Genotype–phenotype correlation in 21 patients with Wolf–Hirschhorn syndrome using high resolution array comparative genome hybridisation (CGH)

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ABSTRACT

Background: The Wolf-Hirschhorn syndrome (WHS) is usually caused by terminal deletions of the short arm of chromosome 4 and is phenotypically defined by growth and mental retardation, seizures, and specific craniofacial manifestations. Large variation is observed in phenotypic expression of these features. In order to compare the phenotype with the genotype, we localised the breakpoints of the 4pter aberrations using a chromosome 4 specific tiling BAC/PAC array.

Methods: In total, DNA from 21 patients was analysed, of which 8 had a cytogenetic visible and 13 a submicroscopic deletion.

Results and conclusion: In addition to classical terminal deletions sized between 1.9 and 30 Mb, we observed the smallest terminal deletion (1.4 Mb) ever reported in a patient with mild WHS stigmata. In addition, we identified and mapped interstitial deletions in four patients. This study positions the genes causing microcephaly, intrauterine and postnatal growth retardation between 0.3 and 1.4 Mb and further refines the regions causing congenital heart disease, cleft lip and/or palate, oligodontia, and hypospadias.

Wolf–Hirschhorn syndrome (WHS), first described by Wolf et al4 and Hirschhorn et al,2 is usually caused by a partial deletion of the short arm of chromosome 4. It is a well known syndrome with growth and mental retardation, microcephaly, seizures, “Greek helmet” facies (fig 1), and major malformations such as cleft lip and/or palate (CL/P), coloboma of the eye, congenital heart defects (CHD), and hypospadias. Large variation is observed in phenotypic expression. These features, especially the facial appearance, change with ageing.3–7 The Pitt–Roger–Danks syndrome was described before.8–10 Although WHS affects around 1 per 50 000 births, it is suspected that the syndrome is more frequent, because not all patients can be diagnosed with a standard chromosomal investigation. In our experience, the incidence of WHS patients is similar to the incidence of Angelman syndrome patients—about 1 per 20 000 births. In case of a clinical suspicion of WHS in a patient with normal chromosomes, additional fluorescence in situ hybridisation (FISH) studies of the subtelomeres and the WHS critical region (WHSCR) are usually performed.

Different genes probably play an important role in the complex phenotype of WHS. One approach to understand the role of different genes is to compare the phenotypes of patients with differently sized deletions and correlating the genotype with the phenotype.5–11 Earlier genotype–phenotype correlation studies identified a WHSCR of approximately 165 kb. This WHSCR covers the entire WHS candidate gene 2 (WHSC2), and part of the WHS candidate gene 1 (WHSC1).12–16 In a recent study, this 165 kb WHSCR was not deleted in one patient with a typical WHS face, which prompted the investigators to assign a new critical region of WHS, “WHSCR2”. This region includes the LETM1 and partially the WHSC1, but not the WHSC2 gene.11 We reported on five mild WHS patients with small deletions of chromosome 4p covering or flanking the WHSCR, which also pinpointed the WHSC1 as the main candidate for causing the facial WHS appearance.12

Genotype–phenotype correlation studies suggest that hemizygosity of genes other than WHSC1 in the region contribute to some of the phenotypic aspects. An overview of the genes in the region and their potential contribution to the WHS phenotype was described before.14 CL/P, CHD, renal abnormalities and severe mental retardation are rare in patients with a small terminal microdeletion, but are common in patients with a larger deletion extending to proximal of 4.4 Mb.15 However, which of these genes is responsible for which phenotype remains unknown, with the exception of LETM1 which is the most likely candidate gene for epilepsy in WHS patients.17

In order to advance phenotype–genotype correlation efforts, we present genotype–phenotype correlations of eight previously reported and 13 new patients with WHS phenotypic features, and characterised the location and size of the deletions by full tiling chromosome 4 BAC array comparative genome hybridisation (CGH) for all 21 patients. Previous genotype–phenotype correlations of this region have been hampered by the presence of other imbalances in part of the WHS patients which likely confused some of the correlations. In this study, only patients with pure 4p deletion were included, which enables us to further refine the 4p phenotypic map.

PATIENTS AND METHODS

Patients

All 21 patients were diagnosed and clinically examined by clinical geneticists in Brussels and
Leuven (Belgium), Groningen (The Netherlands), Stockholm (Sweden), and Paris and Lille (France). A personal and family history was obtained from each patient. Most patients were regularly re-evaluated during several years and follow up data could be recorded. A summary of the clinical features for each patient is provided in tables 1 and 2. Patient 5, 6, 8–11, 20 and 21 have been described elsewhere.\textsuperscript{12 18–22}

### Cytogenetic analysis

Karyotyping was performed on metaphase spreads prepared from peripheral blood lymphocytes by routine standard cytogenetic procedures.\textsuperscript{23}

### Fluorescence in situ hybridisation (FISH)

The deletion for all but two patients was confirmed using the commercial LSI WHS region/CEP 4 control (dual colour) (Abbott Inc, Downers Grove, Illinois, USA). The WHSCR probe covering the entire 165 kb WHSCR between loci D4S166 and D4S3327 (Cytocell Technologies Ltd, Oxford, UK) was used to analyse the metaphase spreads of patient 2. The metaphase spreads of patient 1 have been tested with probe D4S96 (Oncor, Parsippany, New Jersey, USA) located at \(1.2\) Mb from the telomere. Locus specific BAC or PACs were Spectrum Orange labelled as described before.\textsuperscript{23}

### Array CGH

Genome wide array CGH at 1 Mb resolution was performed as described before.\textsuperscript{23} A chromosome 4 tiling BAC array containing 1908 targets was generated as described elsewhere. The DNA from these BAC and PAC targets was obtained from CHORI.\textsuperscript{24} Cy5 labelled patient DNA was co-hybridised versus Cy3 labelled reference DNA of a healthy individual. The fluorescence intensities measured were first background subtracted. Normalisation was performed by dividing each log\(_2\) transformed intensity ratio by the mean of the log\(_2\) transformed intensity ratios of all targets derived from the long arm of chromosome 4. In about one third of the hybridisations, an additional 2D normalisation was performed using Bioconductor (http://www.bioconductor.org).\textsuperscript{25 26} If successive clones have intensity values below 4 \(\times\) SD of all intensity ratios, the region is considered deleted. Hybridisation efficiencies of the chromosome 4 tiling array were around 97%. The average standard deviation of the log\(_2\) intensity ratio per experiment was 0.08. Graphs of the array-CGH analysis of DNA from a patient with a small terminal and a cytogenically visible interstitial deletion are provided in fig 2.

### RESULTS

#### Genotype–phenotype correlation

In this study, we aim to correlate the WHS phenotypes with 4p deletion sizes and therefore excluded patients carrying unbalanced translocations or other chromosomal rearrangements which may influence the phenotypic features.\textsuperscript{11} About one of five WHS patients were a carrier of a cryptic translocation. The presence of unbalanced translocations was excluded by subtelomeric FISH for patients 5, 6, 10, 11, and 21,\textsuperscript{12 20} and both subtelomeric and interstitial chromosomal rearrangements were excluded by 1 Mb array CGH in the other patients. Thus, only those patients with pure 4p deletions were retained in this study. Subsequently, DNA from patients was hybridised on a chromosome 4 tiling array.
Table 1  Clinical findings in nine Wolf–Hirschhorn syndrome (WHS) patients with de novo cytogenetically visible deletions

<table>
<thead>
<tr>
<th>Case</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>17</th>
<th>18</th>
<th>19</th>
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<td>F</td>
<td>F</td>
<td>5/3</td>
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<tr>
<td>Facial appearance</td>
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<td></td>
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</tr>
<tr>
<td>Greek warrior helmet facies</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>7/8</td>
</tr>
<tr>
<td>Hypertelorism</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>8/8</td>
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<tr>
<td>Prominent glabella</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>7/8</td>
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<tr>
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<td>+</td>
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<td>+</td>
<td>+</td>
<td>8/8</td>
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<tr>
<td>Epicanthus</td>
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<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>5/8</td>
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<tr>
<td>Broad nose</td>
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<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>6/8</td>
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<tr>
<td>Beaked nose</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>4/8</td>
</tr>
<tr>
<td>Short philtrum</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Oligodontia</td>
<td>NI</td>
<td>+</td>
<td>NI</td>
<td>−</td>
<td>NI</td>
<td>NI</td>
<td>Too young</td>
<td>Too young</td>
<td>1/2</td>
</tr>
<tr>
<td>Micrognathia</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>8/8</td>
</tr>
<tr>
<td>Downturned shape of the mouth</td>
<td>−/−</td>
<td>−/−</td>
<td>−/−</td>
<td>−/−</td>
<td>−/−</td>
<td>−/−</td>
<td>−/+</td>
<td>−/+</td>
<td>2/8</td>
</tr>
<tr>
<td>Colobomata of iris</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>8/8</td>
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<tr>
<td>Narrow lacrimal ducts</td>
<td>NI</td>
<td>−</td>
<td>NI</td>
<td>−</td>
<td>+</td>
<td>Ni</td>
<td>Ni</td>
<td>+</td>
<td>4/7</td>
</tr>
<tr>
<td>Strabismus</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>Dysplastic ears</td>
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<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>2/8</td>
</tr>
<tr>
<td>Preauricular tag/pit</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>2/8</td>
</tr>
<tr>
<td>Birth</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational weeks at birth (weeks, days)</td>
<td>33</td>
<td>38</td>
<td>38</td>
<td>40,2</td>
<td>38</td>
<td>37</td>
<td>41,5</td>
<td>37,2</td>
<td>38*</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>1180 (&lt;P10)</td>
<td>1630 (&lt;P10)</td>
<td>1900 (&lt;P10)</td>
<td>3660 (P75–P90)</td>
<td>1930 (&lt;P10)</td>
<td>1500 (&lt;P10)</td>
<td>2610 (&lt;P10)</td>
<td>1950 (&lt;P10)</td>
<td>1995*</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>39 (&lt;P10)</td>
<td>42 (&lt;P10)</td>
<td>42 (&lt;P10)</td>
<td>52 (P50–P75)</td>
<td>47.5 (&lt;P10)</td>
<td>Ni</td>
<td>48 (&lt;P10)</td>
<td>42 (&lt;P10)</td>
<td>46*</td>
</tr>
<tr>
<td>IUGR</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>7/8</td>
</tr>
<tr>
<td>OFC (cm)</td>
<td>27 (&lt;P10)</td>
<td>30.5 (&lt;P10)</td>
<td>30 (&lt;P10)</td>
<td>36 weeks: 36.5 (P10–P25)</td>
<td>34.5 (P75–P90)</td>
<td>Ni</td>
<td>Ni</td>
<td>Ni</td>
<td>30.5*</td>
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<tr>
<td>Microcephaly at birth</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>Ni</td>
<td>+</td>
<td>5/7</td>
</tr>
<tr>
<td>Postnatal examination</td>
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<td></td>
<td></td>
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<tr>
<td>Age at examination (years, months)</td>
<td>6</td>
<td>12</td>
<td>7</td>
<td>3,6</td>
<td>5</td>
<td>50</td>
<td>1,6</td>
<td>0,2</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>89 (&lt;P3)</td>
<td>142 (&lt;P3–P25)</td>
<td>&lt;P3</td>
<td>9 (&lt;1SD)</td>
<td>&lt;P3</td>
<td>120 (&lt;P3)</td>
<td>72.5 (P3)</td>
<td>47.5 (&lt;P3)</td>
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<tr>
<td>Weight (kg)</td>
<td>8.0 (&lt;P3)</td>
<td>30 (&lt;P3)</td>
<td>&lt;P3</td>
<td>12.6 (&lt;P3)</td>
<td>25 (&lt;P3)</td>
<td>6.9 (&lt;P3)</td>
<td>2.5 (&lt;P3)</td>
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</tr>
<tr>
<td>OFC (cm)/microcephaly (+/−)</td>
<td>44.2 (&lt;P3)</td>
<td>48 (&lt;P3)</td>
<td>&lt;P3</td>
<td>47.5 (−2SD)</td>
<td>43 (&lt;P3)</td>
<td>46 (&lt;P3)</td>
<td>42.2 (&lt;P3)</td>
<td>35 (&lt;P3)</td>
<td></td>
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<tr>
<td>Mental retardation</td>
<td>Severe</td>
<td>Severe</td>
<td>+</td>
<td>IQ 74</td>
<td>Severe</td>
<td>Profound</td>
<td>Profound</td>
<td>Profound</td>
<td></td>
</tr>
<tr>
<td>Sitting without support (years, months)</td>
<td>3,6</td>
<td>1,5</td>
<td>3,6</td>
<td>1,6</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>4/8</td>
</tr>
<tr>
<td>Walking without support (years, months)</td>
<td>−</td>
<td>3,5</td>
<td>6</td>
<td>3,6</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>3/8</td>
</tr>
<tr>
<td>Speech (years, months)</td>
<td>−</td>
<td>−</td>
<td>7</td>
<td>1,5</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>2/8</td>
</tr>
<tr>
<td>Hypotonia</td>
<td>+</td>
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<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>6/8</td>
</tr>
<tr>
<td>Seizures</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>Congenital heart defects</td>
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<td>−</td>
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<td>+</td>
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<td>−</td>
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<td>3/8</td>
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<tr>
<td>Clubbing of fingers/toes</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>0/8</td>
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</tbody>
</table>

Continued
Of eight patients with cytogenetically visible terminal 4p deletions, six carried a terminal 4p deletion. Patient 17 carried an interstitial deletion 1 Mb upstream of the WHSCR1 from 2.7 to 14.8 Mb (fig 2B), and patient 15 had an interstitial deletion spanning between 1.8 and 10.1 Mb from the telomere. An overview of the genotypes and phenotypes of the eight patients is shown in table 1 and fig 3A. Hypertelorism, prominent glabella, high forehead, short philtrum, typical down turned or carp shaped mouth, and microcephaly were present in all patients. The typical facial features, intrauterine growth retardation (IUGR) and seizures were present in all patients except for patient 17 (2.7 to 14.8 Mb interstitial deletion). In two of these eight patients a CL/P was observed (patient 20 and 21), with terminal deletions of 19.5 Mb and 37 Mb, respectively. Strabismus was present in five of the seven investigated patients (not in patient 15 (1.8 to 10.1 Mb deletion) and 20 (0 to 19.5 Mb deletion)). Colobomata of the iris were observed in three patients with deletions of at least 10.9 Mb from the telomere. Two patients (patients 18 and 21 with, respectively, a 14.8 and 37 Mb terminal deletion) were found to have narrow lacrimal ducts. A pre-auricular tag or pit was observed in patients with deletions of 14.8 Mb and larger. All patients had mental, postnatal growth, and psychomotor retardation. Of six patients who underwent renal ultrasound, a renal defect was only found in one patient (patient 14 with an 8.5 Mb deletion). Fifteen of the 17 investigated patients had sacral dimples. All three boys with a large deletion presented with genital defects. Patients 16 and 18 had hypospadias (10.9 and 14.8 Mb terminal deletions), and patient 17 had a normal migrated left testis and a non-descended right testis (2.7 to 14.8 Mb interstitial deletion). Hypotonia was present in six of eight individuals except patient 15 and 20 (1.8–10.1 and 0–19.5 Mb deletion).

In 13 patients, a submicroscopic deletion was initially detected by FISH. In 11 of them, the deletion was observed by FISH using the locus specific probe detecting the WHSCI deletion. However, in patient 1, the deletion was detected using the Oncor WHS probe located distally from WHSCR1. Subsequently, array-CGH was performed to determine the deletion boundaries. This patient had a 1.4 Mb terminal deletion not including the WHSCR1, confirmed by FISH with the flanking clones RP11-1244E8 (1.35 to 1.42 Mb, deleted) and RP11-1398P2 (1.46 to 1.61 Mb, normal) and the deleted telomeric clone CTC-36P21. In patient 2, FISH using the Cytocell WHS probe presented a weak signal in one chromosome 4 and a strong signal in the other chromosome 4 in all metaphases, suggesting a partial deletion. Array-CGH pinpointed the breakpoint between RP11-21114 (1.91 to 2.10 Mb, deleted) and RP11-318G6 (2.00 to 2.19 Mb, normal) (Ensembl release 42, December 2006). In two patients (patient 6 and 10), small interstitial deletions were observed. An overview of the genotype-phenotype correlation of these 13 patients is shown in table 2 and fig 3B. Patients with deletions of the WHSCR1 all present with the characteristic facial features, except for patient 3. Patient 1 with the 1.4 Mb terminal deletion not involving the WHSCR1 had mild clinical stigmata and no typical WHS face. Patient 10 with the 1.8 to 3.6 Mb interstitial deletion presented with the typical WHS face, but lacked most other WHS stigmata.

**DISCUSSION**

Genotype-phenotype correlation studies may enable the identification of the role of the different 4p genes in the aetiology of WHS. However, several issues remain to be resolved. The number of patients analysed is still limited and the resolution at...
**Table 2** Clinical findings in 13 Wolf–Hirschhorn syndrome (WHS) patients with de novo microdeletions

<table>
<thead>
<tr>
<th>Case:</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<th>10</th>
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<td>F</td>
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<tr>
<td>Facial appearance</td>
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<tr>
<td>Greek warrior helmet facies</td>
<td>–</td>
<td>+</td>
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<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Hypertelorism</td>
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<td>+</td>
<td>Ni</td>
<td>Ni</td>
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<td>Prominent glabella</td>
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<td>Ni</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>Ni</td>
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<td>45 (P25)</td>
<td>49 Ni</td>
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**Walking without support (year/month)**: 1, 6

**NI:** Not investigated

**Hypotonia:**
- Case 1: ++
- Case 2: ++
- Case 3: ++
- Case 4: ++

**Seizures:**
- Case 1: +
- Case 2: +
- Case 3: +
- Case 4: +

**Deafness:**
- Case 1: +
- Case 2: +
- Case 3: +
- Case 4: +

**Congenital heart defects:**
- Small VSD
  - Case 1: +
  - Case 2: +
  - Case 3: +
  - Case 4: +

**Clubbing of fingers/toes:**
- Case 1: +
- Case 2: +
- Case 3: +
- Case 4: +

**Scoliosis/hyperkynosis:**
- Case 1: +
- Case 2: +
- Case 3: +
- Case 4: +

**Club feet:**
- Case 1: +
- Case 2: +
- Case 3: +
- Case 4: +

**Renal anomalies:**
- Left sided kidney duplication
  - Case 1: +
  - Case 2: +
  - Case 3: +
  - Case 4: +

**Genital anomalies:**
- Hypospadias
  - Case 1: +
  - Case 2: +
  - Case 3: +
  - Case 4: +

**Genetic anomalies:**
- Left inguinal hernia
  - Case 1: +
  - Case 2: +
  - Case 3: +
  - Case 4: +

**Skeletal anomalies:**
- Left-sided kidney dolicho-cystic
  - Case 1: +
  - Case 2: +
  - Case 3: +
  - Case 4: +

**Hypothyroidism:**
- Case 1: +
- Case 2: +
- Case 3: +
- Case 4: +

**Dilation of the deletion (Mb):**
- 1.4
  - Case 1: +
  - Case 2: +
  - Case 3: +
  - Case 4: +

**Delineation of the deletion (Mb):**
- 1.9
  - Case 1: +
  - Case 2: +
  - Case 3: +
  - Case 4: +

**Diagnosis by means of FISH:**
- Oncor WHS probe
- Cytocell WHS probe
- Vysis WHS probe
- Vysis FISH subtel.

**Subtelomeric screening by FISH:***
- Subtel. probe
- 1 Mb array

**Last normal clone (interstitial):**
- RP11-20I20(1.1–1.3)
- RP11-318G6(2.0–2.2)
- RP11-478C1(2.1–2.3)
- RP11-478A6(2.4–2.6)

**First deleted clone (interstitial):**
- RP11-386I15(1.1–1.3)
- RP11-262P20(1.8–2.1)
- RP11-386I15(1.3–1.4)
- RP11-21I14(1.9–2.1)

**Last deleted clone (Mb):**
- RP11-318G6(2.0–2.2)
- RP11-478C1(2.1–2.3)
- RP11-318G6(2.0–2.2)
- RP11-478C1(2.1–2.3)

**ASD: atrial septal defect; F, female; FISH, fluorescence in situ hybridisation; IUGR, intrauterine growth retardation; M, male; NI, not investigated; OFC, occipitofrontal circumference; Pst, pulmonic stenosis; VSD, ventricular septal defect.**

*Average.*

[Original article](http://jmg.bmj.com/ Downloaded from group.bmj.com on June 20, 2017)
which the location of the breakpoints has thus far been analysed is low. Second, most genotype–phenotype correlation studies are derived from patients with large cytogenetically visible deletions spanning several Mb. Third, since several of the clinical features of this syndrome have considerable variable expressivity or penetrance, the phenotypic characterisation of many more WHS patients will be required to pinpoint the genes involved in these more rare aspects of the WHS phenotype. Fourth, the role of position effects due to the relocation of the telomeres is unknown. Finally, since WHS is a contiguous gene syndrome, some of the features seen in WHS could result from haploinsufficiency of more than one gene in the region. Unravelling the contribution of each of the genes in the region to these multigenic phenotypes provides a new challenge for geneticists.

This report presents the largest genotype–phenotype correlation analysis of WHS patients thus far. Using a chromosome 4 tiling path array, both interstitial and terminal 4p deletion breakpoints were fine mapped. In addition to the classical terminal deletions, sized between 1.9 and 30 Mb, we identified a 1.4 Mb terminal deletion, the smallest deletion ever to be reported in a patient with WHS phenotypic features. In addition, we identified and mapped four interstitial deletions. Both the patients lacking all classic WHS features and the atypical deletions advanced the dissection of the molecular features leading to the different WHS characteristics.

The main characteristic of WHS is the typical face, usually referred to as a “Greek warrior helmet face”. Previous studies hypothesised hemizygosity of the WHSC1 as the most likely cause of the facial phenotype. This notion was confirmed by the present study. All, except in one patient with a WHSC1 deletion, did have the typical facial features. The patient with the WHSC1 deletion but without the typical face is 25 years old. Because of advancing age the facial features could have coarsened over time (patient 3, fig 1C,D). One other patient (patient 7) has partial facial phenotypic features but was not considered to have the “Greek warrior helmet”. The two patients in this study without the facial WHS features do not have a deletion of WHSC1: one patient had only a 1.4 Mb terminal deletion not covering the WHSC1 (patient 1, fig 1A,B), while another patient had a large interstitial deletion upstream of the WHSC1 (patient 17, fig 1E,F).

The molecular features of WHSC1 suggest that the gene might function as a chromatin remodelling enzyme because SET domains have been shown to function as histone methylases. A deficiency in chromatin remodelling could deregulate the expression of a variety of genes and hence lead to pleiotropic effects. Recent studies indicated that haploinsufficiency of other chromatin remodelling enzymes cause syndromic phenotypes such as the Chromodomain helicase DNA-binding protein 7 (CHD7) gene, causing the CHARGE (Coloboma, Heart anomaly, Choanal atresia, Retardation, Genital and Ear Anomalies) syndrome, the Nipped-B-like (NIPBL) gene, causing the Cornelia de Lange syndrome, and heterozygous mutations in the V-ha-ras Harvey rat sarcoma viral oncogene homologue (HRAS) gene, causing the Costello syndrome. Because of this possibility, we sequenced the WHSC1 gene in five WHS patients without 4p deletions (data not shown). Intriguingly, no mutations were detected in this nor in previous studies. Either mutations elsewhere in the genome can cause phenocopies of the WHS, or WHSC1 is the wrong target gene and another gene in the region is key for the phenotype.

Patient 17, a boy with a large interstitial deletion ranging from 2.7 to 14.8 Mb, has normal growth parameters, he had no seizures, and mental delay was mild with an IQ of 74. Sitting and walking without support and speech were only mildly delayed compared to the other patients with large deletions. In contrast, all patients with large 4pter deletions, including our six patients with large terminal deletions sized between 8.8 and 57 Mb, present with pre- and postnatal growth retardation, the typical face, seizures, microcephaly, severe to profound mental and psychomotor retardation and muscular hypotonia (the latter except patient 20). Hence, haploinsufficiency of genes in the 2.7 Mb 4pter region causes these WHS main features.

Figure 2 Results of the array CGH analysis of two patients. The Y axis represents the log2 of the intensity ratios of the combined dye swap experiments of patient versus control DNA. In the X axis the spotted clones are ordered from the 4p telomere to the centromere. The distance from the 4p telomere is indicated in Mb. The arrows indicate the location of the breakpoints and the distance from the telomere is indicated. (A) DNA from a patient with a small terminal deletion. (B) DNA from a patient with a cytogenetically visible interstitial deletion.
Not only patient 17 (2.7–14.8 Mb deletion), but also patient 10 with an interstitial deletion (1.8–3.6 Mb deletion) has normal growth parameters. A patient with a normal height and a 191 kb deletion encompassing the \textit{WHSC1} and \textit{WHSC2} genes was described.\textsuperscript{16} In contrast, the patient with a 1.4 Mb terminal deletion did present with short stature and IUGR. Therefore, this study locates a short stature candidate region in the terminal 1.4 Mb.

In our previous study, a healthy female with multiple miscarriages carried a 0.3 Mb terminal deletion.\textsuperscript{12} Therefore, the 0.3 Mb terminal region is not a candidate region for the genotypic map. Previously, we speculated that the WHS associated microcephaly might be the result of a contiguous deletion involving at least two genes, one located in a 1.8 Mb terminal region and one between 2.2–2.5 Mb. This hypothesis resulted from patients carrying a 1.9 and 2.2 terminal deletion without microcephaly. This latter observation may also result from incomplete penetrance or genetic modifiers. The microcephaly in the patient with a 1.4 Mb terminal deletion as well as in the other three investigated patients with deletion sizes up to 2.3 Mb is, however, more consistent with a gene localised in this terminal 1.4 Mb region.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{(A) Results of the detected deletions using the full tiling array CGH of chromosome 4 in eight Wolf–Hirschhorn syndrome (WHS) patients with microscopically visible deletions. The bars show the sizes of the deletions. (B) Results of the detected deletions using the full tiling array CGH of chromosome 4 in 13 WHS patients with submicroscopic deletions. The bars show the sizes of the deletions. The thick black bars show the locations of the candidate regions for each phenotypic feature. The red bars show the locations of the critical regions for hypospadias and CL/P defined by Estabrooks et al.\textsuperscript{34}}
\end{figure}
Hemizygosity of LETM1, a ubiquitous Ca\(^{2+}\) binding protein involved in Ca\(^{2+}\) homeostasis, located at 1.8 Mb from the 4p telomere, has been suggested to cause seizures.\(^{11,12}\) However, the patient with an interstitial deletion encompassing LETM1 (patient 6) did not present with seizures, while the patient with the 1.4 Mb terminal deletion did present with seizures. Therefore, another gene in this terminal region may cause the epilepsy.

Besides these main characteristics features, WHS is characterised by several minor features. Oligodontia occurred in six of the nine investigated WHS patients in the present study. Nieminen et al showed that a deletion of the msh homebox 1 gene (MSX1), located at 4.9 Mb, might underlie this defect.\(^{11}\) In contrast to this hypothesis, three patients in this study with deletions in the terminal 2.7 Mb or smaller had oligodontia. Since the carrier of a deletion from 1.1 to 2.5 Mb has oligodontia, the candidate gene has to be sought in this interval.

Cleft lip and palate (CL/P) was described in the literature,\(^{9,10}\) and a candidate region for CL/P has been observed in nine of 29 patients with oligodontia, the candidate gene has to be sought in this interval.

The 1.4 Mb terminal deletion did present with seizures. In contrast to this hypothesis, three patients in this study with the 1.4 Mb terminal deletion did present with seizures. In the present study, four patients from both large and small deletions presented with a CHD when having a deletion of 3.7 to 14.8 Mb. We refined the region for heart defect proximal from 3.7 Mb. In the present study, four of eight patients from both large and small deletions presented with a CHD when having a deletion of 3.7 to 14.8 Mb. We refined the region for heart defect proximal from 3.7 Mb.

In conclusion, WHS is a syndrome with a spectrum of phenotypic features, from very subtle and mild to a wide range of severe aberrations. The genes causing the IUGR, postnatal growth retardation and microcephaly are localised in the 0.3 to 1.4 Mb 4pter region. It is conceivable that a single gene might cause all these features. Finally, this study refines the candidate regions for CHD, CL/P, oligodontia and hypospasias. The phenotypic characterisation of more WHS patients will be required to delineate regions involved in these more rare aspects of the WHS phenotype.

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References:


Genotype-phenotype correlation in 21 patients with Wolf-Hirschhorn syndrome using high resolution array comparative genome hybridisation (CGH)

N M C Maas, G Van Buggenhout, F Hannes, B Thienpont, D Sanlaville, K Kok, A Midro, J Andrieux, B-M Anderlid, J Schoumans, R Hordijk, K Devriendt, J-P Fryns and J R Vermeesch

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