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für Kinder-Onkologie, Hämatologie und Klinische Immunologie
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Germline Mutations in children with malignancies

Dissertation

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To the little owl, the symbol of wisdom in Ancient Athens

Part of this work has been published:

“Genetic predisposition in children with cancer – affected families' acceptance of Trio-WES”

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Summary

Until recently, the presence of childhood cancer was thought to be an isolated event, of unknown origin associated more with fortune than with an underlying disorder. The idea that a hereditary pattern could be involved in the initiation of malignant diseases arose for the first time through the close clinical observation of two doctors, Li and Fraumeni, in 1969. The genetic proof of this theory came years later through the discovery of the responsible gene, the nowadays well-known TP53. In the last decades, the extended use of whole-genome sequencing techniques and the progress in understanding of the human genome led to a change of our belief about the genesis of malignancies.

The recent theory is that cancer and most of all cancer at the age of childhood could be a result of an existing genetic condition, or better predisposition. It is thought that a considerable percentage of childhood malignancies are due to cancer predisposition syndromes (CPS), though not adequately investigated until now. The ratio of CPSs caused by inherited versus de novo germline mutations is also unknown and, thus, the recurrence risk in siblings. Moreover, it is presumed that the probability of a positive family history or a remarkable personal history can be higher at patients with inherited pre-existing germline mutations.

Through an ongoing prospective study performing a three-generation pedigree, a detailed family and personal history of the patients and whole-exome sequencing (WES) of parent-child trios we tried to identify CPSs and inheritance patterns in newly diagnosed patients in our Duesseldorf Pediatric Oncology Centre. The key question of the presented study was – alongside with testing of an underlying CPS - to investigate the acceptance of a genetic testing regarding cancer predisposition among affected families.

Zusammenfassung

Bis Ende des 20. Jahrhunderts galten Krebserkrankungen des Kindesalters als ein isoliertes Ereignis unklarer Genese. Die Urfrage nach dem Warum konnte ebenso wenig wie das Wiederholungsrisiko für ein Geschwisterkind beantwortet werden. In den letzten Dekaden wurde es jedoch aufgrund von Entwicklungen neuer molekulargenetischer Methoden und deren breitere Verfügbar- und Bezahlbarkeit zunehmend möglich, diese Fragen systematisch zu untersuchen. Dabei spielen das fortschreitende Verständnis des menschlichen Genoms sowie Ganzgenomuntersuchungen wie das „whole-genome sequencing“ (WGS) eine essentielle Rolle.

Die logischste Hypothese für die Genese von Krebserkrankungen des Kindesalters ist ein präexistenter genetischer Schaden – quasi eine angeborene Prädisposition. Solche Konstellationen sind bereits für einzelne Tumoridentitäten beschrieben (beispielsweise das Li-Fraumeni Syndrom) und werden daher zusammengefasst als Tumorprädispositionssyndrome (CPS; cancer predisposition syndrome). Dabei ist der genaue Anteil von CPSs unter den kindlichen Krebserkrankungen noch nicht hinreichend untersucht. Zudem muss selbst bei Entdeckung eines CPS geklärt werden, ob es sich wirklich um einen angeborenen genetischen Defekt handelt oder um eine im Kind entstandene de novo Mutation. Nur mit dieser Charakterisierung lässt sich neben dem Warum auch die Frage des Wiederholungsrisikos beantworten.

In der vorliegenden Arbeit wurde im Rahmen einer prospektiven Studie an der Klinik für Kinder-Onkologie und Hämatologie wurden an krebskranke Kinder und deren Eltern mittels Ganzexomsequenzierung (WES; whole-exome sequencing) untersucht. Zusätzlich erfolgte eine umfangreiche klinische Charakterisierung der Patienten und Familien, bestehend aus einem 3-Generationenstammbaum und einer ausführlichen Familien- und Patientenanamnese. Die erste Frage der Studie war, neben der Quantifizierung von CPSs unter den Krebserkrankungen im Kindersalter, die Akzeptanz dieses Studienansatzes und damit von genetischen Untersuchung in Hinblick auf das Vorliegen einer genetischen Prädisposition bei den Familien zu untersuchen.

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LIST OF ABBREVIATIONS

AACR	American Association of Cancer Research
ALL	Acute lymphoblastic leukemia
AML	Acute myeloblastic leukemia
ART	Assisted reproductive technology
BWS	Beckwith-Wiedemann-Syndrome
CMMRD	Constitutional mismatch repair deficiency
CNS	Central nervous system
CPG	Cancer predisposition gene
CPS	Cancer predisposition syndrome
LFS	Li-Fraumeni Syndrome
MAF	Minor allele frequency
NGS	Next generation sequencing
VUS	Variant of unknown significance
WES	Whole-exome sequencing
WGS	Whole-genome sequencing

1. Introduction

In the literature, there is an extended discussion about the genesis and the initiation mechanisms of malignant diseases. Through both theory and experimental models scientists are trying to enlighten this not fully explained pathophysiology of the creation of a malignant clone.

Already in the mid-18th century Virchow made the observation that the teratocarcinomas showed an astonishing resemblance to the embryonic tissues, introducing for the first time the term of `differentiation´ as a characteristic for malignancies. In 1889, Julius Cohnheim made the suggestion of a rapid multiply rhythm of the malignant cells during an early developmental period `the simplest view appears to me undoubtedly to be that in an early stage of embryonic development more cells are produced than are required for building up the part concerned´. The opinion that `cancer is a problem of developmental biology`, that has to do both with the cells as with the surrounding environment was for the first time expressed from G. Barry Pierce et al. in 1978.

In regard to the theory that cancer represents a deviation in normal development, nowadays it is thought that the majority of malignancies origin from a single cell, transformed through genetic and epigenetic changes that lead to the malignant alteration. The neoplastic clone gathers progressively additional mutations, which accumulatively characterize fully the type of the disease. The majority of the human cancers begin as a result of somatic mutations, that deregulate the cell physiological program. In 1971, Alfred Knudson introduced the 'two-hit' theory for the genesis of retinoblastoma, suggesting that the inactivation of both alleles of a specific gene was necessary for retinoblastoma to occur: `Based upon observations on 48 cases of retinoblastoma and published reports, the hypothesis is developed that retinoblastoma is a cancer caused by two mutational events. In the dominantly inherited form, one mutation is inherited via the germinal cells and the second occurs in somatic cells. In the nonhereditary form, both mutations occur in somatic cells.´ [1]

Analogue to the Knudons hypothesis, there is a percentage of malignancies that arise from genetic changes observed on the germline and not on the somatic line. These types of malignancies share common features; they form the so called `cancer predisposition syndromes´ (CPSs), characterized by multiple and repetitive tumour incidents, with onset typically at young age and occasionally additional morphological abnormalities or concomitant diseases. Nowadays, there are several genes on the germline known to be

pathogenic or probably pathogenic for malignancies. They can be both autosomal or recessive inherited and the majority of the known genes are related with proteins that affect the cell cycle and the cell apoptosis. Nevertheless, the idea of predisposition in the tumorigenesis is quite new and our knowledge about the CPSs and the involved gene-list are expected to expand through the next years.

On year 1969 two doctors, Li and Fraumeni, described for the first time the occurrence of malignant diseases in certain families at an unusual young age. Years later a connection was discovered between the malignancies and a specific germline mutation of the gene TP53. The syndrome was named Li-Fraumeni Syndrome (LFS) after the scientists who first made the clinical observation. LFS is the most known and at the same time most frequent genetic CPS; it is inherited in an autosomal-dominant manner. Characteristic malignancies for the LFS are soft tissue tumors, bone tumors, adrenocortical tumors, leukemia and premenopausal breast cancer. The lifelong risk of developing a malignancy is estimated to be 50% before the age of 30 years and 90% before the age of 60 years. The prevalence of the syndrome is between 1 in 20.000 und 1 in 5.000 habitants.

Since then, further CPS like CMMRD, Lynch-Syndrome, DICER-Syndrome or the Gorlin Syndrome were discovered. [2-4] Additionally, the existence of other genetic conditions is proven to be associated with carcinogenesis in the pediatric population; for example, Trisomy 21, [5, 6] Neurofibromatosis type I, [7] or Noonan Syndrome. [8-10] Even inherited immunodeficiencies, like Ataxia telangiectasia and Nijmegen Breakage Syndrome, present a significant predisposition to the occurrence of malignancies. A high proportion of childhood embryonal cancers, such as retinoblastoma and pleuropulmonary blastoma, are caused by germline mutations in RB1 and DICER1, respectively. [4, 11, 12]

It is currently estimated that, overall, approximately 3% of cancers are the result of germline mutations in CPGs. [13] The proportion of children and adolescents with cancer, which is attributable to an underlying cancer predisposition syndrome (CPS), is still unclear. Moreover, the contribution of genetic inheritance to individual cancers is variable. Recent research indicates that a considerable proportion of childhood cancers are due to CPSs estimated at 8.5% of all pediatric malignancies in general. In this study, 1120 patients younger than 20 years of age were included and genetically sequenced. In total, the DNA sequences of 565 genes were analysed, including 60 that have been associated with autosomal-dominant CPS, for the presence of germline mutations. Mutations that were deemed to be pathogenic or probably pathogenic were identified in 95 patients with cancer (8.5%). The report determined

an incidence of 16.0% in patients with solid tumors, 8.6% with brain tumors, and 3.9% with leukemia. The study initially focused on 23 well-known cancer predisposition genes (CGPs) and genes that predispose to pediatric cancer with a high penetrance. The most commonly mutated genes in the affected patients were TP53, APC, BRCA2, NF1, PMS2, RB1, and RUNX1. [14]

However, - in the era of upcoming high-throughput sequencing - it can be supposed that new CPSs will be discovered and, thus, - presumably - the proportion of affected children and their families will increase within the next decades. In addition, to date, the proportion of CPSs caused by inherited versus de novo germline mutations in CPGs and, thus, the risk of recurrence in other children is up to now unknown. According to estimates, the number of de novo mutations comprise up to 25% in TP53 germline mutations (LFS). Although the field of CPSs is a relative new theme of research, the list of the until now known CPSs is quite long, including both predisposition to solid, as well as liquid tumors. An approach of a schematic and approximate classification of the up to now known CPS is seen on the table 1. The first, most frequent and better described predisposition syndrome is the LFS, predisposing to sarcomas, leukemias (typically characterized from hypodiploidy), brain tumors and breast cancers. After that, numerous new CPSs have been discovered that predispose to hematologic malignancies, neuroendocrine or gastrointestinal tumors. Another group of CPSs is associated with overgrowth disorders and are typically related with nephroblastoma, hepatoblastoma or rhabdomyosarcoma. A particular group of CPSs are the syndromes that are associated with DNA repair deficiencies/immunodeficiencies. These syndromes are characterized usually from a high incidence of malignancies, very often even multiple tumors, but require very careful and fine adjusted treatment. Due to the pathologic DNA repair mechanisms, there is an abnormal high sensitivity to chemotherapy and to irradiation; these patients tend to develop severe complications during the treatment, with prolonged aplasia and severe skin and mucosa toxicity. The early recognition of a DNA repair syndrome has a direct impact and an immediate significance for the patient, it is essential for the choice of the treatment plan and can be lifesaving, preventing from severe side effects. On the other hand, the presence of unexplained toxicity and unusual complications can be a sign of an underlying CPS, suggesting further investigation even in institutes where the CPS screening is not part of the clinical routine.

Table 1: Classification of the Cancer Predisposition Syndromes (CPS) (adapted from Kuhlen [15])

1. Li Fraumeni Syndrome	
2. Overgrowth Disorders and Predisposition to Nephroblastoma, Hepatoblastoma	
Beckwith-Wiedemann syndrome	Bohring-Opitz syndrome
Mulibrey nanism	Perlman syndrome
Trisomy 18	Simpson-Golabi Behmel syndrome
WT1-related syndromes (WAGR, Denys-Drash, Frasier)	Sotos syndrome
3. Neurofibromatosis 1 and 2, Schwannomatosis, Predisposition to other Neural Tumors	
4.Gastrointestinal Cancer Syndromes	
APC-related adenomatous polyposis	*MUTYH*-associated polyposis
Peutz-Jeghers Syndrome	Juvenile Polyposis syndrome
5. Constitutional Mismatch Repair Deficiency	
6. Neuroendocrine Tumors	
Von Hippel Lindau	Hereditary Pheochromocytoma/Paraganglioma syndromes
Multiple Endocrine Neoplasia 1	Multiple Endocrine Neoplasia 2A and 2B
Multiple Endocrine Neoplasia 4	*CDC73*-Related (Hyperparathyroid-Jaw Tumor) syndrome
7. Leukemia Predisposition	
PAX5, CEBPA, ETV6, RUNX1, Robertsonian translocation 15;21, ringchromosome 21, other	
8. DNA Repair Syndromes and Immunodeficiency syndromes	
Ataxia Telangiectasia	Bloom syndrome
Dyskeratosis congenital	Fanconi anemia
Nijmegen breakage syndrome	Xeroderma pigmentosa
9. Rasopathies	
10. Other Disorders	
DICER1 syndrome	

The majority of CPGs encode for proteins that affect the cell cycle, apoptosis and differentiation, up to now 114 CPGs have been identified. The inheritance pattern of cancer predisposition is variable; it is autosomal-dominant for 65 CPGs, autosomal-recessive for 28, X-linked for 4, and Y-linked for 1, the SRY gene located on the Y chromosome and associated with prostate malignancies. This list though, represents only partially the responsible genes and is expected to expand the next years because of the extended use of whole-genome sequencing techniques. Most of the recorded mutations involve loss-of-

function mutations and only 11 predispose to cancer as gain-of-function mutations. A minority of the CPGs cause phenotypes in both monoallelic and biallelic mutation pattern. It is also observed that for some of the CPGs, the recessive condition is a more severe manifestation of the dominant condition. For example, biallelic BRCA2, PALB2, MLH1, MSH2, MSH6, and PMS2 mutation carriers is interpreted clinically with high risk of childhood cancer. On the contrary, for the same genes the heterozygous condition predisposes to cancer predisposition in adulthood. It should also be mentioned that the clinical phenotype and severity depends on the mutation type and location of the affected gene. This is adequately described for LFS, where different mutations on the same TP53 gene may lead to variable predisposition to cancer, influence disease penetrance, cancer site and the risk of secondary malignancies. Around 250 different TP53 germline alterations have been reported, 70% missense mutations and 30% other defects (splicing, frameshift, nonsense, intronic etc). Dominant-negative TP53 missense mutations within the DNA-binding domain are related with poorer prognosis. The location of the up to now known CPGs can be seen on the figure below (Figure 1). Through this `predisposition map´ it can be seen that the CPGs are spread through the genome, detected in each and every chromosome; with no association to a specific chromosome. The genes in red colour account for gain-of-function mutations and as seen consist a minority of CPGs and the genes in blue represent for loss-of-function alterations. as well as the connection to the most common pediatric malignancies (Table 2).[16]

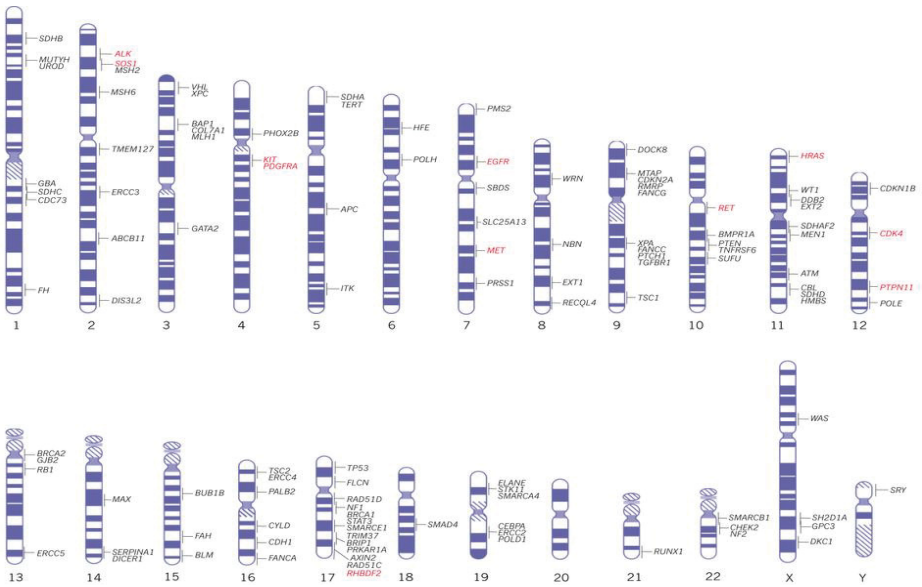
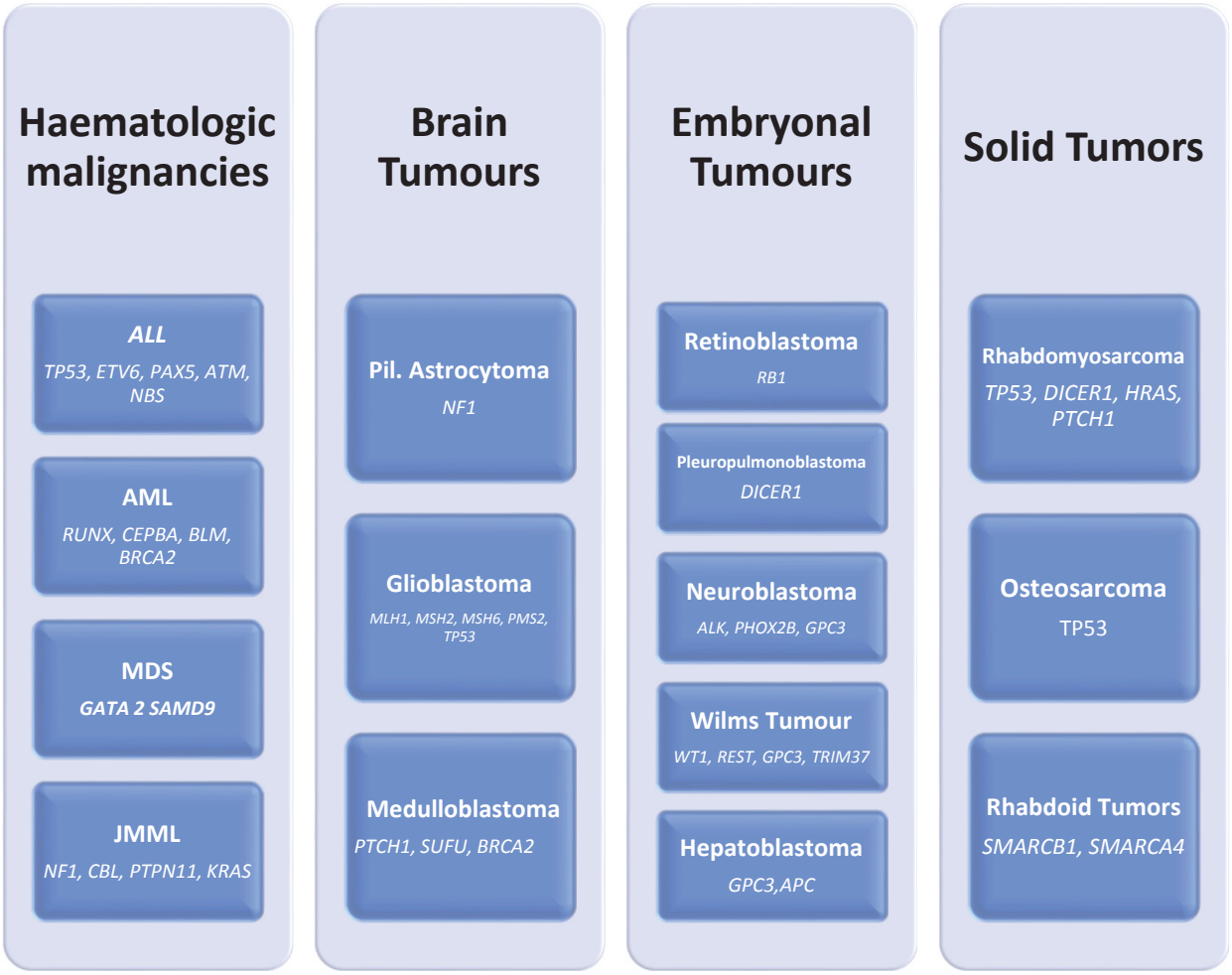


Figure 1: Chromosomal location of 114 Cancer Predisposition Genes (CPGs) taken from [16].

Table 2: Correlation of most common pediatric malignancies to the known CPGs



Although the diagnosis of a CPS can only be secured through genetical analysis, the indication for a further testing or better the suspicion of a genetical predisposition is mainly a clinical decision. On year 2016 Jongmans et al. published a useful tool for the recognition of CPS in childhood based only on clinical criteria. This decision is based on 5 topics: family history, type of malignancy, the presence of ≥2 malignancies, excessive toxicity to chemotherapy and the presence of concomitant congenital or other anomalies (Table 3, taken from Jongmans et al)[17].

Table 3: Clinical tool for the recognition of CPS [17]

Childhood cancer, indication for referral to a clinical geneticist?

If your patient fulfills one or more of the criteria mentioned below (one or more circles filled), he or she may benefit from referral to a clinical geneticist.

1. Family history of the child with cancer

- ○ ≥ 2 malignancies at childhood age (≤ 18 years of age)
- ○ a first degree relative (parent or sibling) with cancer < 45 years of age
- ○ ≥ 2 second degree relatives with cancer < 45 years of age on the same side of the family
- ○ the parents of the child with cancer are related, i.e. consangious

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2. A person with one of these tumors in childhood

- ○ Adrenocortical carcinoma
- ○ Atypical teratoid rhabdoid tumor
- ○ Cerebellar gangliocytoma
- ○ Choroid plexus carcinoma
- ○ Endolymphatic sac tumors
- ○ Hemangioblastoma
- ○ Hepatoblastoma
- ○ JMML
- ○ Low hypodiploid ALL
- ○ Malignant peripheral nerve sheath tumor
- ○ Medullary thyroid carcinoma
- ○ Medulloblastoma
- ○ Optic glioma
- ○ Ovarian sertoli-leydig cell tumor
- ○ Pleuropulmonary blastoma
- ○ Pituitary blastoma
- ○ Pineoblastoma
- ○ Retinoblastoma
- ○ Schwannoma
- ○ Subependymal giant cell tumor

Or ○ A cancer of adult age, i.e. colorectal cancer, ovarian cancer, basal cell carcinoma etc.

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3. ○ **A child with two malignancies** one of those with onset < 18 years of age (unless the 2nd malignancy is consistent in time and/or tissue type with these expected from their treatment regimen).

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4. ○ **A child with cancer and congenital anomalies or other specific symptoms**

Sign	***Think of***
Congenital anomalies	Organs, bones, oral clefting, teeth, eyes, ears, brain, urogenital anomalies, etc.
Facial dysmorphisms	
Intellectual disability	
Aberrant growth	Length, head circumference, birth weight, asymmetric growth
Skin anomalies	Aberrant pigmentation i.e. > 2 café-au-lait spots, vascular skin changes, hypersensitivity for sunlight, multiple benign tumors of the skin
Hematological disorders	Pancytopenia, anemia, thrombocytopenia, neutropenia
Immune deficiency	

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5. ○ **A child with excessive treatment toxicity**

Up to now statistically the malignancies associated to germline mutations - and in this way also to CPGs - account only for a minority of the cancer pathology in general. For the adult oncology this statement is an undisputable issue, but in pediatric oncology the role of germline mutations in the pathogenesis of cancer seems to be underestimated. This hypothesis

is based on a simple rational fact: `Alone the development of a malignancy during the childhood - independent of the existence or not of other co-factors - is a potent sign of a yet unrevealed cancer predisposition or underlying condition.´ In order to understand the role of predisposition in malignancies during childhood it is necessary to collect thorough clinical information from each patient, including a detailed family history, as well as complete genetic data through DNA sequencing. The combination and correlation of both clinical and germline genetic information in large patient cohorts can enlighten our knowledge about the genesis of cancer in childhood.

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CORRECTION

Correction to: Genetic predisposition in children with cancer – affected families' acceptance of Trio-WES

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The original version of this article, unfortunately, contained an error.

The reference citations in the original paper are incorrectly cited to its corresponding bibliographic information.

The original article was corrected.

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ORIGINAL ARTICLE

Genetic predisposition in children with cancer – affected families' acceptance of Trio-WES

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Abstract A considerable percentage of childhood cancers are due to cancer predisposition syndromes (CPS). The ratio of CPSs caused by inherited versus de novo germline mutations and the risk of recurrence in other children are unknown. We initiated a prospective study performing whole-exome sequencing (WES) of parent-child trios in children newly diagnosed with cancer. We initially aimed to determine the interest in and acceptance of trio WES among affected families and to systematically collect demographic, medical, and family history data to analyze whether these point to an underlying CPS. Between January 2015 and December 2016, 83 (88.3%) of 94 families participated; only 11 (11.7%) refused to participate. Five (6.0%) children presented with congenital malignancies and three (3.6%) with tumors with a high likelihood of an underlying CPS. Two (2.5%) families showed malignancies in family members < 18 years, 11 (13.8%) showed relatives < 45 years with cancer, 37 (46.3%) had a positive cancer history, and 14 (17.5%) families had > 1 relative with cancer.

Conclusions: Genetic testing in pediatric oncology is of great interest to the families, and the vast majority opts for investigation into potentially underlying CPSs. Trio sequencing provides unique insights into CPS in pediatric cancers and is increasingly becoming a common approach in modern oncology, and thus, trio sequencing needs also to be integrated routinely into the practice of pediatric oncology.

What is Known:
- *A considerable percentage of childhood cancers are due to cancer predisposition syndromes (CPS).*

What is New:
- *Knowing about an underlying CPS and, thus, the risk of recurrence in other children is of great interest to affected families.*

Keywords Cancer predisposition syndrome · Children · Trio · Whole-exome sequencing

Abbreviations

AACR	Association of Cancer Research
ART	Assisted reproductive technologies

Arndt Borkhardt and Michaela Kuhlen contributed equally to this article.

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BWS	Beckwith-Wiedemann syndrome
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CPG	Cancer predisposition gene
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LFS	Li-Fraumeni syndrome
MAF	Minor allele frequency
NGS	Next generation sequencing
VUS	Variant of unknown significance
WES	Whole-exome sequencing

Introduction

The proportion of children and adolescents with cancer attributable to an underlying cancer predisposition syndrome (CPS) is still unclear. Recent research studies indicate that a considerable percentage of childhood cancers are due to CPSs (16.7% of non-central nervous system (CNS) solid tumors, 8.6% of CNS tumors, and 4.4% of leukemias) [20]. However, in the era of high-throughput sequencing, it might be supposed that new CPSs will be discovered and, thus, the identification of affected children and their families will presumably increase within the next decade [9]. In addition, the ratio of CPSs caused by inherited versus de novo germline mutations in cancer predisposition genes (CPGs) and, thus, the risk of recurrence in other children is almost completely unknown so far. For example, the number of inherited TP53 germline mutations causing Li-Fraumeni syndrome (LFS) is estimated to be as high as 75% [1].

Indeed, mutations in CPGs involved in the DNA repair machinery, including mismatch and double-strand break repair, might have immediate implications on clinical decisions. For instance, LFS patients are highly susceptible to radiation-induced tumorigenesis and alkylating chemotherapy and, thus, have an increased risk of developing secondary cancers [5, 7].

Whole-exome sequencing (WES) of parent-child trios has become a popular strategy to identify causative genetic variants in children with rare diseases [4, 10, 21]. However, it has not been routinely implemented in pediatric oncology as yet. A number of reports on children developing metachronous tumors and families with familial clustering of malignancies suggest that trio sequencing in pediatric oncology can identify underlying CPSs [3, 6, 14].

Consequently, we initiated a monocentric prospective study on CPSs in a cohort of children and adolescents with a newly diagnosed malignancy by trio sequencing of the affected children and their parents. The main objectives of our study were first, to determine the interest in and acceptance of comprehensive clinical and molecular genetic evaluation in a pediatric oncology and hematology department; second, to systematically collect detailed demographic, medical, and family history data from this pediatric cancer cohort to analyze whether these data point to underlying CPSs; and third, to assess the proportion of children affected by either a well-known or suspected underlying CPS including the distribution pattern of contributing CPGs.

Patients and methods

Since January 1st, 2015, an ongoing research study titled "Germline mutations in children with cancer" has been prospectively evaluating children and their parents by WES to test for underlying CPSs. All children (aged 0–18 years) with any newly diagnosed malignancy who were treated at the Department of Pediatric Oncology, Hematology and Clinical Immunology of the University Children's Hospital and their parents were eligible. Families whose children died before the informed consent process was completed were excluded from this analysis. No other exclusion criteria were defined.

Informed consent process

Informed consent was obtained in a multi-step process. In an initial conversation, the child (wherever possible) and the child's parents were informed about the diagnosis and the study by being provided with the study information. In a second step, a few days after the diagnosis, the family was notified about the study aims, benefits, and risks in more detail, including implications for the patient and the entire family, the possibility of incidental findings and variants of unknown significance (VUS), options and preferences regarding how results should be reported, and their "right not to know." In a third step, remaining questions were addressed and written informed consent was obtained. If the family was still undecided about participating, they were given more time for consideration. Pre-test counseling was provided by a pediatrician with a certificate in genetic counseling for genetic testing in pediatrics, as stipulated in the German gene diagnostic law. In cases where the family did not speak sufficient German, the informed consent process was performed with the help of a professional translator.

Medical history and three-generation pedigree

Demographic data and the child's medical history of previous malignancies and pre-existing conditions were collected through means of a standardized in-depth interview by a pediatrician (Supplement). This interview collected information regarding pregnancy, delivery, postnatal adaptation, development during early childhood, congenital anomalies, and other specific symptoms. As references for the comparison of birth data and data on assisted conception, the Annual Report (2016) of the Federal Statistical Office (Destatis) and the

Annual Book (2015) of the German IVF Registry (DIR) were used.

All patients were thoroughly examined by a pediatrician, with particular attention to congenital anomalies and signs or conditions suggesting an underlying syndrome. Additionally, information on tumor/leukemia features pointing to an underlying germline defect was recorded and excessive toxicity to cancer therapy was prospectively evaluated.

Three-generation pedigrees (patient, parents, grandparents, siblings, uncles, and aunts) were constructed for each participating family, including information on birth date, deceased, age at and cause of death, symptoms.

DNA for WES analysis was extracted pre-therapeutically either from peripheral blood in patients with solid tumors or from skin biopsies using fibroblasts in patients with leukemia or lymphoma. Peripheral-blood-derived DNA from the parents was used for WES.

A bioinformatic pipeline was established based on data analysis published by the St. Judes study group and a constantly updated gene list currently comprising 2224 genes including the 565 known cancer-predisposing genes, which were summarized by Zhang et al. [20]. To identify only relevant single nucleotide variants (SNVs) by WES, we defined the following analysis criteria: (1) a high-quality DNA sequencing coverage of ≥ 250-fold (to additionally identify parental mosaicism); (2) variants with a minor allele frequency (MAF) below 10%; (3) SNVs in any of the 2224 genes of the cancer gene list with non-synonymous coding changes; (4) in silico prediction tools (SIFT and PolyPhen) considering the identified variant as (probably/possibly) damaging or deleterious for protein function; and (5) a CADD (combined annotation-dependent depletion) [8] score > 10. An overview of the bioinformatic pipeline is given in Fig. 1.

For this analysis, the data of patients enrolled between 1 January 2015, and 31 December 2016, were examined. The study was approved by the Ethics Committee of Heinrich Heine University, Duesseldorf, Germany (study number 4886).

Results

Between 1 January 2015, and 31 December 2016, 94 families of children and adolescents with a newly diagnosed malignancy were asked to participate in the study. Of these, 83 (88.3%) families agreed to participate in the study and 11 (11.7%) families refused to participate. Reasons for refusal were fear of the results in six cases (four of them with a positive cancer history in the family), uncertainty and mental overload in three families, and cultural objections in two families (none of them with a positive familial cancer history). In one of these cases, the adolescent patient refused to participate (due to fear) while both parents wanted to participate.

In 11 families, only one parent was available, due to either a lack of contact information (nine cases) or one parent having already died (two cases, both due to cancer). In one family, consent to participate in the study was given by both parents for the child but one parent (with a highly suspicious familial cancer history) refused to be tested him-/herself. Thus, in these cases, only duo sequencing was feasible. Details on recruitment and refusal are depicted in Fig. 2.

Patient characteristics and medical history

Demographic characteristics of participating families are listed in detail in Table 1. The mean number of children per family was 2.4 (range 1–6).

In 80 (96.4%) of 83 families, complete details on medical history including birth date, cause of death, and, in the case of a positive cancer history, also the type of cancer were available for further analysis in a three-generation pedigree. In addition to the information provided by the families, in some cases we also asked for and received medical records of the affected family members. Of these 80 children, three (3.8%) children's parents reported the use of assisted reproductive technologies (ART), three (3.8%) children presented with congenital heart defects, four (5.0%) children with café-au-lait spots, six (7.5%) children with pre-existing conditions (Asperger's syndrome, attention deficit hyperactivity disorder, depression, strabismus, splenic cyst, and hemangioma), and two (2.5%) children with a history of a previous malignancy (further details are given in Table 2).

Comparing these data with data from the Federal Statistical Office and the German IVF Registry, no differences were observed in parental age, prematurity, or the number of reported ARTs used [16].

Of 83 children, most were diagnosed with leukemias (28, 33.7%) and brain tumors (19, 22.9%) (Fig. 3a). Three (3.6%) presented with tumors including hypodiploid ALL, plexus carcinoma, and pleuropulmonary blastoma (PPB) with a high likelihood of an underlying germline defect. Six (7.2%) children developed excessive toxicity (grade 4 mucositis, neurotoxicity, veno-occlusive disease, hyperammonemia, and grade 5 respiratory failure) to cancer therapy, which was either particularly long-lasting or developed in a therapy regimen normally not associated with that kind of toxicity according to expert clinical experience. In two of these children, a CPS was subsequently diagnosed. Five (6.0%) children presented with congenital leukemias or tumors (Table 3).

Three-generation pedigree

Three-generation pedigrees revealed malignancies in family members under the age of 18 years in two (2.5%) of 80

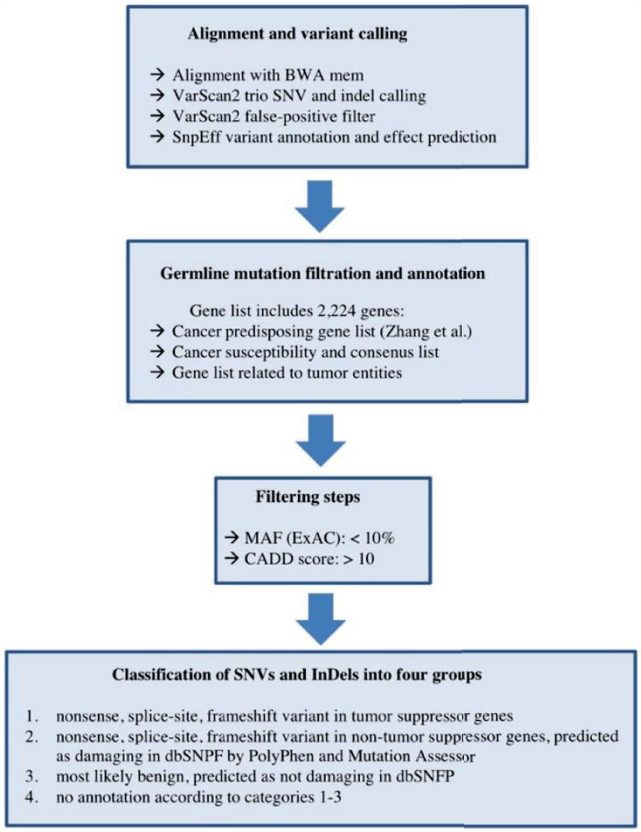

Fig. 1 Overview of the bioinformatic pipeline

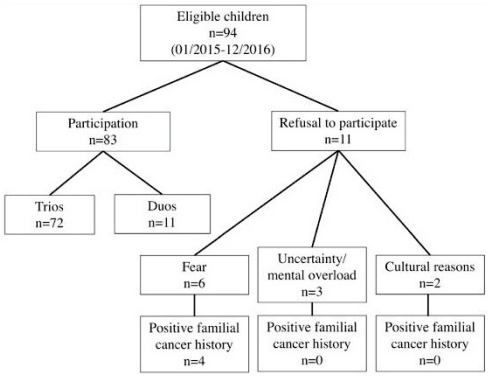

Fig. 2 Overview on participation and refusal reasons of families with children with a newly diagnosed malignancy (n = 94)

families, relatives with cancer before the age of 45 years in 11 (13.8%) families, any cancer history in 37 (46.3%) families, and more than one relative with cancer in 14 (17.5%) families

Table 1 Demographic characteristics of participating patients and their families (n = 83)

Gender	
Male	54 (65.1%)
Female	29 (34.9%)
Age at onset in years, median (range)	6.0 (birth–18.0 years)
Parental age in years, median (range)	
Father	34.3 (20.2–50.3)
Mother	29.9 (18.0–48.8)
Siblings, median (range)	
None	23 (27.7%)
1–2	47 (56.6%)
≥ 3	13 (15.7%)

Table 2 Details on medical history of the patients (n = 80)

Assisted reproductive technology	
No	76 (95.0%)
Hormonal	1 (1.3%)
IVF/ICSI	3 (3.8%)
Abnormalities during pregnancy	
No	62 (77.5%)
Yes	18 (22.5%)
Small for gestational age	
No	75 (93.8%)
Yes	5 (6.3%)
Prematurely born	
No	72 (90.0%)
Yes	8 (10.0%)
Postpartal adaptation	
Regular	70 (87.5%)
Remarkable	10 (12.5%)
Development in early childhood	
Regular	70 (87.5%)
Remarkable	7 (8.8%)
Not applicable	3 (3.8%)
Congenital anomalies	
No	73 (91.3%)
Yes	7 (8.8%)
Pre-existing conditions other than congenital anomalies	
No	74 (92.5%)
Yes	6 (7.5%)
History of previous malignancies	
No	78 (97.5%)
Yes	2 (2.5%)

(Table 4). No parents were consanguineous. More precisely, first- or second-degree relatives presented with (mostly premenopausal) breast cancer (including one father) in 16 (20.0%) families, sarcoma in four (5.0%) families, leukemia/lymphoma in four (5.0%) families, and colon cancer in ten (12.5%) families. An overview of cancer diagnoses in first- or second-degree relatives is given in Fig. 3b.

Reporting of results

In case of an underlying CPS, validation of the identified mutation was carried out by Sanger sequencing before the results were reported to the families. To exclude sample swap, confirmation of the mutation was performed using a second peripheral blood sample by Sanger sequencing.

The treating pediatrician told the parents when the results were ready. If the parents wanted to know the results, a member of the study team met with the parents and, wherever possible, with the child to explain and discuss the results. In total, 82 (98.8%) of 83 families wanted to be informed about

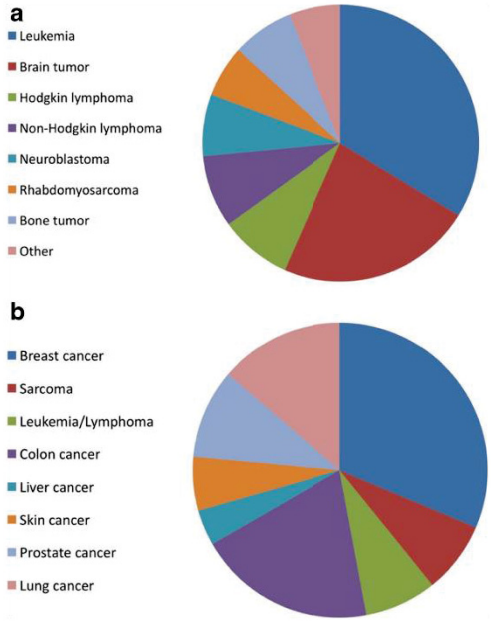

Fig. 3 **a** Overview of the diagnoses of children with cancer enrolled in the study (n = 83). **b** Overview of cancer diagnoses in first- or second-degree relatives. Same cancer entities were counted just once per family

the results, and none of them changed their minds when the results were available. Due to a positive family history and fear, one family did not want to be informed. When a CPS was identified, an appointment with a genetic counselor was recommended to the parents. If a CPS was then diagnosed, the affected child was integrated into a cancer surveillance program either according to published recommendations (for LFS) or individually conceptualized and subsequently adapted according to the recommendations of the Cancer Predisposition Workshop of the American Association of

Table 3 Tumor specifics and cancer therapy tolerance in participating children and adolescents (n = 83)

Congenital tumor	
No	78 (94.0%)
Yes	5 (6.0%)
Tumor with high likelihood of germline defect	
No	79 (95.2%)
Yes	3 (3.6%)
Not applicable	1 (1.2%)
Excessive toxicity to cancer therapy	
No	74 (89.2%)
Yes	6 (7.2%)
Not applicable	3 (3.6%)

Table 4 Details on three-generation pedigree ($n = 80$)

Malignancies in family members under the age of 18 years	2 (2.5%)
Relatives with cancer > 18–45 years of age	11 (13.8%)
≥ 2 first- or second-degree relatives in the same parental lineage with cancer under the age of 45 years	3 (3.8%)
Any cancer history	37 (46.3%)
More than 1 relative with cancer	14 (17.5%)
Deaths due to cancer	22 (27.5%)

Cancer research (AACR) [11, 12]. Examples of children in whom diagnosis of a CPS led to adaptation of cancer therapy and/or inclusion in a cancer surveillance program, respectively, are given in Table 5.

As examples, brief descriptions of three families are given.

Case #1: The 8-month-old boy was diagnosed with plexus carcinoma, a rare tumor with a high likelihood of an underlying germline defect. The parents did not have other children. The family history was highly suggestive of an existing CPS, including three second-degree relatives with osteosarcoma diagnosed under the age of 45 years and premenopausal breast cancer in the paternal lineage. However, genetic counseling had not been performed so far. Trio WES analysis confirmed a heterozygous germline mutation in TP53 (p. Gly245Ser, c.733G>A), suggesting LFS, which is transmitted by the father and predicted to be pathogenic and disease causing. Response to international treatment recommendations (CPT SIOP 2009 including vincristine, cyclophosphamide, etoposide, doxorubicine, cisplatin, and actinomycin) was poor. Thus, normal radiotherapy was indicated. Due to an underlying LFS, an individual treatment concept omitting radiotherapy was created, which was based on drug resistance testing of the tumor (including bortezomib) and high-dose chemotherapy (including thiotepa, carboplatin, and etoposide) with autologous stem cell transplantation. On day + 120, the boy is well without evidence of relapse.

Case #2: The 13-month-old girl presented with desmoplastic medulloblastoma and skin features reminiscent of constitutional mismatch repair deficiency (CMMRD). The non-consanguineous parents (both under the age of 30 years) and an older sister were healthy, as were three generations of the family. Two inherited homozygous VUS of *MSH2* (c.274C>G, p.Leu92Val) and *MSH6* (c.2426_2428delTAG, p.Val809del) were identified by WES and, thus, further raised the suspicion of CMMRD. As a differential diagnosis, germline mutations in *POLD1* and *POLE* were ruled out [19]. Tumor microsatellite instability testing and immunohistochemistry analysis were inconclusive. Therefore, in collaboration with the CMMRD consortium [18], functional analyses were initiated to confirm diagnosis of CMMRD (Fremerey et al. submitted). The girl was treated according to the HIT guidance protocol without radiotherapy due to her young age. Two years onwards, the girl is well and in radiological complete remission.

Case #3: The 10.5-year-old boy was diagnosed with periosteal osteosarcoma. His medical history was remarkable, with embryonal rhabdomyosarcoma of the thoracic wall at the age of 1.75 years. At that time, treatment comprised alkylating agents but no radiotherapy. His parents and dizygotic twin brother were healthy. An uncle in the paternal lineage was deceased due to cancer (further details were not available). Applying the abovementioned criteria for single nucleotide variants (SNVs) analysis, we did not detect any SNV fulfilling these conditions.

Discussion

Here, we evaluated the interest in and acceptance of comprehensive clinical and molecular genetic screening for an

Table 5 Examples of adaptation of cancer therapy and/or inclusion in a cancer surveillance program after diagnosis of a CPS by trio WES

CPS and type of cancer	Adaptation of cancer therapy	Cancer surveillance program according to
Li-Fraumeni syndrome in hypodiploid ALL	Omission of cranial irradiation; instead, administration of additional intrathecal chemotherapy	Kratz et al. Clin Canc Res (2017)
Li-Fraumeni syndrome in plexus carcinoma	Omission of cranial irradiation; instead, high-dose chemotherapy with autologous stem cell transplantation	Kratz et al. Clin Canc Res (2017)
Constitutional mismatch repair deficiency in medulloblastoma	None	Tabori et al. Clin Canc Res (2017)
Dicer syndrome in pleuropulmonary blastoma	None	Schultz et al. Clin Canc Res (2017)
Gorlin syndrome	None	Schultz et al. Clin Canc Res (2017)
Neurofibromatosis type I in glioma	Omission of cranial irradiation; instead, systemic chemotherapy	Evans et al. Clin Canc Res (2017)

underlying CPS, including trio whole-exome sequencing of parents with a child diagnosed with cancer. Our data suggest that knowledge of an underlying CPS is of great interest to the families in our sample and that the vast majority of parents do not claim their right not to know. Instead, most families participated immediately, as they hoped to find a reasonable explanation for why their child had been struck with such an extraordinarily rare event in childhood and to learn about the risk of recurrence in their other children.

Whenever next generation sequencing (NGS) is initiated, according to the gene diagnostic law, the treating physician is obligated to discuss the full range of benefits, risks, and alternatives of this particular genetic test including the potential to reveal gene abnormalities related to other disorders. However, disclosing the diagnosis of cancer is overwhelming and dramatically limits the child's/parents' receptivity. Consequently, in the daily clinical routine of pediatric oncology, this leads to the imperative necessity for a time-consuming multi-step process as depicted above. Indeed, the decision-making process of the families sometimes takes a few months. In our sample, only a minority of families exercised their "right not to know". These were either families with a highly suspicious familial cancer history or families who refused to participate due to their cultural background. This is in line with the empirical expert knowledge that parents frequently ask whether their other children have an increased risk of developing cancer.

Identifying children with a hereditary CPS by trio WES has far-reaching consequences that extend beyond providing cancer care for the child. Close and more distant relatives might likewise be affected despite being young and as-yet asymptomatic. Disclosing a hereditary CPS in these relatives might be clinically relevant and even lifesaving on the one hand, as it provides the excellent possibility to initiate early cancer surveillance programs [11]. On the other hand, it constitutes an enormous life-long burden of knowledge and might deeply affect quality of life and family planning. In this context, the potential advantages and drawbacks as well as personal autonomy regarding the "right not to know" must be discussed in detail before initiating trio WES. An appointment with a genetic counselor was strongly recommended to families in which an underlying CPS was diagnosed. However, a discussion about further genetic testing of additional family members must consider that, in contrast to standards for genetic testing in adults, predictive testing in children is recommended only when the disease is associated with childhood onset, and only with available effective screening and/or intervention options [2, 13]. Refraining from predictive testing in childhood allows the child to make this decision autonomously when reaching adulthood. As one child had already developed cancer, disease onset during childhood is given in all families. Nevertheless, cancer surveillance programs which are advantageous to survival exist only for LFS to date, but still need to be established and proved to be beneficial for other CPS.

We could not identify features in pregnancy, delivery, congenital anomalies, postnatal adaptation, or development during early childhood that pointed towards an underlying CPS. Moreover, neither parental age nor ART seems to be associated with an increased cancer risk in our study cohort. This is in line with previous findings that children born after ART are not at increased cancer risk [15, 17].

However, 3–8% of the children presented with suggestive clinical features (e.g., café-au-lait spots), tumors with a high likelihood of an underlying germline defect, or excessive toxicity to cancer therapy. In addition, in a remarkable number of families, the three-generation pedigree revealed a highly suggestive family history. Thus, although this is an observational study with respective limitations, our preliminary findings demonstrate that a thorough clinical examination and in-depth family history might point towards an underlying CPS, which is in contrast to previous findings by Zhang et al. [20].

However, a highly suggestive medical history, such as one including metachronous tumors, or an unremarkable family history could both be misleading. The latter is of particular importance, as the number of de novo TP53 germline mutations causing LFS is estimated to be as high as 25% [1]. Notably, the proportion of CPSs caused by de novo germline mutations in other CPGs is so far completely unknown.

Thus, our study provides a highly valuable resource to determine the type, frequency, and the de novo mutation rate of CPSs in a cohort of newly diagnosed pediatric cancer patients and may eventually identify novel CPSs in the future.

Conclusions

In pediatric oncology, testing for an underlying CPS seems to be more important to the affected families than exercising their right of not knowing. In order to gain better insights into the ratio between inherited risk alleles found throughout the family and acquired de novo mutations, trio sequencing needs to be integrated routinely into the practice of pediatric oncology

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Authors' contributions TB collected the data and first drafted the manuscript. JT assisted in data management, was responsible for sample management, and revised the manuscript for important intellectual content. EV helped in collecting the data and assisted in data management. MD contributed to the WES analysis. DW contributed to genetic

counseling. AB designed and supervised the project and critically reviewed and revised the manuscript for important intellectual content. MK designed and supervised the project, supervised data collection and analysis, and first drafted the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Compliance with ethical standards The study was approved by the Ethics Committee of Heinrich Heine University, Duesseldorf, Germany (study number 4886).

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent Informed consent was obtained from the parents and legal guardians respectively.

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3. Discussion

a. Acceptance of the study

In our study, we observed an unexpectedly high interest of participation of the affected families (83 out of 94 families, 88%). This acceptance aroused from two main reasons: a) firstly, almost all families wanted to find an explanation, a possible cause of the disease and b) secondly, the families wanted to know the risk of re-occurrence in siblings; the participation at the study was considered as a kind of prevention-act for the rest of the family. The reason of denial was often fear, arousing from a positive family history or insecurity and overwhelming feelings about the situation in general. Moreover, most of the families submitted their consent to the genetic test very soon after the discussion with the study personnel, also indicative of the great acceptance, with almost no hesitations. As to the acceptance of the results the majority of the families wanted to be informed immediately in case of an underlying CPS. In total, 82 (98.8%) of 83 families wanted to find out the results and be advised, and none of them changed their minds when the results were available. Due to a positive family history and fear, one family did not want to be informed. Past studies investigating the psychological impact of LFS in affected individuals and families showed that although the families are, as expected, exposed to a significant psychological burden due to disease and loss experience, the information about the disease and the attachment to a screening program fulfilled the families with a sense of confidence, safety and empowerment. [18, 19] An augmentation of genetic testing in CPS is expected to rise further along with the progress of preventive measures and the discovery of new effective treatment possibilities. It is argued that, if little can be offered by way of prevention, few will want to know their risk status.

The impression of the up to date data of the participants is that complications during pregnancy, delivery, postnatal adaptation and development during early childhood are not associated with a CPS. Moreover, parental age at conception and ART seem also to be irrelevant, as already suggested from previous studies.

The 3-generation pedigree revealed any cancer history in about half of the patients (37; 46.3%), and more than one relative with cancer in 14 (17.5%) families. As to relevant clinical signs, 3-8% of the children presented with syndrome associated clinical features (e.g. café-au-

lait spots), tumors with a high likelihood of an underlying germline defect, or excessive toxicity to cancer therapy.

As a conclusion, although the application of genetic analysis with Next Generation Sequencing (NGS) is related with some skepticism, in our study simply having a better understanding of why cancer occurred was highly valued from the family perspective.

b. CPS detection and personalized medicine

The identification of CPGs has both a substantial impact on the recognition of tumorigenesis patterns and a significant clinical utility. Indeed, such a discovery can transform medical care in multiple levels, including future cancer prevention, as well as the planning and adjustment of the current treatment. The benefits of determining if a cancer is due to a CPS are incontrovertible. During the treatment the diagnosis of a CPS can lead to treatment modification and a so called more `personalized medicine´. For example, vismodegib, a hedgehog pathway inhibitor, has shown responses in basal-cell nevus syndrome patients with PTCH1 mutations´. [20] On the contrary, temozolomid a front line chemotherapeutic drug for the intracranial high-grade gliomas is unlikely to be of benefit and may actually promote neoplastic progression in MSH6 mutation carriers. There is also growing evidence on the effectivity of immunotherapies, for example checkpoint inhibitors for the treatment of CMMRD-related tumors. [21] For the LFS patients the avoidance or replacement of the radiotherapy is of high importance and lead to a significant life prolongation. Preclinical data suggest that TP53 mutations enhance radiosensitivity in vitro and in vivo and the few clinical observations showed that Li-Fraumeni families were at a higher risk of secondary radio-induced malignancies. [22, 23] Nowadays, the most promising therapeutic approach is that of synthetic lethality, applying PARP inhibitors to destroy tumour cells deficient in double strand break repair by homologous recombination, such as cells mutated for the breast cancer early onset genes BRCA1 or BRCA2. Several trials have provided proof of principle in achieving synthetic lethality of PARP inhibition in the setting of BRCA deficiency in human cancer. Currently, phase III clinical trials are in progress for the treatment of breast and ovarian cancers with BRCA mutations and the PARP inhibitor olaparib has been approved for advanced ovarian cancers with germline BRCA mutation. [24-27] Additionally, management

of non-cancer associated problems can also be important, for example certain WT1 mutations could result in insidious renal dysfunction which requires monitoring and early intervention.

c. Screening and prevention after CPS diagnosis

The benefits of CPS detection are not limited to a treatment modification. The knowledge of an underlying germline defect can lead to individualized and risk-adapted screening protocols for the early detection of further malignancies or abnormalities. The prevention of future malignancies can be achieved according to the CPS on one hand with strict surveillance programs or at some cases more radically with surgical removal of the at-risk tissue, as in the case of thyroid in RET mutation carriers. [28, 29] the colon in APC carriers [30] and Lynch syndrome patients [31], or with bilateral mastectomy in BRCA1 and BRCA2 mutation carriers. [32-34] There are not definite recommendations for every known CPS regarding the type and the frequency of control, so that the aftercare program can vary from institution to institution. Despite the rarity of the CPSs though there is an ongoing effort to standardize the surveillance program, for example for the Gorlin Syndrome surveillance MRI in the first years of life, regular dermatologic examinations lifelong and sun protection are recommended. [35] For the LFS carriers that account for the majority of the affected CPS patients, very clear guidelines for both childhood and adulthood were created, that include a combination of physical examination, blood tests and imaging, based on clinical data from multiple studies. [36-38] The current recommendations can be seen in detail in Table 4 and consist a modification of the Toronto protocol. The importance of attaching to a screening program for the survival of the LFS patients was proven through an 11-year study in 3 different oncologic centers comparing two groups of LFS carriers, LFS carriers undergoing a surveillance program according to the Toronto protocol and LFS carriers following no screening measures. An improved overall survival (OS) was observed in individuals undergoing surveillance: 5-year OS 88.8% versus 59.6% (surveillance vs. no surveillance groups). [39]

Table 4: Recommended LFS screening protocol (based on the Toronto Protocol, with modifications) [40]

Children (birth to age 18 years)
General assessment
• Complete physical examination every 3–4 months, including blood pressure, anthropometric measurements plotted on a growth curve (with particular attention to rapid acceleration in weight or height), Cushingoid appearance, signs of virilization (pubic hair, axillary moisture, adult body odor, androgenic hair loss, clitoromegaly, or penile growth), and full neurologic assessment
• Prompt assessment with primary care physician for any medical concerns
ACC
• US of abdomen and pelvis every 3–4 months
• In case of unsatisfactory US, blood tests[a, b] may be performed every 3–4 months: total testosterone, dehydroepiandrosterone sulfate, and androstenedione
Brain tumor
• Annual brain MRI (first MRI with contrast; thereafter without contrast if previous MRI normal and no new abnormality)
Soft tissue and bone sarcoma
• Annual WBMRI
Adults
General assessment
• Complete physical examination every 6 months
• Prompt assessment with primary care physician for any medical concerns
Breast cancer
• Breast awareness (age 18 years onward)
• Clinical breast examination twice a year (age 20 years onward)
• Annual breast MRI screening (ages 20–75)
• Consider risk-reducing bilateral mastectomy

Brain tumor (age 18 years onward)
• Annual brain MRI (first MRI with contrast; thereafter without contrast if previous MRI normal)
Soft tissue and bone sarcoma (age 18 years onward)
• Annual WBMRI[c]
• US of abdomen and pelvis every 12 months
Gastrointestinal cancer (age 25 years onward)
• Upper endoscopy and colonoscopy every 2–5 years
Melanoma (age 18 years onward)
• Annual dermatologic examination

d. Study Limitations in research and clinical practice

Our study was the first prospective study in the field of germline cancer predisposition that combined detailed clinical data - including a 3-generation pedigree - and genetic data from WES of parents and affected children. Therefore, the information and the observations derived enriched our knowledge of the tumorigenesis and the role of germline mutations in malignancies of the childhood. Nevertheless, it is worth mentioning that such a study has, by definition, some limitations that mainly originate from the rarity of the malignant diseases in childhood and the up to now insufficient knowledge about the CPGs and their clinical consequences. Another issue is the correct evaluation of the genetic findings, which we tried to overcome by classifying as CPS only the mutations that are damaging or probably damaging and ignoring the more ambiguous mutations, with the risk, of course, of false negative genetic results. It is also increasingly apparent that many other mechanisms are likely to play a role. Genetic and epigenetic cancer predisposing post-zygotic events have been identified, for example H19 hypermethylation first described in children with Wilms tumor as well as in patients with Hepatoblastoma. [41, 42] Apart from genetic mutations, there are genetic modifiers that are thought to influence the severity of the CPSs, such as MDM2 polymorphisms for the LF carriers [43, 44] or the accumulation of copy number variations (CNV) [45], so that the direct correlation between genotype and phenotype may be

much more perplex than expected. Additionally, one emerging area is the role of mosaic mutations, particularly in individuals with multiple cancers. The germline comprises a lineage of different cellular contexts, from the zygote to the gamete. Post-zygotic mutations can potentially lead to germline mosaicism, that are more difficult to be identified. [46] In clinical setting this lack of experience regarding the interpretation, as well as the prognostic value of the genetic results, can lead to uncertainty about the recommendations and the advice to be offered to the affected families. The moral issues and questions that arise from the - at least up to now - vague cases are multiple, as the diagnosis and communication of a CPS to the affected individuals based only on evidence should be compared to the benefit and the prevention possibilities. As already mentioned there are different guidelines for the surveillance of patients with CPS and it is shown in multiple studies that the compliance to a follow up program leads to significant life prolongation for CPG carriers.

e. Ethical Aspects

The broad application of genetic testing especially in the field of pediatric cancer arises significant ethical, legal, and social aspects for several reasons. First, alone the performance of a so vital genetic test with results that could potentially affect lifelong the carrier, based only on the consent of the parents is an issue of discussion. The incapacity of decision making in childhood leads to the complete dependency of our young patients from the parents. The surrogate decision making works generally unproblematic because parents decide for the best interest of their children, in cases referring to therapy and diagnostic. In the case of a genetic cancer predisposition test though, the decision of the parents does not always represent the wish and personal view of the child. Thus, it can be more complex and controversial as it is a decision not based on logical arguments and scientific facts; it is a totally individual choice of the carrier to seek for strong prognostic information regarding the risk of cancer disease, or to avoid the psychological burden of such a knowledge. And in this case the choice of the parents, even if their intentions are focused on the well-being of the child, will not always represent the personality and life attitude of their child and future adult. In order to minimize the risk of `unwanted´ consent both parents and child (whenever possible) must be informed in detail. The American Society of Human Genetics (ASHG) has suggested, `Counseling and communication with the child and family about genetic testing should include the following components: 1) assessment of the significance of the potential benefits and harms of the test,

2) determination of the decision-making capacity of the child, and 3) advocacy on behalf of the interests of the child´. Recommendations concerning the approach of the patients and their families, as well as a full presentation of the ethical, legal and psychosocial aspects of genetic testing are nowadays created and available from several institutions, like the American College of Medical Genetics and Genomics, the `EURAT´ project of the Marsilius Kolleg of Heidelberg University and the Leopoldina National Academy of Sciences Germany. [47-50]

Another issue is that a cancer predisposition genetic testing can not only affect the life of the individual, but also that of the whole family (parents, siblings, extended family) with both positive consequences, like the opportunity to initiate early beneficial cancer surveillance program, as well as negative results, varying from insecurity, fear, psychological burden up to social stigmatization.

Moreover, the information acquired from the extended genetic analysis such as whole-exome, or whole-genome sequencing is complex; each mutation needs to be evaluated and arranged in pathogenic, probably pathogenic or non-damaging. The interpretation of the genetic findings could be very challenging and require expertise and special care. The extended use of WGS analysis is relatively new and we have only limited experience about the significance of every genetic alteration, so that over- or underestimation of the pathogenity of the genetic findings can be expected in cases of newly discovered mutations. Even after detailed evaluation, the correlation to the phenotype is unclear, as the clinical expression and the risk for malignancy associated with the same mutation can be extremely variable. The genetic information and its impact on the individual's present and future health status has mainly a probabilistic character, because even the most experienced geneticists can only express presumptions about the cancer risk of the carrier, based on possibilities from the up to now known and relative limited data.

f. Outlook

Through this prospective both clinical and genetic study we could try to approach the correlation of the phenotype – genotype in malignancies of the childhood. One important question that must be investigated is to which point there is a reliable correlation between clinical features (including the 3-generation pedigree) and the detection of CPS related

germline mutations. Could a skilled physician rely on the thorough clinical examination and family history to detect a CPS? And if so, which are the decisive clinical signs? The hypothesis is that a detailed, oriented clinical history and physical examination can indicate the cases where a CPS is suspected, but on the contrary a completely unremarkable family and personal history is not powerful enough to exclude the presence of an underlying CPS.

The proportion of CPSs caused by de novo germline mutations (DNM) is not yet thoroughly investigated. Numerous studies try to examine the proportion and the mechanisms of the DNMs in general (not only the CPS associated) and up to now there are indications that the frequency of the DNMs is analogue related to the paternal age. [51] As to the most studied CPS, the LFS, the number of de novo TP53 germline mutations causing LFS is estimated to reach the number of 25%. [52] Another possible explanation for the disharmony between genotype and phenotype could be the nonappearance of the full clinical spectrum of certain CPS at the time of diagnosis, due to the young age of parents for example or the absence of siblings (from the parental side).

Of the numerous recorded clinical data, decisive significance for the detection of a CPS have: a) the family history with one or more cancer cases at young age (under 45 or less), as well as the personal history of more than one malignancies, b) the type of malignancy, c) the presence of other anomalies, more specifically skin anomalies, congenital malformations and growth abnormalities, and d) the age of diagnosis, especially congenital malignant tumors. These conclusions are in line with previous studies that also indicate the importance of clinical evaluation and family history in the detection of CPS. The recognition though of the clinical particularities in the context of a CPS can be a real challenge even for a skilled pediatric oncologist, as the identification of fine congenital anomalies can be missed or underestimated. It is also important to perform a thorough clinical examination before the application of chemotherapy, because it could be almost impossible during the treatment to evaluate and distinguish preexisting skin depigmentation lesions or nail anomalies from chemotherapy secondary effects.

In our opinion the combination of WES of parent-child, the documentation of a three-generation pedigree and a detailed history of the patient and the family could confirm the diagnosis of a CPS when suspected and lead to the detection of new CPSs, in cases where none of the already known CPGs are involved, but the family history is highly indicative.

It is necessary to perform a comprehensive evaluation of known CPGs in large patient and population cohorts so that the clinical phenotype, genotype-phenotype associations, genetic and non-genetic modifying factors and contribution to cancer can be clarified.

4. Conclusions

In conclusion, the integration of genetic testing for CPS, through WES of parents and affected children, in combination with the systematic recordation of a 3-generation pedigree to the daily routine in every pediatric oncology department can only be of benefit; on one hand directly for each and every family and on the other hand prospectively by providing valuable information about the mechanisms of tumorigenesis in childhood, as well as the inheritance patterns. Provided the high acceptance recorded in our study, it is to assume that the establishment of such a practice can be proceeded with no perplexity in each institution.

The difficulties that arise through the rarity of the CPSs and our insufficient knowledge can be overcome through improved networks and registries of mutation carriers. Through large patient cohorts and collaboration, we can better define the role of inheritance in cancer of childhood, describe the precise correlation between the genotype and clinical phenotype and finally provide more personalized care and new therapeutic options.

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