Cancer predisposition in children: genetics, phenotypes & screening

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Citation for published version (APA):

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Structural genome variations in individuals with childhood cancer and tumor predisposition syndromes

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Chapter 3

Abstract

Background

Previous studies have shown a high prevalence of syndromes in children with cancer. We described four patterns of co-occurring morphological abnormalities indicating new tumor predisposition syndromes. These patterns were named after their key-abnormalities: blepharophimosis (BP), epicanthal folds (EF), asymmetric lower limbs (LLA) and Sydney creases (SC) pattern. The purpose of our study was to identify structural genomic variants possibly involved in these tumor predisposition syndromes.

Patients and methods

In 49 probands (13 from BP, nine from EF, 20 from LLA and seven from SC patterns respectively) karyotyping was performed. Copy number variation (CNV) in genomic DNA of the probands was analyzed to detect microdeletions/-duplications using SNP array. FISH and quantitative-polymerase chain reaction (q-PCR) experiments were done to validate events identified by cytogenetic and CNV analysis.

Results

Cytogenetic analysis showed an inherited inversion of chromosome 15, inv(15)(q25q26) in a proband with LLA-pattern. Evaluation of the genes at the breakpoints made it unlikely that these explained the phenotype and tumor in this patient. Eleven CNV events met our inclusion criteria; three inherited CNV events involved an oncogene. A duplication involving \( BCL9 \) was identified in a proband diagnosed with Burkitt lymphoma. A duplication involving \( PCM1 \) was identified in a proband diagnosed with pre-B-ALL. Both probands showed the EF-pattern of morphological abnormalities. A deletion involving \( TRA@ \) was identified in two probands from the BP-pattern diagnosed with rhabdomyosarcoma and pre-B-ALL respectively.

Conclusions

We report on structural genomic variants in pediatric cancer patients with newly recognized tumor predisposition syndromes. We identify three CNV events which we suggest to be susceptibility loci.
Introduction

Genetic syndromes can be associated with an increased risk for tumor development. In such tumor predisposition syndromes, the constitutional molecular defects that lead to the specific phenotype play an important role in oncogenesis¹. Previous studies from our center showed a significantly increased incidence of morphological abnormalities and high prevalence of syndromes in a cohort of 1073 childhood cancer probands compared to 1007 healthy schoolchildren². Furthermore, we described four new patterns of co-occurring morphological abnormalities as tumor predisposition syndromes³. These were named after their key abnormalities: ‘Blepharophimosis (BP) pattern’, ‘epicanthal folds (EF) pattern’, ‘asymmetric lower limbs (LLA) pattern’ and ‘Sydney creases (SC) pattern’ (Table 1). The aim of the present project is to identify the structural genomic

<table>
<thead>
<tr>
<th>Name pattern of co-occurring morphological abnormalities</th>
<th>Morphological abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLEPHAROPHIMOSIS PATTERN (n=10)</td>
<td>≥2 of the following:</td>
</tr>
<tr>
<td></td>
<td>Blepharophimosis</td>
</tr>
<tr>
<td></td>
<td>Patchy hypopigmentation</td>
</tr>
<tr>
<td></td>
<td>Café-au-lait spots</td>
</tr>
<tr>
<td></td>
<td>Increased angulation of the spine</td>
</tr>
<tr>
<td>ASYMMETRIC LOWER LIMBS PATTERN (n=17)</td>
<td>≥2 of the following:</td>
</tr>
<tr>
<td></td>
<td>Asymmetric lower limbs</td>
</tr>
<tr>
<td></td>
<td>Tall stature</td>
</tr>
<tr>
<td></td>
<td>Ptosis</td>
</tr>
<tr>
<td></td>
<td>Hypoplastic malae</td>
</tr>
<tr>
<td></td>
<td>Pectus carinatum or pectus excavatum</td>
</tr>
<tr>
<td>EPICANTHAL FOLDS PATTERN (n=8)</td>
<td>≥3 of the following:</td>
</tr>
<tr>
<td></td>
<td>Epicanthal folds</td>
</tr>
<tr>
<td></td>
<td>Flat, broad, nose</td>
</tr>
<tr>
<td></td>
<td>Full lips</td>
</tr>
<tr>
<td></td>
<td>Prominent ears</td>
</tr>
<tr>
<td></td>
<td>Café-au-lait spots</td>
</tr>
<tr>
<td></td>
<td>Hyperlaxity of joints</td>
</tr>
<tr>
<td>SYDNEY CREASE PATTERN (n=6)</td>
<td>≥3 of the following:</td>
</tr>
<tr>
<td></td>
<td>Sydney crease</td>
</tr>
<tr>
<td></td>
<td>Full lateral parts of eyelids</td>
</tr>
<tr>
<td></td>
<td>Microdontia</td>
</tr>
<tr>
<td></td>
<td>Clinodactyly digitus V</td>
</tr>
<tr>
<td></td>
<td>Hypermobility of small joints</td>
</tr>
</tbody>
</table>

Table 1: Summary of patterns of statistically significant co-occurring morphological abnormalities and number of patients in each pattern. The total of 41 probands that were included for our analyses were selected from a large prospective cohort of childhood cancer probands and all showed one of the four patterns of statistically significant co-occurring morphological abnormalities. The table shows the criteria for each of the patterns and the number of patients included from each pattern, based on the morphological examination by Merks et al. (partly described in ³).
variants that may cause these tumor predisposition syndromes. Copy number variants (CNVs) are structural variants that affect copy count number such as deletions, duplications and insertions.

Until now, the importance of CNVs to cancer predisposition has been explored only in part. An increased number of CNVs in the genome of individuals with Li-Fraumeni syndrome was reported 4 and 40% of cancer genes reported in the ‘census of human cancer genes’ 5 are altered by CNVs 6. Genes involved in pathogenic CNV events are candidates for the association with the corresponding phenotype 6. In this project we used conventional cytogenetics and SNP arrays to identify inversions, deletions and duplications in genes which may explain both malignancy and morphological abnormalities in the new tumor predisposition syndromes.

Patients and methods

Probands
Forty-nine Caucasian probands, previously designated as showing one of the four patterns of morphological abnormalities 3 were asked to participate. Probands and parents with a written consent were included. Blood was collected from probands and if possible from their parents.

Karyotyping
Cell cultures and sediments for karyotyping using G-banding were performed for all probands using local standard protocols. For all chromosome analyses, about 550 bands per haploid karyotype were visible. Karyotypes were described according to ISCN classification (2009) 7.

Whole-genome high-resolution SNP array
SNP array was carried out using the Illumina® Bead Chip 660W SNP array (Illumina, San Diego, CA, USA) in a certified laboratory (Service XS, Leiden, the Netherlands) according to the manufacturer’s protocol. For the Illumina® Bead Chip 660W SNP array 660.000 probes are spotted on the array, leading to a median spacing of 2.3 kb. The call rate was 0.99 or higher for all samples, excluding CNV markers.

SNP array analysis
Data for each bead chip were self-normalized in Genomestudio GT (Illumina) using information contained within the array. Copy numbers were estimated for each individual by comparison with a common reference set of 200 samples from the HapMAP project (www.hapmap.org/downloads/raw_data) supplied by Illumina and visualized in Nexus
Copy Number™ software package (version 4, Biodiscovery, El Segundo, CA, USA). For analysis, SNP-Rank segmentation method was used with significance thresholds of $1 \times 10^{-5}$ and log-ratio thresholds of 0.2 and -0.2 for duplications and deletions, respectively. The maximum contiguous probe spacing was 1000 kb and the minimum number of probes per segment was set to five, limiting CNV detection to sizes $> 10.6$ kb. Comparison was performed with reference sets of Shaikh et al. 8 (2026 control subjects), Pinto et al. 9 (776 control subjects), Jacobsson et al. 10 (485 control subjects) and Simon-Sanchez et al. 11 (272 control subjects). CNV events in which LogR and BAF read-outs correlated, which were not reported in the reference sets, with a size $> 120$ Kb from probands of Caucasian ancestry, or that involved an (onco)gene, were included for validation. The process of CNV analysis is illustrated in Figure 1.

Validation using fluorescent in-situ hybridization and/or relative-quantitative PCR analysis

CNV events found to be eligible for validation were validated using FISH and/or quantitative-polymerase chain reaction (q-PCR) in accordance with common protocols 12. An example of validation steps in a CNV event is shown in Figure 2. Duplications are often difficult to distinguish using FISH. If FISH was inconclusive, q-PCR was used. If parents’ material was limited to DNA and no cells were available to perform...
Figure 2 A, B, C: Example of validation steps for one CNV event in a proband.
A: The SNP array read out is shown for region 1q21.1 for proband number 2. An increase of the LogR (intensity) is observed together with a separation in the B-allele frequency, consistent with a duplication event. The location of the BAC probes RP11-102F23 (green signal) and RP11-30I17 (red signal) are depicted schematically.
B: FISH experiment using a BAC-probe in the region of the duplication. There is a hyperintense green signal on the aberrant chromosome 1 and a clear interphase signal, meaning that the duplication can be confirmed by BAC-FISH.
C: Quantitative-PCR experiment for proband and parents, primers were chosen in the region of the duplication. The relative quantification bar for the proband is marked “PIN2 (proband)”, the RQ bars in both mother and father are marked “PIN2m (mother)” and “PIN2d (father)” respectively. The three outer right RQ bars correspond to controls (Control1, Control2, Control3). Compared to controls, the signal in proband PIN2 and her mother is significantly increased. This confirms a duplication in their DNA at this location. The duplication in this proband is inherited from mother.

FISH, q-PCR was primarily used. For FISH experiments, BAC clones were selected from the UCSC Genome Browser (http://genome.ucsc.edu/build 36.1, hg18), purchased from BACPAC resources center (Oakland, CA, USA) and labelled (Random Prime labelling system; Invitrogen corporation) with Bio-16-d UTP (biotin, red) or Dig-11-dUTP (digoxigenin, green), Roche Applied Science, Indianapolis, IN, USA.
For qualitative real-time PCR, primer pairs were designed from unique sequences within the minimal deleted or duplicated regions using Primer Express® Software version 3 (Applied
Biosystems, Carlsbad, CA, USA). The nucleotide BLAST algorithm at NCBI (http://www.ncbi.nlm.nih.gov/BLAST/) was used to confirm that each PCR amplification product was unique. Experiments were designed with the C14orf145 region as control locus. In the 35 cycle q-PCR assays, sybergreen was used as fluorochrome (KAPA SYBR® FAST q-PCR Master Mix, Kapa Biosystems).

The Decipher Database (http://decipher.sanger.ac.uk/, version 5.1) was used to compare phenotypes in probands to patients with the same CNV.

Results

Karyotyping

Conventional karyotyping showed a normal 46,XX/XY karyotype in 48/49 probands. Proband 43 (PIN43), who had a Wilms tumor at the age of four and the LLA-pattern of morphological abnormalities, was found to have an inversion of chromosome 15; inv (15)(q25q26) (Figure 3). Further analysis of the parents showed that this inversion was inherited from father, who has had no malignancies and did not show any morphological abnormalities of the LLA-pattern. The family history of father’s side was blank for malignancies. Breakpoints of the inversion were characterized using BAC-FISH and showed BAC RP11-1105N16 (15q25.2) to span the proximal breakpoint. There are several genes in this region: C15orf40, BTBD1 (OMIM 608530), TM6SF1 (OMIM 606562) and FAM103A1 (OMIM 614547). BAC probe RP11-794C20 (15q26.3) spanned the distal breakpoint. ADAMTS17 (OMIM 607511) is located in this region. None of the genes at the breakpoint regions, including ADAMTS17 have been reported in relation to the morphological abnormalities and/or malignancy observed in this proband. We were not able to test the status of ADAMTS17 in tumor tissue of proband. Because of the asymmetric lower limbs in combination with the Wilms tumor, Beckwith-Wiedemann syndrome loci (methylation at KCNQ1OT1 (LIT1) and H19) were analyzed, these tested normal.

SNP array/CNV analysis

In total 604 CNV events were identified in 49 probands. Most events (n = 466) were not considered to be real CNVs as there was no good correlation between BAF and LogR, likely due to technical artefacts. We detected 138 ‘realistic events’ (i.e. CNV events with a good correlation of LogR and BAF): an average of 2.8 (138/49) CNV events per subject (median size 106 kb; average size 215 kb). Of the latter, 138 only CNV events that met the other inclusion criteria (see ‘Patients and Methods’ section) were included, leading to eleven CNV events eligible for validation. All eleven CNV events were found to be present in germ-line material of the probands, and all had been inherited from a healthy parent and thus not specific to the probands, except for two cases in whom parental material was not available (Table 2).
The eleven events detected in 10 probands were included for analysis (Table 2). Here we only discuss the CNV events involving a (possible) oncogene and the CNV events that occurred in more individuals with the same pattern of morphological abnormalities. In the single patient of whom tumor material was available, the CNV event was present in the tumor material as well. Additional information on the family histories concerning cancer

<table>
<thead>
<tr>
<th>Proband</th>
<th>Pattern of co-occurring morphological abnormalities</th>
<th>Type of malignancy</th>
<th>Event</th>
<th>Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIN2</td>
<td>Epicanthal folds syndrome</td>
<td>Burkitt lymphoma</td>
<td>Dupl 1q</td>
<td>1592297</td>
</tr>
<tr>
<td>PIN21</td>
<td>Epicanthal folds syndrome</td>
<td>Pre-B-acute lymphoblastic leukemia</td>
<td>Dupl8p</td>
<td>188909</td>
</tr>
<tr>
<td>PIN12</td>
<td>Blepharophimosis syndrome</td>
<td>Embryonal rhabdomyosarcoma</td>
<td>Del 14q</td>
<td>400054</td>
</tr>
<tr>
<td>PIN14</td>
<td>Blepharophimosis syndrome</td>
<td>Pre-B-acute lymphoblastic leukemia and leiomyosarcoma (adult age)</td>
<td>Del 14q</td>
<td>231673</td>
</tr>
<tr>
<td>PIN19</td>
<td>Asymmetric lower limbs syndrome</td>
<td>Ependymoma</td>
<td>Dupl 12q</td>
<td>372032</td>
</tr>
<tr>
<td>PIN19</td>
<td>Asymmetric lower limbs syndrome</td>
<td>Ependymoma</td>
<td>Dupl 5q</td>
<td>178949</td>
</tr>
<tr>
<td>PIN27</td>
<td>Asymmetric lower limbs syndrome</td>
<td>Malignant fibrous histiocytoma</td>
<td>Dupl 21q</td>
<td>180078</td>
</tr>
<tr>
<td>PIN40</td>
<td>Asymmetric lower limbs syndrome</td>
<td>Endodermal sinus tumour testis</td>
<td>Dupl 8p</td>
<td>188909</td>
</tr>
<tr>
<td>PIN37</td>
<td>Asymmetric lower limbs syndrome</td>
<td>Embryonal rhabdomyosarcoma</td>
<td>Del 20q</td>
<td>11249</td>
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<tr>
<td>PIN30</td>
<td>Asymmetric lower limbs syndrome</td>
<td>T-cell acute lymphoblastic leukemia</td>
<td>Del 20q</td>
<td>10352</td>
</tr>
<tr>
<td>PIN31</td>
<td>Sydney crease syndrome</td>
<td>Pre-B-acute lymphoblastic leukemia</td>
<td>Del 3q</td>
<td>282702</td>
</tr>
</tbody>
</table>

Table 2: Summary of CNV events included for validation and their results. All events could be validated in germline material and were indeed present. Heritability could not be established in one proband because of lack of parental material. Tumor material on behalf of validation of the event in the tumor was only available for one proband. N/A= not available

Validation of CNV events

The eleven events detected in 10 probands were included for analysis (Table 2). Here we only discuss the CNV events involving a (possible) oncogene and the CNV events that occurred in more individuals with the same pattern of morphological abnormalities. In the single patient of whom tumor material was available, the CNV event was present in the tumor material as well. Additional information on the family histories concerning cancer
Table 2: Summary of CNV events included for validation and their results. All events could be validated in germline material and were indeed present. Heritability could not be established in one proband because of lack of parental material. Tumor material on behalf of validation of the event in the tumor was only available for one proband. N/A = not available

<table>
<thead>
<tr>
<th>RefSeq gene involved</th>
<th>Event validated in germline</th>
<th>Heritability</th>
<th>Event validated in tumour</th>
<th>Relationship predisposition</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCL9, NBPF11, NBPF24, PRKAB2, CHD1L, FMO5, ACP6, GJA5, GJA8, GPR89B, GPR89C</td>
<td>yes; present</td>
<td>Maternal inheritance</td>
<td>N/A</td>
<td>Likely</td>
</tr>
<tr>
<td>MTUS1, FGL1, PCM1</td>
<td>yes; present</td>
<td>Maternal inheritance</td>
<td>N/A</td>
<td>Likely</td>
</tr>
<tr>
<td>TRA@</td>
<td>yes; present</td>
<td>Maternal inheritance</td>
<td>N/A</td>
<td>Unlikely</td>
</tr>
<tr>
<td>TRA@</td>
<td>yes; present</td>
<td>Maternal inheritance</td>
<td>N/A</td>
<td>Likely</td>
</tr>
<tr>
<td>KRT1, KRT3, KRT4, KRT8, KRT18, KRT76, KRT77, KRT78, KRT79, EIF4B, TENC</td>
<td>yes; present</td>
<td>Maternal inheritance</td>
<td>N/A</td>
<td>Unlikely</td>
</tr>
<tr>
<td>ARL15</td>
<td>yes; present</td>
<td>Paternal inheritance</td>
<td>N/A</td>
<td>Unlikely</td>
</tr>
<tr>
<td>KCNE1, KCNE2, FAM165B, RCAN1</td>
<td>yes; present</td>
<td>Paternal inheritance</td>
<td>yes; present</td>
<td>Unlikely</td>
</tr>
<tr>
<td>FGL1, MTUS1, PCM1</td>
<td>yes; present</td>
<td>Maternal inheritance</td>
<td>N/A</td>
<td>Unlikely</td>
</tr>
<tr>
<td>BCAS1</td>
<td>yes; present</td>
<td>N/A</td>
<td>N/A</td>
<td>Unlikely</td>
</tr>
<tr>
<td>BCAS1</td>
<td>yes; present</td>
<td>Maternal inheritance</td>
<td>N/A</td>
<td>Unlikely</td>
</tr>
<tr>
<td>RARRES1, MFSD1</td>
<td>yes; present</td>
<td>N/A</td>
<td>N/A</td>
<td>Unlikely</td>
</tr>
</tbody>
</table>

and morphological abnormalities in the families of probands is presented in Supplementary Table 1.

CNV events including oncogenes

Proband 2 (PIN2, EF-pattern) showed a duplication on 1q21.1 involving BCL9 (OMIM 609004). This proband has had a Burkitt lymphoma (Table 2, Figure 2). She inherited the duplication 1q21.1 from her mother, who has had no malignancies and only showed the broad nasal tip of the ‘EF pattern’.

Proband 21 (PIN21, EF-pattern) and proband 40 (PIN40, LLA-pattern) had a duplication on 8p22, both maternally inherited. The mother of PIN40 has had no malignancies and
did not show any morphological abnormalities of the LLA-pattern, PIN21 has had pre-B acute lymphoblastic leukemia, PIN40 has had an endodermal sinus tumor of the testis. The duplication involved $PCM1$ (OMIM 600299), $FGL1$ (OMIM 605776) and $MTUS1$ (OMIM 609589).

CNVs occurring in more individuals with the same pattern of morphological abnormalities

Two probands with the BP-pattern of morphological abnormalities shared a hemizygous deletion involving $TRA@$ (OMIM 186880) (Table 2). One (PIN12) has had an embryonal...
rhabdomyosarcoma, the other (PIN14) has had pre-B acute lymphoblastic leukemia as a child and a leiomyosarcoma at adult age. In both cases the CNV was maternally inherited. The mother of PIN12 has had no malignancies and did not show any morphological abnormalities of the BP-pattern. The mother of PIN14 has had mamma carcinoma, she did not show any morphological abnormalities of the BP-pattern.

Two probands of the LLA-pattern of morphological abnormalities shared a CNV event involving the oncogene BCAS1 (OMIM 602968). Proband 30 (PIN30) had a homozygous deletion and had suffered from T-cell acute lymphoblastic leukemia; proband 37 (PIN37) had a hemizygous deletion and had suffered from an embryonal rhabdomyosarcoma. In PIN30, the event was maternally inherited. The mother has had no malignancies and she did not show any morphological abnormalities of the LLA-pattern. In PIN37 inheritance could not be established because both parents’ material were unavailable. Information on malignancies and morphological abnormalities in the father was unavailable. The mother has had no malignancies and she did not show any morphological abnormalities of the LLA-pattern.

Discussion

In this project we use SNP array and karyotyping in combination with FISH and q-PCR to identify CNV events in genes which may explain both the tumor and phenotypes in four newly delineated tumor predisposition syndromes.

We did not find a de novo CNV event that could explain for both tumor and dysmorphic phenotypes. However, the duplication identified in PIN2 involving BCL9 (1q21.1) may be part of the explanation for the Burkitt lymphoma in her, even though translocations and not duplications involving BCL9 have been reported in B-cell malignancies/lymphoma. Unfortunately no tumor material was present to further investigate this possible relationship. Several authors have reported on patients with a (micro)duplication in this region. (Micro)duplications involving #1q21.1 have been reported in patients with congenital heart disease and ocular, neuromuscular and skeletal abnormalities. Brunetti et al. mentioned hypertelorism and frontal bossing as main facial morphological abnormalities in #1q21.1 microduplications. The phenotype of PIN2 did not show any overlap with these features, nor with the phenotypes of the 27 patients that are listed in the Decipher Database. Because detailed morphological signs on the patients in the Decipher Database were lacking, we remain unsure whether the duplication in BCL9 may explain the epicanthal folds pattern of morphological abnormalities.

In two probands who have had pre-B acute lymphoblastic leukemia (PIN21) and endodermal sinus tumor of the testis (PIN40) respectively, a duplication on chromosome 8p22 involving PCM1, FGL1 and MTUS1 was found. PCM1 encodes a component of centriolar satellites,
its 5 prime portion is known to be involved in rearrangements of the RET tyrosine kinase domain in papillary thyroid carcinoma. The duplication involving PCM1 may be associated with the acute lymphoblastic leukemia in PIN21, as chromosomal aberrations involving PCM1 are reported to be associated with a variety of haematological malignancies such as chronic myeloid leukemia and T-cell lymphoma. Proband 40 has had an endodermal sinus tumor of the testis and PCM1 is not known to be involved in such tumors. FGL1 is known to be a mitogenic active factor for hepatocytes, may play a tumor suppressive role in hepatocellular carcinoma, and is suggested to act as acute phase reactant. FGL1 is not known to be involved in endodermal sinus tumor of the testis. MTUS1 encodes a protein that interacts with the Angiotensin II receptor and seems to act as tumor suppressor in prostate cancer, colon cancer and breast cancer with poor prognosis. In the Decipher Database seven patients are listed with a duplication of the same region. This database reports only on major malformations, which did not overlap with the phenotype in our probands, and morphological details were lacking, so data are insufficient to determine if the CNV event explains for the asymmetric lower limbs pattern.

We found a hemizygous deletion involving TRA@ at chromosome 14q11.2 in two probands (PIN12 and PIN14) of the BP-pattern. Proband 14 has had pre-B acute lymphoblastic leukemia and a leiomyosarcoma at the age of 30. Inversions in TRA@ are known to occur somatically in T-cell chronic lymphoblastic leukemia and translocations involving TRA@ are detected somatically in T-cell malignancies of patients with ataxia telangiectasia. As TRA@ is known to be somatically involved in human T-cell leukemias and lymphomas, it seems plausible that the constitutional hemizygous deletion in TRA@ could have contributed to the susceptibility to develop acute lymphoblastic leukemia in PIN14. No tumor material was available so we were not able to test the status of TRA@ in bone marrow of PIN14. There have been no reports of TRA@ involvement in leiomyosarcoma. Because PIN14 received total body irradiation, the leiomyosarcoma might be secondary to the irradiation. A few reports have described patients with a 14q11.2 deletion, and the phenotypes in these patients did not show significant overlap with our patients. In the Decipher database four patients with a TRA@ deletion are listed, but phenotype information is too limited to decide whether overlap with probands 12 or 14 exists.

In two probands of the LLA-pattern (diagnosed with T-cell acute lymphoblastic leukemia and embryonal rhabdomyosarcoma respectively) a deletion involving BCAS1 was identified. BCAS1 is located in a region harbouring copy number gains in breast cancer and other tumors, but no association with acute lymphoblastic leukemia or rhabdomyosarcoma has been reported so far. There have been no reports on patients with a germ-line deletion in BCAS1. BCAS1 is highly expressed in most breast cancer cell lines in which its region is amplified, while in the present probands a deletion was found. We remain unsure whether an association exists between the deleted BCAS1 and the phenotype.
In all present cases, the specific CNV event was inherited from one of the parents. Finding *de novo* CNV events would make the relation between the events and the phenotype including malignancy strong. However, finding inherited CNV events does not necessarily exclude them as being causative. Various publications have discussed mechanisms explaining this phenomenon \(^{33,34}\). Several syndromes have shown that not all carriers of the genetic defect exhibit the full phenotype (‘incomplete penetrance’). Also, there is the phenomenon of variable expressivity, the extent to which a genotype exhibits its phenotype expression. Both incomplete penetrance and variable expression can be explained by modifier genes, genes that alter the expression of other genes. These modifier genes can affect the threshold for trait expression, leading to a larger or smaller proportion of individuals affected by a certain event and thus affect penetrance. Also, modifier genes can affect the range of phenotypes associated with a certain event and thus lead to variable expressivity. Other explanations for incomplete phenotypes in parents carrying the same genomic variant could be allelic variation or complex environmental and genetic interaction \(^{35}\). Translating this to oncogenesis, the CNV can be a susceptibility locus; having this CNV event makes that particular individual more susceptible for developing cancer, but still other factors are needed to actually develop a malignancy. We hypothesize that the duplications involving *BCL9*, *PCM1* and the deletion involving *TRA@* may be such susceptibility loci.

Interestingly enough, the events seem to be predominantly maternally inherited. However, because of the relatively small size of the cohort this might result from mere coincidence. One could try to confirm these findings in a second validation cohort. However, low numbers of patients fulfilling the criteria for the patterns of morphological abnormalities may make this a challenging exercise. If indeed there were to be a predominantly maternal inheritance, this could be explained by mitochondrial inheritance or an epigenetic effect such as maternal imprinting. In this study we did not investigate epigenetic processes such as methylation status.

To further elaborate the relation between the identified CNV events and the malignancy and morphological abnormalities in the proband, several strategies could be followed. Further analyses of the tumors (if they would have been available) could have shown if the specific event was retained in the tumor and thus of importance for tumorigenesis and identification of a second hit of the remaining allele. In functional experiments involving the gene of interest in tumor cell-lines matching the histiotype of the proband’s tumor, silencing by shRNA and/or upregulation by transfection could be useful.

Recently, sequencing techniques such as next generation sequencing (NGS) have become widely available, \(^{36,37}\) and have become an important tool in genetic and genomic analysis. The present approach using a SNP array to detect deletions and duplications and conventional cytogenetics to identify large inversions and translocations allow us to identify
most structural variations at acceptable cost. However, the present approach has limitations in detecting structural variations such as small insertions or inversions and point mutations. In part of the patients, there could be a polygenic cause of the childhood malignancy and morphological abnormalities. We think the additional value of NGS rests in the identification of second genomic variants that could not be detected in the current approach. However, more detail comes with a price: namely the presence of individual variants (both inherited or de novo) which may not be related to the syndrome of the proband. NGS might also identify variations in the probands who were found negative for variations in the current approach. Therefore, a subsequent study using NGS techniques has now been initiated.

Acknowledgements

We thank our patients and their parents for their kind cooperation in our study. This study was funded by the ‘Tom Voûte Fund’. This study makes use of data generated by the DECIPHER Consortium. A full list of centres who contributed to the generation of the data is available from http://decipher.sanger.ac.uk and via email from decipher@sanger.ac.uk. Funding for the project was provided by the Wellcome Trust. Those who carried out the original analysis and collection of the Data bear no responsibility for the further analysis or interpretation of it by the Recipient or its Registered Users.

Role of the funding source

This study was funded by the “Tom Voûte Fund”. The funder had no role in the design of the study nor in the data collection, analysis or preparation of the manuscript.
References


## Chapter 3

### Supplementary data

<table>
<thead>
<tr>
<th>Proband</th>
<th>Pattern of co-occurring morphological abnormalities (proband)</th>
<th>Type of malignancy (proband)</th>
<th>Malignancies in family (age at diagnosis in years)</th>
</tr>
</thead>
</table>
| PIN2    | Epicanthal folds syndrome                                     | Burkitt lymphoma            | \begin{align*}S_1\text{MM:} & \text{mamma carcinoma (>50)} \\
|         |                                                               |                             | \text{S}_2\text{MM:} & \text{mamma carcinoma (>50)} \\
|         |                                                               |                             | \text{SPP:} & \text{mamma carcinoma (40)} \\
|         |                                                               |                             | \text{FsFP:} & \text{carcinoma of unknown origin (40)} \end{align*} |
| PIN21   | Epicanthal folds syndrome                                     | Pre-B-acute lymphoblastic leukemia | \begin{align*}S_1\text{MM:} & \text{leukemia (>80)} \\
|         |                                                               |                             | \text{S}_2\text{MM:} & \text{mamma carcinoma (>50)} \\
|         |                                                               |                             | \text{S}_3\text{MM:} & \text{pancreas carcinoma (>70)} \\
|         |                                                               |                             | \text{F}_1\text{MM:} & \text{lung carcinoma (70), smoker} \\
|         |                                                               |                             | \text{F}_2\text{MM:} & \text{oesophagus carcinoma (age unknown)} \\
|         |                                                               |                             | \text{F}_3\text{MM:} & \text{gastric carcinoma (>50)} \\
|         |                                                               |                             | \text{FeSM:} & \text{brain tumor of unknown origin (34)} \\
|         |                                                               |                             | \text{S}_1\text{MP:} & \text{mamma carcinoma (>50)} \\
|         |                                                               |                             | \text{S}_2\text{MP:} & \text{mamma carcinoma (>50)} \\
|         |                                                               |                             | \text{S}_2\text{MP:} & \text{mamma carcinoma (>50)} \\
|         |                                                               |                             | \text{S}_4\text{MP:} & \text{mamma carcinoma (>50)} \end{align*} |
| PIN12   | Blepharophimosis syndrome                                     | Embryonal rhabdomyosarcoma   | \begin{align*}\text{FP:} & \text{acute leukemia (65)} \\
|         |                                                               |                             | \text{SP:} & \text{lung carcinoma (70), breast carcinoma (70)} \\
|         |                                                               |                             | \text{PP:} & \text{lung carcinoma (>90)} \\
|         |                                                               |                             | \text{MP:} & \text{renal carcinoma (>60), colorectal carcinoma (>80)} \\
|         |                                                               |                             | \text{SPM:} & \text{chronic leukemia (>60)} \\
|         |                                                               |                             | \text{PPM:} & \text{leukemia (>60)} \end{align*} |
| PIN14   | Blepharophimosis syndrome                                     | Pre-B-acute lymphoblastic leukemia and leiomyosarcoma (adult age) | \begin{align*}\text{M:} & \text{mamma carcinoma (55)} \\
|         |                                                               |                             | \text{PP:} & \text{carcinoma of unknown origin (40)} \end{align*} |
| PIN19   | Asymmetric lower limbs syndrome                               | Ependymoma                  | \begin{align*}\text{SM:} & \text{thyroid carcinoma (49)} \\
|         |                                                               |                             | \text{FeFM:} & \text{leukemia (9)} \\
|         |                                                               |                             | \text{PM:} & \text{lymphoma (>50)} \\
|         |                                                               |                             | \text{FPM:} & \text{lung carcinoma (>40), smoker} \\
|         |                                                               |                             | \text{SPM:} & \text{ovarian carcinoma (60)} \\
|         |                                                               |                             | \text{S}_1\text{MM:} & \text{leukemia (48)} \\
|         |                                                               |                             | \text{S}_2\text{MM:} & \text{cervix carcinoma (65)} \\
|         |                                                               |                             | \text{F}_1\text{MM:} & \text{gastric carcinoma (45)} \\
|         |                                                               |                             | \text{F}_2\text{MM:} & \text{oesophagus carcinoma (65)} \\
|         |                                                               |                             | \text{F}_3\text{MM:} & \text{lung carcinoma (45)} \\
|         |                                                               |                             | \text{F}_4\text{MM:} & \text{lung carcinoma (55), smoker} \\
|         |                                                               |                             | \text{Fe}_5\text{MM:} & \text{lung carcinoma (55)} \\
|         |                                                               |                             | \text{FeFM\text{MM:}} & \text{carcinoma of unknown origin (64)} \\
|         |                                                               |                             | \text{FsFM\text{MM:}} & \text{lung carcinoma (50), smoker} \\
|         |                                                               |                             | \text{FPP:} & \text{colorectal carcinoma (58)} \end{align*} |
## Morphological abnormalities in parents overlapping with pattern in the proband and noticeable morphological abnormalities in rest of family

<table>
<thead>
<tr>
<th>M:</th>
<th>broad nasal tip</th>
</tr>
</thead>
<tbody>
<tr>
<td>P:</td>
<td>full lips</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>M:</th>
<th>broad nasal tip</th>
</tr>
</thead>
<tbody>
<tr>
<td>P:</td>
<td>prominent ears</td>
</tr>
<tr>
<td>S:</td>
<td>large hyperpigmented area over face, neck, breast, arm</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>M:</th>
<th>none</th>
</tr>
</thead>
<tbody>
<tr>
<td>P:</td>
<td>none</td>
</tr>
<tr>
<td>FP:</td>
<td>extra toe on one foot</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>M:</th>
<th>none</th>
</tr>
</thead>
<tbody>
<tr>
<td>P:</td>
<td>none</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>M:</th>
<th>none</th>
</tr>
</thead>
<tbody>
<tr>
<td>P:</td>
<td>hypoplastic malae</td>
</tr>
</tbody>
</table>
### PIN27: Asymmetric lower limbs syndrome
Malignant fibrous histiocytoma

- **MM:** mamma carcinoma (>50)  
- **SMM:** oesophagus carcinoma (>60)

### PIN40: Asymmetric lower limbs syndrome
Endodermal sinus tumor testis

- **MM:** gastric carcinoma (41), breast carcinoma (41)  
- **SPM:** colorectal carcinoma (68)  
- **SP:** mamma carcinoma (45)  
- **F₁P:** pancreas carcinoma (>50)  
- **F₂P:** gastric carcinoma (60)  
- **PP:** lymphoma (60)

### PIN37: Asymmetric lower limbs syndrome
Embryonal rhabdomyosarcoma

- **SMM:** carcinoma of unknown origin (>50)

### PIN30: Asymmetric lower limbs syndrome
T-cell acute lymphoblastic leukemia

- **F₁M:** prostate carcinoma (59)  
- **F₂M:** lung carcinoma (>50), smoker  
- **FsFM:** carcinoma of unknown origin (32)  
- **PM:** pancreas carcinoma (>70)  
- **SMM:** mamma carcinoma (66)  
- **SMMM:** mamma carcinoma (>70)  
- **SP:** mamma carcinoma (over 50)  
- **SMP:** carcinoma of unknown origin (>50)

### PIN31: Sydney crease syndrome
Pre-B-acute lymphoblastic leukemia

- **M:** chronic myeloid leukemia (47)

### Supplementary Table 1:
Family history concerning malignancies and morphological abnormalities which overlap with pattern of proband and noticeable morphological abnormalities in rest of family. Relatives were described using French nomenclature: **M**= mother, **P**= father, **F**= brother, **S**= sister, **Fs**= son, **Fe**= daughter. For example for a cousin who is the son of the brother of mother of proband (=son of uncle mother’s side): **FsFM**.
### Structural genome variations in childhood cancer

#### PIN27 Asymmetric lower limbs syndrome
- **Malignant fibrous histiocytoma**
- **MM:**
- **F1P:**
- **F2P:**
- **PP:**
- **SMM:**
- **SMMM:**
- **SP:**
- **SMP:**

- **M:** none
- **P:** hypoplastic malae
- **FeSM:** spina bifida

- **M:** none
- **P:** none

- **M:** none
- **P:** hypoplastic malae
- **FeSM:** spina bifida

- **M:** none
- **P:** hypoplastic malae
- **FeSM:** spina bifida

#### PIN40 Asymmetric lower limbs syndrome
- **Endodermal sinus tumor of testis**
- **MM:**
- **SPM:**
- **SP:**
- **F1P:**
- **F2P:**
- **PP:**
- **SMM:**
- **SMMM:**
- **SP:**
- **SMP:**

- **M:** none
- **P:** hypoplastic malae
- **FeSM:** spina bifida

- **M:** none
- **P:** hypoplastic malae
- **FeSM:** spina bifida

- **M:** none
- **P:** hypoplastic malae
- **FeSM:** spina bifida

- **M:** none
- **P:** information not available

#### PIN37 Asymmetric lower limbs syndrome
- **Embryonal rhabdomyosarcoma**
- **SMM:** carcinoma of unknown origin (>50)

- **M:** none
- **P:** information not available

#### PIN30 Asymmetric lower limbs syndrome
- **T-cell acute lymphoblastic leukemia**
- **F1M:**
- **F2M:**
- **FsFM:**
- **PM:**
- **SMM:**
- **SMMM:**
- **SP:**
- **SMP:**

- **M:** none
- **P:** hypoplastic malae
- **FeSM:** spina bifida

- **M:** none
- **P:** hypoplastic malae
- **FeSM:** spina bifida

- **M:** none
- **P:** hypoplastic malae
- **FeSM:** spina bifida

- **M:** none
- **P:** information not available

#### PIN31 Sydney crease syndrome
- **Pre-B-acute lymphoblastic leukemia**
- **M:** chronic myeloid leukemia (47)
- **MM:** melanoma (78)
- **P:** none
- **FMM:** colorectal carcinoma (70)
- **FPM:** prostate carcinoma (78)
- **MPM:** multiple myeloma (age unknown)
- **PP:** chronic myeloid leukemia (>50)

### Supplementary Table 1:
Family history concerning malignancies and morphological abnormalities which overlap with pattern of proband and noticeable morphological abnormalities in rest of family. Relatives were described using French nomenclature: M= mother, P= father, F= brother, S= sister, Fs= son, Fe= daughter.

For example for a cousin who is the son of the brother of mother of proband (=son of uncle mother's side): FsFM.