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A double cryptic chromosome imbalance is an important factor to explain phenotypic variability in Wolf–Hirschhorn syndrome

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A total of five Wolf–Hirschhorn syndrome (WHS) patient with a 4p16.3 *de novo* microdeletion was referred because of genotype–phenotype inconsistencies, first explained as phenotypic variability of the WHS. The actual deletion size was found to be about 12 Mb in three patients, 5 Mb in another one and 20 Mb in the last one, leading us to hypothesize the presence of an extrachromosome segment on the deleted 4p. A der(4)(4qter→p16.1::8p23→pter) chromosome, resulting from an unbalanced *de novo* translocation was, in fact, detected in four patients and a der(4)(4qter→q32::4p15.3→qter) in the last. Unbalanced t(4;8) translocations were maternal in origin, the rec(4p;4q) was paternal. With the purpose of verifying frequency and specificity of this phenomenon, we investigated yet another group of 20 WHS patients with *de novo* large deletions (*n* = 13) or microdeletions (*n* = 7) and with apparently straightforward genotype–phenotype correlations. The rearrangement was paternal in origin, and occurred as a single anomaly in 19 out of 20 patients. In the remaining patient, the deleted chromosome 4 was maternally derived and consisted of a der(4)(4qter→4p16.3::8p23→8pter). In conclusions, we observed that 20% (5/25) of *de novo* WHS-associated rearrangements were maternal in origin and 80% (20/25) were paternal. All the maternally derived rearrangements were *de novo* unbalanced t(4;8) translocations and showed specific clinical phenotypes. Paternally derived rearrangements were usually isolated deletions. It can be inferred that a double, cryptic chromosome imbalance is an important factor for phenotypic variability in WHS. It acts either by masking the actual deletion size or by doubling a quantitative change of the genome.
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Introduction

Wolf–Hirschhorn syndrome (WHS [MIM 194190] is a contiguous gene syndrome caused by a partial deficiency

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of chromosome arm 4p, with critically deleted region in 4p16.3.[1,2] Large deletions detectable by conventional cytogenetic methods cause a severe phenotype of severe psychomotor and growth retardation, congenital hypotonia, seizures, major malformations, and a characteristic facial appearance, with high forehead, large and protruding eyes, hypertelorism, prominent nasal bridge and down-turned mouth. In several WHS patients, 4p16.3 microdeletions are

detected only by molecular probes. Microdeletions tend to be associated with a milder phenotype, with no major malformations, mild psychomotor delay and normal head circumference in some case.[1,3–11] Although the severity of the clinical presentation usually correlates with the size of the deletion, phenotypic variability represents a hallmark of this condition, suggesting the role of other factors in the determination of the phenotype. *De novo* 4p rearrangements more often occur in paternal meiosis[12,13] and are largely assumed to be isolated deletions. However, unbalanced *de novo* t(4p;8p) translocations have been detected with unexpected high frequency in WHS patients and, whenever tested, they were maternal in origin.[10,14,15] It was recently demonstrated that the breakpoints of t(4;8) translocations fall within 4p and 8p olfactory receptors (OR)-gene clusters, with the involvement of two different OR-gene clusters in 4p at an average distance of 5 and 14 Mb from the telomere.[16] Frequency, specificity and, more importantly, clinical relevance of unbalanced *de novo* translocations in WHS are currently unclear. Genetic factors for phenotypic variability of the WHS are also still unknown. In the present report, we make an attempt to clarify these points.

Patients and methods
Patients
In all, 25 patients with WHS (10 boys and 15 girls), all carrying *de novo* 4p rearrangements, age 1–24 years, were analyzed clinically and genetically, including parental origin of the deleted chromosome 4. Patients 1, 8, 13, 15, 44, 46 and MG (Tables 1 and 3) were reported previously.[1,11] However, the assessment of the basic genetic defect with molecular cytogenetics methods including not only chromosome 4p analysis but also all subtelomeric regions analysis, their correlation with parental origin and the clinical outcome of the newly characterized rearrangements are reported here for the first time.

We offer detailed clinical descriptions on five previously unreported patients with apparent microdeletions and a WHS phenotype more severe than expected, and one additional unreported patient with unbalanced translocation first diagnosed as isolated deletion.

All the parents had normal chromosomes.

Patient 52: This is a 5-year-old boy born normally at 39 weeks of gestational age with a weight of 1800 g (−4 SD), length of 47 cm (−2 SD) and occipital frontal circumference (OFC) of 32 cm (−2 SD). The postnatal course was characterized by failure to thrive and poor suck. An atrial septal defect (ASD) was diagnosed at birth. At $2\frac{1}{2}$ years, he had his first episode of generalized seizures, recurring several times as unilateral myoclonic seizures. He sat at 3 years, walked without support at $4\frac{1}{2}$ years and spoke the first few words at 3 years. When we first saw him at 5 years, his language was still limited to a few words, although he was open to his

environment and was interested in things around him. Weight was 11 kg (−3.5 SD), height 95 cm (−3 SD) and OFC 48.6 cm (−2 SD). He presented with a typical WHS facial phenotype (Figure 1). A *de novo* der(4)(4qter→4p16.1::8p23→8pter) chromosome was detected in this patient, with a 4p deletion interval of 5 Mb.

Patient 74: This 24-year-old woman was born normally at term. Birth weight was 2120 g (−3 SD), length 45 cm (−3 SD) and OFC 30.5 cm (−3 SD). She had no major malformations, walked unsupported at $2\frac{1}{2}$ years and spoke the first words at 2 years. She experienced generalized seizures during the first 12 years. This patient presented with mild mental retardation. At 24 years, language was very rich, she could write and read and was able to hold a job. Weight was 30.3 kg (−5 SD), height 133 cm (−5 SD) and OFC 49 cm (−5 SD). A *de novo* unbalanced t(4;8)(p16.3;p23) translocation was also detected in this patient, with a 4p deletion of about 5 Mb.

Patient 30: This is an 18-year-old young woman, born at 35 weeks by cesarean section because of fetal distress, with a birth weight of 1700 g (−2 SD), length of 40 cm (3 SD) and OFC of 30 cm (−2 SD). Postnatally, she had persistent failure to thrive. Febrile seizures occurred when she was a child. Clinical signs included glaucoma on the left, bilateral retinal coloboma and microphthalmia on the right. Unspecified renal dysplasia was reported. Precocious puberty occurred at 9 years.

At 18 years, she manifested severe psychomotor delay and marked growth retardation. Language was absent and she could not walk. Length was 128 cm (−6 SD), weight 43.5 kg (−2.5 SD) and OFC 49 cm (−4 SD).

A der(4)(4qter→4p16.1::8p23→8pter) derivative chromosome was also diagnosed in this patient, but with a 4p deletion interval of 12 Mb.
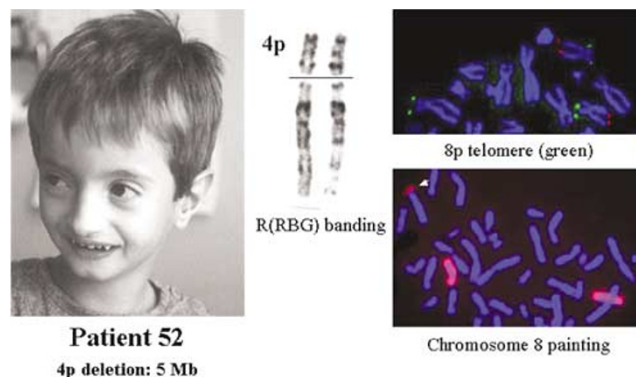

Figure 1 Facial appearance, conventional cytogenetic and molecular cytogenetic findings in patient 52, with a 5 Mb 4p deletion. FISH result with subtelomeric probes is showed on the right (top). Red signals identify the 8q telomere (two copies), green signals the 8p (three copies, one of which is in 4p). FISH result with an eight-specific painting probe is also showed on the right (bottom).

Patient 39: This 16-year-old boy was born normally at term. Weight was 2050 g (−3 SD), length 47 cm (−2 SD) and OFC was not recorded. Intrauterine growth retardation was evident during and after the 6th month of pregnancy. Neonatally, he experienced respiratory distress. Malformations included hypospadias, unilateral iris coloboma and ASD (surgically corrected at 8 years). Generalized seizures began at the age of 6 months. He sat at 1 year, walked without support at $2\frac{6}{12}$ years. At 16 years language was absent. Weight was 44 kg (−2 SD), height 148 cm (−3.5 SD) and OFC 51 cm (−4 SD). Facial appearance was fully consistent with the WHS (Figure 2). The same genomic rearrangement as in case 30 was also detected in this patient.

Patient 40. This is a 16-month-old girl, born normally at term. Birth weight was 2055 g (−3 SD), length 45 cm (−3 SD) and OFC 33 cm (−1 SD). Neonatally, she experienced gastroesophageal reflux and was found to have bilateral renal hypoplasia and pulmonary stenosis. Febrile seizures occurred from early on. At 16 months she could sit unsupported, but language was absent. Her weight was 6400 g (−4 SD), length 69 cm (−4 SD) and OFC 45 cm (−1.5 SD). The 4p rearrangement originated from a t(4p;8p) translocation and the deletion spanned about 12 Mb, as in patients 30 and 39.

Patient 45: This 4-year-old boy was born at 38 weeks by cesarean section because of podalic presentation. Birth weight was 1750 g (−4 SD), length 45 cm (−3 SD) and OFC 32 cm (−1.5 SD). Intrauterine growth retardation was apparent during and after the 6th month. Clinical

Figure 2 Facial appearance, conventional cytogenetic and molecular cytogenetic findings in patient 39, with a 12 Mb 4p deletion. Iris coloboma is evident on the right. Please note that chromosomes are apparently normal. FISH results on the right (top) refer to an 8p-specific subtelomeric probe. With an eight-specific painting probe the fluorescent signal in 4p appears of a similar extent as in patient 52 (right side, bottom).

manifestations included cleft lip and palate and microtia on the left, hypospadias, ASD, unilateral renal hypoplasia and coloboma of the optic nerve on the left. Hypoplastic corpus callosum was apparent on brain MRI. At 4 years, he presented with severe mental retardation. He could not raise his head, language was absent. Weight was 6750 g (−5 SD), length 86 cm (−4 SD) and OFC 41.4 cm (−6.5 SD). Seizures never occurred, although EEG anomalies, as typically observed in WHS,[16] were observed from early on.

The der(4)(qter→q32::p15.3→qter) in this patient caused partial 4p deletion and partial 4q duplication, both spanning about 20 Mb.

Conventional and molecular cytogenetics
Chromosome analysis (average 600 bands) was performed on peripheral blood lymphocytes by R(RBG) banding. At least 20 metaphase spreads were scored on each patient.

FISH analyses were carried out on metaphase chromosomes with several molecular probes.

Probes specific for chromosome arm 4p: a set of cosmids or BACs spanning the chromosome region from 4p15 to 4pter was hybridized according to standard procedures.[17] In each patient, FISH analyses were performed with the subtelomeric probe for D4S3359 (Vysis) and with probes 190b4 and 184d6 falling within the WHSCR-2.[1] Individual patients were analyzed further with a series of probes that were specifically selected from the following: 266E24, 390C19, 497E20, 778B12, 481K22, 332D24, 565F20, 358C18, 367J11, MSX1, 228a7, 21f12, 247f6, 241c2, 185f6, 139h8, 79f5, 33c6, 124h12 (10d12), 108f12, 141a8, 174g8, 19h1, 190b4, pC385.12 and pC678. Probes tested in individual patients are listed in Table 1. Parents' chromosomes were analyzed with probe pC678 (locus D4S96), which was found to be deleted in all patients.

Deletion sizes were established according to the 'Ensembl' mapping and the mapping reported by Baxendale et al.[18]

Probes specific for all chromosome arms: commercially available subtelomeric probes (Vysis) were tested according to the manufacturer's instructions. FISH analyses were first performed with the 8p subtelomeric probe and, if negative, with probes for all other chromosomes. Unbalanced t(4p;8p) translocations were also analyzed with an eight-specific painting probe.

Microsatellite analysis
DNA from patients and their parents was extracted from peripheral blood samples by conventional methods. Familial segregation analysis of the following PCR-amplified polymorphic microsatellites was performed according to standard procedures: D4S2936, D4S3030, D4S1614, D4S127, D4S412, D4S3369, D4S432 and D4S404 (Eurobio). Microsatellites were chosen in individual patients on the basis of the deletion interval.
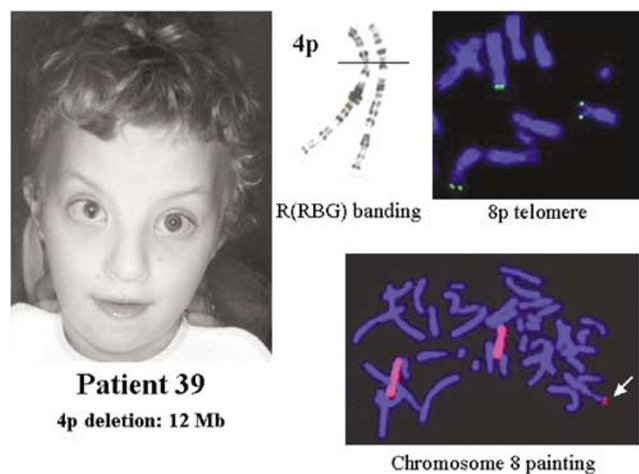
Table 1 Cosmids and BACs for 4p15pter tested by FISH

Locus	Cosmid/BAC	Distance from 4pter (Mb)	37	1	36	45	41	30* 32 33 39* 40* 42 73 82	8	35 52*	31 74*	44	77	46	13	75	15	MG
								(Patient)										
S3359	Tel		–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
S96	pC678								–		–	–	–	–			–	–
S98_FGFR3	pC385.12	1.8																–
S332 7	190b4	1.9							–							–	–	–
	19h1	1.9										–		–	–		–	+
	174g8											–		–	–		–	+
	141a8	2.2													–		–	
	108f12	2.2												–	–	–	+/–	+
	124h12	2.2														–	+	
S43	33c6	2.25							–	–				–		+	+	
	79f5	2.5												–	–			
	139h8	2.6													–			
	185f6	2.6													–			
	241c2	2.7													+			
S182	247f6	2.8									–			+				
S180	21f12	3.3							–	–	–	–	+	+				
S81	228a7	3.7									–		+	+	+	+		
MSX1	MSX1	4.7							–	–	+	+	+	+	+	+		
	367J11	7			–	–	–	–	–	+								
	358C18	9				–	–		+									
	565F20	15			–	–	–	+										
	332D24	20			+		+		+									
	481K22	22		–		+												
	778B12	25		+	+	+	+											
	497E20	27	–			+												
	390C19	30	+	+														
	266E24	30	+															

Distances were approximated up to 50 kb.
Coincident evaluations refer to overlapping cosmids.
+/–: A weak fluorescent signal on one chromosome 4 and a strong signal on the homologous.
*Maternally derived deletions.
Clinical data of patients 1, 8, 13, 15, 44, 46 and MG were previously reported (Zollino *et al*, 2000; Zollino *et al*, 2003).[1] Subtelomeric regions analysis in these patients was not carried out before.
Patients are ordered according to the size of the deletion.

Results

Chromosomes and parental origin of the rearrangement

On conventional cytogenetics, a large 4p deletion, with proximal breakpoint varying from p15.1 to p16.1 was detected in 13 patients. A preliminary diagnosis of microdeletion was carried out in the remaining 12 patients, based on findings of apparently normal chromosomes and hemizygosity of 4p16.3 on FISH analysis. All deletions either large or small were terminal.

Large deletions: Although the deletion interval on 4p proved to be quite different in different patients, also at a molecular level, comprising between 8.5 and 30 Mb, the proximal breakpoint of the rearrangement was at about 12 Mb from the telomere in five cases. Conventional cytogenetics was fully concordant with these results.

When all these patients were investigated with subtelomeric probes, extra signals on the deleted chromosome 4 were never detected. By microsatellite analysis, these deletions were demonstrated to be paternal in origin.

Microdeletions: On further FISH analyses with probes specific for chromosome arm 4p, six apparent microdeletions of 12 in total were found to measure about 5 Mb in two patients, 12 Mb in three patients and 20 Mb in the last. Thus, molecular cytogenetics was not concordant with conventional cytogenetics.

In the remaining six patients with a preliminary diagnosis of microdeletion, the extent of the 4p deletion comprised between 3.5 and 1.9 Mb, as expected.

When all of these patients were investigated with subtelomeric probes, a t(4;8)(p16.1;p23) unbalanced *de novo* translocation was detected in the five patients with either 5 or 12 Mb deletions. On FISH analysis with an eight-specific painting probe, the translocated 8p segment appeared to have a similar extent in all of these patients (Figures 1 and 2), and was judged to span about 8 Mb. All

these rearrangements were maternal in origin. A der(4)(4q-ter→q32::p15.3→qter), leading to partial 4q trisomy and partial 4p deficiency, was ascertained in the 20 Mb deleted patient. Going back to conventional cytogenetics at a better resolution, an atypical banding pattern in 4p was evident, consistent with that of the 4q32qter chromosome region. This double intrachromosomal rearrangement was of paternal origin.

Microdeletions comprised between 3.5 and 1.9 Mb were all single anomalies and paternally derived.

Genotype–phenotype correlations

Dividing individual phenotypes into a 'classical' or a 'mild' form, as suggested previously,[1] we found that paternally derived deletions resulted in a clinical phenotype fully consistent with the extent of the 4p deletion as established by means of conventional cytogenetics and of FISH analysis with a single molecular probe. Of the paternally derived rearrangements, six were first diagnosed as micro-deletions. On further genetic analysis, 5/6 (83%) were confirmed as microdeletions (<3.6 Mb) giving rise to the 'mild' form of the WHS phenotype. The only exception was patient 45, whose genomic rearrangement actually consisted of a der(4)(qter→q32::p15.3→qter), thus implying a double quantitative change of the genome, with a 4p deletion size of about 20 Mb.

Patient 15, with an isolated 2.2 Mb paternal deletion, presented with severe psychomotor delay, no major malformations and normal head circumference.[11] Her psychomotor development was reasonably good until 17 months, when she suffered from repeated epileptic status, with two episodes of cardiac arrest. Therefore, the severity of mental retardation in this patient was considered secondary to severe critical episods.

All of the paternally derived deletions that were easily detectable by conventional cytogenetics were associated with 'classical' WHS, although variability was noted among patients, mostly depending on the individual deletion size.

Maternally derived deletions were associated with the 'classical' form of the WHS in most cases (5/6) and were usually mistaken as microdeletions. As reported above, the totality of maternally derived deletions were actually derivative chromosomes, resulting from unbalanced t(4;8)(p16.1;p23) cryptic translocations. The severity of the clinical presentation was correlated with the extent of the 4p deletion, being more severe in patients 30, 39 and 40 with the largest deletions (12 Mb), than in patient 52 and 74 with a 5 Mb deletion. A summary of these observations is provided in Tables 2 and 3.

Discussion

WHS is widely recognized as a severe condition, in which a large 4p deletion of several megabases is usually detected by conventional cytogenetics. In patients with large

Table 2 Clinical data of patients with double chromosome imbalance (Tot: 6)

Patient	Sex	Age (years)	Weight (kg) (SD)	Length (cm) (SD)	OFC (cm) (SD)	CHD	Midline defects	Coloboma	Renal abnorm.	Seizures	Language	Independent walking (years)	Phenotype
52	M	5	11 (−3.5)	95 (−3)	48.6 (−2)					Yes	Poor	$4\frac{6}{12}$	Classical-Mild
74	F	24	30.3 (−5)	133 (−5)	49 (−2)					Yes	Fluent	$2\frac{6}{12}$	Mild
30	F	18	43.5 (−2.5)	128 (−6)	49 (−2)	ASD	Hypospadias	Retinal	+	Yes	Absent	Absent	Classical
39	M	16	44 (−2)	148 (−3.5)	51 (−4)	Pulmonary stenosis		Iris		Yes	Absent	$2\frac{6}{12}$	Classical
40	F	$1\frac{6}{12}$	6.4 (−4)	69 (−4)	45 (−1.5)				+	Yes	Absent	Absent	Classical
45	M	4	6.75 (−5)	86 (−4)	41.4 (−6.5)	ASD	Hypospadias	Optic nerve	+	EEG+	Absent	Absent	Classical

SD: standard deviation.
ASD: atrial septal defect.

Table 3 Clinical data of patients with isolated deletion (Tot: 19)

Patient	Sex	Age (years)	Weight (kg) (SD)	Length (cm) (SD)	OFC (cm) (SD)	CHD	Midline defects	Coloboma	Renal abnorm.	Seizures	Language	Independent walking (years)	Del 4p (Mb)	Phenotype
1	F	7	13 (−3.5)	102 (−3.5)	45.2 (−4.5)	ASD VSD, PDA	Cleft palate			Yes	Absent	Absent	>22	Classical
8	M	$8\frac{4}{12}$	20.8 (−2)	114.5 (−3.5)	48.3 (−3.5)		Hypospadias		Pelvis dilatation	Yes	Absent	$2\frac{3}{12}$	8	Classical
13	M	13	41 (50th)	152 (50th)	51 (−2)					Yes	Fluent	$1\frac{8}{12}$	2.6	Mild
15	F	10	21.5 (−2)	126 (−1.5)	52 (50th)					Yes	Few words	6	2.2	*Classical
31	F	3	8.3 (−3.5)	82 (−5)	45.7 (−3)	ASD	Cleft uvula			Yes	Few words	$2\frac{6}{12}$	4.5	Classical–mild
32	F	6	11.7 (−4)	101 (−3)	42 (−7)	ASD		Iris	Renal hypoplasia	Yes	Absent	Absent	12	Classical
33	M	13	19 (−4)	120 (−5)	48.5 (−3.5)		Cleft palate Hypospadias			Yes	Absent	With support	12	Classical
35	F	20	22 (−6.5)	128 (−6)	49 (−4)		Cleft palate		Pelvis duplication	Yes	Few words	5	6	Classical
36	F	4	10.2 (−4)	91 (−3)	42 (−6.5)			Iris		Yes	Absent	Absent	18	Classical
37	F	$7\frac{6}{12}$	10.6 (−5.5)	94 (−4)	42 (−8)		Schisis S1	Iris Optic nerve	Renal hypoplasia	Yes	Absent	Absent	28	Classical
41	F	15	26 (−4)	127 (−5.5)	46 (−6.5)	VSD				Yes	Absent	Absent	18	Classical
42	F	18	45 (−2)	126 (−6)	48.5 (−4)	VSD		Iris		Yes	Absent	Absent	12	Classical
44	M	11	22 (−3)	124 (−3)	47.5 (−3)		Hypospadias			Yes	Good	3	3.5	Mild
46	F	1	7.7 (−2)	70 (−2)	42 (−3)		Cleft palate			Yes			2.6	Mild
73	M	$0\frac{4}{12}$	4.35 (−3)	57 (−3)	37.5 (−4)	Pulmonary stenosis	Hypospadias			Yes			12	Mild
75	F	$2\frac{4}{12}$	8.5 (−3)	85 (−1)	43 (−4.5)					Yes	10 words	$2\frac{5}{12}$	2.2	Mild
77	F	13	49 (50th)	142.5 (−2)	53 (50th)					Yes	Fluent	$1\frac{6}{12}$	2.5	Mild
82	M	2	5.5 (−5.5)	75 (−4)	42 (−6)			Iris	Renal hypoplasia	Yes	Absent	Absent	12	Classical
MG	M	$2\frac{6}{12}$	7 (−4)	78 (−4)	44.5 (−4)					Yes	Few words	2	1.9	Mild

VSD = ventricular septal defect.
PDA = patent ductus arteriosus.
*Severe MR secondary to epileptic status (see 'Genotype–phenotype correlations' section).
Patients 1, 8, 13, 15, 44, 46 and MG were previously reported (Zollino et al, 2000; Zollino et al, 2003).[1]

Table 4 Double chromosome anomaly in WHS: comparison between conventional and molecular cytogenetics

Patient	WHS phenotype	Parental origin	Conventional cytogenetics	Molecular cytogenetics	4p deletion size (Mb)
52	Classical–mild	Maternal	46,XY	der(4)(4qter→4p16.1::8p23→8pter)	5
74	Mild	Maternal	46,XX	der(4)(4qter→4p16.3::8p23→8pter)	5
30	Classical	Maternal	46,XX	der(4)(4qter→4p16.1::8p23→8pter)	12
39	Classical	Maternal	46,XY	der(4)(4qter→4p16.1::8p23→8pter)	12
40	Classical	Maternal	46,XX	der(4)(4qter→4p16.1::8p23→8pter)	12
45	Classical	Paternal	46,XY	der(4)(qter→q32::p15.3→qter)	20

deletions language is usually absent or limited to a few words, independent walking occurs on average very late in infancy, interaction with the environment is very poor (Table 3).

The development of molecular cytogenetics in the last few years has allowed the detection of several 4p16.3 microdeletions in apparently normal chromosomes.[1,3–9,11] Small deletions tend to be associated with a milder phenotype, without major malformations and possibly normal head circumference. In particular, patients with microdeletions usually reach independent walking by the age of 3 years, are able to form complex sentences with a relatively rich vocabulary and present with a lively and social behavior. With the purpose of proper genetic diagnosis and counseling, we previously proposed to divide WHS into a 'classical' and a 'mild' form, depending on the severity of the clinical presentation, which, in turn, appeared strongly correlated with the size of the deletion.[1] There is substantial evidence that genotype–phenotype correlations in WHS mostly depend on the apparent extent of the deletion; however, most clinicians involved in this field experienced cases that lack such correlations. Additional genetic factors have a role in determining the final phenotype, most likely. However, none of these factors has been characterized yet. Looking at a routinary cytogenetic analysis, *de novo* microdeletions are diagnosed when chromosomes are apparently normal and hemizygosity of the WHS critical region in 4p16.3 is proven with one molecular probe.

WHS-associated genomic rearrangements arise as *de novo* events in most cases, usually on the *paternally* derived chromosome 4.[12,13] *De novo* 4p deletions are reasonably assumed to be single chromosome anomalies. However, unbalanced *de novo* translocations, above all t(4;8)(p16.1;p23) translocations, were detected recently with an unexpectedly high frequency in WHS[10,14,15] and, whenever investigated, they were of maternal origin. It was recently demonstrated that WHS-associated t(4p;8p) translocations occur within OR-gene clusters on both 4p and 8p, with the involvement of two distinct OR-gene clusters in 4p at a distance of about 5 and 14 Mb from the telomere and of a unique OR-gene cluster in 8p.[15] Such double chromosome abnormalities may account for phenotypic variability of the WHS, although frequency and specificity of this phenomenon, molecular aspects of WHS-associated unbalanced translocations and their clinical relevance are still unclear. We report on six WHS patients with either a *de novo* unbalanced translocation [t(4;8) in five cases] or an intrachromosomal double rearrangement [der(4) (qter→p15.3::q33→qter) in one patient] from a total sample of 25 cases. Five of these double rearrangements were initially mistaken as microdeletions, in spite of a severe clinical presentation. Genotype–phenotype inconsistencies were initially attributed to the 'well-known' phenotypic variability of the WHS. Surprisingly, the extent of the deletion, which was expected to be smaller than 3.5 Mb, did span 12 Mb in three patients (30, 39 and 40), 20 Mb in another patient (45) and 5 Mb in two (52 and 74) (Table 4). Individual phenotypes were now fully consistent with the deletion size. With respect to t(4;8) translocations, we observed that the breakpoint in 8p recurred in the same region, the trisomic 8p segment being judged to include about 8 Mb in each patient. On the contrary, breakpoints in 4p occurred at two different sites, mostly at a distance of 12 Mb from the telomere, but also at 5 Mb, thus implying a different extent of the 4p deletion. Our observations confirm those recently reported by Giglio *et al*.[15] We found that, depending on the 4p deletion interval on the derivative chromosome, patients with unbalanced t(4p;8p) translocations present with clinical phenotypes of different severity. We also found that nearly all *de novo* unbalanced t(4p;8p) translocations caused large deletions easily mistaken as microdeletions. They were never detected in patients in whom a large 4p deletion was already diagnosed by conventional cytogenetics. A preliminary diagnosis of microdeletion was made also in patient 45, who presented with a severe WHS phenotype. A 20 Mb 4p deletion, masked by a large 4q segment, was detected in this patient, and clinical signs were now fully consistent.

We conclude that a double, cryptic chromosome imbalance is an important factor explaining the phenotypic variability of WHS. It acts either by masking the actual extent of the 4p deletion, causing a misinterpretation of the cytogenetic data and by adding a second aneuploidy. Phenotypically, patients with unbalanced translocations presented with a typical WHS phenotype, in agreement with the well-known evidence that deletions are more

deleterious than duplications. However, we can reasonably consider that the concomitant partial trisomy does affect the degree of mental retardation. We also found that head circumference was normal in two out five patients with partial 8p trisomy, in spite of a 4p deletion of 12 and 5 Mb, respectively. There is evidence that small trisomies of different chromosome arms give rise to macrocephaly.[19,20]

It is worth noting that paternally derived deletions were usually isolated, with only one double chromosome rearrangement arising from an intrachromosomal recombination. Accordingly, no genotype–phenotype inconsistencies were noted in most patients with a paternally derived rearrangement. On the contrary, all WHS-associated unbalanced translocations originated in the maternal meiosis. They were usually cryptic and accounted for phenotype–genotype inconsistencies. Based on these data, we can expect that about 20% of patients with apparent microdeletions will present with a seemingly inconsistent severe phenotype.

A new diagnostic test for WHS-associated microdeletions is strongly recommended. It should include at least two molecular probes, one falling within the WHS critical region, the latter specific for the 8p telomere.

Finally, our observations demonstrate that mechanisms causing genomic rearrangements in maternal and paternal meiosis are different.

Electronic database information

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for WHS [MIM 194190] Ensembl Human Map, http://www.ensembl.org).

References

1 Zollino M, Lecce R, Fischetto R *et al*: Mapping the Wolf–Hirschhorn syndrome phenotype outside the currently accepted WHS critical region and defining a new critical region, WHSCR-2. *Am J Hum Genet* 2003; **72**: 590–597.

2 Wright TJ, Ricke DO, Denison K *et al*: A transcript map of the newly defined 165 kb Wolf–Hirschhorn syndrome critical region. *Hum Mol Genet* 1997; **6**: 317–324.

3 Gandelman KY, Gibson L, Meyn MS, Yang-Feng TL: Molecular definition of the smallest region of deletion overlap in the Wolf–Hirschhorn syndrome. *Am J Hum Genet* 1992; **51**: 571–578.

4 Johnson WP, Altherr MR, Blake JM, Keppen LD: FISH detection of Wolf–Hirschhorn syndrome: exclusion of D4F26 as critical site. *Am J Med Genet* 1994; **52**: 70–74.

5 Clemens M, Martsolf JT, Rogers JG, Mowery-Rushton P, Surti U, McPherson E: Pitt–Rogers–Danks syndrome: the result of a 4p microdeletion. *Am J Med Genet* 1996; **66**: 95–100.

6 Lindeman-Kusse MC, Van Haeringen A, Hoorweg-Nijman JJG, Brunner HG: Cytogenetic abnormalities in two new patients with Pitt–Rogers–Danks phenotype. *Am J Med Genet* 1996; **66**: 104–112.

7 Fang YY, Bain S, Haan EA *et al*: High resolution characterization of an interstitial deletion of less than 1.9 Mb at 4p16.3 associated with Wolf–Hirschhorn syndrome. *Am J Med Genet* 1997; **71**: 453–457.

8 Partington MW, Fagan K, Soubjaki V, Turner G: Translocations involving 4p16.3 in three families: deletion causing the Pitt–Rogers–Danks syndrome and duplication resulting in a new overgrowth syndrome. *J Med Genet* 1997; **34**: 719–728.

9 Wieczorek D, Krause M, Majewski F *et al*: Effect of the size of the deletion and clinical manifestation in Wolf–Hirschhorn syndrome: analysis of 13 patients with a de novo deletion. *Eur J Hum Genet* 2000; **8**: 519–526.

10 Wieczorek D, Krause M, Majiewski F *et al*: Unexpected high frequency of the novo unbalanced translocation in patients with Wolf–Hirschhorn syndrome (WHS). *J Med Genet* 2000; **37**: 798–804.

11 Zollino M, Di Stefano C, Zampino G *et al*: Genotype–phenotype correlations and clinical diagnostic criteria in Wolf–Hirschhorn syndrome. *Am J Med Genet* 2000; **94**: 254–261.

12 Tupler R, Bortotto L, Buhler EM *et al*: Paternal origin of the de novo deleted chromosome 4 in Wolf–Hirschhorn syndrome. *J Med Genet* 1992; **29**: 53–55.

13 Dallapiccola B, Mandich P, Bellone E *et al*: Parental origin of chromosome 4p deletion in Wolf–Hirschhorn syndrome. *Am J Med Genet* 1993; **47**: 921–924.

14 Tonnies H, Stumm M, Neumann L *et al*: Two further cases of WHS with unbalanced *de novo* translocation t(4;8) characterised by CGH and FISH. *J Med Genet* 2001; **38**: e21.

15 Giglio S, Calvari V, Gregato G *et al*: Heterozygous submicroscopic inversions involving olfactory receptor-gene clusters mediate the recurrent t(4;8)(p16;p23) translocation. *Am J Hum Genet* 2002; **71**: 276–285.

16 Sgrò V, Riva E, Canevini MP *et al*: 4p– syndrome: a chromosomal disorder associated with a particular EEG pattern. *Epilepsia* 1995; **36**: 1206–1214.

17 Lichter P, Chang CJ, Call K *et al*: High resolution mapping of human chromosome 11 by *in situ* hybridization with cosmid clones. *Science* 1990; **247**: 64–69.

18 Baxendale S, MacDonald ME, Mott R *et al*: A cosmid contig and high resolution restriction map of the 2 megabase region containing the Huntington's disease gene. *Nat Genet* 1993; **4**: 181–186.

19 Opitz JM, Patau K: A partial trisomy 5p syndrome; in Bergsma D (ed): *New Chromosomal and Malformation Syndromes*, Vol. 11 Miami, FL: Symposia Specialist for the National Foundation-March of Dimes: Birth Defects; 1975, pp 191–200.

20 Concolino D, Cinti R, Ferraro L, Moricca MT, Strisciuglio P: Partial trisomy 1(q42-qter): a new case with a mild phenotype. *J Med Genet* 1998; **35**: 75–77.