Prevalence and Patterns of Morphological Abnormalities in Patients With Childhood Cancer

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Context Constitutional gene defects predispose to cancer in children. Such tumor predisposition syndromes can be recognized by specific patterns of morphological abnormalities.

Objectives To assess the prevalence of morphological abnormalities in a large cohort of patients with childhood cancer and to identify new tumor predisposition syndromes.

Design, Setting, and Participants Patients were recruited from Emma Children’s Hospital, Academic Medical Center, Amsterdam, the Netherlands, between January 2000 and March 2003. A total of 1073 patients underwent a physical examination directed at 683 morphological abnormalities. The patient cohort consisted of 898 long-term survivors of childhood cancer and 175 newly diagnosed pediatric patients with cancer. The control group consisted of 1007 schoolchildren examined in an identical way. Mean ages of patients and controls were 21.2 and 10.4 years, respectively.

Main Outcome Measures Prevalence and patterns of morphological abnormalities in patients compared with controls. To prevent age bias, only age-independent abnormalities were used for overall prevalence analysis. Patients younger than 9 years were excluded from the pattern analysis. The sample was restricted to white patients to prevent ethnicity bias.

Results Morphological abnormalities were significantly more prevalent in pediatric patients with cancer. Major abnormalities were present in 26.8% of patients vs 15.5% of controls ($P < .001$) and minor anomalies in 65.1% of patients vs 56.2% of controls ($P < .001$). Three or more minor anomalies were detected in 15.2% of patients vs 8.3% in controls ($P < .001$). Forty-two patients were diagnosed with an established tumor predisposition syndrome. Multivariate analyses showed 14 morphological abnormalities to occur significantly more often in the patient group. For 2 of these (blepharophimosis and asymmetric lower limbs), we identified statistically significant patterns of co-occurring morphological abnormalities suggestive of new tumor predisposition syndromes. Thirty-four patients fit 1 of the 2 novel tumor predisposition patterns.

Conclusions Pediatric patients with cancer show a significantly higher prevalence of morphological abnormalities compared with controls. Specific patterns of morphological abnormalities indicate possible unrecognized tumor predisposition syndromes, but validation in an independent sample is needed.

JAMA. 2008;299(1):61-69 www.jama.com

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MORPHOLOGICAL ABNORMALITIES IN CHILDHOOD CANCER

Figure 1. Schematic Depiction of the Terminology and Classification System of Morphological Abnormalities

Boxes highlighted in blue indicate categories of morphological abnormalities included in this analysis. Classification based on Merks et al.21

Methods

Patients and Controls
Between January 2000 and March 2003, we examined 898 consecutive patients visiting the Clinic for Late Effects of Childhood Cancer in the Emma Children's Hospital, Academic Medical Center (AMC), Amsterdam, the Netherlands, and 175 children newly diagnosed with cancer (aged 0-18 years) in the Emma Children's Hospital, AMC, Amsterdam, the Netherlands.

In 1996, the Emma Children's Hospital, AMC started a clinic for the assessment of late effects of childhood cancer treatment.16 During the study period, 1056 survivors of childhood cancer visited the Late Effects Clinic. A total of 924 consecutive patients at the Late Effects Clinic were invited to participate (those not invited being observed at the same time as another patient and could not be examined by the primary investigators); of these, 898 entered the study (97%). During the same period, 285 children were newly diagnosed with cancer in the center, of whom 175 were invited to participate; all entered the study. There were no exclusion criteria.

Written informed consent was obtained from patients aged 16 years or older, patients and parents if the patient was older than 12 years but younger than 16 years, or the parents if the patient was 12 years or younger. The oncological case histories were retrieved from the medical records and included the nature of the tumor, therapeutic regimens, and family history. Ethnicity was registered, because it can influence the external phenotype of patients. Ethnicity was classified by the primary investigator (J.H.M.M.) as white or nonwhite, based on checking the parents and grandparents of each patient. If one of the parents or grandparents of each patient was partly nonwhite, the patient was classified as nonwhite. Permission for the study was obtained from the Medical Ethical Committee of the hospital.

From March 2004 to June 2006, 1007 controls were examined and analyzed in an identical way. Controls were recruited from the city of Haarlem and the surrounding semirural and rural area, because the region has been reported to have a health profile representative of the Netherlands.17,18 In addition, a large proportion of patients referred to the Emma Children's Hospital originates from this area. We recruited children born in 1993-1994 who were undergoing a general preventive health screening and invited entire 6th, 7th, and 8th grade classes from schools within the study area. Three hundred seventy-four children (participation rate, 96.4%) were recruited from the preventive health screening and 633 children from the schools (participation rate, 92.4%). All children and their parents received in advance written and oral information about the study, and written approval was obtained from all parents. Further details on acquisition, methodology, and results of the control cohort are presented elsewhere.15

Terminology and Classification
Definitions were set for all morphological abnormalities that can be scored by body surface examination.19-21 A hierarchical tree was built using the London Dysmorphology Data Base as a model,20 comprising 29 major anatomical areas, subdivided into 98 different structures, and containing 683 morphological abnormalities. All morphological abnormalities were classified according to their presumed pathogenesis21 and divided into major abnormalities and minor variants (Figure 1; detailed definitions are available elsewhere).21 Major abnormalities and minor anomalies are the most potent indicators of abnormal development3,22,23; therefore, all subsequent analyses were directed at those 2 subclasses.

Clinical Examination
Both primary investigators (J.H.M.M. and H.M.O., a pediatrician and physician, respectively) were trained in clinical morphology by a pediatrician-clinical geneti-
tor did not, resulting in a items positive where the first investigator scored 85 and the second investigator did not. This resulted in a disagreement on 178 items: the first investigator scored 27 items as abnormal where the first investigator did not, and the second investigator scored 85 items positive where the first investigator did not, resulting in a k score of 0.85.

Data Analysis
Morphological findings can show an age-dependent prevalence. Some, such as macular stains, can be more prevalent in infants and children and decrease in prevalence thereafter, while others, such as prominent lower jaw, arise only later on, especially during puberty. We used only the 214 age-independent morphological abnormalities for comparison because of the difference in mean age between controls (prepubertal) and patients with cancer (mainly adolescents). These 214 presumed age-independent morphological abnormalities were all confirmed to be age independent in the patient group by comparing their prevalence in the different age groups (≤8 years, 9-14 years, 15-21 years, or ≥22 years; data available from the author on request).

To prevent bias introduced by different ethnic backgrounds, only white cases and controls were included in analyses. First, overall prevalence data of age-independent major abnormalities and minor anomalies were generated in the white pediatric cancer cohort and compared with white controls. Second, prevalences of age-independent morphological abnormalities were analyzed univariately by using Fisher exact test, comparing their prevalence in patients with those in controls. Statistical significance was considered to exist if 2-tailed P < .05. We corrected for multiple testing by applying the method of Benjamini and Hochberg, which minimizes the false discovery rate.

Third, a multivariate logistic regression analysis was performed (S-PLUS 2000; MathSoft, Inc, Cambridge, Massachusetts) of patients vs controls, with relevant age-independent morphological abnormalities. We defined morphological abnormalities as relevant if the univariate P value of the Fisher exact test was less than .20. Morphological abnormalities that were observed in only 1 individual (or in none) were excluded from the multivariate analyses. The number of morphological abnormalities tested in the logistic model relative to the number of patients was high, inhibiting reliable estimates using the logistic regression model. Therefore, we used a penalized logistic regression analysis. The penalty parameter was estimated by bootstrap cross-validation.

Finally, we used a customized stepwise statistical cluster analysis to identify patterns in the cancer group starting from the morphological abnormalities that were independently and significantly associated with childhood cancer in the multivariate analysis (lead-abnormalities). First, co-occurring morphological abnormalities were selected based on their significant correlation with each lead-abnormality (Pearson correlation). Second, for each lead-abnormality and its set of significantly correlating morphological abnormalities, we calculated the cumulative number of these abnormalities present per individual and compared its distribution between the patient and control groups. Third, the distributions were dichotomized at that number of abnormalities per individual that showed the best discrimination between patients and controls. Individuals having more abnormalities than this threshold were considered to carry the syndrome associated with the lead-abnormality. Finally, we included in the description of the syndrome only those morphological abnormalities that occurred with more than 15% frequency in the candidate syndrome patients.

To describe new syndromes to their full extent, it is important to identify the complete pattern of anomalies composing the syndrome, including anomalies that show an increasing or decreasing prevalence with age. Therefore, both age-independent and age-dependent morphological abnormalities were included in the analysis. To avoid any possible bias introduced by the inclusion of morphological abnormalities that are more frequent at a younger age, patients younger than the control group (<9 years; n = 112) were excluded from pattern analyses. To avoid bias introduced by the inclusion of age-dependent morphological abnormalities that are more frequent at an older
age, all morphological abnormalities identified as part of a putative new pattern were checked for increasing frequency with age, and when positive were excluded from the pattern.

**RESULTS**

The characteristics of patients and controls are shown in Table 1. The median age at examination of the patients was 21 years (range, 0-52 years) compared with 11 years (range, 8-14 years) for controls. In newly diagnosed patients with cancer, the median age was 6 years (range, 0-18 years). The sex ratio (male:female) was 1.13 in patients and 0.93 in controls.

### Table 1. Characteristics of Patients and Controls

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Controls (n = 1007)</th>
<th>Total Group (N = 1073)</th>
<th>Late-Effects Cohort (n = 898)</th>
<th>Newly Diagnosed (n = 175)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>7.2 (4.9)</td>
<td>7.3 (4.9)</td>
<td>6.5 (5.2)</td>
<td>6.5 (5.2)</td>
</tr>
<tr>
<td>At diagnosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex ratio (male:female)</td>
<td>1.13</td>
<td>1.07</td>
<td>1.46</td>
<td></td>
</tr>
<tr>
<td>Ethnicity, No. (%)</td>
<td>White</td>
<td>923 (91.7)</td>
<td>941 (87.7)</td>
<td>813 (90.5)</td>
</tr>
<tr>
<td></td>
<td>Nonwhite</td>
<td>84 (8.3)</td>
<td>132 (12.3)</td>
<td>85 (9.5)</td>
</tr>
<tr>
<td>Tumor groups, No. (%)</td>
<td>(Ganglio)neuroblastoma</td>
<td>69 (6.4)</td>
<td>56 (6.2)</td>
<td>13 (7.4)</td>
</tr>
<tr>
<td></td>
<td>Germ cell tumor</td>
<td>28 (2.6)</td>
<td>20 (2.2)</td>
<td>8 (4.6)</td>
</tr>
<tr>
<td></td>
<td>Rhabdomyosarcoma</td>
<td>89 (8.3)</td>
<td>78 (8.7)</td>
<td>11 (6.3)</td>
</tr>
<tr>
<td></td>
<td>Nephroblastoma</td>
<td>137 (12.8)</td>
<td>121 (13.5)</td>
<td>16 (9.1)</td>
</tr>
<tr>
<td></td>
<td>Osteosarcoma</td>
<td>54 (5.0)</td>
<td>47 (5.2)</td>
<td>7 (4.0)</td>
</tr>
<tr>
<td></td>
<td>Ewing sarcoma</td>
<td>46 (4.3)</td>
<td>33 (3.7)</td>
<td>13 (7.4)</td>
</tr>
<tr>
<td></td>
<td>Medulloblastoma</td>
<td>27 (2.5)</td>
<td>24 (2.7)</td>
<td>3 (1.7)</td>
</tr>
<tr>
<td></td>
<td>Non-Hodgkin lymphoma</td>
<td>126 (11.7)</td>
<td>111 (12.4)</td>
<td>15 (8.6)</td>
</tr>
<tr>
<td></td>
<td>Hodgkin disease</td>
<td>90 (8.4)</td>
<td>77 (8.6)</td>
<td>13 (7.4)</td>
</tr>
<tr>
<td></td>
<td>Acute lymphoblastic leukemia</td>
<td>195 (18.2)</td>
<td>163 (18.2)</td>
<td>32 (18.3)</td>
</tr>
<tr>
<td></td>
<td>Acute myeloid leukemia</td>
<td>26 (2.4)</td>
<td>20 (2.2)</td>
<td>6 (3.4)</td>
</tr>
<tr>
<td></td>
<td>Other malignancies</td>
<td>186 (17.3)</td>
<td>148 (16.5)</td>
<td>38 (21.7)</td>
</tr>
</tbody>
</table>

The median age was 6 years (range, 0-18 years). There were no differences in the type of major abnormalities or minor anomalies in newly diagnosed patients compared with controls.

### Occurrence of Morphological Abnormalities in Patients With Cancer

Both major abnormalities and minor anomalies were significantly more prevalent in the pediatric cancer group (per 1000 cases, patients had 268 major abnormalities and controls had 155 abnormalities, $P < .001$; in total, 1252 per 1000 minor anomalies were found in patients compared with 898 in controls, $P < .001$). There was no difference in frequency of major abnormalities or minor anomalies in newly diagnosed patients vs patients observed at the Clinic for Late Effects of Childhood Cancer. Furthermore, pediatric patients with cancer had a significantly higher occurrence of combinations of major abnormalities or minor anomalies (FIGURE 2). One or more major abnormalities were present in 26.8% of individual patients (15.5% in controls, $P < .001$), 2 or more abnormalities in 9.1% of patients (6.3% in controls, $P < .001$), and 3 or more abnormalities were found in 5.1% of patients (1.6% in controls, $P < .001$), and 3 or more abnormalities were found in 0.9% compared with none in controls ($P = .008$). One or more minor anomalies were found in 65.1% of individual patients (56.2% in controls, $P < .001$), 2 or more minor anomalies in 32.8% of patients (22.1% in controls, $P < .001$), and 3 or more minor anomalies were found in 15.2% of patients compared with 8.3% in controls ($P < .001$).

In 42 patients (3.9%), an established clinical genetic syndrome was diagnosed.\(^{26}\) The above described patterns and statistical significance were unchanged when these 42 patients were excluded from the analyses. As the goal of the study was to discover novel tumor predisposition syndromes, further analyses were performed in the 903 white patients without an established clinical genetic syndrome.

### Univariate and Multivariate Analysis of Morphological Abnormalities Associated With Childhood Cancer

In a univariate analysis, 28 of the 214 age-independent morphological abnormalities were found to occur significantly ($P < .05$) more often in patients compared with controls (TABLE 2). Correction for multiple testing resulted in 13 morphological abnormalities indicating statistical significance ($P < .05$). These occurred 2.0 to 29.6 times more frequently in patients compared with controls, or were not detected at all in controls. Multiple logistic regression analysis showed 14 age-independent morphological abnormalities that were independently and significantly ($P < .05$) associated with childhood cancer. These specific morphological abnormalities occurred 376 times in 316 pediatric patients with cancer compared with 69 times in 68 controls ($P < .001$).

### Identification of Patterns of Morphological Abnormalities

Pattern identification was undertaken by stepwise statistical analyses of other morphological abnormalities significantly co-occurring with each of those
14 morphological abnormalities in individual patients aged 9 years or older. For 2 of these morphological abnormalities (blepharophimosis and asymmetric lower limbs), patterns of co-occurring morphological abnormalities were identified in multiple individual patients (FIGURE 3 and FIGURE 4).

The blepharophimosis pattern (Figure 3) consists of blepharophimosis, increased anterior-posterior angulation of the spine, patchy hypopigmentation of the skin, and multiple café-au-lait spots. Thirteen patients had 2 or more abnormalities from this pattern compared with no controls ($P < .001$).

The asymmetric lower limb pattern (Figure 4) consists of asymmetric lower limbs, tall stature, midface hypoplasia, ptosis, and pectus carinatum or excavatum. Twenty-one patients had 2 or more abnormalities from this pattern compared with 2 controls ($P < .001$).

None of the 2 patterns were significantly associated with a particular type of tumor. The patterns were checked for a variety of possible confounding factors. Mean age of the patients within each of the 2 syndromes was similar to that in the general cancer group (19.6 years vs 21.2 years). Sex differed significantly in the asymmetric lower limbs syndrome (19 patients were males and only 2 were females, $P = .001$). Comparing the combined prevalence of 2 or more morphological abnormalities in the asymmetric lower limb syndrome in individual male patients ($n=19$) with male controls ($n=2$), the prevalence of the syndrome remained significantly higher ($P = .001$). Male predominance for the asymmetric lower limb tumor pattern might indicate that this candidate tumor predisposition syndrome could be X-linked.

### COMMENT

We have demonstrated a significantly higher prevalence of morphological abnormalities in a large cohort of patients with childhood cancer compared with controls. In addition, we found patterns of co-occurring morphological abnormalities suggesting novel tumor predisposition syndromes, although an independent validation sample is needed to confirm these findings. These results show an important role of constitutional genetic defects and/or prenatal environmental factors in pediatric oncogenesis.

These findings are supported by our earlier observations of a higher incidence of established clinical genetic syndromes in the same cancer group. Most of these syndromes were already known to be associated with an increased risk for childhood cancer. In 42 pediatric patients with cancer (3.9%), we diagnosed an established clinical genetic syndrome, which is much higher than in the general Dutch population ($<1\%$). Early studies describe a high prevalence of minor anomalies in pediatric patients with cancer. The study by Mehes et al examined 106 pediatric patients with cancer, 81 healthy siblings, and 106 controls with infectious diseases. The prevalence of minor anomalies was significantly higher in the patients with malignant disease and their siblings compared with controls ($69.2\%$ of the patients, $63.0\%$ of the siblings, and $34.6\%$ of the controls had at least 1 minor anomaly). When 2 or more minor anomalies were considered, the prevalence data were $36.5\%$, $29.6\%$, and $12.5\%$, respectively. The prevalence of major malformations did not differ between patients and controls. The major drawback of the study by Mehes et al is the small group size, reducing the precision of the frequency estimations. Furthermore, only 57 minor anomalies and major malformations were screened for, which could lead to underestimation of the prevalence of morphological abnormalities, and limits the possibility of detecting patterns of co-occurring morphological abnormalities. The higher prevalence of minor anomalies in siblings indicates that the external phenotype in patients and their siblings may be highly penetrant, while tumor development shows a relatively low penetrance.

For comparison of data in patients with childhood cancer and controls, we used the same standardized methodology in a study of an unselected control group ($n=1007$) of schoolchildren representative of the Dutch population. A limitation of our study is the difference in age between patients (0-52 years) and controls (8-14 years). Therefore, we only used age-independent morphological abnormalities in our statistical analyses. All 214 selected age-independent morpho-
Morphological abnormalities were confirmed to be age independent in the patient group. For pattern analysis, age-dependent morphological abnormalities were also included. To avoid age bias, we excluded patients younger than 9 years and we checked all morphological abnormalities from the new patterns for frequency changes with age. None of the

Table 2. Morphological Abnormalities Occurring Significantly More Frequently in White Pediatric Patients With Cancer vs Controls

<table>
<thead>
<tr>
<th>Morphological Abnormality</th>
<th>Definition</th>
<th>Controls (n = 923)</th>
<th>Pediatric Patients With Cancer (n = 903)</th>
<th>Ratio of Patients With Cancer to Controls (95% CI)</th>
<th>Univariate P Value</th>
<th>Univariate (FDR Corrected) P Value</th>
<th>Multivariate P Value</th>
<th>Type of Morphological Abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blepharophimosis</td>
<td>Static reduction in the distance between upper and lower eyelid that gives the palpebral fissures a narrowed slit-like appearance</td>
<td>6</td>
<td>65</td>
<td>11.1 (4.8-25.4)</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>Minor anomaly</td>
</tr>
<tr>
<td>Asymmetric lower limbs</td>
<td>A difference in measured length of the long axis ≥ 2 SD for sex and age</td>
<td>2</td>
<td>58</td>
<td>29.6 (7.3-121.0)</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>Minor anomaly</td>
</tr>
<tr>
<td>Sydney crease</td>
<td>Extension of the proximal transverse crease (5 finger crease) to the ulnar edge of the palm</td>
<td>3</td>
<td>30</td>
<td>10.2 (3.1-33.4)</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>Minor anomaly</td>
</tr>
<tr>
<td>Broad foot</td>
<td>A foot that appears disproportionately wide for its length, and for which the measured width is &gt;95th percentile for age</td>
<td>3</td>
<td>26</td>
<td>8.9 (2.7-29.2)</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>.006</td>
<td>Minor anomaly</td>
</tr>
<tr>
<td>Isolated short metatarsals</td>
<td>Diminished length of an isolated metatarsal, with resultant proximal displacement of associated toe</td>
<td>0</td>
<td>14</td>
<td>∞ (1.8-∞)</td>
<td>&lt;.001</td>
<td>.002</td>
<td>.03</td>
<td>Minor anomaly</td>
</tr>
<tr>
<td>Short distal phalanx of thumb</td>
<td>Short distance from the end of the thumb to the most distal interphalangeal crease or DIPJ flexion point</td>
<td>0</td>
<td>13</td>
<td>∞ (1.6-∞)</td>
<td>&lt;.001</td>
<td>.004</td>
<td>.01</td>
<td>Minor anomaly</td>
</tr>
<tr>
<td>Port-wine stain</td>
<td>A darkly colored angioma of the skin that is usually minimally or not raised</td>
<td>2</td>
<td>18</td>
<td>9.2 (2.1-39.5)</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>.006</td>
<td>Major abnormality</td>
</tr>
<tr>
<td>Hyperconvex nails</td>
<td>When viewed on end (with the digit tip pointing toward the examiner’s eye), the curve of the nail forms a tighter curve of convexity</td>
<td>0</td>
<td>12</td>
<td>∞ (1.5-∞)</td>
<td>&lt;.001</td>
<td>.006</td>
<td>.02</td>
<td>Minor abnormality</td>
</tr>
<tr>
<td>Retrognathia</td>
<td>Lower jaw appears small when viewed from the side but not from the front</td>
<td>16</td>
<td>43</td>
<td>2.8 (1.6-4.8)</td>
<td>&lt;.001</td>
<td>.007</td>
<td>.04</td>
<td>Minor abnormality</td>
</tr>
<tr>
<td>Hypoplastic alae nasi</td>
<td>Alae nasi are thinned, deficient, or excessively arched</td>
<td>6</td>
<td>25</td>
<td>4.3 (1.8-10.3)</td>
<td>&lt;.001</td>
<td>.009</td>
<td>&lt;.001</td>
<td>Minor abnormality</td>
</tr>
<tr>
<td>Prominent ears</td>
<td>Ear projects more than normally from the skull; angle between the posterior aspect of the pinna and the mastoid plane of the skull is &gt;2 SD for age</td>
<td>24</td>
<td>52</td>
<td>2.2 (1.4-3.6)</td>
<td>&lt;.001</td>
<td>.02</td>
<td>.06</td>
<td>Minor abnormality</td>
</tr>
<tr>
<td>Broad hand</td>
<td>Width of the palm appears disproportionately wide for the length: for children aged 0-4 years, palm width is &gt;2 SD above the mean; for children aged 4-16 years, palm width is &gt;95th percentile</td>
<td>2</td>
<td>15</td>
<td>7.7 (1.8-33.4)</td>
<td>.001</td>
<td>.02</td>
<td>.10</td>
<td>Minor abnormality</td>
</tr>
<tr>
<td>Scoliosis</td>
<td>Lateral curvature of the spine along its long axis</td>
<td>12</td>
<td>32</td>
<td>2.7 (1.4-5.3)</td>
<td>.002</td>
<td>.03</td>
<td>.06</td>
<td>Major abnormality</td>
</tr>
<tr>
<td>Hypertelorism</td>
<td>Measured distance between the pupils is &gt;95th percentile for age</td>
<td>26</td>
<td>51</td>
<td>2.0 (1.3-3.2)</td>
<td>.004</td>
<td>.07</td>
<td>.02</td>
<td>Minor abnormality</td>
</tr>
<tr>
<td>Tall stature (proportionate)</td>
<td>Height is &gt;97th percentile for age and sex, with normal ratio of trunk and limbs</td>
<td>3</td>
<td>15</td>
<td>5.1 (1.5-17.6)</td>
<td>.007</td>
<td>.09</td>
<td>.04</td>
<td>Minor abnormality</td>
</tr>
<tr>
<td>Microcephaly</td>
<td>Measured occipito-frontal head circumference is &lt;3rd percentile for sex and age</td>
<td>3</td>
<td>18</td>
<td>6.1 (1.8-20.8)</td>
<td>.01</td>
<td>.14</td>
<td>.02</td>
<td>Minor abnormality</td>
</tr>
<tr>
<td>Macrocephaly</td>
<td>Measured occipito-frontal head circumference is &gt;97th percentile for sex and age</td>
<td>3</td>
<td>13</td>
<td>4.4 (1.3-15.5)</td>
<td>.01</td>
<td>.14</td>
<td>.03</td>
<td>Minor abnormality</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; DIPJ, distal interphalangeal joint; FDR, false discovery rate.

*Twenty-eight morphological abnormalities occurred significantly more frequently in 903 white pediatric patients with cancer vs 923 white controls in univariate analysis (Fisher exact test, P < .05). The 13 morphological abnormalities that remained significant after controlling for multiple testing (FDR-corrected) are listed. Multiple regression analysis showed 14 morphological abnormalities, which independently from one another were significantly associated with childhood cancer. Although there is considerable overlap between outcomes of both analyses, there are differences. Ranking of morphological abnormalities is in order of decreasing significance in the univariate analysis.
morphological abnormalities composing the new patterns showed an increase in frequency with age. It is therefore unlikely that our results are biased by the difference in age distribution between patients and controls. A control population of age-matched controls would have been preferable, because this would have allowed the inclusion of age-dependent morphological abnormalities in the comparisons and might have identified even more overrepresented abnormalities and patterns. However, it was logistically impossible to recruit a sufficiently large control group of adults representative of the general population, not biased for socioeconomic background and education level, and with a sufficiently high participation rate.

A second limitation is that the observers were not blinded as to whether the participants were patients or controls, because the groups were examined in different periods. However, the majority of patients with childhood cancer are recognizable, due to the presence of scars from central venous catheters, tumor resections, or late effects of radiotherapy (growth deformities, skin changes, and alopecia). Using hospital controls was considered. However, this would have introduced bias of significant magnitude; many controls from within a hospital population have disorders with a possible developmental origin, which may show in the phenotype. To prevent observer bias, morphological abnormalities were only scored when clearly present. In case of doubt, photographs were taken and discussed with the clinical geneticist. Furthermore, 11% of controls and 7% of patients were scored independently by 2 observers, resulting in high k scores.

The access to the Clinic for Late Effects of Childhood Cancer in the Emma Children’s Hospital allowed us to examine a large cohort of patients with childhood cancer. The predominance of childhood cancer survivors introduced a survival bias, which is to some extent reflected in the spectrum of malignancies represented. However, there were no significant differences in type of malignancies between survivors and patients with newly diagnosed cancer. Certain tumor groups that are overrepresented may be explained by survival bias, like the predominance of nephroblastomas and lymphomas (12.7% and 20.6%, respectively, in our cohort vs 6.4% and 11.0%, respectively, in the annual childhood cancer incidence in

**Figure 3. Blepharophimosis Predisposition Syndrome: Prevalence per 1000 Cases of Combinations of Morphological Abnormalities and Frequency of Individual Morphological Abnormalities**

<table>
<thead>
<tr>
<th>Prevalence per 1000 Cases of</th>
<th>Frequency of Morphological Abnormalities in 13 Candidate Syndrome Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Morphological Abnormalities</td>
<td>Pediatric patients with cancer</td>
</tr>
<tr>
<td>0</td>
<td>Blepharophimosis</td>
</tr>
<tr>
<td>1</td>
<td>Increased anterior-posterior angulation of spine</td>
</tr>
<tr>
<td>2</td>
<td>Patchy skin hypopigmentation</td>
</tr>
<tr>
<td>3</td>
<td>Multiple café-au-lait spots</td>
</tr>
</tbody>
</table>

Analysis performed on white children aged 9 years or older, 815 with cancer and 919 controls. Patients with 2 or more morphological abnormalities from each pattern were considered candidates for the syndrome. For the cumulative prevalence of combinations of pattern abnormalities, Fisher exact test was used. Prevalences were significantly higher in patients with cancer than 1 or more and 2 or more morphological abnormalities ($P < .001$) and in those with 3 or more abnormalities ($P = .05$). For a short definition of blepharophimosis, see Table 2. The definition of increased anterior-posterior angulation of the spine is increased posterior curvature of the thoracic spine, increased anterior curvature of the lumbar spine, or both. Patchy skin hypopigmentation is circumscribed areas of decreased skin pigmentation, and multiple café-au-lait spots are circumscribed light-brown colored macular skin areas.

**Figure 4. Asymmetric Lower Limbs Predisposition Syndrome: Prevalence per 1000 Cases of Combinations of Morphological Abnormalities and Frequency of Individual Morphological Abnormalities**

<table>
<thead>
<tr>
<th>Prevalence per 1000 Cases of</th>
<th>Frequency of Morphological Abnormalities in 21 Candidate Syndrome Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Morphological Abnormalities</td>
<td>Pediatric patients with cancer</td>
</tr>
<tr>
<td>0</td>
<td>Asymmetric lower limbs</td>
</tr>
<tr>
<td>1</td>
<td>Tall stature</td>
</tr>
<tr>
<td>2</td>
<td>Midface hypoplasia/hypoplastic malae</td>
</tr>
<tr>
<td>3</td>
<td>Ptosis</td>
</tr>
<tr>
<td>4</td>
<td>Pectus carinatum or excavatum</td>
</tr>
</tbody>
</table>

Analysis performed on white children aged 9 years or older, 815 with cancer and 919 controls. Patients with 2 or more morphological abnormalities from each pattern were considered candidates for the syndrome. For the cumulative prevalence of combinations of pattern abnormalities, Fisher exact test was used. Prevalences were significantly higher in patients with cancer with 1 or more and 2 or more morphological abnormalities ($P < .001$) and in those with 3 or more abnormalities ($P = .05$). For short definitions of asymmetric lower limbs and tall stature, see Table 2. The definition of midface hypoplasia is infraorbital and perianal flatness, vertical shortening, or both; and hypoplastic malae is concavity of the face, reduced nasolabial angle/flat malar bones, or both. Ptosis is bilateral or unilateral drooping of the upper eyelid (blepharon), which obscures major portions of the upper half of the iris and sometimes the pupil. Pectus carinatum is abnormal usually asymmetrical protrusion of the chest and pectus excavatum is depression (a portion of) the sternum.

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(Reprinted) JAMA, January 2, 2008—Vol 299, No. 1
Western Europe). Other differences are center specific; for example, central nervous system tumors are historically less frequently observed in the Emma Children’s Hospital, explaining the relative paucity of central nervous system tumors in our cancer cohort (6.7% vs 21.6% in Western Europe). Furthermore, a significant influence of age bias is unlikely, because patients newly diagnosed with cancer had a similar distribution of morphological abnormalities as childhood cancer survivors.

We limited our study population to white patients, because ethnicity can influence the relative prevalence of tumor types in the cohort and the prevalence of morphological abnormalities. To our knowledge, there are no studies available comparing the frequencies of morphological abnormalities in various populations. The frequency of syndromes among various populations is in general remarkably similar and only shows differences when obvious exogenous influences are present or in the presence of founder effects. We estimate many of our findings will hold for nonwhite populations, but future studies are needed before our findings can be generalized to other ethnic groups.

The associations between patterns of morphological abnormalities and childhood cancer could be helpful in identifying underlying genetic and environmental defects. First, associations between tumors and established syndromes for which causative genes are known suggest a role for those genes in tumorigenesis. For example, PTPN11 (OMIM 1607830) was first identified as a gene causing Noonan syndrome when constitutionally mutated. Noonan syndrome was known to co-occur with juvenile myelomonocytic leukemia and subsequently somatic mutations of PTPN11 in juvenile myelomonocytic leukemia were recognized.

Second, the patterns of morphological abnormalities in pediatric patients with cancer may show overlap with the phenotype of already known syndromes and may point to defects in either the same gene or in genes functioning within the same developmental pathway as the known syndromes. This can be illustrated by the resemblance in phenotype of Rubinstein-Taybi syndrome and Saethre-Chotzen syndrome (caused by genes CBP [OMIM 600140] and TWIST [OMIM 601622], respectively), both acting in the Sonic Hedgehog- Patched-Gli pathway.

Third, data mining tools may be helpful to translate childhood cancer-related patterns of morphological abnormalities into candidate genes in either human or mouse. This can be illustrated by the recognition of a strong resemblance in phenotype of the mouse bleb mutants to the symptoms in Frasier syndrome, eventually leading to the identification of the causative gene FRAS1 (OMIM 1607830) in both man and mouse.

We conclude that the high incidence of single and combined morphological abnormalities in pediatric patients with cancer indicates that constitutional genetic defects play a more important role in pediatric oncogenesis than is currently estimated.

Furthermore, the detection of patterns of morphological abnormalities allows identification of new tumor predisposition syndromes.

Author Contributions: Dr Merks had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Merks, Zwinderman, Caron, Hennekam.

Acquisition of data: Merks, Özgen, Hennekam.

Analysis and interpretation of data: Merks, Özgen, Koster, Zwinderman, Caron, Hennekam.

Drafting of the manuscript: Merks, Caron, Hennekam.

Critical revision of the manuscript for important intellectual content: Merks, Özgen, Koster, Zwinderman, Caron, Hennekam.

Statistical analysis: Merks, Koster, Zwinderman.

Obtained Funding: Caron.

Administrative, technical, or material support: Merks, Özgen, Koster, Zwinderman, Caron, Hennekam.

Financial Disclosures: None reported.

Funding/Support: Dr Merks is a fellow of the Academic Medical Center Meelmeejer Fund. This research was in part funded by the Foundation of Pediatric Cancer Research (Stichting Kindergeneeskundig Kankeronderzoek), Amsterdam, the Netherlands.

Role of the Sponsor: The Foundation of Pediatric Cancer Research had no role in the design and conduct of the study, in the collection, management, analysis, and interpretation of the data, in the preparation, review, or approval of the manuscript.

Additional Contributions: We thank our colleague pediatric oncologists and medical oncologists who were involved in patient accrual. Rogier Verslegers, PhD, Department of Human Genetics, University of Amsterdam, Academic Medical Center, Amsterdam, the Netherlands, and Andrew Copp, PhD, Institute of Child Health, University College London, London, England, provided critical reading of the manuscript. Richard Heinen, MSc, Clinic for Late Effects of Childhood Cancer Study Group, Academic Medical Center, Amsterdam, the Netherlands, provided help in data managing. No compensation was received by any of the mentioned persons. We are grateful to the patients and to their parents for giving their permission to participate in the study.

Solitary, meditative observation is the first step in the poetry of research, in the formation of scientific fantasies, the reality of which we then test with the tools of logic, mathematics, physics and chemistry. —Theodore Billroth (1829-1894)