General introduction
Introduction

1.1 Definition and incidence of tumor predisposition syndromes

Approximately 1 in 600 children is diagnosed with a malignancy before the age of 15 in the developed world\(^1\). In the Netherlands, yearly around 600 new cases of childhood cancer are diagnosed. The childhood cancer registry of the Dutch Childhood Oncology Group (DCOG) also known as Dutch foundation for pediatric oncology Stichting Kinderoncologie Nederland (‘SKION basisregistratie’) registered 602 new cases of childhood cancer in the year 2011\(^2\), the diagnoses are classified according to the International Classification of Childhood Cancer (ICCC)\(^3\). The largest group of cases comprise the leukemias/myeloproliferative diseases/myelodysplastic diseases (ICCC diagnostic group I, 25%), followed by CNS/miscellaneous intracranial/intraspinal/neoplasms (ICCC diagnostic group III, 21%) and lymphomas and reticuloendothelial neoplasms (ICCC diagnostic group II, 13%). Of the solid tumors, rhabdomyosarcoma, Wilms’ tumors (nephroblastoma), neuroblastoma and hepatoblastoma comprise 10% of tumors. Malignant bone tumors (Ewing sarcoma and osteosarcoma) comprise 5.5% of tumors.

An important difference between solid tumors in adulthood and childhood is that in adults the vast majority of tumors are epithelial cancers. In adult (mainly epithelial-type) cancers, environmental factors contribute most to the susceptibility for developing cancer and inherited genetic factors only play a minor role\(^4\). In adult cancer, about 5-10% of tumors is said to be caused by genetic predisposition\(^5\), these reported percentages vary widely in childhood cancer\(^6\). Adults have been exposed to several environmental factors that could have caused successive genetic hits. Most children only have had limited exposure to potential oncogenic environmental factors so that in children genetic factors are thought to be of higher importance.

Genes and their corresponding proteins have (almost) invariably a dual function, one prenatally and one postnatally: a gene that steers the formation of an organ or body part (developmental gene) during embryogenesis, frequently becomes a gene that is involved in growth regulation after birth\(^7\). Therefore, an altered developmental gene causes an unusual phenotype or syndrome prenatally and may go along with an increased risk to develop cancer in the same individual postnatally\(^8,9\).

A tumor predisposition syndrome can be defined as a recognizable combination of features (i.e. related to morphological manifestations, tumor, family history or a combination) with a common cause, leading to an increased susceptibility for developing cancer. The gene alterations responsible for the tumor predisposition syndrome are not always identified in these tumor predisposition syndromes, so that it can also be clinical diagnosis. In these tumor predisposition syndromes, the same constitutional molecular defects that lead to the clinical phenotype predispose the patient to develop specific cancers\(^10\). For example, in ‘Gorlin syndrome’ or ‘basal nevus syndrome’ [OMIM 109400] mutations in the *PTCH-1* or...
SUFU gene cause morphological manifestations such as overgrowth, short metacarpals, rib defects, broad face and dental abnormalities on one hand and lead to an increased risk of medulloblastoma and rhabdomyosarcoma on the other hand 7.

Few studies have been done into the prevalence or incidence of tumor predisposition in childhood cancer. One of the first studies giving an estimate of tumor predisposition in childhood cancer was a case record study performed by Narod et al. In their study, the files of 16.5 thousand childhood cancer cases from the UK were reviewed. In more than 3% of the cases, a genetic condition was reported and when family history was taken in account (whenever available) this was more than 4% 11. An important drawback in the study by Narod et al. is that case record studies may easily underestimate the percentage of tumor predisposition because of under reporting or under recognition. Another study, performed by Knapke et al, reviewed the family history, clinical history and tumor type to study the eligibility for genetics evaluation in a cohort of 370 survivors of childhood cancer. They found an indication for further genetic evaluation in almost 30% of the patients 12.

The study performed by Knapke et al has a few drawbacks too. Firstly, studying a cohort of survivors may lead to underestimation of the incidence of tumor predisposition. Tumor predisposition syndromes may lead to a poorer prognosis, so that childhood cancer survivors are less likely to have a tumor predisposition syndrome. Secondly, studying purely indications of further genetic evaluation might lead to an overestimation of incidence as only part of the patients will have a tumor predisposition syndrome.

In both of the studies by Narod and Knapke, morphological examination was not taking into account, whereas morphological abnormalities might be indicators of underlying tumor predisposition syndromes 13,10, 14.

The only prospective study into the incidence of tumor predisposition that has been based on the combination of morphological examination, patient history and family history is performed by Merks et al. In this study, 1073 childhood cancer patients underwent a physical examination. This study cohort comprised of 898 (84%) long-term survivors (children who are in remission for more than five years) and 175 (16%) prospectively recruited newly diagnosed patients. A known syndrome was diagnosed in 42 patients (3.9%) and in another 35 patients (3.3%), a syndrome was suspected. This means they identified a total of more than 7% of (possible) tumor predisposition in their cohort of childhood cancer patients 15. Strengths of this study are that it focused on morphology and tumor predisposition syndromes in childhood cancer which makes the data reliable. Although this study included newly diagnosed patients (16%), a disadvantage is that the majority of patients (84%) were long-term survivors.
1.2 Patterns of morphological abnormalities in childhood cancer patients, a selected cohort

Although few studies have been done into the prevalence or incidence of tumor predisposition syndromes in childhood cancer, more studies have been done on the prevalence of morphological abnormalities or congenital anomalies in childhood cancer patients, based on different methodologies. These included a review of death certificates, examination of cancer registries, record linkage studies, or interviews. Because in these studies no morphological examinations were performed, these analyses are mostly confined to major abnormalities. However, many syndromes manifest themselves rather as combinations of minor anomalies. Several studies have been done on the prevalence of minor anomalies in childhood cancer patients, but these were all small patient cohorts. As mentioned in the previous paragraph, the cohort Merks et al. is unique in its cohort size and because not only medical history and family history were noted, but also patients underwent a physical examination directed to 683 morphological abnormalities. The prevalence and patterns of morphological abnormalities were compared with controls, 1007 schoolchildren from the same geographical region who underwent the same physical examination.

A higher incidence of both major abnormalities (28.8% in patients compared to 15.5% in controls, P<0.001) and minor anomalies (65.1% in patients compared to 56.2% in controls, P<0.001) was found in childhood cancer patients compared to their health peers. Also, a combination of three or more minor anomalies was found in 15.2% of the childhood cancer patients compared to 8.3% in controls (P<0.001). The fact that morphological abnormalities are more prevalent in childhood cancer patients, supports tumor predisposition syndromes as being an explanation for part of the cases of childhood cancer.

Multiple logistic regression analysis showed a selection of independent morphological abnormalities that were significantly associated with childhood cancer. By performing a stepwise cluster analysis, four new patterns of co-occurring morphological abnormalities indicative of tumor predisposition syndromes were established (for detailed methodology, please refer to Merks et al 2008). These patterns were named after their key-abnormalities: ‘blepharophimosis (BP) pattern’, ‘epicanthal folds (EF) pattern’, ‘asymmetric lower limbs (LLA) pattern’ and ‘Sydney creases (SC) pattern’. Patients, designated as showing one of the four patterns of morphological abnormalities formed the basis of the cohort described in the Chapters 3, 4 and 6 of this thesis. (Table 1, patterns and their morphological abnormalities).

1.3 Cancer as a genetic disease

Although environmental factors have a principle role in causing sporadic cancer, cancer is in essence a genetic disease. It takes multiple genetic or epigenetic changes for a somatic cell and its progeny to develop into a cancer. This multistep process leads to changes in gene activity that enables the neoplastic clone to survive, proliferate and disseminate.
During this process cells acquire themselves capabilities, described in the iconic article ‘Hallmarks of Cancer’ by Hanahan and Weinberg 39. These capabilities or ‘hallmarks’ include self-sufficiency of growth signals, insensitivity to anti-growth signals, tissue invasion and metastasis, limitless replicative potential, sustained angiogenesis and evading apoptosis 39. More recently, this concept has been expanded with so called ‘enabling characteristics’ that underly these hallmarks (genome instability and mutation as well tumor-promoting inflammation) and ‘emerging hallmarks’ for which evidence is accumulating (deregulating cellular energetics and avoiding immune destruction). Also the so called ‘microenvironment’ of a tumor, which is constructed during tumorigenesis and consists of different cell types, contributes to the biology of tumors 40 (Figure 1).

**Figure 1:** The hallmarks of cancer; emerging hallmarks and enabling characteristics. Adapted from ‘Hallmarks of cancer, the next generation’ D. Hanahan and R. A. Weinberg, Cell, Volume 144, Issue 5, 4 March 2011, Pages 646–674. Reprinted with permission.

In tumor predisposition, inherited alteration in cancer predisposition genes (i.e. oncogenes, tumor-suppressor genes and stability genes) make an individual more susceptible for developing a cancer 38. Sometimes, four types of genetic predisposition are being distinguished 1. Firstly, predisposition through highly penetrant genes; these usually lead to familiar clusters of malignancies with a clear mode of inheritance. Examples of these include retinoblastoma predisposition syndrome [OMIM 180200] and LiFraumeni syndrome [OMIM
151623]. Secondly, syndromes which present primarily with morphological abnormalities but also lead to an increased cancer risk. Examples of these include Beckwith-Wiedemann syndrome [OMIM 130650] and Denys-Drash syndrome [OMIM 194080]. Thirdly, genes with lower penetrance, which means that not all carriers are affected. In these cases, familial clustering is usually not evident although there might be first degree relatives that are both affected. It is hypothesized that such genes could play an important role (or actually might comprise the largest category of inherited predisposition) in causing (childhood) cancer 1, 41. Examples of these include the efforts to define susceptibility loci for ALL42, 43. Lastly, normal genomic variants of genes may exist that act as genetic modifiers by influencing response to environmental factors or have effect on 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 1, 41, 44.

1.4 Advances in genetic research

Genetic testing for congenital disorders and/or tumor predisposition syndromes has switched from mainly cytogenetic techniques (at chromosome level) to mainly molecular techniques (at DNA level) over the last 10 to 15 years.

To study alterations at chromosome level, karyotyping using G-banding has proven to be a highly reliable cytogenetic technique for identifying chromosomal aberrations such as translocations, inversions, duplications and deletions. However, the resolution of karyotyping is limited to 5-10 Mb. Consequently, chromosomal aberrations of smaller size cannot be detected using karyotyping 45, 46. FISH allows identification of smaller chromosomal aberrations but because of the relatively large size of most probes (100-150 kb), smaller deletions and duplications cannot be detected 45, 46.

To study alterations at DNA level, different high density microarray techniques such as oligo-array and SNP (Single Nucleotide Polymorphism) array have become available. Among other things, the number of probes on the array chip as well as the spacing of the probes, determines the coverage and resolution. For example on the platform used in Chapter 3 (Illumina® Bead Chip 660W SNP array), almost 660,000 probes are spotted on the array, leading to a median genomic coverage of 1,0 (mean genomic coverage 0,92) and a median spacing of 2,3 kb (mean spacing 4,4 kb). SNP arrays can be used to detect genome-wide associations (GWA) of a specific polymorphism in a cohort of patients with the same disease. Therefore, a study cohort of sufficient size as well as a second, independent, so called ‘replication cohort’ are required to establish the association between a SNP and a certain disease. However, SNP arrays can also be used to detect loss (deletion) or gain (duplication), also known as Copy Number Variations (CNVs) in a single individual 45, 46. CNVs are structurally variable regions of DNA and occur in all individuals. Most of the CNVs are “common”, which means that they are shared by other individuals. Therefore, to distinguish
the “pathogenic” CNVs from these “common” CNVs, the use of a representative reference set is necessary. Until now, the importance of CNVs to cancer predisposition has not been explored fully. However, Shlien et al showed that the number of CNVs in the genome of individuals with Li-Fraumeni cancer predisposition syndrome was highly significant increased compared to healthy individuals 47. These results suggest that screening individuals and families predisposed for cancer for CNVs may identify individuals with an abnormally high number of these events, possibly contributing to tumor development 47. The genes that are involved in a pathogenic CNV event can be a candidate for the association with the corresponding clinical phenotype 48. Using a CNV approach has limitations in detecting structural variations such as small insertions or inversions and point mutations. These changes at the level of individual nucleotides can be detected by DNA sequence analysis 49. Direct sequence techniques, in which single genes or gene panels are studied, are labour-intensive. Over the last five years, genome-wide sequencing using next-generation sequencing techniques has proven to be an efficient and effective alternative for discovering new genes involved in diseases 50-53. More recently, next generation sequencing techniques are also introduced for diagnostic purposes. A recent whole-exome study amongst unselected patients who were referred for genetic evaluation of mainly neurologic disorders (including developmental delay, autism spectrum disorder, speech delay and intellectual disability) or neurologic disorders in combination with a disorder in another organ-system, led to a molecular diagnosis in 25% of the patients 54.

At the time of the molecular studies described in part I of this thesis, next generation sequencing techniques were not yet as easily carried out as they currently are. Therefore, our approach described in Chapter 3 of this thesis was confined to using SNP array to detect deletions and duplications and conventional cytogenetics to identify large inversions and translocations. Recently, we started a whole-exome study in a selection of probands showing one of the patterns of co-occurring morphological abnormalities, in whom we had not identified a structural genomic variant using our approach as described in Chapter 3. However current results and validation of these analyses are pending so they could not be included as a separate chapter. Next generation sequencing will be discussed in the General Discussion in part III of this thesis.

1.5 Advances in phenotypic research

In the diagnosis of genetic conditions, morphological abnormalities are often a first clue55. Precise phenotypic descriptions are necessary to define homogeneous study populations and subsequently compare patients with controls and establish genotype-phenotype relations 56. In both studying genotype-phenotype relations and evaluation of large patient populations as well as on individual level, it is essential to use the same nomenclature for morphological abnormalities. Therefore, in 2009, an international group of clinicians working in the field of dysmorphology has initiated the standardization of terms
used to describe human morphology. Firstly, to have the disposal of uniform and internationally accepted terms to describe the human morphological phenotype, is essential to formalize and standardize the clinical assessment of a proband. Secondly, using uniform nomenclature for morphological abnormalities identified in a proband will help in better description of (tumor predisposition) syndrome patients facilitating exchange of knowledge and experiences in recognition of (tumor predisposition) syndromes. Lastly, the definition of patient groups homogeneous in phenotype before performing molecular diagnostics increases the chance of finding the responsible genetic defect.

Traditionally, direct anthropometry has been an important tool in analysing a patient’s phenotype. However, disadvantages include inter-observer variability, impossibility to repeat or perform additional measurements in absence of the subject and the necessity of a subject to sit still. These are important limitations for performing direct anthropometry in pediatric patients. Two-dimensional photography compensates somewhat for these limitations, but the quality and the usability of a 2D picture is highly dependent of the experience of the photographer and cooperation, pose and lighting of the subject. In contrast, 3D photography offers a fast capture of images, independent of the subject’s pose. Also, it allows for repeated measurements without requiring the presence or prolonged cooperation of the subject. In 3D photography, photogrammetric and laser scanning devices are the most commonly used. These devices capture tens to hundreds of thousands of 3D point on a human face. The captured faces are annotated with anatomical landmarks and these landmarks, can be used for linear and angular measurements. However, when performing surface-based shape analysis, so called dense surface models (DSMs) need to be created. Following manual landmarking, collections of face surfaces are used to compute DSMs. Dense surface modelling techniques introduce tens of thousands of densely corresponded points as quasi-landmarks across the surface of the face. The captured faces are annotated with anatomical landmarks and these landmarks, can be used for linear and angular measurements. During this process, surfaces of the faces are pulled together to generate a dense surface correspondence of mesh points across all of the surfaces. In other words, landmarks guide the formation of a dense correspondence between a common set of points across all face surfaces in the study group. Once the dense correspondence has been made, as many as 10,000 points are used as landmarks. After landmarking and construction of a dense surface correspondence, principal components analysis (PCA) of shape differences from the mean face were computed in order to build dense surface models (DSMs) (Figure 2). A DSM consists typically of sufficient PCA modes responsible for 99% of shape variation in the target population. Moreover, every face used to build the DSM can be reconstructed as a weighted linear sum of the DSM modes. 3D Analysis of facial morphology using dense surface modeling has successfully delineated the facial phenotype of genetic conditions such as Noonan, Williams, Smith-Magenis and 22q11 deletion syndromes. In each of these
conditions, Dense surface modeling techniques have attained high rates of discrimination between the faces of unaffected and syndromic groups 67. Using these highly sensitive models of facial dysmorphism, it is then possible to detect fine-grained differences in atypical patients and assist genotype-phenotype studies 69.

![3D surface scanning of the face](image)

**Figure 2:** 3D surface scanning of the face. (A) Multiple views of a 3D photogrammetric face image. (B) Mesh of 3D points on the face annotated with landmarks. (C) Three face surfaces depicting the use of landmarks to warp the faces close together prior to generating a dense surface correspondence of mesh points across all of the surfaces. (D) Polyhedral depiction of face shape using 3D landmarks. Courtesy of Prof. Peter Hammond, Figure ‘3D surface scanning of the face’, reprinted with permission from ‘The use of 3D face shape modelling in dysmorphology’ Arch Dis Child 2007; 92: 1120-1126

### 1.6 Importance of recognition of tumor predisposition syndromes

The diagnosis of a specific tumour predisposition syndrome in patients with childhood cancer is important and clinically relevant because it can affect management and optimize care for patients and their families 15, 70-72. Firstly, diagnosing a tumor predisposition syndrome may allow screening for subsequent malignancies. Early detection of a malignancy may improve the cancer prognosis and therefore, could be lifesaving 15, 70, 72. Secondly, it can facilitate pre-symptomatic identification of other relatives at risk for malignancies who can
be screened if appropriate. Thirdly, some syndrome-associated malignancies require specific treatment strategies. Fourthly, better knowledge of the underlying syndrome may guide care for non-malignancy manifestations. It might contribute to familial understanding and acceptance and might provide better insight in recurrence risks and prognosis. Lastly, recognizing and increased knowledge of tumor predisposition syndromes will improve our understanding of normal development and the pathophysiology of cancer.

Even though testing for tumor predisposition syndromes can be beneficial and might lead to better care and cure for childhood cancer patients, few ethical considerations should be taken into account when testing children for tumor predisposition. These include the distinction between diagnostic and pre-symptomatic testing in children. Pre-symptomatic testing in children should only be performed when a positive test result will have medical consequences. Genetic testing can have important social and psychological consequences for a child. The genetic counselling of testing for tumor predisposition syndromes should include benefits and harm of testing and should carefully be considered with the parents and child amongst others. Several recommendations have been proposed for the counselling of genetic testing for tumor predisposition syndromes in children. These give guidance to professionals to help parents and children in their process of informed decision making.

Merks et al. showed that half of the tumor predisposition syndromes diagnoses in childhood cancer patients had been missed during routine care, despite intensive pediatric care and follow-up. This illustrates that it can be difficult to diagnose a tumor predisposition syndrome in childhood cancer patients. Many tumor predisposition syndromes have a low incidence, so that not every caretaker is familiar with the phenotype which makes it less likely that the caregiver will recognize the tumor predisposition syndrome. Also, most pediatricians are not specifically trained in performing a morphological examination. Furthermore, in many tumor predisposition syndromes morphological abnormalities and other features can be subtle, so that they are easily missed.

Therefore, we and others recommended that all children diagnosed with a malignancy should be assessed by a clinical geneticist or a pediatrician skilled in clinical morphology. Because in many countries there is limited access to such consultations and genetic consultations can be a low priority in acutely ill patients, a screening instrument could be a reliable aid in assuring that all childhood cancer patients at risk for having a tumour predisposition syndrome can be recognized and referred.

To our knowledge, only two efforts have been done to develop such a screening instrument. Jongmans et al. developed a referral test, to be completed by the pediatrician involved. Based on five criteria, the test determines whether referral for further clinical genetic evaluation is indicated. We developed a screening instrument which consists of a screening form (to be completed by a genetic counsellor or nurse), together with 2D and 3D pictures of the patient. The completed screening instrument can serve as a condensed clinical genetic evaluation of
the patient. Based on the completed screening form and pictures, the clinical geneticist can then select those suspected to have a syndrome for a full genetic consultation in an efficient manner. This guarantees that the possibility of a tumor predisposition syndrome is considered in all childhood cancer patients. The design of our screening instrument as well as the pilot validation are described in part III of this thesis.

1.7 Objective and outline of this thesis

The objectives of this thesis were

1. To identify the structural genomic variants that might underlie the four patterns of co-occurring morphological abnormalities
2. To further delineate the morphological abnormalities in patients from the patterns of co-occurring morphological abnormalities, using 3D photography and determine the added value of 3D photography in characterization of facial morphology
3. To develop a screening instrument for known tumor predisposition syndromes

The patterns of co-occurring morphological abnormalities indicative of new tumor predisposition syndromes are described in Table 1, the establishment of this cohort is explained in Chapter 1.2: Patterns of morphological abnormalities in childhood cancer patients, a selected cohort.

Case illustration of a tumor predisposition syndrome in childhood cancer

Chapter 2 describes a patient with molecularly proven PTEN Hamartoma Tumor Syndrome and Gorham-Stout phenomenon. Gorham-Stout phenomenon has not been reported before in a patient with a constitutional mutation in the PTEN gene.

Part I. Genetic aspects of tumor predisposition syndromes in childhood cancer

In Chapter 3 we describe the structural genome variations (microdeletions and microduplications) in individuals showing the patterns of co-occurring morphological abnormalities indicative of new tumor predisposition syndromes.
Name pattern of co-occurring morphological abnormalities | Morphological abnormalities
---|---
BLEPHAROPHIMOSIS PATTERN (n=10) | ≥2 of the following: Blepharophimosis, Patchy hypopigmentation, Café-au-lait spots, Increased angulation of the spine
ASYMMETRIC LOWER LIMBS PATTERN (n=17) | ≥2 of the following: Asymmetric lower limbs, Tall stature, Ptosis, Hypoplastic malae, Pectus carinatum or pectus excavatum
EPICANTHAL FOLDS PATTERN (n=8) | ≥3 of the following: Epicanthal folds, Flat, broad, nose, Full lips, Prominent ears, Café-au-lait spots, Hyperlaxity of joints
SYDNEY CREASE PATTERN (n=6) | ≥3 of the following: Sydney crease, Full lateral parts of eyelids, Microdontia, Clinodactyly digitus V, Hypermobility of small joints

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 in Chapter 3, 4 and 6 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 10).

**Part II. Phenotypic aspects of tumor predisposition syndromes in childhood cancer**

Originally, we used a UK-Dutch control group in our 3D analyses. In order to test whether the use of a combined UK-Dutch control group was justified or not, in Chapter 4 we use 3D photography to assess differences in face shape between individuals from the UK and the Netherlands. The added value of 3D shape analysis to characterize facial morphology in childhood cancer patients from the four patterns of morphological abnormalities is illustrated in Chapter 5. We compare overall facial dysmorphism and dysmorphism of a
localized malar region between patients and controls. Also, we study differences in facial asymmetry in patients and controls. Chapter 6 reviews the clinical and molecular aspects of syndromes associated with brain tumors in children, thereby facilitating recognition of syndromes in children with a brain tumor and early diagnosis of brain tumours in children with syndromes.

Part III. Screening for tumor predisposition syndromes in childhood cancer

In Chapter 7 the development of a clinical screening instrument for tumor predisposition syndromes in childhood cancer and its first clinical validation is described.

Part IV General discussion

Chapter 8 summarizes the main findings, conclusions, strengths and limitations of the studies in this thesis. Furthermore, recommendations for further studies within the field of tumor predisposition syndromes in childhood cancer are provided.

Acknowledgement

The author wishes to thank professor Douglas Hanahan (Lausanne, Switzerland/San Francisco, USA) and professor Robert Weinberg (Cambridge, USA) for their feedback on Figure 1.
References


64. Farkas, L. Antropometry of the head and face (Raven Press, New York, 1998).


