients with *de novo* deletions

Distance from 4pter	*Mikrosatellite marker*	*Cosmid*	*YAC*	*1 46,XY*	*2 46,XX*	*3 46,XY*	*4 46,XX*	*5 46,XY, del(4) (p16.2)*	*6 46,XY, del(4) (p16.2)*	*7 46,XX, del(4) (p16.2)*	*8 46,XX, del(4) (p16.1)*	*9 46,XX, del(4) (p15.3)*	*10 46,XX, del(4) (p15.3)*	*11 46,XX, del(4) (p15.3)*	*12 46,XY, del(4) (p15.2)*	*13 46,XX, del(4) (p15.2)*
–200 kb		pC847.351 (D4F26)		del	del	del	del	del	del	del	del	del	del	del	del	del
–300 kb		CD2 (D4S90)		del	del	del										
–1.2 Mb		pC678 (D4S96)		del	del	del	del	del	del	del	del	del	del	del	del	del
–1.8 Mb		pC385.12 (D4S98)		del	del	del								del		
–1.9 Mb	L75B9 Rep.			M	P		P			P		n.i.	P	P	M	P
–2.0 Mb	D4S1182			n.i.	n.i.		n.i.			P		P	n.i.	n.i	M	n.i.
–2.2 Mb	D4S43	33c6 (D4S43)		del	del	P	n.i.	M		P		n.i.		P	M	P
–2.8 Mb		24716 (D4S182)		n.d.	n.d.	del	del					del				
–3 Mb	Hu4/Hu5			n.d.	n.d.	n.i.				P		P	P		M	n.i.
–3.3 Mb		21f12 (D4S180)		n.d.	n.d.	n.d.	del			del		del				
–3.8 Mb	A2AR			n.i.	n.i.					n.i.		P	n.i.		n.i.	n.i
–3.8 Mb		228a7 (D4S81)		n.d.	n.d.	n.d.	del	del	del	del	del	del	del	del	del	del
–5.5 Mb	Hox 7						n.d.			n.i.		P	n.i	n.i	n.i.	n.i.
–5.5 Mb			424H7					del	del							
–7 Mb			877G6					del		del					del	
–8.8 Mb	D4S2366			n.d.	n.d.	n.d.	n.d.	M		P		P	P	P	n.i.	n.i.
–9 Mb			911D6					del	del	del	del					
–10.6 Mb			392C3					n.d.	del	del	del					
–13.0 Mb			405D10					n.d.	n.d.	n.d.	del				del	
–14 Mb			794D12					n.d.	n.d.	n.d.	del					
–16.5 Mb	D4S403			n.d.			n.i.	n.d.	n.d.	n.d.		P	P		n.i.	P
–19.8 Mb			225D2							n.d.	n.d.	n.d.	del	del	del	
–21.5 Mb			435A11										del	del		
–23.0 Mb	D4S2639			n.i.	n.d.	n.i.	n.d.		n.d.	n.d.		n.d.	n.d.	del	n.i.	P
–27 Mb			400F9							n.d.	n.d.			n.d.	del	
–29 Mb			848G12							n.d.				n.d.	del	del
–31 Mb			796D3							n.d.				n.d.	n.d.	del
–34.0 Mb	D4S2397			n.d.			n.d.		n.d.	n.d.		n.d.	n.d.	n.i.	n.d.	n.d.

del: deleted; n.d.: not deleted; n.i.: not informative; M: maternally deleted; P: paternally deleted.

non-specific hybridisation. Hybridisation was detected using monoclonal anti-digoxin, anti-mouse IgG FITC conjugate and anti-rabbit IgG FITC conjugate (Sigma Immuno Chemicals, St Louis, USA), and counterstained using propidiumiodide or DAPI. A cosmid derived from D4S96 and the whole chromosome paint 4 (AGS, Heidelberg, Germany/Oncor, Gaithersburg, VA, USA) were hybridised according to the manufacturer's instructions. For all hybridisations, clone pGXba11/340[25] was used as centromeric control for human chromosome 4. The results of FISH analysis are listed in Table 3. For patients 6 and 8, DNA of the parents was not available, so only FISH was used to determine the size of the deletion.

FISH staining with a whole chromosome paint 4 (AGS/Oncor) gave hybridisation signals along the entire length of both chromosomes 4 in all 13 patients. Therefore, unbalanced translocations within the detection limit of wcp 4 were excluded in this group of patients. Smaller *de novo* translocations involving the short arm of one chromosome 4 could not be excluded with this technique. Cryptic translocations in the parents were excluded performing FISH with at least one cosmid (D4S96 or CD2) in combination with clone pGXba11/340 as control.

Cell lines, genomic DNA isolation, and DNA probe analysis of the patients and their parents

Genomic DNA was prepared from peripheral blood lymphocytes or EBV transformed lymphoblastoid cell lines from patients and their parents according to previously described techniques.[26,27]

Genotyping of chromosome 4p was performed using the following previously described microsatellite markers: D4S1182,[28] D4S43,[28] ADRA2C,[21] HOX7,[29,30] D4S403,[31] D4S2366, D4S2639 and D4S2397. A new microsatellite, 75B9Rep, was generated by us and is described elsewhere.[32,15] The Hu4/Hu5 primer pair amplifies the CAG repeat of the Huntington gene.[33,34] In patients 1, 5 and 12 the maternal allele was absent, in contrast to patients 2, 3, 4, 7, 9, 10, 11,

and 13, where the paternal allele was missing. The smallest deletion in this group of patients comprises 2.2–2.8 Mb in patients 1 and 2, the largest deletion nearly 30 Mb in patient 13. None of our patients had an interstitial deletion.

Statistical evaluation

For statistical analysis, Spearman correlation coefficient and the Fisher test were applied. A level of confidence of $\delta = 0.05$ was used to assume a statistically significant difference.

Results

We compared the clinical findings in this group of WHS patients with the size of the deletion. The characteristic facial phenotype is present in all patients, reflecting that the suggested diagnosis was due to the facial gestalt. Cleft lip/palate is present only in 3/13 patients (23.1%) and absent in patients with a terminal deletion smaller than 9 Mb. Preauricular pits/tags are reported in our patients in the same frequency as cleft lip/palate and are only present in patients with deletions larger than 10 Mb.

The standard deviations of birth weight and birth length correlate with the size of the deletion ($P < 0.05$), whereas head circumference at birth does not correlate. Interestingly, head circumference in older patients correlates with the size of the deletion ($P < 0.05$). The number of patients who learned to sit, walk and talk is too small to perform statistical analysis. However, it appears to us that patients with smaller deletions probably have a better developmental outcome than patients with large deletions. Patients 1, 3, and 4 are able to sit, walk and speak individual words; patient 2 was too young for us to assess these abilities. Patients 12 and 13 with the largest deletions at the age of 8 and 10 years, respectively, are neither able to sit or to communicate. Muscular hypotonia and seizures are present in nearly all patients independent of the size of the deletion. Deafness is present in three patients independent of the size of the deletion. Formal hearing testing has not been performed. Congenital heart defects were present in four patients (30.7%) with size of the deletion ranging from nearly 16 to 29 Mb. However, in the patient with the largest deletion (patient 13), no heart defect has been recognised. Genital and renal anomalies are present in patients with small and large deletions. Colobomata of iris are lacking in patients with deletions smaller than 10 Mb.

The origin of deletion could be defined in 11 patients; 3/11 patients had a deletion of maternal origin, whereas in 8/11 patients the deletion was of paternal origin. It is noteworthy that all female WHS patients with known parental origin of deletion (n = 6) have deletion of paternal origin, whereas in 3/4 male WHS patients the deletion was of maternal origin. This result is statistically significant ($P < 0.05$). We could not identify statistically significant differences between the eight female and five male patients in respect of body measurements, clinical findings, or size of deletion.

Discussion

The Wolf-Hirschhorn syndrome (WHS) is a well known multiple congenital anomaly/mental retardation syndrome due to the partial deletion of the short arm of chromosome 4 involving at least a 165 kb segment of 4p16.3.[6] Interstitial deletions of 4p proximal to 4p16, cause a phenotype distinct from WHS.[35–37]

Different mechanisms cause the deletion responsible in WHS: *de novo* simple deletions, unbalanced translocations due to familial translocations, or *de novo* complex chromosomal rearrangements such as unbalanced translocations result in 4p deletions. Centerwall *et al*[38] estimated the frequency of unbalanced translocations in WHS to be 5%; Lurie *et al*[10] reviewed the literature and showed that 13% of the reported cases with WHS were caused by parental chromosomal aberrations. They concluded that the genetic structure of 4p- does not differ from that of other autosomal deletion syndromes. Thus, of 22 cases of WHS we identified three (13.6%) caused by familial translocations, which is consistent with the data from the literature. Six of 22 patients had a *de novo* unbalanced translocation, five of them with a *de novo* translocation t (4p;8p).[15] In the remaining 13 patients described here in more detail, *de novo* deletions were detected.

The size of the terminal deletions in our patients ranged from nearly 2.5 Mb to 31 Mb. The smallest deletion is submicroscopic at a banding resolution of 400 bands per haploid genome, the largest having a breakpoint in 4p15.2. This variability of cytogenetic visible deletions is well known.[39]

The frequency of clinical findings and mean body measurements in comparison with data in the literature are listed in Table 3. We could not identify a statistically significant difference between males and females on the one hand and paternally and maternally inherited deletions on the other. Female liveborn WHS patients are more frequent than males, which has been documented in all reviews. An average 39 weeks gestation at birth in our group of patients agrees with the data of Wilson *et al*.[3] Birth weight ranged from 1580 g (patient 3) to 2750 g (patient 4) in our patients consistent with all reviews estimating an average birth weight of 2000 g (Table 2). Head circumference at birth is microcephalic with 31 cm in most of our WHS patients and in those described in the literature. Postnatal short stature is reported with a frequency of 76% to 100% and postnatal microcephaly is present in almost all reported patients. Estabrooks *et al*[4] estimated the frequency of microcephaly to be lower (64%). This might be due to the fact that they described WHS patients with small deletions. Mental retardation in patients in the literature was reported in more than 83%. Unfortunately, no data about the mental development of the patients with small deletions are included in some recent reports. Cleft lip and palate were more frequently observed in older reviews,[40,41] this may be due to larger deletions in those patients. The same may be true for congenital heart

Table 3 Frequency of clinical findings in our WHS patients compared with data in the literature

	Johnson et al[40] *n = 43 (review)*	*Rivas* et al[41] *n = 37 (review)*	*Wilson* et al[3] *n = 13*	*De Grouchy*[53]	*Schinzel*[54]	*Estabrooks* et al[4] *n = 11*	*Battaglia* et al[5] *n = 15*	*Our data n = 13*
Sex	n.r.	21f/16m	8f/5m	f/m: 2/1 ratio	f/m: 2/1 ratio	6f/5m	12f/3m	8f/5m
Characteristic facial findings								
Hypertelorism	74%	88%	100%	n.r.	n.r.	64%	100%	100%
Prominent glabella	47%	50%	92%	n.r.	n.r.	55%	n.r.	62%
Broad nose	64%	n.r.	100%	n.r.	n.r.	64%	n.r.	100%
Beaked nose	64%	n.r.	n.r.	n.r.	n.r.	55%	n.r.	62%
Short philtrum	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	100%	100%
Micrognathia	69%	73%	100%	n.r.	n.r.	45%	100%	100%
Downturned corners of the mouth	n.r.	n.r.	100%	n.r.	n.r.	45%	100%	77%
Cleft lip/palate	57%	85%[a]	31%	n.r.	15%	27%	46.6%	23%
Dysplastic ears	69%	43%	92%	n.r.	n.r.	55%	93.3%	92%
Preauricular tag/pit	n.r.	n.r.	84%	n.r.	>20%	9%	n.r.	23%
Gestational weeks at birth (mean)	n.r.	n.r.	39	n.r.	n.r.	n.r.		39
Birth weight in g (mean)	n.r.	<2000	1870	2000	2000	n.r.	n.r.	2241
[SD] (mean)								2.6
Birth length in cm (mean)	n.r.	n.r.	n.r.	44	n.r.	n.r.	n.r.	46.7
[SD] (mean)								–1.8
OFC at birth in cm (mean)	n.r.	n.r.	n.r.	30.7	31	n.r.	n.r.	31.2
[SD] (mean)								–3.2
Postnatal short stature	76%	n.r.	100%	n.r.	n.r.	64%	100%	83%
Postnatal stature in [SD] (mean)	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	–3.8
Postnatal microcephaly	91%	90%	100%	n.r.	n.r.	64%	100%	100%
Postnatal OFC in [SD] (mean)	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	–4.1
Mental retardation	100%	83%	100%	n.r.	n.r.	91%	100%	100%
Sitting without support	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	46%
Walking without support	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	26.7%	31%
Speech	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	23%
Muscular hypotonia	n.r.	60%	100%	n.r.	n.r.	64%	93.3%	92%
Seizures	47%	53%	92%	n.r.	n.r.	55%	86.6%	85%
Clinical findings								
Coloboma	n.r.	28%	15%	n.r.	n.r.	n.r.	n.r.	33%
Strabism	36%	55%	83%	n.r.	n.r.	27%	n.r.	62%
Deafness	n.r.	n.r.	n.r.	n.r.	n.r.	9%	33.3%	23%
Congenital heart defect	55%	60%	23%	>50%	n.r.	18%	33.3%	31%
Clubbing of fingers/toes	n.r.	n.r.	77%	n.r.	n.r.	45%	n.r.	46%
Scoliosis	66%	n.r.	23%	n.r.	n.r.	18%	13.3%	15%
Club feet	n.r.	63%	53%	n.r.	n.r.	18%	n.r.	15%
Renal anomalies	n.r.	n.r.	n.r.	n.r.	n.r.	18%	13.3%	23%
Genital anomalies	64%	n.r.	80%	n.r.	n.r.	36%	n.r.	31%
Sacral dimple	33%	35%	100%	n.r.	n.r.	27%	n.r.	8.3%

f: female; m: male; n.r.: not reported; [a]including all palatal anomalies, not only cleft lip/palate.

defects.[40,41] However, in more recent papers, where the deletions have been characterised with molecular methods, clinical data were not described in detail and photographs were lacking, which adds to a controversial debate if the variability of the clinical spectrum seen in WHS is reflected by the size of the deletion.[6,42]

We correlated the clinical findings with the size of the deletion and came to the following conclusions: cleft lip/palate, preauricular pits/tags, and colobomata were missing in patients with deletions smaller than 9 Mb and congenital heart defects were absent in patients with deletions smaller than 16 Mb. These conclusions are supported by other reported patients with small deletions, who lacked these clinical findings, eg the patients of Albiez *et al*,[43] Gandelmann *et al*,[42] and Johnson *et al*.[40] Johnson *et al*[14] hypothesised that cardiac and lip and palatal defects are due to deletions located more proximally. Fang *et al*[44] added renal abnormalities and hearing loss to this list.

Reduced length and height correlate with the size of the deletion, which is also true of head circumferences in older patients. To our knowledge in this respect, there are no detailed literature data for comparison. The number of patients who are able to walk or to talk is too small to argue whether the degree of developmental delay correlates with the size of the deletion, as suggested by Johnson *et al*.[40] Estabrooks *et al*[4,45] hypothesised that the size of the deletion

and clinical severity do not correlate. This may be due to the fact that these authors included WHS patients only with small deletions. Moreover, they also concluded that severe mental retardation appears to be associated with more distal deletions, and mild mental retardation appears to be associated with a more proximal, interstitial 4p16 deletion as by Fang *et al*.[44]

Estabrooks *et al*[4] performed a preliminary phenotypic map of chromosome 4p16 based on 11 WHS patients with deletions smaller than 8 Mb. Lurie and Opitz[46] question the attempt to map nonspecific findings common to many different forms of segmental autosomal aneuploidies to a specific region. Estabrooks *et al*[4] also included patients with additional autosomal trisomic segments, which may have an influence on the phenotype. In concordance with their data, we agree that preauricular pits/tags and congenital heart defects are present only in patients with larger deletions. Hearing loss was present in one patient with a small deletion in our group of patients and is in contrast to this map. These authors[4] suggest that seizures may be mapped to the distal region because they are present in nearly all patients. We do not agree with cleft lip/palate being localised distally as discussed above, because of the absence of this sign in our patients with small deletions.

In this paper we do not differentiate between patients with Pitt-Rogers-Danks and Wolf-Hirschhorn syndrome. These conditions are both caused by deletions in the same region on chromosome 4p16.3.[47] Clinical phenotype is so similar and may change in one person at different ages. Thus, we agree that these conditions are not distinct entities.[48]

In conclusion, the standard deviations of birth weight, birth length, and postnatal head circumference, and the severity of mental retardation correlate with the size of the deletion.

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