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Comprehensive analysis of Wolf–Hirschhorn syndrome using array CGH indicates a high prevalence of translocations

Sarah T South[*,1,4], Heidi Whitby[1], Agatino Battaglia[1,2], John C Carey[1] and Arthur R Brothman[1,3,4]

[1]Division of Medical Genetics, Department of Pediatrics, University of Utah, Salt Lake City, UT, USA; [2]The Stella Maris Clinical Research Institute, University of Pisa, Calambrone, Pisa, Italy; [3]Departments of Human Genetics and Pathology, University of Utah, Salt Lake City, UT, USA; [4]ARUP Laboratories, Salt Lake City, UT, USA

Wolf–Hirschhorn syndrome (WHS) is caused by deletions involving chromosome region 4p16.3. The minimal diagnostic criteria include mild-to-severe mental retardation, hypotonia, growth delay and a distinctive facial appearance. Variable manifestations include feeding difficulties, seizures and major congenital anomalies. Clinical variation may be explained by variation in the size of the deletion. However, in addition to having a deletion involving 4p16.3, previous studies indicate that approximately 15% of WHS patients are also duplicated for another chromosome region due to an unbalanced translocation. It is likely that the prevalence of unbalanced translocations resulting in WHS is underestimated since they can be missed using conventional chromosome analyses such as karyotyping and WHS-specific fluorescence in situ hybridization (FISH). Therefore, we hypothesized that some of the clinical variation may be due to an unrecognized and unbalanced translocation. Array comparative genomic hybridization (aCGH) is a new technology that can analyze the entire genome at a significantly higher resolution over conventional cytogenetics to characterize unbalanced rearrangements. We used aCGH to analyze 33 patients with WHS and found a much higher than expected frequency of unbalanced translocations (15/33, 45%). Seven of these 15 cases were cryptic translocations not detected by a previous karyotype combined with WHS-specific FISH. Three of these 15 cases had an unbalanced translocation involving the short arm of an acrocentric chromosome and were not detected by either aCGH or subtelomere FISH. Analysis of clinical manifestations of each patient also revealed that patients with an unbalanced translocation often presented with exceptions to some expected phenotypes.
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Introduction

Wolf–Hirschhorn syndrome (WHS) affects at least 1/50 000 live births and presents with a broad range of clinical

*Correspondence: Dr ST South, Clinical Cytogenetics, 1C210 SOM, 30 North 1900 East, University of Utah Health Sciences Center, Salt Lake City, UT 84132-2117, USA.
Tel: +1 801 581 5524; Fax: +1 801 585 5241;
E-mail: sarah.south@hsc.utah.edu

manifestations (MIM no. 194190). The minimal diagnostic criteria are mild-to-severe mental retardation, hypotonia, growth delay, and a distinctive facial appearance. Variable clinical manifestations include severe feeding difficulties, seizures, antibody deficiency, and major congenital anomalies such as skeletal anomalies, heart lesions, oral facial clefts, sensoineural deafness, and genitourinary tract defects.[1]

WHS is caused by deletions involving 4p16.3. Different mechanisms leading to the deletion of 4p16.3 include cytogenetically visible de novo 4p terminal and interstitial

deletions (50–60%), *de novo* microdeletions detected by fluorescence *in situ* hybridization (FISH) using a probe for the critical region (25–30%), or an unbalanced translocation either *de novo* or inherited from a familial balanced translocation (approximately 15%).[1,2]

A portion of the clinical variation appears to be due to differences in the size of the deletion on 4p.[3–5] However, many patients do not fit into a strict correlation between size of the deletion and severity of the syndrome.[6,7] Allelic variation in the non-deleted 4p region as well as different genetic backgrounds could modify the phenotype of similarly sized and located deletions. Another potential explanation for the variability in clinical manifestations is the presence of an unbalanced translocation resulting in a 4p deletion. In these cases, the deleted 4p also contains material from another chromosome, making this patient partially trisomic for that material. Multiple patients with atypical WHS clinical manifestations have been shown to carry unbalanced translocations involving 4p.[8–13]

Unbalanced translocations may be missed by both a karyotype and routine FISH analysis of the region due to the limitations of these techniques. A karyotype has a limit of resolution in the 5–10 Mb range. Therefore, unbalanced translocations resulting in changes smaller than 5–10 Mb are usually missed by the karyotype. Furthermore, a karyotype can also miss alterations that do not change the banding pattern of the chromosomes. FISH analyzes only small (approximately 100 kb) regions of the genome at a time. Therefore, rearrangements involving regions outside of this small area are missed. Whole chromosome painting of chromosome 4 has been recommended previously for all patients with an apparently *de novo* deletion to exclude a cryptic translocation.[14] However, this method cannot reliably detect translocations involving the distal ends of chromosomes.

The prevalence of unbalanced translocations resulting in WHS is likely underestimated since unbalanced translocations can be missed using conventional methods. In contrast, array comparative genomic hybridization (aCGH) can evaluate the entire genome for deletions and duplications at a much higher resolution than a karyotype (eg: ≤ 1 *vs* 5–10 Mb resolution respectively).[15–17] aCGH is also comparable to performing thousands of FISH experiments simultaneously. Therefore, we analyzed 33 patients with WHS for cryptic chromosome rearrangements using aCGH to assess both the prevalence of unbalanced translocations among WHS patients and to determine the role any additional chromosomal material may have in the patient's phenotype.

Materials and methods

Participants were recruited through the United States national support group for WHS (4p-Support Group) and

through WHS medical specialists at the University of Utah (Dr John Carey) and at the University of Pisa (Dr Agatino Battaglia). All participants were diagnosed previously with WHS and shown by either a karyotype or FISH analysis to have a deletion involving 4p16.3. To avoid a selection bias, all patients with WHS interested in participating in this study were analyzed, regardless of any previous genetic findings. One patient, 003, has been described previously.[18] This study was approved by our institutional review board and informed consent was obtained for each patient.

aCGH analysis was performed on genomic DNA extracted from peripheral blood using the Spectral Genomics 2600 array platform and following the manufacturer's protocol (Spectral Genomics, Perkin Elmer Corporation, Waltham, MA, USA). This platform has an average spacing of one bacterial artificial chromosome (BAC) clone every 1 Mb with increased density near the subtelomeric regions of each chromosome. Cy3-dCTP and the Cy5-dCTP were purchased from Amersham BioSciences (Buckinghamshire, UK). Scanning was performed with Axon's GenePix 4000B microarray scanner and the images were analyzed with SpectralWare 2.2.

FISH analysis narrowed the breakpoint on chromosome 11p in three cases. FISH was performed using BACs CTD-2226C18 and RP11-120E20, which were identified using the May 2004 assembly of the University of California at Santa Cruz Human Genome Browser and purchased from Invitrogen Corporation (Carlsbad, CA, USA). The method used to grow, label and hybridize these BAC clones has been described previously.[19]

FISH was also performed using a commercially available WHS-specific probe, an acrocentric p-arm-specific probe, D15S11 (15q11.2), and D15Z1(15 centromere) (Vysis, Downers Grove, IL, USA). Commercially available probes for the chromosome 13/21 centromeres and the 14/22 centromeres (Cytocell, Rainbow Scientific Inc., Windsor, CT, USA) were also used for FISH studies.

Clinical phenotype was assessed using a 15-page questionnaire sent to the parents or primary care provider, plus a physical examination in our two centers whenever possible.

Results

Thirty-four unselected patients with WHS were enrolled in the study and analyzed for a genomic imbalance using aCGH. Sixteen of these patients consented to publication of a facial photograph (Figure 1). The clinical phenotype was accessed in 32 of these patients and is summarized in Table 1. Two patients (867 and 648) are related and have the same genetic imbalance by common descent. Therefore, in determining the prevalence of the various types of alterations leading to a 4p deletion, their result is considered only one time, making the denominator for our prevalence study 33 rather than 34.

Eighteen of the 33 patients analyzed for the prevalence study were determined to have only a 4p monosomy without evidence of an unbalanced translocation (Figure 2). Each patient had an apparently terminal deletion that encompassed the two proposed critical regions for WHS.[20–26] As expected, the size of the deletion varied within our study group from just over 2 Mb to greater than 20 Mb. There also appeared to be some grouping of patients into common breakpoints; however, since aCGH does not determine the breakpoint at the nucleotide level, it is possible that the breakpoints within these groupings do vary at a higher resolution.

Fifteen of the 33 patients had an unbalanced translocation leading to both a 4p monosomy and a partial trisomy for another chromosome arm (Figure 3). Again the size of the 4p monosomy varied, but none was larger than 10 Mb. Each patient's deletion was apparently terminal and involved the two critical regions in 4p16.3. There were various partner chromosomes for the unbalanced translocation; however, some chromosome arms were represented at an increased frequency, including 8p (involved in 7/15 cases), 11p (involved in 3/15 cases), and 15p (involved in 2/15 cases). There also appeared to be some grouping of

patients into common breakpoints, particularly around 3.4–4.9 and 8.3–9.7 Mb. Five of the unbalanced translocations were determined to be inherited from a balanced parental translocation, eight were apparently de novo, and either one or both parents were unavailable for analysis in the remaining two cases (Table 1).

Seven of 15 unbalanced translocations were cryptic in that they were not identified by karyotype analysis or FISH with a WHS-specific probe (Table 2). Three of these seven cases were identified as unbalanced translocations by previous clinical testing using a subtelomere FISH assay; whereas, the other four unbalanced translocations were unexpected and are new findings.

Three of the 15 unbalanced translocations were not identified by the aCGH platform. These three cases involved translocations with the short arm of an acrocentric chromosome (Table 3). Due to the highly repetitive sequence on both the stalk and satellite regions of the acrocentric short arms, these regions are not represented on the aCGH platforms. Therefore, the aCGH procedure identified only a 4p monosomy in these three cases. The unbalanced translocation in patient 842 was identified only after parental studies using a WHS-specific probe-

Figure 1 Facial appearance of 16 study participants with WHS. Patient ID no. is noted in the upper left-hand corner of each photograph. WHS, Wolf–Hirschhorn syndrome.

Table 1 Clinical manifestations of WHS patients

ID no.	Age (years)	Del(4p) size (Mb)	Origin	GR	Microcephaly	SZ	Heart defect	GU defect	CL/P	Skeletal defects	Hearing loss	Ocular defects	Verbal	Walk
217	8.5	2.1–2.2		+	n/a	+	−	−	−	−	+	+	−	−
387	2.5	2.3–2.7		+	+	+	−	−	+	−	−	+	−	−
038	5.0	3.4–4.9		+	+	+	ASD	+	+	−	−	−	+	+
498	7.2	3.4–4.9		+	n/a	+	−	−	−	−	−	+	+	+
973	11.0	3.4–4.9		±	+	+	−	−	−	−	−	−	+	+
105	6.1	4.9–6.3		+	+	+	−	−	−	−	−	−	−	−
959	4.2	4.9–6.3		+	n/a	+	PFO	−	−	+	−	+	+	+
434	4.0	4.9–6.3		+	+	+	ASD/PFO	−	−	−	−	−	−	−
675	4.5	7.1–8.1		+	n/a	+	PS	+	+	−	−	+	+	+
033	25.8	8.3–9.7		+	+	+	−	−	−	−	+	−	−	+
396	2.0	12.6–13.0		+	+	+	PS	+	−	−	−	−	−	−
216	29.0	14.8–16.0		+	n/a	+	DV	+	−	+	+	−	−	+
199	1.0	16.5–17.3		+	+	+	−	−	−	−	+	+	−	−
500	13.0	20.4–21.9		+	+	+	ASD/VSD	−	−	+	+	+	−	−
284	15.5	20.4–21.9		+	n/a	+	−	−	−	−	+	−	−	+
496	4.5	20.4–21.9		+	+	+	VSD/PS	+	−	−	+	+	−	−
231	8.5	22.1–24.8		+	+	+	ASD	−	−	+	−	+	−	−
636	5.1	3.1–3.2; dup15p	mat	−	n/a	+	−	+	−	+	−	+	+	+
842	3.3	3.1–3.2; dup15p	mat	−	+	+	−	−	−	−	−	+	−	+
867	3.5	3.4–4.9; dup11p 2.9–3.6	mat	+	−	+	−	−	−	+	+	−	−	−
648	38.0	3.4–4.9; dup11p 2.9–3.6	pat	−	−	+	−	+	−	+	−	−	−	+
372	10.0	3.4–4.9; dup11p 2.9–3.6	pat	−	−	+	−	−	−	+	−	+	−	−
415	1.2	3.4–4.9; dup7p 6.4–7.5	n/a	+	+	+	+	+	−	−	+	+	−	−
312	5.1	3.4–4.9; dup8p 6.4–8.6	dn	+	n/a	+	PS	+	−	−	−	+	+	+
214	4.0	3.4–4.9; dup8p 6.4–8.6	dn	−	+	+	PFO	+	−	−	+	+	−	−
828	1.5	3.4–4.9; dup8p 6.4–8.6	dn	+	−	−	−	+	−	+	−	−	−	−
003	1.0	4.9–6.5; dup11p 1.3–1.4	dn	−	−	+	PFO	−	−	−	−	+	−	−
265	15.8	6.5–7.0; dup14p or 22p	dn	+	+	+	ASD/BAV	+	+	+	−	−	−	+
999	41.0	7.1–8.1; dup8p 6.1–8.6	n/a	+	+	+	−	−	−	−	+	+	−	−
311	8.8	8.3–9.7; dup8p 4.8–6.0	dn	+	n/a	+	−	+	−	+	−	−	−	−
412	4.0	8.3–9.7; dup8p 6.4–8.6	dn	+	+	+	ASD/PS	+	−	−	+	−	−	−
400	10.1	8.3–9.7; dup8p 8.8–8.9	dn	+	+	+	ASD	−	−	−	−	+	−	+

Abbreviations: ASD, atrial septal defect; BAV, bicuspid aortic valve; CL/P, cleft lip/palate; dn, *de novo* translocation; DV, dextroversion; GR, growth retardation; GU, genitourinary; mat, inherited from maternal translocation; n/a, not available; pat, inherited from paternal translocation; PFO, patent foraman ovale; PS, pulmonic stenosis; SZ, seizures; VSD, ventricular septal defect; WHS, Wolf–Hirschhorn syndrome.
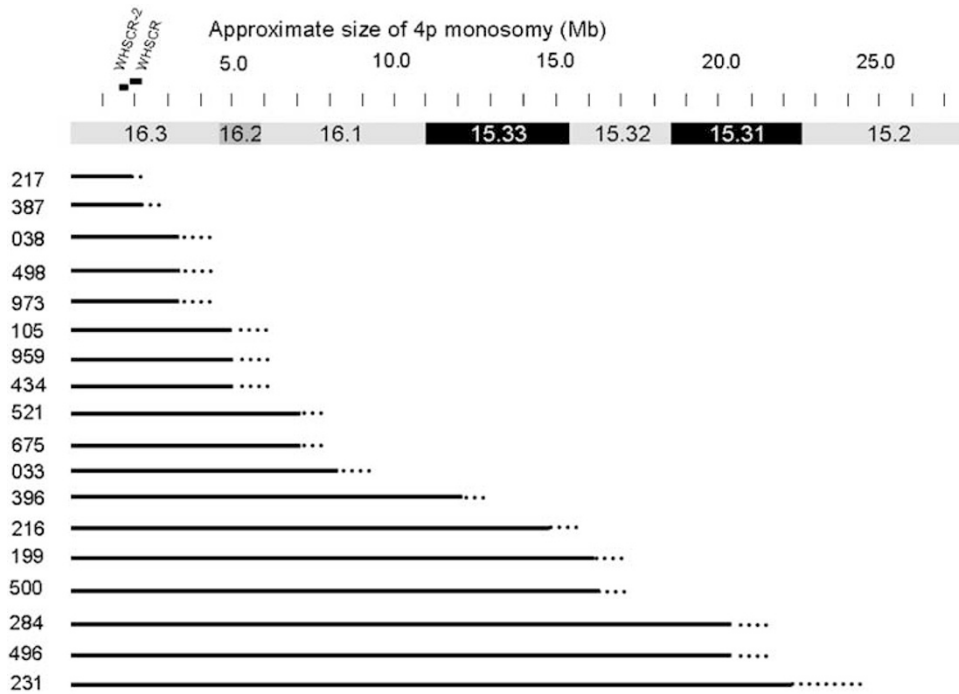
Figure 2 Approximate 4p deletion size as determined by aCGH for each study participant with only a 4p deletion. Solid lines represent the region known to be deleted whereas the dashed line represents the unknown deletion boundary between the most proximal BAC deleted and the most distal BAC not deleted. Partial ideogram of the 4p region with megabase markers and the location of the two WHS critical regions, WHSCR and WHSCR-2, are shown along the top. Patient ID nos. are listed to the left of each patient's result. aCGH, array comparative genomic hybridization; BAC, bacterial artificial chromosome; WHS, Wolf–Hirschhorn syndrome.
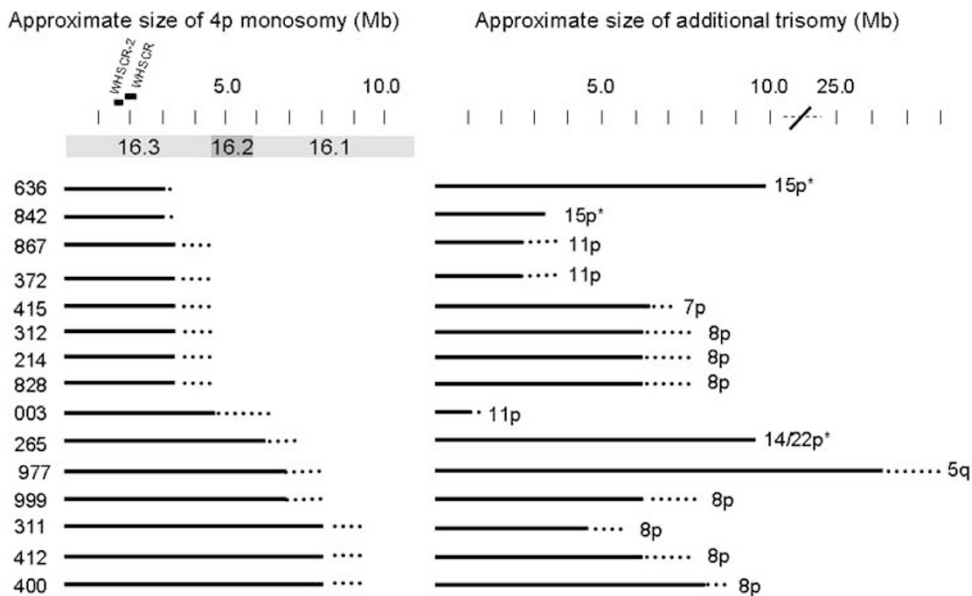

Figure 3 Approximate deletion and duplication sizes for each study participant with an unbalanced translocation as determined by aCGH and FISH. Solid lines represent the region known to be deleted/duplicated whereas the dashed line represents the unknown deletion/duplication boundary between the most proximal BAC deleted/duplicated and the most distal BAC with a normal hybridization pattern. Asterisks (*) indicate the approximate size of the duplication as an estimate based on FISH results and the average size of the acrocentric p-arms. Partial ideogram of the 4p region with megabase markers and the location of the two WHS critical regions, WHSCR and WHSCR-2, are shown along the top left side. Patient ID nos. are listed to the left of each patient's result. aCGH, array comparative genomic hybridization; BAC, bacterial artificial chromosome; FISH, fluorescence *in situ* hybridization; WHS, Wolf–Hirschhorn syndrome.

revealed 4p16.3 material on the short arm of one chromosome 15 in this patient's mother, suggesting that the mother carried a balanced translocation between 4p and 15p. Subsequent FISH studies in our laboratory on this patient showed acrocentric short arm material on her derivative 4, confirming her unbalanced translocation. The remaining two patients had previous karyotype studies that identified material of unknown origin on distal 4p. In patient 636, this material was identified as acrocentric short arm in origin through silver staining of the nucleolar organizing region (NOR) regions. Using FISH, this patient's mother was determined to have a balanced translocation between 4p and 15p and subsequent FISH studies in our laboratory showed that the derivative chromosome 4 was positive for chromosome 15 satellite III DNA. For patient 265, the material of unknown origin was determined by FISH in our laboratory to be either chromosome 14 or 22 alpha satellite DNA. This translocation was not characterized further since this FISH probe cannot distinguish between the two highly similar centromeric regions of chromosomes 14 and 22, and neither parent was found to carry a translocation involving 4p.

Discussion

The use of aCGH to characterize a deletion syndrome such as WHS was shown to have some very clear advantages. aCGH successfully detected a monosomy of 4p in each patient shown previously to have a 4p deletion by either karyotype or FISH. Furthermore, aCGH revealed a cryptic unbalanced translocation in seven of our patients. Three of these cryptic unbalanced translocations had been identified previously by subtelomere FISH screening. However, aCGH provided the added information on approximate size and therefore genetic imbalance of both the deletion and duplication, information that is not revealed through a subtelomeric FISH assay.

This study revealed unbalanced translocations in patients with WHS at a frequency higher than expected. The largest study (based on 108 cases) on the rate of unbalanced translocations in WHS indicated that approximately 13% of WHS cases had a derivative 4 due to a parental translocation and approximately 1.6% of cases had a *de novo* unbalanced translocation, making the total approximately 15%.[2] Other smaller studies (based on 22–25 cases) have indicated that the rate of unbalanced

Table 2 Cryptic unbalanced translocations detected by aCGH

ID no.	Previous karyotype	Previous FISH	aCGH
867	46,XX	ish del(4)(p16.3)(WHS−), der(4)t(4;11)(4pter−;11pter+)	arr cgh 4p16.3(RP1-36P21→RP11-357G3) × 1,11p15.5(RP1-44H16→RP11-371C18) × 3
372	46,XY	ish del(4)(p16.3)(WHS−), der(4)t(4;11)(4pter−;11pter+)	arr cgh 4p16.3(RP1-36P21→RP11-357G3) × 1,11p15.5(RP1-44H16→RP11-371C18) × 3
415	46,XY	ish del(4)(p16.3)(WHS−), der(4)t(4;7)(4pter−;7pter+)	arr cgh 4p16.3(RP1-36P21→RP11-357G3) × 1, 7p22.3p22.1(RP1-164D18→RP11-161C7) × 3
003	46,XX, del(4)(p16.3)	ish del(4)(p16.3)(WHS−)	arr cgh 4p16.3p16.2(RP1-36P21→RP11-91B20) × 1, 11p15.5(RP1-44H16→RP11-371C18) × 3
311	46,XX, inv(4)(p15.32p16.3)	ish del(4)(p16.3)(WHS−)	arr cgh 4p16.3p16.1(RP1-36P21→RP11-101J14) × 1, 8p23.3p23.2(RP11-91J19→RP11-1K11) × 3
412	46,XX	ish del(4)(p16.3)(WHS−)	arr cgh 4p16.3p16.1(RP1-36P21→RP11-101J14) × 1,8p23.3p23.1(RP4-580L5→RP11-780O22) × 3
400	46,XX	ish del(4)(p16.3)(WHS−)	arr cgh 4p16.3p16.1(RP1-36P21→RP11-101J14) × 1, 8p23.3p23.1(RP11-91J19→RP11-18L2) × 3

Abbreviations: aCGH, array comparative genomic hybridization; FISH, fluorescence in situ hybridization.

Table 3 Unbalanced translocations involving acrocentric p-arms missed by aCGH

ID no.	Previous karyotype	Previous FISH	aCGH	Subsequent FISH
842	46,XX	FISH on patient: ish del(4)(p16.3)(4pter−,WHS−) FISH on mother: ish t(4;15)(p16.3;p11.2)(WHS−;WHS+)	arr cgh 4p16.3(RP1-36P21→ RP11-520M5) × 1	ish der(4)(D15Z1−,D15S11−,acro p-arm+)
636	46,XY,der(4)t(4;?) (p16.2; p11.2)	ish der(4)t(4;?)(p16.2;p11.2) (D4S96−)der(4p) positive for AgNORs and C-banding	arr cgh 4p16.3(RP1-36P21→ RP11-520M5) × 1	ish der(4)(D15Z1+, D15S11−)
265	46,XY,der(4)t(4;?) (p16.1; ?)	none	arr cgh 4p16.3p16.1(RP1-36P21→RP11-89K12) × 1	ish der(4)(D13Z1/D21Z1−,D14Z1/ D22Z1+,D15Z1−)

Abbreviations: aCGH, array comparative genomic hybridization; AgNORs, silver nitrate staining for the nucleolar organizing regions; FISH, fluorescence *in situ* hybridization.

translocations in WHS may be closer to 25%.[8,14] The initial large study was based primarily on cytogenetically visible translocations. Therefore, with the use of new techniques that improve the detection rate of cryptic alterations such as aCGH, perhaps it is not surprising that the true incidence is higher. We found that 15/33 patients (45%) had an unbalanced translocation and seven of these 15 cases were cryptic translocations. Our rate also agrees with the rate of unbalanced translocations involving 4p identified in a large analysis of the frequency and patterns of subtelomere rearrangements by Ravnan et al.[27] A total of 11 688 individuals with developmental disabilities and a normal karyotype were analyzed by subtelomere FISH. Fifteen patients had deletions of the 4p subtelomere probe and in 7 of these 15 (46.6%), the 4p deletion was part of an unbalanced translocation. The concurrence of these two rates of unbalanced translocations involving 4p supports the conclusion that the rate of unbalanced translocations in WHS is certainly higher than reported previously and is approximately 45%.

This study also revealed limitations in the use of aCGH for characterization of unbalanced translocations. aCGH did not recognize unbalanced translocations involving the short arms of the acrocentric chromosomes because these regions are not represented on aCGH platforms. These translocations would also be missed using the commercially available subtelomere screening assay since this kit does not include FISH probes for the acrocentric short arms. Translocations involving the acrocentric short arms are not rare events; indeed, 20% (3/15) of our translocation cases involved acrocentric short arms. The study by Ravnan et al[27] looking at the rate of cryptic chromosome imbalances using a subtelomeric FISH analysis, found that of 145 unbalanced translocations identified, 17 (11.7%) involved a duplication onto an acrocentric short arm. Since the subtelomere assay could detect only a duplication on an acrocentric short arm and not the reciprocal alteration (a duplication of an acrocentric short arm on another chromosome), and both would be predicted to occur at equal frequencies, it is reasonable to expect that the frequency of unbalanced translocations involving an acrocentric short arm is twice that found in the study, around 23%. Two of the three cases in our study were cytogenetically visible. However, one of the three cases in our study had a cryptic unbalanced translocation with 15p that was revealed only by parental studies.

The grouping of patients into common breakpoint regions on 4p, particularly in the translocation cases, suggests that there may be some underlying genomic architecture that predisposes these regions to either deletions or translocations. The March 2006 assembly of the University of California at Santa Cruz human genome browser shows two gene-poor and segmental duplication-rich regions between 3.9 to 4.2 Mb and 8.85 to 9.45 Mb from 4pter. Many of these segmental duplications also map to chromosomes 8 and 11, which is

consistent with the increased frequency noted for translocations between these two chromosomes and 4p.

Previous studies have also implicated the olfactory receptor (OR) gene clusters in the recurrent translocation between 4p and 8p[28] and breakpoints on 4p-terminal deletions without a translocation.[29] There are four clusters of OR genes on 4p at 3.9, 4.2, 9.1, and 9.4 Mb from 4pter;[30] regions that coincide with our clusters of 4p breakpoints. There are also OR gene clusters on 8p at 7.1, 7.4, 7.6, and 7.9 Mb from 8pter as well as on 11p at 3.4, 3.6, 4.1, and 5.0 Mb from 11pter. These OR gene clusters map within two of our t(4p;11p) cases and five of our t(4p;8p) cases. However, it should be noted that the OR genes are present in almost all human chromosomes and there are currently 135 recognized OR gene clusters. In addition, we have not shown that our breakpoints occur within these OR gene clusters. Therefore, the presence of OR gene clusters alone may not explain completely the increased frequency of these translocations.

A number of investigators have looked for correlations between clinical manifestations and deletion size in WHS.[3–8] For some clinical manifestations, a correlation has been made, but there are often reports of atypical patients that appear to have either a more or less severe clinical course than predicted by deletion size alone. We hypothesized that some of these cases may be due to unrecognized unbalanced translocations, where the trisomic material may be responsible for the deviation from the expected clinical manifestation. Cryptic unbalanced translocations have been identified previously in patients with WHS and genotype–phenotype inconsistencies.[8] Therefore, we compared the clinical data available for 32 of our patients with the expected genotype–phenotype correlations and did find that patients with an unbalanced translocation often presented with some exceptions to the expected clinical course.

Microcephaly is reported in almost all cases of WHS. However, we obtained head circumference data for 23 of our patients and five of these 23 did not have microcephaly. Interestingly, all five of these cases had cryptic unbalanced translocations, four cases involving 11p duplication and one case involving 8p duplication.

Heart defects have also been associated with a 4p deletion breakpoint within or proximal to band 4p16.2.[3] Sixteen of the 32 patients in our study had heart defects and four of these patients had deletion breakpoints within 4p16.3, which is distal to 4p16.2. Three of these four patients with heart defects and smaller-sized deletions had unbalanced translocations, and one of these was cryptic.

Hearing loss has been associated with 4p deletions greater than 6 Mb.[5] Thirteen of 32 patients in our study reported hearing loss. Four of these thirteen had 4p deletions smaller than 6 Mb and three of these four patients had unbalanced translocations, two of which were cryptic. Another patient with hearing loss had a 4p

deletion between 4.9 and 6.5 Mb and also had a cryptic unbalanced translocation.

In conclusion, aCGH detected successfully a deletion of 4p in each patient diagnosed previously with a 4p deletion, and in a subset of patients (7/33) aCGH also detected an additional duplication of another region not detected by chromosome analysis plus WHS-specific FISH. However, aCGH analysis does not identify unbalanced translocations involving the acrocentric p-arms. Given this limitation, optimal characterization of the genetic imbalance in a patient with WHS should involve both a standard karyotype analysis and aCGH. Furthermore, parents should be studied for cryptic translocations that may confer a significant recurrence risk.

Finally, unbalanced translocations in patients with WHS were more common than reported previously (45 *vs* 15% respectively). Patients with an unbalanced translocation often presented with exceptions to some expected clinical manifestations, which are likely due to modification of the phenotype by the trisomic material.

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