Cancer susceptibility syndromes in children in the area of broad clinical use of massive parallel sequencing

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Abstract  Children diagnosed with cancer are considered for inherited cancer susceptibility testing according to well-established clinical criteria. With increasing efforts to personalize cancer medicine, comprehensive genome analyses will find its way into daily clinical routine in pediatric oncology. Whole genome and exome sequencing unavoidably generates incidental findings. The somatic “molecular make-up” of a tumor genome may suggest a germline mutation in a cancer susceptibility syndrome. At least two mechanisms are well-known, (a) chromothripsis (Li-Fraumeni syndrome) and (b) a high total number of mutational events which exceeds that of other samples of the same tumor type (defective DNA mismatch repair). Hence, pediatricians are faced with the fact that genetic events within the tumor genome itself can point toward underlying germline cancer susceptibility. Whenever genetic testing including next-generation sequencing (NGS) is initiated, the pediatrician has to inform about the benefits, risks, and alternatives, discuss the possibility of incidental findings and its disclosure, and to obtain informed consent prior to testing.

Conclusions: Genetic testing and translational research in pediatric oncology can incidentally uncover an underlying cancer susceptibility syndrome with implications for the entire family. Pediatricians should therefore increase their awareness of chances and risks that accompany the increasingly wide clinical implementation of NGS platforms.

What is Known:
• The proportion of cancers in children attributable to an underlying genetic syndrome or inherited susceptibility is unclear.
• Pediatricians consider children diagnosed with cancer for inherited cancer susceptibility according to well-established clinical criteria.

What is New:
• Genetic testing of tumor samples can incidentally uncover an underlying cancer susceptibility syndrome.
• Findings in tumor genetics can be indicative that the tumor arose on the basis of the child’s germline alteration, (a) chromothripsis and (b) a high total number of mutational events which exceeds that of other samples of the same tumor type.

Keywords  Cancer susceptibility syndrome · Hereditary · Childhood · Next-generation sequencing · Chromothripsis · Mutation rate

Abbreviations

ALL  Acute lymphoblastic leukemia
CMMR-D  Constitutional mismatch repair-deficiency
CSS  Cancer susceptibility syndromes
DGV  Database of genomic variants
INFORM  Individualized therapy for relapsed malignancies in childhood
LFS  Li-Fraumeni syndrome
LOH  Loss of heterozygosity
Mb  Megabase
NGS  Next-generation sequencing
SHH-MB  Sonic-Hedgehog medulloblastoma
SNPs  Single nucleotide polymorphisms
SNVs  Single nucleotide variants
Introduction

Until now, the proportion of cancers in children and adolescents attributable to an underlying genetic syndrome or inherited susceptibility is unclear. In the early 1990s, the inherited fraction of childhood cancer was estimated at 1–10 % [29]. A recent report from the Pediatric Cancer Genome Project/St. Jude Children’s Research Hospital determined an incidence of 16.0 % in patients with solid tumors, 8.6 % with brain tumors, and 3.9 % with leukemias. The report initially focused on 23 well-known cancer predisposition genes and 8 genes that predispose to pediatric cancer with a high penetrance [47]. The most frequently affected genes included TP53, APC, and BRCA2. Additional analyses were expanded to 565 genes that are known to play a role in various steps and pathways of cellular transformation. Identifiable variants were classified as pathologic, likely pathologic, uncertain significance, likely benign, and benign. Taking the larger gene-set into account, the overall prevalence of an inherited mutation increased only slightly, with a pathologic or likely pathologic variant being detected in 8.6 % of all patients and 4.6 % of patients with leukemia. However, the spectrum of tumors sequenced was not numerically representative of the spectrum of childhood tumors, and the mutation frequencies may be skewed accordingly. In a hereditary cancer risk assessment study in survivors of childhood cancer, a genetic counselor considered 29 % of the survivors as eligible for further genetics evaluation [19].

However, in the era of high-throughput sequencing in which new cancer susceptibility syndromes (CSS) and mechanisms are increasingly discovered—did we so far maybe just see the tip of the iceberg?

Current clinical approach to CSS

Pediatric oncologists consider children diagnosed with cancer and their families for inherited cancer susceptibility according to well-established criteria [20]. These comprise patient-specific constellations including (i) rare tumors commonly associated with cancer predisposition (e.g., adrenocortical carcinoma), (ii) bilateral or multifocal tumors (e.g., Wilms tumor), (iii) cancer diagnosis at a younger than expected age (e.g., thyroid carcinoma), (iv) multiple synchronous or metachronous tumors, (v) additional conditions (e.g., axillary freckling) indicative of an underlying syndrome, and (vi) suspicious family features. These might include (a) familial clustering of the same or closely related cancers, (b) cancer diagnoses in two or more first-degree relatives, (c) tumor patterns associated with a specific cancer predisposition syndrome, (d) exceptional young age at diagnosis, (e) sibling with childhood cancer, and (f) consanguineous parents.

Li-Fraumeni syndrome (LFS) is one of the most striking familial cancer predisposition syndromes. It is clinically and genetically heterogeneous and characterized by autosomal dominant inheritance and early onset of tumors, multiple tumors within one individual, and multiple affected family members. LFS presents with a variety of tumor types with soft tissue sarcomas, osteosarcomas, breast cancer, brain tumors, leukemia, and adrenocortical carcinoma being the most common tumor types. Comprehensive surveillance protocols have been implemented and proven efficiency in terms of superior survival [46]. Table 1 lists common hereditary cancer susceptibility syndromes sorted by the underlying mechanism. The American College of Medical Genetics and Genomics and the National Society of Genetic Counselors just published the latest referral indications for cancer predisposition assessment [13]. However, due to de novo mutations, incomplete penetrance of inherited mutations, and variable phenotype/genotype correlations, the family history may not in all cases be helpful. For example, up to 25 % de novo events of TP53 mutations are reported in Li-Fraumeni syndrome [6]. In most other cases of CSS, however, the proportion of inherited susceptibility versus de novo mutations remains unknown.

Personalized medicine

With the ongoing efforts to personalize cancer medicine, comprehensive genome analyses will increasingly find its way into daily clinical routine in pediatric oncology. In the recently established German INdividualized therapy FOR Relapsed Malignancies in childhood (INFORM) project, this idea has been introduced for pediatric patients with relapsed or refractory high-risk disease without further standard of care therapy options. Individual tumor samples are characterized on the molecular level by next-generation sequencing (NGS) to establish a “fingerprint” of the tumor to identify promising targets for a successful relapse therapy [10].

Other such examples in which the detection of specific mutations has already led to a change of therapy of course also exist. Recently, a new leukemia subtype of high-risk B-precursor acute lymphoblastic leukemia (ALL), called Ph-like ALL, was characterized. Besides its Ph- or BCR-ABL-like transcriptional profile, no translocation t(9;22) or BCR/ABL rearrangement, respectively, is present. Instead, multiple other genetic alterations can be detected, which are potentially druggable by tyrosine kinase inhibitors or other targeted therapies [18, 24, 36, 37]. In pediatric low-grade astrocytoma, the BRAF V600E-mutation was identified as a frequent genomic aberration activating the MAPK pathway. Tumors carrying this mutation show significantly increased BRAF and CCND1 levels [33]. Since its discovery, the BRAF V600E-mutation has been described in an increasing number of pediatric central nervous system (CNS) tumors [8, 11, 40, 41]. Targeted therapies such as the BRAF inhibitor vemurafenib and MEK1/2 inhibitors are available and some encouraging examples of effective therapies even in very aggressive tumor
| Syndrome | Gene(s) | Inheritance | Clinical characteristics | Tumor types | Cancer risk |
|----------|---------|-------------|--------------------------|-------------|------------|
| DNA damage repair defects/genetic instability | | | | | |
| Ataxia telangiectasia (AT) * | ATM | AR | Progressive ataxia, central nervous system degeneration, growth deficiency, ocular and facial telangiectasia, immunodeficiency, infertility, premature aging | Leukemia, lymphoma, carcinoma | 10–38 % overall cancer risk 70-fold increased leukemia risk (T-ALL, T-PLL) 250-fold increased lymphoma risk (B cell) |
| Bloom syndrome (BS) | BLM | AR | Short stature, immunodeficiency, malar rash, microcephaly, high-pitched voice, hypogonadism | Leukemia, lymphoma | 50 % overall cancer risk 15 % leukemia risk 15 % lymphoma risk |
| Constitutional mismatch repair-deficiency syndrome (CMMR-D) | MLH1, MSH2, MSH6, PMS2 | AR | Multiple café au lait (CAL) spots, features reminiscent of NF1 | Pediatric brain tumors, colorectal cancers, ALL, AML, lymphoma, early onset gastrointestinal or gynecological cancers | |
| Fanconi anemia (FA) | FANCA, C, D1, D2, E, F, G, I, J, L, M, RAD51C, SLX4/BTBD12, FANCB | X-linked | Bone marrow failure, growth failure, radial ray abnormalities, renal abnormalities, CAL spots, hypopigmentation, congenital heart disease, microphthalmia, ear anomalies/deafness, hypogonadism; up to 25 % phenotypically normal | Leukemia (MDS, AML), squamous cell carcinoma, gynecological tumors, brain tumors, Wilms tumor, neuroblastoma | 25 % cumulative risk of hematologic malignancy by age 45 7 % MDS 9 % (500-fold increased risk of) AML |
| Li-Fraumeni syndrome (LFS) | TP53 | AD | Up to 25 % de novo mutations beyond classical LFS malignancies phenotypically normal | Soft tissue sarcoma, osteosarcoma, breast cancer, adenocortical carcinoma (ACC), leukemia, brain tumors (glioblastoma multiforme, high-grade astrocytoma/primitive neuroectodermal tumor, medulloblastoma, chordoid plexus carcinoma) | 90 % lifetime risk to develop cancer 1–3 % ALL (hypodiploid ALL) |
| Nijmegen breakage syndrome (NBS) | NBS1 | AR | Microcephaly, prominent midface, receding mandible, CAL, recurrent infections, bone marrow failure | NHL, DLBCL, Burkitt lymphoma, T-LBL/-ALL, AML, Hodgkin lymphoma, medulloblastoma, thalassemia/sarcoma | 40 % cancer risk by the age of 20 years |
| Bone marrow failure (BMF) syndromes: ribosome biogenesis and/or telomere maintenance anomalies | | | | | |
| Congenital amegakaryocytic thrombocytopenia (CAMT) type I/II | MPL | AR | Thrombocytopenia and megalakaryocytopenia with no physical anomalies | MDS/AML | Unknown |
| Diamond blackfan anemia (DBA) | RPS19, RPS24, RPS17, RPL5, RPL5A, RPL11, RPS7, RPS26, RPS10, GATA1 | AD | Majority sporadic normochromic macrocytic anemia, reticulocytopenia, and nearly absent erythroid progenitors in the bone marrow, growth retardation, craniofacial, upper limb, heart, and urinary system congenital malformations, persistence of hemoglobin F | Adenocarcinoma of the colon, sarcoma, genital cancer, MDS/AML, ALL | 5.4 %-fold increased cancer risk |
| Dyskeratosis congenital (DC) | DKC1, CTC1, TERC, TERT, TINF2, NOP10, NHP2, WRAF53 | X-linked | Triad of abnormal skin pigmentation, nail dystrophy, and leukoklastic of the oral mucosa | MDS/AML | 3–33 % leukemia risk |
| Shwachman-Diamond syndrome (SDS) | SBDS | AR (considered) | Exocrine pancreatic insufficiency, hematologic abnormalities (pancytopenia), skeletal abnormalities | MDS/AML, ALL | 5–24 % leukemia risk |
| Severe congenital neutropenia (SCN) (Kostmann syndrome) * | ELANE, HAX1 | AD AR | Congenital neutropenia, recurrent/persistent infections, omphalitis | MDS/AML | 8–25 % leukemia risk |
| Syndrome                                                                 | Gene(s)                                         | Inheritance | Clinical characteristics                                                                 | Tumor types                                      | Cancer risk                                                                 |
|------------------------------------------------------------------------|-------------------------------------------------|-------------|-----------------------------------------------------------------------------------------|-------------------------------------------------|-----------------------------------------------------------------------------|
| Thrombocytopenia and absent radii syndrome (TAR)                        | RBM8A and/or microdeletion 1q21.1               | Unclear     | Reduction in the number of platelets and absence of the radius                          | MDS/AML                                         | Unknown                                                                     |
| Cell cycle/differentiation defects (RAS pathway dysfunction)           |                                                 |             |                                                                                         |                                                 |                                                                             |
| CBL syndrome                                                           | CBL                                            | AD          | Dysmorphic facial features, short neck, developmental delay, hyperextensible joints,    | JMML                                            | Unknown                                                                     |
| Neurofibromatosis type 1 (NF1)                                         | NF1, SPRED1                                     | AD          | CAL, axillary/inguinal freckling, Lisch nodules, bony dysplasia, seizures, learning      | CMML/JMML, AML, neurofibroma, optic pathway      | 200-500-fold increased JMML risk 11 % MDS 5-fold increased brain tumor     |
|                                                                       |                                                 |             | difficulties, splenoid wing abnormalities                                               | glioma, peripheral nerve sheath tumor, astrocytoma, paraganglioma/pheochromocytoma | almost 100 % neurofibroma risk                                              |
| Noonan/Noonan-like syndrome                                            | PTPN11, HRAS, KRAS, NRAS, RAF1, SOS1, BRAF, SHOC2, MEPK1 | AD          | Short stature, short webbed neck, lymphedema, hypertelorism, coarse faces, CAL,          | Self-resolving myeloproliferative disease (MPD/TMD) and JMML, CMML, ALL, neuroblastoma, rhadomyosarcoma | MPDJMML in pts with PTPN11                                                  |
| Transcription factors/pure familial leukemia syndromes                |                                                 |             |                                                                                         |                                                 |                                                                             |
| Familial CEBPA leukemia                                                | CEBPA                                          | AD          | None                                                                                     | MDS/AML                                         | FAB M1/M2 highly penetrant                                                |
| Familial ETV6 / ALL syndrome                                          | ETV6                                           | AD          | Thrombocytopenia                                                                          | MDS/AML                                         | Unknown                                                                     |
| Familial platelet disorder with predisposition to myeloid malignancy (PD/AML) | RUNX1 (dominant)                                | AD          | Mild to moderate thrombocytopenia, platelet function abnormalities                        | MDS/AML                                         | 35 % AML risk                                                               |
| Familial PAX5 syndrome                                                 | PAX5                                           | AD          | None                                                                                     | ALL                                             | 30 % penetrance in PAX5 SNP allele carriers PAX5 c.547G>A                  |
| MonoMac                                                                | GATA2                                          | AD          | Monocytopenia, NK cell lymphopenia, infections                                           | MDS/AML                                         | 50 % leukemia risk                                                          |
| Immunodeficiencies                                                     |                                                 |             |                                                                                         |                                                 |                                                                             |
| Wiskott-Aldrich syndrome (WAS)                                         | WAS                                            | X-linked    | Eczema, thrombocytopenia, immunodeficiency                                              | Diffuse large B cell lymphomas, non-Hodgkin’s   | 5–13 % lymphoid malignancies                                              |
|                                                                       |                                                 |             |                                                                                         | lymphoma of larynx, leukemia, cerebellar astrocytoma, Kaposi sarcoma, smooth muscle tumors |                                                                             |
| X-linked lymphoproliferative (XLP) syndrome type I / II                | SH2D1A XIAP, SAP                               | X-linked    | Severe immune dysregulation often after viral infection, typically with Epstein-Barr    | Hemophagocytic lymphohistiocytosis (HLH), non-Hodgkin lymphoma | Unknown                                                                     |
|                                                                       |                                                 |             | virus (EBV), severe or fatal mononucleosis, acquired hypergamaglobulinemia, (HLH),      |                                                 |                                                                             |
|                                                                       |                                                 |             | lymphomatoid granulomatosis is                                                          |                                                 |                                                                             |
| Autoimmune lymphoproliferative syndrome (ALPS) type IA/B/II            | CD95 CD95L CASP10 IL12RB1                       | AD AR in ALPSIA | Lymphadenopathy with hepatosplenomegaly and autoimmune cytopenias, hypergamaglobulinemia | Hodgkin (HL) and non-Hodgkin (NHL) lymphoma, carcinoma (thyroid, breast, skin, tongue, liver), multiple neoplastic lesions (thyroid/breast adenomas, glomas) | 14-fold NHL risk 51-fold HL risk                                          |
| IL-2-inducible T cell kinase deficiency                                | ITK                                            | AR          | Fever, lymphadenopathy, splenomegaly, EBV associated lymphoproliferation                  | Hodgkin lymphoma,                               | Unknown                                                                     |
| Familial mosaic monosomy 7                                             | Unknown                                        | Unknown     | Early-childhood onset of bone marrow insufficiency / failure                             | MDS, AML                                        | Very high, fatal outcome                                                  |

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| Syndrome | Gene(s) | Inheritance | Clinical characteristics | Tumor types | Cancer risk |
|----------|---------|-------------|--------------------------|-------------|-------------|
| **Beckwith-Wiedemann syndrome** (BWS) | p57, H19, LIT1, ICR1, CDKN1C, NSD1 | complex (AD, genomic imprinting, pUPD) | Overgrowth syndrome, macrodactyly, omphalocele, hemihypertrophy, neonatal hypoglycemia | Wilms tumor, hepatoblastoma, adrenal carcinoma, rhabdomyosarcoma | 8.6% cancer risk, depending on subtypes |
| **Cowden syndrome type I-VI (CWS)** | PTEN, SDHB, SDHD, KLLN | AD | Hamartomatous polyps of the gastrointestinal tract, mucocutaneous lesions, cobblestone-like papules of the gingiva and buccal mucosa, multiple facial trichilemmomas | Dysplastic gangliocytoma of the cerebellum (Lhermitte-Duclos), colon, breast and thyroid cancer | Lifetime risk 25-30% breast cancer 10% thyroid cancer 5-10% endometrial/uterine cancer |
| **Denys-Drash syndrome (DDS)** | WT1 (dominant) | Usually sporadic | Diffuse mesangial sclerosis leading to early endstage renal disease, disorder of sexual development in XY patients | Wilms tumor, gonadoblastoma | Almost 100% Wilms tumor |
| **Down syndrome (DS)** | Trisomy 21 | n.a. | Facial dysmorphism, mental retardation, hypotonia, congenital heart disease | TMD, AML, ALL | 10% TMD, 1-2% AML/AML 10-20-fold increased leukemia risk 500-fold increased risk of AMKL |
| **Familial Adenomatous Polyposis (FAP) syndrome** | APC | AD | Intestinal polyposis, osteomas, fibromas, sebaceous cysts, dental abnormalities | Colon, thyroid, stomach, and intestinal cancer, hepatoblastoma, desmoid tumors, medulloblastoma | Almost 100% colorectal cancer |
| **Familial neuroblastoma** | ALK, PHOX2B | AD | None | Neuroblastoma | Unknown |
| **Familial Pleuropulmonary blastoma tumor predisposition syndrome** | DICER1 | AD | Pulmonary cysts, multilobar goiter | PPB, cystic nephroma, Sertoli-Leydig cell tumors, rhabdomyosarcoma, supratentorial primitive neuroectodermal tumor, intracranial medulloepithelioma | Variable penetrance, exact rest unknown |
| **Hereditary paragangliomas and pheochromocytoma syndrome (HPPS)** | SDHB | AD | None | Paraganglioma, pheochromocytoma, renal, thyroid | >70% with metastatic disease 12% GISTs |
| **Multiple endocrine neoplasia type I (MEN1)** | MEN1 | AD | None | Pancreatic islet cell tumor, pituitary adenoma, parathyroid adenoma | 10% carcinoid tumors |
| **Multiple endocrine neoplasia type II (MEN2A, MEN2B)** | RET | AD | Mucosal neuroma (intestinal tract, tongue, lips), marfanoid habitus | Medullary thyroid carcinoma, pheochromocytoma, parathyroid hyperplasia | 100% risk of developing medullary thyroid carcinoma in MEN2A |
| **Nevoid basal cell carcinoma syndrome (NBCCS) / Gorlin syndrome** | PTCH1, 2, SUFU | AD | Macrocystic hygroma, palmar or plantar pits, rib abnormalities, ectopic calcification of the falk cerebri | Basal cell carcinoma, desmoplastic medulloblastoma, ovarian fibromas | 90% basal-cell carcinoma, 5% medulloblastoma |
| **Peutz-Jeghers syndrome (PJS)** | STK11 | AD | Melanocytic macules of the lips, buccal mucosa, digits, multiple gastrointestinal hamartomatous polyps | Intestinal, ovarian, pancreatic, breast cancers | 55% gastrointestinal cancer 45% breast cancer |
| **Familial retinoblastoma syndrome (RB)** | RB1 | AD | Leukocoria | Retinoblastoma, osteosarcoma, melanoma, glioma, carcinoma | 80% bilateral retinoblastoma 20% unilateral retinoblastoma |
| **Rhabdoid tumor predisposition syndrome** | SMARCB1/INI1 | Unclear, up to 21% de novo mutations | None | Rhabdoid tumor, medulloblastoma, choroid plexus tumor, schwannoma | Penetration unclear |
| **Rubinstein-Taybi syndrome (RSTS)** | CREBBP | AD | Short stature, learning difficulties, distinctive facial features, broad thumbs and first toes, microcephaly, growth retardation | Neuroblastoma, medulloblastoma, oligodendrogloma, meningioma, pheochromocytoma, rhabdomyosarcoma, kienymyosarcoma, leukemia, lymphoma | Unknown |
types have already been reported, such as the successful treatment of a 12-year-old child with relapsed glioblastoma multiforme with vemurafenib [38]. With the identification of a highly recurrent genetic alteration and its resulting fusion protein in ependymoma, the C11orf95-RELA protein, a further potentially druggable target was identified and specific therapy will hopefully be available in the near future [31]. We might also hypothesize that children with hereditary cancer syndromes like the so-called rasopathies might soon benefit from targeted therapy, as the underlying genetic alterations are highly recurrent [1, 9].

Next-generation sequencing

Due to rapid technical advances in the field of NGS, tumor (including leukemia) genomes can nowadays comprehensively be analyzed within few days. Today’s state of the art in high-throughput sequencing already allows the usage of whole genome sequencing for research projects and of whole exome sequencing for daily clinical routine. However, the likelihood of identifying contemplable mutations is highly dependent on the relative ability of the sequencing approach to find these mutations. Computational processing, analyzing, and interpreting the massive amounts of data and genetic variants produced by NGS still remains challenging and requires comparisons with databases such as dbSNP and 1000 genomes project [3, 16]. Another valuable resource in interpreting own experimental data is the ExAC browser provided by the Broad Institute at www.exac.broadinstitute.org. It meanwhile provides exome data from more than 60,000 unrelated individuals. Before definitive conclusions can be drawn, the functional consequences of identified mutations on protein structure and function often have to be demonstrated experimentally [43]. In addition, a frequent conceptual misunderstanding relates to the fact that even a mutation with profound impact on protein function does not automatically proves its pathogenicity and disease-causing effect.

Each of us carries an average of approximately 3000 single nucleotide polymorphisms (SNPs) in terms of individual SNPs. To generate a personal cancer genome signature for molecular targeted therapy, it is important to discriminate between these individual SNPs and somatic (tumor) mutations. Thus, comparing the NGS data of tumor versus germline DNA is a condition sine qua non to identify the somatically acquired genetic variants of the tumor.

However, NGS not only generates focused genetic results with precise clinical implications for treatment but also so-called incidental findings with possible, limited, or unknown clinical impact or might even uncover an underlying susceptibility to cancer and other hereditary diseases. Such incidental findings are divided into “anticipatable” and “unanticipatable” ones. The former is
a finding that is known to be associated with the test and is possible to be found. The latter could not have been anticipated given the current state of scientific knowledge [17]. Hence, treating physicians will increasingly be faced with such incidental genetic findings and the difficulties of interpreting and reporting these results.

Moreover, the pediatric oncologist is confronted with one new situation in particular: the fact that genetic events within the tumor genome itself can point toward underlying germline cancer susceptibility. Thus, even if not initially aimed to detect a CSS, the somatic “molecular make-up” of the tumor genome may suggest a germline mutation in a CSS gene.

Up to now, there are two well-known findings in tumor genetics which can be indicative that the tumor arose on the basis of the child’s germline alteration, (a) chromothripsis and (b) a high total number of mutational events which exceeds that of other samples of the same tumor type.

(a) The phenomenon of **chromothripsis** was first reported by Stephens in 2011 [44]. The term “chromothripsis” (“chromo” from chromosome; “thripsis” for shattering into pieces) describes the shattering of a chromosome or a chromosomal region into tens to hundreds of pieces and locally clustered reassembling of some of the genomic fragments while others are lost to the cell.

According to Stephens [44], chromothripsis is defined by six features: (1) rearrangements localized within the genome, (2) oscillating changes of the copy number profile between one and two copies, whereby (3) loss of heterozygosity (LOH) causes a copy number of one, and retaining heterozygosity a copy number of two, (4) clustering of breakpoints across the chromosome, (5) conjunction of two remote chromosome fragments, and (6) joining rearrangements between two chromosome arms with clustering at the breakpoints. Rapid oscillations between copy number states one and two within the whole or parts of the chromosome characterizes the copy number profile in case of chromothripsis.

In contrast to common theories of cancer evolution through progressive accumulation of genomic alterations such as oncogene activation and tumor suppressor loss through environmental and lifestyle factors in adults, chromothripsis as a single catastrophic event might be involved in the development of a variety of cancers in childhood. It can cause the formation of new gene fusions, disruption of tumor suppressors, and amplification of oncogenes [35, 44]. In adults, 2–3 % of all cancers show evidence of chromothripsis; in bone cancers, this incidence is especially high with 25 % [44]. The impact of chromothripsis on cancer gene function and cancer development in childhood has already been demonstrated for many different tumor entities, e.g., ALL, AML, ependymoma, medulloblastoma, neuroblastoma, and retinoblastoma [4, 23, 26, 28, 30, 31, 35]. In addition, chromothripsis has been associated with poor prognosis in neuroblastoma [28]. A list of pediatric tumors, in which chromothripsis has been described, is given in Table 2. Conversely, alterations in TP53 have been shown for low-hypodiploid ALL but without chromothriptic pattern [15].

(b) To provide a comprehensive landscape of somatic genomic alterations (termed mutational signatures) in cancer genomes, numerous cancers have been profiled by DNA sequencing [2, 34, 45]. The occurring genomic alterations are presumably caused by defective DNA replication or repair and exogenous or endogenous mutagen exposure and include substitutions, insertions or deletions, rearrangements, copy number alterations, completely new sequences from exogenous sources, and combinations of all these possibilities. The prevalence of such mutations is highly variable between cancer (sub)types [2, 22]. Due to extensive exposure to carcinogens, small cell lung cancer (tobacco) and malignant melanoma (ultraviolet light) show the highest somatic mutation prevalence (over 100/Megabase (Mb)). In contrast, the mutation rate in pediatric cancers is lowest (0.1/Mb; approximately one change across the entire exome) as chronic mutagenic exposure plays a minor part in carcinogenesis in childhood [22]. An outline of mutation frequencies in various (pediatric) cancer types is given in Table 3.

Alexandrov et al. [2] described a mutational signature with very large numbers of substitutions and small indels, the latter at short nucleotide repeats and with overlapping microhomology at breakpoint junctions, termed “microsatellite instability,” which is characteristic of cancers with defective DNA mismatch repair and may suggest constitutional mismatch repair-deficiency syndrome (CMMR-D) in childhood.

As was shown by Rausch et al. [35], the single nucleotide variant (SNV) rate of children with Sonic-Hedgehog medulloblastoma (SHH-MB) is clearly higher (24 tumor-specific SNVs) in the case of inherited TP53 mutations compared to sporadic pediatric medulloblastoma samples (average 5.7 non-synonymous SNVs per sample; [32]). Thus, comparing the patient’s SNV with the average SNV rate of a given tumor entity, an increased mutation frequency (SNV rate) detected by NGS of the tumor again may point to an underlying CSS (Li-Fraumeni syndrome).

**Ethical and legal issues**

“Are our other children at an increased risk of developing cancer?” Parents of a child diagnosed with cancer frequently raise this question. Up to now, pediatric oncologists mostly
reassure them that cancer in children usually is not hereditary but an exceptionally bad stroke of fate. However, will this statement still hold true in the future with ever-increasing knowledge about underlying cancer predisposition syndromes and inherited cancer susceptibilities in childhood?

The incidental finding of chromothripsis and its association with Li-Fraumeni syndrome in SHH-MB patients very well demonstrates the far-reaching consequences of translational research and genetic testing in pediatric oncology with its challenges for scientists, treating physicians, and the affected child and his entire family.

By detecting chromothripsis in a tumor, further genetic testing for germline p53 mutations is highly advisable as this phenomenon might be attributable to an underlying Li-Fraumeni syndrome. The latter obviously represents an important piece of clinical information as it will guide treatment, surveillance, and further early cancer screening programs [21, 46].

According to the recommendations of national and international human genetic societies and the legislation of most European countries, prior to genetic testing, the child (wherever possible) and the parents must be informed in detail, preferences as

### Table 2 Examples of (pediatric) tumors associated with chromothripsis

| Tumor                                      | References                                                                 |
|--------------------------------------------|---------------------------------------------------------------------------|
| Burkitt lymphoma *                         | Sarova et al., Cancer Genet 2014                                          |
| Brain tumors                               |                                                                           |
| • Ependymoma                               | • Parker et al., Nature 2014                                              |
| • High-grade gliomas                       | • Zhao et al., Neuro Oncol 2014                                           |
| • Medulloblastoma                          | • Rausch et al., Cell 2012                                                |
| • Sonic-Hedgehog Group 3                   | • Northcott et al., Nature 2012                                           |
| Hodgkin lymphoma *                         | Nagel et al., Genes Chromosomes Cancer 2013                                |
| Leukemia                                   |                                                                           |
| • AML                                      | • Rausch et al., Cell 2012                                                |
| • ALL (iAMP21)                              | • Harrison et al., Blood 2015; Li et al., Nature 2014                     |
| Neuroblastoma                              | Ambros et al., Frontiers in Oncology 2014; Boeva et al., PLoS One 2013; Molenaar et al., Nature 2012 |

Osteosarcoma *

Phaeochromocytoma (PCC) / Paranglioma (PGL) *

Retinoblastoma

*Described in adult tumor samples

### Table 3 Examples of mutation frequencies in (pediatric) tumors

| Malignancy                        | Mutations (range)                                                        | Reference                                                                 |
|-----------------------------------|--------------------------------------------------------------------------|--------------------------------------------------------------------------|
| AML *                             | 0.37 per Mb (0.01–10) of coding sequence                                 | Lawrence et al., Nature 2013                                             |
| Ependymoma, intracranial b         | 12.8±10.6 mutations (range 5 to 34) per tumor                            | Bettegowda et al., Oncotarget 2013                                       |
| Ependymoma, spinal cord b          | 12.9±6.4 mutations (range 2 to 23) per tumor                             | Bettegowda et al., Oncotarget 2013                                       |
| Ewing b                           | 0.15 per Mb of coding sequence                                           | Brohl et al., PLoS Genet 2014                                           |
| Glioblastoma multiforme b         | 1.4 per Mb                                                                | Cancer Genome Atlas Research Network, Nature 2008                         |
| Glioblastoma, non-brainstem pediatric | 23.5±11.2 mutations (range 4–46) per tumor                                | Bettegowda et al., Oncotarget 2013                                       |
| MDS b                             | 3 (0–12) mutations per sample in 104 cancer genes                        | Haferlach et al., Leukemia 2014                                          |
| Medulloblastoma                   | 8.3 non-synonymous SNVs per sample                                       | Parsons et al., Science 2011                                              |
| Neuroblastoma                     | 0.35 non-silent mutations per megabase                                    | Pugh et al., Nature 2012                                                 |
| Rhabdoid cancers                  | 0.19 per Mb (0–0.45) of coding regions                                   | Lee et al., J Clin Invest 2012                                           |
| Xanthoastroctoma, pleomorphic b    | 9.5±8.5 mutations (range 1 to 28) per tumor                              | Bettegowda et al., Oncotarget 2013                                       |

*a Tumor samples not specified

b Described in adult tumor samples
to which findings should be reported must be assessed. This is a well-established standard of care for targeted molecular testing an affected individual or suspected carrier for a specific hereditary condition. However, NGS is likely, apart from the initial indication to perform it, to uncover incidental findings, such as an underlying CSS as well as non-cancer-related germline mutations (e.g., CFTR, Huntington’s disease) with varying clinical importance for the patient. In order to comply with the aforementioned recommendations, this would require extensive genetic counseling of the child/parents of a child diagnosed with cancer undergoing NGS of the tumor prior to testing, which would have to encompass both incidental findings with possible, limited, or unknown clinical impact and numerous results unrelated to the indication for NGS [42]. We believe that this is highly impractical in the daily life of a pediatric hematologist-oncologist as disclosing the diagnosis of cancer itself is overwhelming and dramatically limits the child’s/parents’ receptivity, and NGS of the tumor often has to be initiated at the time of diagnosis. However, whenever NGS is initiated, the treating physician has an obligation to discuss the full range of generated data and the possibility of incidental findings and its disclosure with the child/parents. Furthermore, the ordering physician is responsible for obtaining informed consent and providing pre- and post-test counseling. Thus, regarding the child’s/parents’ autonomy and both their right to access all NGS data and their “right not to know,” they should be informed of the benefits, risks, and alternatives of genetic testing in detail [5, 7, 12]. When the patient/parent refuses to be informed about incidental findings, even if disclosure leads to beneficial interventions, the physician must ensure that adequate information has preceded this refusal. However, most clinicians do not have sufficient training in NGS and need to be extensively trained for clinical translation and reporting of NGS data.

In contrast to the standards for genetic testing in adults, predictive testing in pediatric patients is only recommended when the disease is associated with childhood onset and only with available effective screening and/or intervention options [7, 39]. Refraining from predictive testing of children allows them to autonomously make this decision once they reach adulthood.

Last but not least, identifying children with hereditary cancer predispositions has immediate consequences for the entire family (siblings, parents, and extended family) [20, 25, 42]. Due to the young age of the index patient, potentially affected relatives might as well be young and yet asymptomatic. Having been tested themselves might—depending on the outcome—influence their family planning but will of course also provide an excellent opportunity to initiate early cancer surveillance programs which they will benefit from. However, genetic testing and tumor surveillance can have deeply affecting psychological consequences for the child and the family, emotional support should thus be in place for the families.

Clear legislation on returning genetic results in oncology are still missing. Lolkema et al. have thoroughly addressed the accompanying ethical, legal, and counseling challenges [25]. Comprehensive ethical recommendations on how to report research results to patients and parents are, for example, given by the American College of Medical Genetics and Genomics, the Boston Children’s Hospital, the American Academy of Pediatrics, the “EURAT” (Ethical and legal aspects of whole human genome sequencing) project of the Marsilius Kolleg of Heidelberg University, and the Leopoldina National Academy of Sciences Germany [5, 12, 14, 27, 39]. However, their practical implementation in day-to-day clinical life remains challenging.

Conclusions

Genetic testing and translational research in pediatric oncology provides new and exciting insights into the evolution and pathogenesis of childhood cancer. On the other hand, it can incidentally uncover an underlying cancer susceptibility syndrome with implications not only for the child but also for the entire family. Pediatric oncologists should therefore increase their awareness of chances and risks that accompany the increasingly wide clinical implementation of NGS platforms [42, 43].

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Authors’ contributions MK screened the literature, collected the data, and drafted the manuscript. AB revised the manuscript. Both authors read and approved the final manuscript.

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