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Cancer risk and predisposition in families with childhood cancer

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By increasing our understanding of the genetic background of childhood cancer, it is possible to improve early diagnosis and treatment. This thesis examines the risk of cancer in relatives of children cancer and the presence of known genetic variants that predispose childhood cancer, with the purpose of identifying families likely to harbor genetic aberrations that have previously not been identified.

FACULTY OF MEDICINE

LUND UNIVERSITY

Cancer risk and predisposition in families with childhood cancer

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LUND
UNIVERSITY

DOCTORAL DISSERTATION

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METHODS: Pediatric cancer patients included in the Lund Childhood Cancer Genetic study provided blood samples after informed consent. The Swedish Population- and Cancer Register were used to identify relatives with cancer diagnoses up to the third degree of relation. The relative risk for relatives was calculated. Illumina HiSeq 2500 was used to sequence DNA from patient blood for 22 cancer predisposition genes.

RESULTS: Study I: 41/528 families (7.8%) had multiple pediatric cancer cases up to the third degree of relation. Related children with cancer often had the same cancer diagnosis and were more likely to be female. Study II: We report an increased risk for adult cancer (SIR 1.07) and pediatric cancer (SIR 1.48) in 16,137 relatives of 757 children with cancer up to the third degree of relation. The results were unchanged when excluding 30 families of children with known germline pathogenic variants. Study III: Among 790 children with cancer, 3.8% carried one of the 22 most frequent pathogenic germline variants, resulting in 4.9% when correcting for diagnosis distribution. The prevalence of pathogenic variants for childhood cancer diagnoses was in line with recent whole genomic sequencing studies.

CONCLUSIONS: Family members of children with cancer have an increased cancer risk compared to the general population, which is not explained by known pathogenic germline variants alone. This thesis supports the need for further studies on genomics as well as other potential causes of pediatric and familial cancer.

Key words: familiar cancer, childhood cancer, cancer, pediatric cancer, heredity, genetics, cancer predisposition

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2021

To all my teachers in life

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Included publications

This thesis is based on the following papers:

I. **Stjernfelt KJ**, von Stedingk K, Wiebe T, Hjorth L, Olsson H, Øra I. Predominance of girls with cancer in families with multiple childhood cancer cases. BMC Cancer. 2017 Dec 19;17(1):868

II. **Stjernfelt KJ**, von Stedingk K, Wiebe T, Hjorth L, Kristoffersson U, Stenmark-Askmalm M, Olsson H, Øra I. Increased Cancer Risk in Families with Pediatric Cancer Is Associated with Gender, Age, Diagnosis, and Degree of Relation to the Child. Cancer Epidemiol Biomarkers Prev. 2020 Nov; 29(11): 2171-2179

III. von Stedingk K, **Stjernfelt KJ**, Kvist A, Wahlström C, Kristoffersson U, Stenmark-Askmalm M, Wiebe T, Hjorth L, Koster J, Olsson H, Øra I. Prevalence of germline pathogenic variants in 22 cancer susceptibility genes in Swedish pediatric cancer patients. Sci Rep. 2021 Mar 5;11(1):5307

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Abbreviations

ACT	Adrenocortical tumors
ALL	Acute lymphocytic leukemia
AML	Acute myeloid leukemia
AMKL	Acute megakaryocytic leukemia
CI	Confidence interval
FAP	Familial adenomatous polyposis
FDR	False discovery rate
FPD/AML	Familial platelet disorder with predisposition to acute myelogenous leukemia
LCCG-study	Lund Childhood Cancer Genetic Study
LFS	Li-Fraumeni Syndrome
NF1	Neurofibromatosis type 1
NHL	Non-Hodgkin lymphoma
RB	Retinoblastoma
SIR	Standardized incidence ratio
SNV	Single nucleoid variant
TKI	Tyrosine kinase inhibitors
WES	Whole exome sequencing
WGS	Whole genome sequencing

Genetic glossary

allele   – version of a gene at a given location along a chromosome

autosomal dominant   – expressed in individuals who have one copy of a pathogenic variant at a particular locus on one of the 22 pairs of autosomes

autosomal recessive   – requires biallelic pathogenic variants at a particular locus on one of the 22 pairs of autosomes to express an observable phenotype

autosome   – chromosomes that are not one of the two sex chromosomes

congenital   – present at birth

constitutional variant   – a variant that is present in all somatic and germline cells; can be passed on to offspring

epigenetic   – alterations to DNA nucleotides or proteins that control gene expression but do not alter the DNA sequence

gene   – the basic unit of heredity; DNA arranged in a linear manner along a chromosome.

genotype   – the set of alleles at a single locus, or the set of alleles at multiple loci

germline   – the cell line from which egg or sperm cells (gametes) are derived

heterozygote   – two different alleles at a particular locus, one of which is usually pathogenic

homozygous   – two identical alleles at the same locus

imprinting – the process by which maternally and paternally derived chromosomes are epigenetically modified to affect the expression of a certain gene or genes on those chromosomes depending on their parental origin

locus – the physical site or location of a specific gene on a chromosome

loss of heterozygosity – loss of one of the two alleles at a locus

pathogenic variant – an alteration in a gene that is associated with an abnormal phenotype or increased disease risk

penetrance – the proportion of individuals with a pathogenic variant causing a particular disorder who exhibit clinical findings of that disorder

phenotype – the observable characteristics of the expression of a gene

polygenic – condition caused by the additive contributions of variants in multiple genes at different loci

Abstract

BACKGROUND: Recent whole genome sequencing studies report that up to 6% of the childhood cancer population harbour a pathogenic variant. Identification of families with hereditary cancer may improve early detection of cancer as well as treatment outcome.

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CONCLUSIONS: Family members of children with cancer have an increased cancer risk compared to the general population, which is not explained by known pathogenic germline variants alone. This thesis supports the need for further studies on genomics as well as other potential causes of pediatric and familial cancer.

Introduction

Childhood cancer is a rare disease, with approximately 350 cases below 18 years of which 300 below 15 years of age diagnosed annually in Sweden, a number that has been stable during the last decades (1, 2). Before the 1960s, a cancer diagnosis during childhood was seen as a death sentence and the only therapy available was surgery. With the development of chemotherapy, risk-adapted standardized therapeutic protocols, and new diagnostic measures introduced since the 1960s, there has been a drastic increase in survival (1-6). However, during the last decade, therapeutic improvement has become more dependent on unraveling the genetic background to childhood cancer as well as the cancer genome for targeting cancer treatment (2, 3).

It is unknown to what extent childhood cancer is attributable to chance mutations, environmental factors, or inherited pathogenic genetic variants. The hereditary ratio has been estimated to be up to 10% (7-13), which is most probably an underestimation as it is based on the impact of identified single-gene variants shown to be likely pathogenic. This does not account for the potential contribution of low penetrant genetic variants. Childhood cancer is probably not the result of one single pathogenic variant in the majority of cases, but the contribution of many interacting variants (3, 14), which complicates the identification of new genetic variants involved in the carcinogenic process.

It has been theorized that the heredity of childhood cancer will increase as childhood cancer survival increases, as more potential carriers of pathogenic variants survive and will have the chance to reproduce (15). However, on a population level, this may not change the incidence in the short perspective.

In the process of investigating the molecular pathways involved in the development of childhood cancer, new targets for treatment might be revealed (2, 3, 11). This would have the greatest impact for cancers with low long-term survival ratios and those resistant to conventional treatment (16). Using genetic profiling of childhood cancer, we can obtain more precise risk assessments and this will result in more effective treatment protocols with less toxicity, something already implemented for some childhood cancer types (3, 17-19)

Identification of germline genetic variants associated with increased cancer risk allows for continuous screening for cancer and identification of family members at risk (20-22). Surveillance of patients at risk enables earlier diagnosis and treatment, which decreases both mortality and morbidity (23-25).

To identify new, potentially pathogenic variants, genome-wide sequencing methods, such as whole genome sequencing (WGS) and whole exome sequencing (WES), are being used. This has led to the identification of new, clinically relevant variants during the last decade (26-29).

Familial cancers have a few features distinguishing them from more sporadic types. The most well- known feature is having a family history of cancer, a cluster of cancer diagnoses within the family (15). Hereditary cancer, as seen in adults, often debuts at a lower age and often is multifocal (15, 30-32). Some specific and rare types of tumors (15) are a strong indicator of underlying genetic aberrations, as is a second primary tumor in a patient with childhood cancer (31, 33).

To identify families with an increased probability of harboring underlying pathogenic germline variants, it is important to collect information on the family history of cancer and the characteristics of those affected by cancer, as well as their type of cancer. As self-reported family history of cancer has been shown to underestimate the number of cancer cases in the family (34, 35), it is important to use objective methods to identify cancers among relatives. Verified official databases, such as the Swedish Population- and Cancer Registry, are a useful resource for conducting precise epidemiological research.

In this thesis, we have investigated hereditary patterns of cancer in extended family trees of children with cancer, as well as the presence of potential inherited pathogenic variants in these children, with the aim to identify frequency and patterns of cancer in these families when compared to families without.

Background

Cancer has most likely existed far longer than humanity itself, as it is present in other species that have walked this earth longer than us (36-38). Some of the earliest findings are from dinosaur fossils and bones (37, 38). It is clear that cancer has been with humans throughout history.

Egyptian manuscripts from 1600 BC, the so-called "Edwin Smith Papyrus," describe the presence of what seems to have been breast cancer, and the lack of a cure (39). Tumors or tumor-like erosions of bones, and sometimes of soft tissues in mummies, have been identified from all epochs of our history (40).

It happens to be that the oldest identified case of cancer in a human is that of a child. A male Celt who was about 15 years old and lived in about 800–600 BC passed away with a tumor in his humerus, which pathologically and radiographically was consistent with a possible osteosarcoma or chondrosarcoma (40). The word "cancer" to describe the disease was first used by Hippocrates (460–370 BC), often considered to have been the "Father of Medicine." Hippocrates used the terms carcinos and carcinoma, stemming from the Greek word for "crab" (39).

The treatment of cancer in children was originally limited to surgery. Improvement in survival occurred only after the introduction of chemotherapy for acute lymphocytic leukemia (ALL) (41). In the 1940s, Dr. Sidney Farber had observed what he believed was accelerated growth of pediatric tumors, including ALL, induced by folic acid. This led to the synthesis of aminopterin, a folate antagonist, which was effective in treatment of childhood ALL. In 1948, Farber and associates reported that aminopterin had induced remission in ten out of 16 children with ALL (42). This study was one the events that led to the establishment of Pediatric Oncology as a subspecialty (41).

Incidence and diagnosis distribution

Childhood cancer is a rare occurrence. The incidence of children diagnosed before 15 years of age in Sweden is 16.0 per 100 000 person years at risk (1). Internationally, the incidence of childhood cancer is 13.6 cases per 100 000 person years at risk, while the incidence in northern Europe is 14.8 (43). There are obvious problems with comparing incidence rates between different countries, as there are great variations in population coverage and methods of diagnosis, as well as in the definition of "childhood cancer." Most often, "childhood cancer" is defined as a cancer diagnosis before 15 or 18 years of age, the former being based on a rough estimate of the passing of puberty and the latter on the cultural and social definition of adulthood. This discrepancy can create problems when comparing different studies with different age cohorts, mainly for bone tumors, lymphomas, and carcinomas, all of which have an incidence peak after 14 years of age, but also for germ cell tumors, which have a double incidence peak.

In Sweden and internationally, there is a significant predominance of boys with cancer, with an incidence of 16.9 in Sweden and 15.1 internationally, compared to 15.0 and 12.9, respectively, for girls (1, 44). The reason for this difference still remains unknown.

Childhood cancer is histologically diverse and can be found in any organ of the body and is therefore categorized according to histology rather than location, as is the norm for adult cancer. It is commonly divided into twelve different subgroups based on morphology, according to the World Health Organization (WHO) International Childhood Cancer Classification, third edition (ICCC3 classification), from 2005. This differs from the diagnosis distribution among adults in whom the majority of cancers derive from epithelial cells and are carcinomas, which only account for 1.8% of pediatric cancers (1, 45).

The most common childhood cancer is leukemia (most commonly, ALL and acute myeloid leukemia (AML)), followed by central nervous system (CNS) tumors and lymphoma/histiocytosis. Together, these diagnoses make up more than two-thirds of all cancer cases in children (45) while the same diagnoses in adults account for less than 1% of all cases (46). The remaining childhood cancer diagnoses are embryonal cancers, i.e., neuroblastoma, retinoblastoma (RB), nephroblastoma, hepatoblastoma, rhabdomyosarcoma, and germ cell tumors, which are all rare in adults, and bone sarcomas and carcinomas, which are more frequent in adults (46). These diagnoses make up the remaining 30% of all childhood cancers (45).
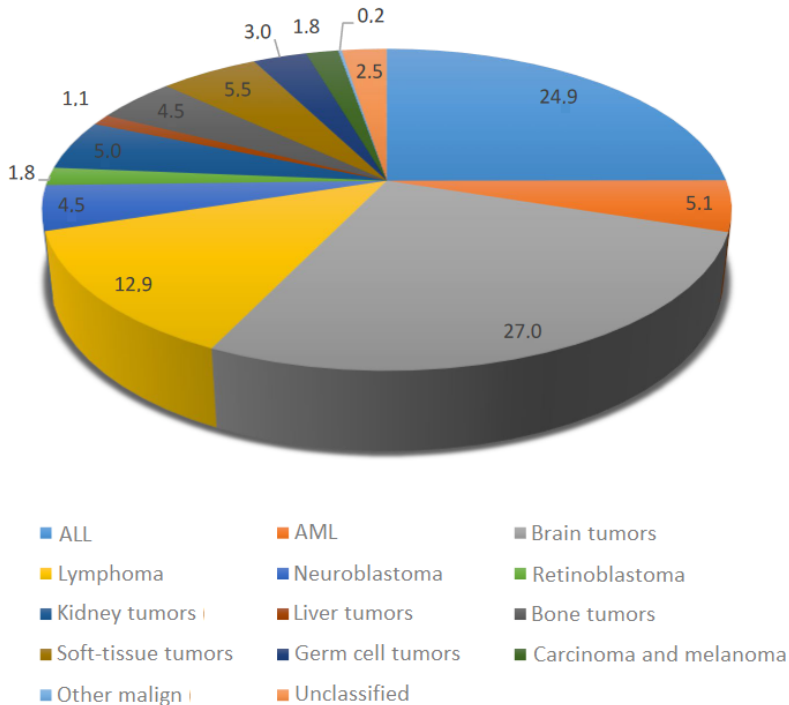
Figure 1. Childhood cancer diagnosis distribution in Sweden.

Abbreviations: ALL = acute lymphocytic leukemia; AML = acute myeloid leukemia. Data from the Swedish

Childhood Cancer Registry.

Children with cancer are most often diagnosed before 5 years of age (1), although the mean age at onset varies between the different cancer types. Leukemia, neuroblastomas, renal tumors, hepatoblastoma, and RBs are often diagnosed at an early age and have an incidence spike before 5 years of age (1, 26, 47). Carcinomas, bone tumors, and lymphomas have an increased incidence in adolescence. CNS tumors, soft tissue sarcomas, and some subgroups such as AML and non-Hodgkin lymphoma (NHL) have a more even age distribution. Germ cell tumors are most common during infancy and adolescence (1, 26, 47).

Childhood cancer treatment and survival in Sweden

During the early 1960s, most children with cancer in Sweden were treated with surgery alone and the majority of those did not survive (Figure 2). Those who were treated with chemotherapy or focused radiotherapy most often had disseminated disease at diagnosis, and therefore mortality rates were very high (1, 48). Chemotherapy, initially based on mustard derivatives (49), was introduced in the 1960s. With its wider use, the survival from several types of cancer, specifically Hodgkin lymphoma and leukemia, increased drastically (1).

Initially, Swedish childhood cancer patients were treated at the pediatric ward of the local hospital and there was a lack of treatment guidelines and centralized pediatric cancer centers. Consequently, treatment regimens varied greatly across the country. In 1967, the Swedish Childhood Leukemia Group was founded with the aim to standardize childhood leukemia treatment. This formed the groundwork for the Nordic countries' treatment protocols for children with leukemia. The standardized protocols allow for evaluation of treatment effect, with treatment intensification or de-escalation when needed (48). Since the introduction of chemotherapy, standardized national and international protocols have been the main factors associated with increased survival in childhood cancer after the 1960s and 1970s (Figure 1).

After the 1990s, there was a stagnation in survival rates for most types of childhood cancer. However, AML has seen a recent increase in survival, which is accredited to timely improvement and intensification of treatment protocols (45). Generally, it is believed that conventional therapy (surgery, current chemotherapy options, and radiotherapy) is unlikely to yield additional improvement in survival for the patient groups which currently have low cure rates (2).

In 2001, the complete human genome was published (50, 51). Since then, knowledge in genomics has evolved, and keeps on evolving, including a deeper understanding and development of cancer genomics. With increased knowledge of cancer morphology and genomics, risk assessment, and treatment stratification of different subtypes of childhood cancer, survival has improved. Through identification of different childhood cancer subgroups by their genotype, it has been possible to adapt and improve treatment protocols. Examples are chemotherapy protocols for pediatric leukemia patients, where the intensity of treatment is based on risk factors including cytogenetic profile of the cancer cells (3, 17-19).
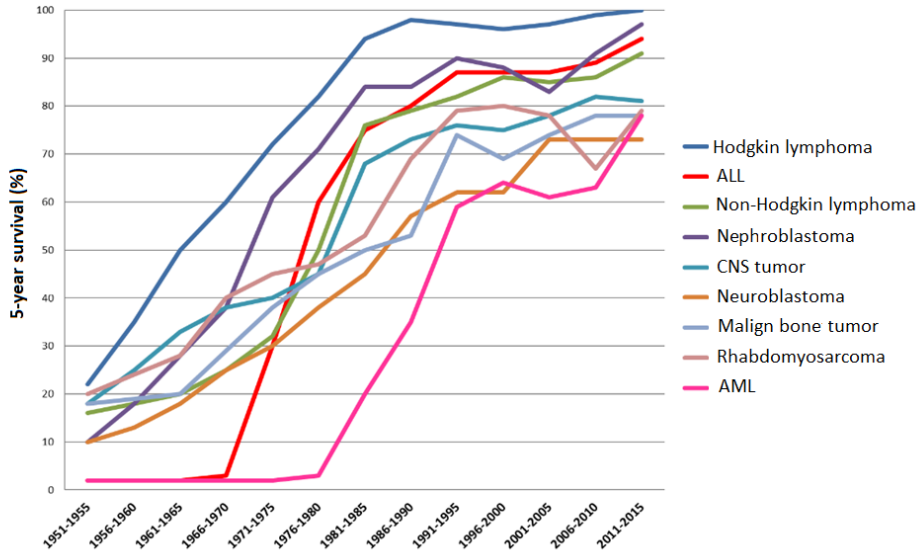
Figure 2. Five-year survival over time.

Abbreviations: ALL = acute lymphocytic leukemia; AML = acute myeloid leukemia; CNS = central nervous system.

Data from the Swedish Childhood Cancer Registry.

Targeted therapies against genomic characteristics of the cancer cells have been introduced, partly as complement to conventional childhood cancer treatment, for example using monoclonal antibodies. One of the first examples was the introduction of tyrosine kinase inhibitors (TKIs) as complement to chemotherapy in rare types of ALL (52, 53) and AML (54), or the use of rituximab, a CD20 antibody, in treatment of Burkitt's lymphoma (55, 56).

While targeted therapies may be highly effective in specific types of childhood cancer, they clearly will not work for all patients. This treatment approach is mostly used in tumors that are dependent on, or are driven by, the molecule targeted by the drug (3). However, in the future, it may be possible for patients to be treated according to their own, unique cancer genetic profile, resulting in higher efficacy and minimal toxicity.

Etiology

Cancer cells derive from normal human cells that accumulate genetic and epigenetic mutations, which enable them to become tumorigenic and, ultimately, malignant (3, 57-59). Random mutations in the cell genome can sometimes lead to new biological capabilities necessary for tumor development. Six such mutations, or biological "hallmarks," were presented by Hanahan & Weinberg et al. in 2000 (57) and consists of:

- sustaining proliferative signaling, often related to changes in the response to exogenous mitogenic stimuli involving tyrosine kinase receptors;

- evading growth suppressors, often due to changes in the anti-proliferative set of controls regulated in part by the *RB* gene;

- resisting cell death, often due to changes in the apoptotic pathway regulated in part by p53;

- enabling replicative immortality, often related to changes in the telomerase pathway, which regulates telomere maintenance;

- inducing angiogenesis, which provides a blood supply for the growing tumor; and

- activating invasion and metastasis, which enables a cancer cell to invade normal tissues.

Furthermore, underlying genome instability in these cells drives the acquisition of additional hallmarks further. It has been estimated that as few as four or as many as seven discrete mutations may be necessary for these changes in humans for cancer to occur (60-62).

Two additional hallmarks for human cancer development were later suggested: reprogramming of energy metabolism, and evading immune destruction (63). These different attributes can be seen in various degrees in all types of cancer, but the specific mutations through which they are acquired differ greatly.

Within a malignant tumor, multiple distinct types of cells interact with each other, including non-malignant cells that get recruited to assist tumor growth. Thus, the development of a tumor is not only dependent on the sum of traits in cells acquired by genetic aberrations, but also on the "*tumor microenvironment*" in which these cells proliferate and interact (63).

The mechanisms that drive accumulation of mutations in the cell's genome are believed to differ between adult and childhood cancer. In adult cancer, we believe

the primary contributor to be the accumulation of exposures and toxic insults that over decades lead to the development of malignant cells (3). Childhood cancers, however, with an obvious shorter period of potential exposure, are believed to be more dependent on other mechanisms (64), such as inherited or acquired genetic variants predisposing for cancer, as well as in utero exposure (3, 65, 66).

As children are in the process of normal growth and development, their cells undergo a complex program of division and differentiation. This gives opportunities for the rise of oncogenic mutations in cell populations that are less vulnerable, or silent, in the adult population (3). This could explain why embryonal tumors, such as neuroblastoma, RBs, embryonal rhabdomyosarcomas, and germ cell tumors, have their peak incidence during infancy (1, 64) and why osteosarcoma and Ewing sarcoma have their peak during the skeletal growth spurt in adolescence (3).

The different factors known to predispose for the development of childhood cancer can be divided into environmental, intrinsic, and genetic factors, as discussed below. Genetic factors will be discussed in depth in the section "Childhood cancer genetics."

Environmental factors

As childhood cancers are rare, it is challenging to conduct etiological investigations. Consequently, few environmental risk factors have been identified. Prior chemotherapy and high dose radiotherapy are the two most well-known and documented risk factors (67-70).

As in many diseases, the role of infections has been discussed as a trigger for cancer development. The Epstein-Barr virus is well known to increase the risk for lymphoma in adults (71, 72), but no decisive studies on the correlation between infections and childhood cancer have been conducted so far. For ALL, the most common childhood cancer and therefore the largest available cohort, it has been hypothesized that a child's immune system that has not been exposed to common infections early on is less developed and consequently more prone to have an unregulated reaction leading to the development of ALL (73). This is supported by the findings that breastfeeding (74, 75) and day care attendance (76, 77) are associated with lower risk for ALL. Moreover, exposure to pesticides has been shown to be related to an increased risk for ALL (78-80).

Several other risk factors have been studied, such as maternal coffee, alcohol, and vitamin consumption (81-83), cigarette smoking of mothers and fathers (84, 85), and exposure to electromagnetic fields (86). However, so far, these studies have been inconclusive (26).

Intrinsic factors

Some physical characteristics are associated with increased childhood cancer risk. Both high birth weight and being large for gestational age are associated with increased risk for childhood cancer (87), in particular for ALL (88, 89), CNS tumors (90), neuroblastoma (91), and Wilms' tumor (WT) (92). This has been theorized to be due to exposure to growth hormones (93) or genetic factors determining birth weight (94). High parental age has also been shown to increase the risk for childhood cancer in offspring (95). There is a slightly lower risk for childhood cancer in twins compared to singletons (96), but whether this is attributable to lower birth weight is unclear.

Birth defects have been shown to correlate with increased risk for childhood cancer, both when the child has a chromosomal anomaly (97-104) and when not (97-99, 101, 104). However, as both childhood cancer and congenital birth defects are very rare, no specific associations have been found.

A shared underlying genetic cause could explain the correlation between childhood cancer and congenital birth defects, possibly through variants in genes regulating embryogenesis (105). Indeed, embryonal cancers such as RBs, neuroblastomas, soft tissue sarcomas, and germ cell tumors have been reported to be specifically associated with congenital defects (98, 100), but brain tumors and leukemia to a smaller extent.

Childhood cancer genetics

The genetic background can explain causality of childhood cancer in the minority of cases. However, studies of genetic variants in cancer cells and in somatic cells of children with cancer might reveal information on the genesis of childhood cancer and potentially uncover targets for treatments.

This thesis focuses mainly on familial cancer and constitutional genetic variants, the latter being either inherited or de novo mutations. These germline mutations are present in all somatic cells of the affected person and can be passed on to offspring. We do not in detail discuss the specific mutations that are acquired during life and accumulated in cancer cells.

History of cancer genomics

Throughout history, different theories have been developed to explain the origin of cancer, including imbalance in body fluids, degeneration of the lymph, trauma, and chronic inflammation (39, 106).

The first insights into the role of the cell's genome for cancer genesis were obtained in the late 19th (107) and early 20th century (108). By using microscopes, researchers were able to observe bizarre chromosomal aberrations in dividing cancer cells; this led to the first suggestions that changes in the hereditary material could cause tumors.

In the 1940s, DNA was determined to be the carrier of inheritance (109) and in 1953 its structure was defined (110). When agents that were shown to cause chromosome aberration as well as mutations in DNA were also seen to increase the risk for cancer, the theory that genetic aberrations drive the development of cancer was strengthened (59, 111).

One of the first defined cancer-associated chromosome abnormalities was the Philadelphia translocation, which occurs between chromosomes 9 and 22 (112, 113). It was discovered in 1960 as a shortened chromosome 22 in cancer cells among patients with chronic myeloid leukemia, and, in accordance with the terminology of those days, named after the city where it was discovered.

One of the most well-known gene with genomic aberrations related to cancer, the TP53 gene and its coded protein p53 were identified simultaneously by several different research teams in 1979 (114-118). Pathogenic TP53 variants were later shown to be present not only in cancer cells: in 1990, TP53 was identified in somatic cells of patients with Li-Fraumeni syndrome (LFS) as well (119), a cancer predisposition syndrome that was observed in families with aggregation of breast cancer and childhood sarcoma and leukemia (120).

The first gene to be associated with an inherited syndrome was RB1 (121), identified in 1986 in patients with RB, hence its name. Retinoblastoma had already been suggested to be both a hereditary and a non-hereditary type, where children with multiple bilateral tumors were suspected to have hereditary cancer (122). To explain this, Alfred G. Knudson suggested the two-hit model in 1971, which is described in greater depth later in this thesis.

Following the discovery of the RB1 mutation, other highly penetrant pathogenic, somatic genetic variants associated with increased pediatric cancer risk were revealed, and several of the most common cancer-predisposing variants in children were identified during the following decade. In 1990, mutations in the NF1 gene were identified in patients with neurofibromatosis type 1 (123), and in 1991, mutations in the APC gene were identified in patients with familial adenomatous

colon polyposis (FAP) (124). In the same year, RUNX1 mutations were identified in patients with AML (125), while MLH1, MSH2, MSH6, and PMS2 mutations were all identified in patients with Lynch's syndrome in 1994 (126). Further, mutations in BRCA1 and BRCA2 were identified in 1994 and 1995, respectively, both shown to be susceptibility genes for hereditary breast and ovarian cancer (127, 128).

Impact of heredity on childhood cancer

In this context, "heredity" is defined as a genetic variant that has been passed down either from the parents to the affected child or as a de novo mutation in the oocyte or sperm before fertilization. Thus, even children without a family history of cancer can have a hereditary predisposition for childhood cancer.

Previous studies have estimated that up to 10% of childhood cancers are due to cancer predisposing germline mutations (7-13). However, their findings were based on already known genetic variants, mainly high penetrance mutations in single genes and predisposition syndromes, and do not account for the effect of potential genetic variants not yet discovered. As a comparison, adult cancers caused by known pathogenic genetic variants account for less than 5% (129), although a statistical estimate based on twin studies in the Nordic countries attributes 33% of cancer risk to heredity (130). Due to the rareness of childhood cancer, similar studies on childhood cancer are difficult, but it is reasonable to assume that the genetic component far exceeds that of already described pathogenic variants.

The fact that more genetic variants have been detected to be causal in childhood cancer compared to adult cancer does not prove that childhood cancer has a stronger hereditary component; it could theoretically also be due to genetic variants being easier to identify in childhood cancer. However, it is generally argued that heredity has a higher impact on childhood cancer risk compared to cancer in adults (32, 131, 132) because children have had shorter environmental exposure. It has also been shown that, among adults, younger age of onset correlates to higher cancer risk among relatives (30-32), which also indicates that cancer at an early age is more likely to be caused by inherited pathogenic genetic variants.

There are differences in hereditary ratios of specific cancer diagnoses, i.e., the fraction of cancer cases attributed to known inherited pathogenic genetic variants varies depending on the type of childhood cancer. For example, adrenocortical carcinomas are thought to be caused by heredity in 50–80% of cases, while the figure for optic gliomas, RBs, and pheochromocytomas is 40% or higher (133-

135). The most common types of childhood cancer, leukemia and CNS tumors, have a much lower hereditary ratio, of <5% and <10%, respectively (12, 13).

Genetic mechanisms of inheritance

There are different mechanisms of genetic inheritance, the most simple being monogenic inheritance, which means that the inherited phenotype (or risk for cancer, which this thesis focuses on) is dependent on variation in one single gene.

In the cell, every gene has a specific location on the chromosome called a "locus" (place or position), and as chromosomes come in pairs, every gene is represented on two loci. The genetic variation of the locus is called "allele." The patterns of inheritance depend on whether the allele has a dominant or recessive effect. Whether the locus is on an autosomal chromosome or a sex chromosome also affects this pattern. An autosomal dominantly inherited variant only needs one defect allele to give rise to a change in phenotype. Therefore, if one of the parents carries the gene, there is a 50% risk for each offspring to get the pathological variant (see Figure 3).

Parent with gene defect

	A	a
A	AA (Normal)	Aa (Pathological)
A	AA (Normal)	Aa (Pathological)

A = normal allele
a = defect allele

Parent without defect

Figure 3. Autosomal dominant inheritance.

By contrast, for autosomal recessive inheritance, the offspring needs defects in both alleles for a pathogenic phenotype to occur, one inherited from each parent. With two parents being carriers of the defect gene, there is a 25% probability of offspring to inherit two normal alleles, 50% to inherit one normal and one defect

25

allele, and 25% to receive two defect alleles, thus giving rise to a pathological phenotype (see Figure 4).

Parent with gene defect

	A	a
A	AA (Normal)	Aa (Carrier)
a	Aa (Carrier)	aa (Pathological)

(Row labels: Parent with gene defect — A, a)

A = normal allele
a = defect allele
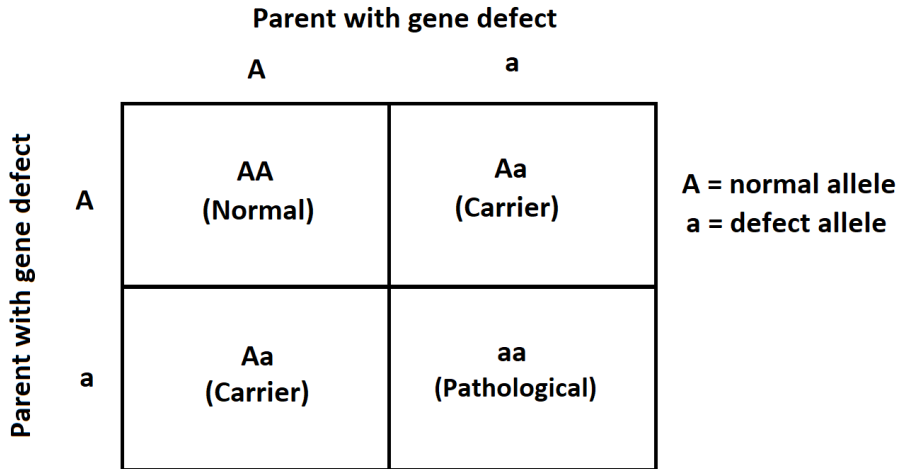
Figure 4. Autosomal recessive inheritance.

An inherited pathogenic variant may not be present in the phenotype at birth, but may manifest later in life. The term "penetrance" is used to indicate the number of carriers of a pathogenic variant who manifest symptoms. The penetrance of a specific disease may therefore increase with age. For example, if an inherited variant reveals a phenotypic manifestation in 80% of cases the penetrance for this variant is 80%.

Even though the monogenic inheritance of high penetrance germline variants is the most common mechanism for inherited childhood cancer risk (136), childhood cancer predisposition syndromes and childhood cancer-associated syndromes are inherited in other manners as well (132). Other examples are chromosomal abnormalities, such as Down syndrome (137), epigenetic disorders such as imprinting errors (138), and polygenetic/complex inheritance. The inherited risk for a specific type of tumor might be attributable to one mechanism or to the sum of several different mechanisms. For example, WT has been shown to be derived from multiple different genetic disorders affecting distinct molecular pathways (139). It is important to be familiar with the underlying genetic mechanisms of inheritance in different types of cancers when deciphering potential cancer predispositions from data on family history of cancer.

Two-hit theory

The "two-hit theory" was proposed by Knudson in 1971 (122). The theory explains why individuals with inherited cancer get cancer at an earlier age, and more often develop multiple cancers and at different sites, compared to individuals with sporadic cancer.

The theory is that, on a cellular level, two mutations, one on each allele, are required for cancer to develop, i.e., a recessive pattern. For sporadic cancer, two separate mutations have to occur within the same cell on both alleles for cancer to develop, and therefore this occurs later in life.

In inherited forms, one pathogenic variant is inherited and found in all cells and all tissues, whereas the second is an acquired somatic mutation found only in tumor cells (140). Therefore, individuals born with one pathogenic variant of the gene in all cells (those with an inherited predisposition) are much more likely to develop cancer at an earlier age and develop bilateral/multiple tumors as they only need one sporadic mutation for cancer to develop (see Figure 5).

When the RB1 gene was identified in 1986, this theory was confirmed and is now considered to be the paradigm for dominantly inherited tumor syndromes. These syndromes are most often caused by pathogenic variants in one allele of a tumor suppressor or DNA repair gene (141).
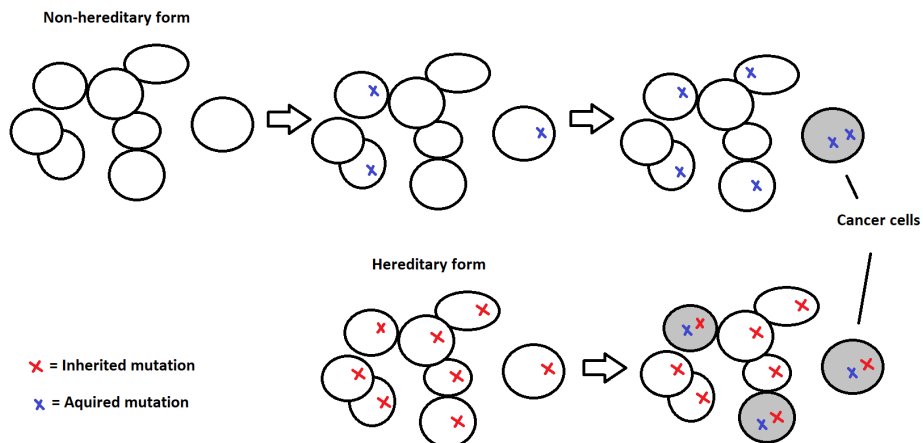
Figure 5. Visualization of the two-hit theory.

Hereditary cancer syndromes

A hereditary cancer syndrome is an inherited disorder in which there is a higher than normal risk of certain types of cancer. These syndromes are caused by mutations in certain genes passed from parents to children.

There are multiple suggestions for when to suspect a hereditary predisposition to cancer. They can generally be summarized as follows (15, 142):

(a) family history of cancer of the same type;

(b) different types of cancers in the same patient;

(c) multifocal or bilateral cancers;

(d) early age at diagnosis;

(e) specific physical findings (such as café au lait spots in neurofibromatosis type 1 (NF1), and macroglossia in Beckwith-Wiedemann syndrome (BWS)); and

(f) specific tumors such as pleuropulmonary blastoma in DICER1 syndrome, malignant rhabdoid tumors, and adrenocortical carcinoma in LFS.

The factors listed above increase the probability of an inherited cancer predisposition, but are not proof of it. A hereditary cancer syndrome is identified through either –

(a) specific phenotypic features, as in classic LFS; or

(b) the identification of a specific pathogenic variant.

The most common hereditary childhood cancer predisposition and childhood cancer-related syndromes are summarized in Table 1. Although many of them usually present in adult age, they may also cause childhood cancer. As previously discussed, all these syndromes combined contribute to less than 10% of all childhood cancer cases (7-13); however, they have specific molecular pathways and mechanisms that are common in several other cancer types (3), and they have been important for understanding the development of childhood cancer. Some of these will be explained in depth in the following section and used as examples for the mechanism of inheritance they represent.

Table 1. Inherited cancer predisposition syndromes.

Syndrome	Gene(s)	Neoplasm(s)/location(s)
Ataxia-telangiectasia	ATM	leukemia, lymphoma, breast, ovarian
Basal cell nevus syndrome (Gorlin syndrome)	PTCH	basal cell carcinoma, medulloblastoma
Beckwith-Wiedemann syndrome	*CDKN1C, H19, IGF2, KNBQOT1*	WT, neuroblastoma, hepatoblastoma, rhabdomyosarcoma
Birt-Hogg-Dubé syndrome	*FLCN*	kidney
Bloom syndrome	*BLM*	leukemia, lymphoma, WT, colon, breast, cervix
Congenital central hypoventilation syndrome	*PHOX2B*	neuroblastoma, ganglioneuroma, ganglioneuroblastoma
Costello	*HRAS*	papilloma, rhabdomyosarcoma, neuroblastoma, transitional cell carcinoma
Cowden syndrome	*PTEN*	breast, thyroid, kidney, glioblastoma
Dyskeratosis congenita	*DKC1, TERC, TERT*	leukemia, esophagus
Dysplastic nevus syndrome	*CDKN2A and others*	melanoma, pancreatic
Hereditary breast/ovarian cancer	*BRCA1, BRCA2*	breast, ovarian, prostate pancreatic
Hereditary non-polyposis colon cancer (Lynch syndrome)	MLH1, MSH2, PMS2, MSH6	colon, uterine, gastric, endometrial, small bowel, sebaceous gland
Fanconi anemia	FAN-CA-FANCN and others	leukemia, hepatocellular, esophagus, head and neck, cervix, WT, medulloblastoma, neuroblastoma, embryonal tumors
Familial acute myeloid leukemia	RUNX1 and others	leukemia
Familial paraganglioma/pheochromocytoma	SDHB, SDHAF2, SDHC, SDHD	paraganglioma, pheochromocytoma, neuroblastoma
LFS	TP53	leukemia, lymphoma, breast cancer, osteosarcoma, brain tumors, neuroblastoma, adrenocortical carcinoma, soft tissue sarcoma
Multiple endocrine neoplasia type 1	MEN1	parathyroid, pancreas, gastrinomas, insulinoma, carcinoid tumors
Multiple endocrine neoplasia types 2A	RET	thyroid medulla, pheochromocytoma
Neurofibromatosis type 1	NF1	MPNST, pheochromocytoma, astrocytoma, glioma, leukemia
Neurofibromatosis type 2	NF2	astrocytoma, melanoma, meningioma
Noonan syndrome	PTPN11, SOS1, RAF1, KRAS	leukemia, neuroblastoma, rhabdomyosarcoma
Nijmegen breakage syndrome	NBS1	lymphoma, leukemia
Peutz-Jeghers syndrome	LKB1	stomach, small intestine, colon, pancreas, uterine,
Familial adenomatous polyposis	APC	colon, small intestine, thyroid, pancreas, hepatoblastoma, medulloblastoma
RB	RB	RB, osteosarcoma, melanoma, pinealoblastoma, lung
Von Hippel-Lindau syndrome	VHL	renal cell carcinoma, pancreatic islet cell tumors, pheochromocytoma
Werner syndrome	WRN	leukemia, melanoma, osteosarcoma, thyroid
WAGR syndrome	WT1	WT
Weaver syndrome	EZH2	neuroblastoma
Wiskott-Aldrich syndrome	WASP	leukemia, lymphoma
Xeroderma pigmentosum	Many	basal cell, melanoma, stomach, leukemia

Adapted from *Pizzo and Poplack's Pediatric Oncology*, 8th edition, S. Blaney, L. Helman, P. Adamson, Wolters Kluwer 2021. (3)

Abbreviations: LFS = Li-Fraumeni syndrome; MPNST = malignant peripheral nerve sheath tumor; RB = retinoblastoma; WAGR = WT, aniridia, genital abnormalities, and mental retardation; WT = Wilms' tumor.

Autosomal dominant disorders

Most single-gene cancer predisposition syndromes are autosomal dominantly inherited, and the majority of them are due to mutations in tumor suppressor genes (143). The gene has a 50% risk of being inherited, evenly from both the mother and the father, and to both daughters and sons. Due to incomplete penetrance, the phenotypical expression of the variant can sometimes seem to "skip" a generation.

Hereditary retinoblastoma

The first observed gene responsible for a childhood cancer predisposition syndrome was RB1 (121), a tumor suppressor gene. Those carrying a variant of this gene go with a primarily increased risk for RB, hence its name, but also carry increased risk for osteosarcoma and malignant melanoma in childhood, as well as other tumors later in life (144, 145). Even for children with RB of the hereditary type, the mutation is most often de novo; thus, the parents are unaffected and siblings are rarely affected. However, a potential offspring of the child with RB has a 45% risk of developing the same tumor (146).

Li-Fraumeni syndrome

The most well-known inherited cancer predisposing syndrome is probably LFS, which is caused by a pathogenic variant in the *TP53* gene in 60–80% of cases (147, 148). Traditionally, LFS has been defined as a proband with sarcoma diagnosed below 45 years of age, with a first-degree relative with any cancer below 45 years of age, in addition to another first- or second-degree relative with either any cancer under 45 years or a sarcoma at any age (149, 150). Several different tumors are associated with LSF and mutations in *TP53*, including breast cancers, brain tumors, leukemia, adrenocortical carcinomas, gastric cancers, lymphoma, and colorectal cancers (147). For children, the most common cancers are osteosarcoma, adrenocortical carcinoma, medulloblastoma, choroid plexus carcinoma, anaplastic rhabdomyosarcoma, and acute lymphoblastic leukemia (Table 1).

As in the case with *RB1*, *TP53* is a classic tumor suppressor gene and would be expected to lead to malignant transformation in the cell through so-called "loss of heterozygosity," meaning that the "healthy" gene is damaged or deleted, in line with the previously mentioned two-hit theory. However, this has been shown to occur in only 50% of cases (151, 152), and the mechanisms for malignant transformation of the cell in the remaining cases are still unknown.

Familial neuroblastoma

In addition to cancer predisposition syndromes inherited through tumor suppressor genes, variants in oncogenes are often inherited in an autosomal dominant pattern (3). A mutation in an inherited "inactive" proto-oncogene can result in transformation to an active oncogene. Thus, this process does not require a mutation in the second allele, and does not follow the two-hit theory.

One of the recently discovered variants in an oncogene in pediatric cancer is *ALK*, which was discovered in 2008 and is associated with familial neuroblastoma (123). A variant linked to familial neuroblastoma was first localized to chromosome 2p23 but was later identified to be a missense mutation specifically in the *ALK* proto-oncogene that converted it to an oncogene (153). This variation in the *ALK* gene has incomplete penetrance and many carriers do not develop a tumor. Moreover, the malignant potential of the tumor varies, from more benign ganglioneuromas to advanced neuroblastomas.

Familial leukemia

Even though leukemia is the most common cancer in children (1, 2), it has been associated with very few germline mutations (12, 13). Some other cancer predisposition syndromes in children, such as NF1, LFS, and DS, are associated with increased leukemia risk, but specific predispositions for leukemia are rare (154). One such predisposition is familial platelet disorder (FPD) with a predisposition to AML (FPD/AML). It is autosomal dominantly inherited and is characterized by thrombocytopenia and a high risk of development of AML. One of the genes identified is the *RUNX1* gene (155, 156). This variant seems partly to follow the two-hit theory (157), but it is likely that other mechanisms are involved as well (3).

Familial adenomatous polyposis

Familial adenomatous polyposis is mainly known for the extensive growth of polyps in the colon and a 90% risk of developing colon cancer throughout life (158). However, FAP is also associated with a 400-fold increased risk for hepatoblastoma in children (159), as well as an increased risk for thyroid cancer in adolescence (160). The APC gene (named after "adenomatous polyposis coli," another name for FAP) is estimated to be the causative mutation in 85–90% of families with FAP (161), and approximately 10–15% of hepatoblastoma patients carry the variant (162, 163). It is therefore important to carefully explore the family history of cancer of patients with hepatoblastoma to identify those at risk of having FAP.

Neurofibromatosis type 1

Neurofibromatosis type 1 is the most common genetic disorder in the general population (164). It is associated with a number of clinical features such as café au lait spots, axillary freckling, Lisch nodules of the iris, and neurofibromas (164). Patients with NF1 develop benign tumors including neurofibromas, gliomas of the optic tract, other low-grade gliomas, and pheochromocytomas. However, these can develop into malign tumors. The childhood cancers primarily associated with NF1 are optic gliomas and malignant peripheral nerve sheath tumors (MPNSTs) (165-167), but risk of AML is also increased (168, 169).

Neurofibromatosis type 1 is associated with a mutation in the large NF1 gene, which is believed to be a tumor suppressor gene (170) that inhibits the Ras protein (3). As a variant in a tumor suppressing gene, it seems to follow the two-hit theory, as tumors associated with NF1 show loss of heterozygosity (165, 171).

Autosomal recessive disorders

In disorders inherited recessively, two pathogenic variants need to be inherited, one maternal and one paternal. The rarity of inheritance of these two events makes the associated disorders appear to be sporadic. Even when both parents carry the variants, there is only a 25% risk that the child inherits both, and children affected by the disorder often have no family history of the disease.

Many of these disorders are caused by variants in genes that encode DNA repair enzymes or DNA damage checkpoint genes, leading to increased sensitivity to spontaneous and exogenous DNA damage (3). Therefore, patients with these types of syndromes are often more sensitive to external environmental factors.

Patients with xeroderma pigmentosum, a rare DNA repair defect syndrome, are at increased risk for skin cancer from sun exposure (172, 173). The sensitivity can also affect the choice of treatment.

Ataxia-telangiactasia (AT) is a recessive disorder with development of truncal ataxia in childhood (174) and is associated with an increased risk for leukemia and lymphomas in children (175). These children are significantly more sensitive to radio- and chemotherapy, and consequently need specific treatment regimens (176). Even though AT is recessive in children, adult females with AT have an increased risk for breast cancer which is inherited dominantly.

Chromosomal abnormalities

Down syndrome

The most common chromosomal abnormality related to childhood cancers is Down syndrome (177). Individuals with Downs syndrome have a 20-fold increased risk of developing leukemia compared to the general population (178). They are more likely to develop AML, and also the rare subtype acute megakaryocytic leukemia (AMKL). This results in an almost 400-fold excess of AMKL in children with DS compared to children without. Unfortunately, AML and AMKL have a worse outcome than ALL, the most common type of leukemia and childhood cancer.

Even when children with Down syndrome have ALL they show a lower 5-year survival (179) and are more prone to treatment-related toxicity (180).

Sex chromosome abnormalities

Sex chromosome abnormalities comprise a group of disorders that result from numerical and structural aberrations of the X and Y chromosomes, many of which lead to an increased risk of childhood cancer. Any phenotypically female child who carries parts of the Y chromosome has an estimated 25% increased risk of gonadoblastoma (181); the TSPY gene on the Y chromosome has been suggested to be the responsible gene (182). It is recommended for gonads to be removed in girls and women with these chromosomal disorders (183) as the gonads are in most cases non-functional.

WAGR syndrome

Specific chromosomal deletions are associated with increased cancer risk in childhood. Depending on the size of the deletion, neighboring genes to the one that leads to increased cancer risk may also be affected, resulting in varied specific features for different disorders (3). The WAGR syndrome is named after potential components of the disorder: WT, aniridia, genital abnormalities, and mental retardation. It is associated with deletions at 11p13 (184). The WT1 gene lies in the WAGR interval and when deleted this leads to the development of WT. Deletions of other parts of the WAGR interval cause other symptoms of the syndrome (185, 186).

Imprinting errors and overgrowth disorders

Beckwith-Wiedemann syndrome

Loss of imprinting can cause two genes to be activated when only one should be. In Beckwith-Wiedemann syndrome, this can cause hemihyperplasia and an increased risk of developing abdominal tumors such as hepatoblastoma and WT (187). Affected genes are located on 11p15. They include paternally expressed (maternally imprinted) insulin-like growth factor 2 gene (IGF2), and RNA transcript KCNQ1OT1 (188, 189).

Disorders due to imprinting errors often have an unusual pattern of inheritance, as carriers show symptoms depending on whether they have inherited the gene paternally or maternally. Thus, a gene can be inactive for generations as long as it is inherited paternally or maternally and can thus be mistaken for have a "sporadic" pattern.

Familial cancer risk

Family history of cancer is an important risk factor for most types of adult (190) and childhood cancers (15, 191), and continuous research of genetic aberrations is undertaken to explain the increased risk of cancer in relatives of cancer patients.

The cancer risk for relatives of childhood cancer patients has been investigated in many studies during the past decades (31, 192-203). Initially, it was deemed unlikely that first-degree relatives (parents, offspring, and siblings) had an increased cancer risk when known predisposition syndromes were excluded (192-198). However, more recent, population-based studies show that a moderately increased cancer risk for adult relatives does exist (31, 199-202).

A family history of childhood cancer seems to have a much higher effect on childhood cancer, compared to adult cancer, among relatives. Already early studies showed a nearly twofold increase in childhood cancer incidence among first-degree relatives (195, 204, 205), which was confirmed in later studies as well (200-202).

The cancer risk for more distant relatives has only been examined in a handful of studies. Risk among relatives for the three largest groups of childhood cancer – leukemia (206-209), CNS tumors (210, 211), and lymphomas (203, 208) – has been studied as far as second-degree relatives. Relatives of children with leukemia

and lymphomas did show an increased risk, but relatives to children with CNS tumors did not. Few studies examined the cancer risk for relatives up to the second degree to children with all types of cancer (200, 201), and only one also included third-degree relatives (202). Two studies showed a generally increased risk for relatives up to the second degree of relation (200, 202).

Early on, twins were studied with regard to childhood cancer risk. Twin concordance was mainly reported for hematological cancers, such as leukemia and lymphoma (212, 213), and for RB (213), a cancer with one of the highest estimated hereditary factors (134). However, for hematological cancers, it is suspected that this finding is not explained by a shared genetic predisposition to cancer, but by an in utero spread of cancer cells between the twins (214, 215). Twin birth is, however, also strongly correlated with low birth weight, which has been shown to have a lower risk of most types of childhood cancer (87-92); this could partly explain the unaffected cancer risk among twin siblings of children with cancer.

Whole genome sequencing and childhood cancer

It was suggested early on that most high penetrance genes predisposing for cancer in childhood have already been discovered as they are the most likely to be identified: they have distinct patterns of inheritance and are monogenic (216). Indeed, the most common variants were discovered early. However, through genome-wide association studies it has been possible to continuously identify less common and less penetrant mutations, and the list of childhood cancer predisposing genes is continuously growing. With WGS analyses, it is possible to compare hundreds of thousands of single nucleoid variants (SNVs) in the search for pathogenic variants causative for cancer. Due to the extremely large number of comparisons, an incredibly high degree of statistical significance is needed, generally $p < 5 \times 10^{-8}$ (26), and therefore very large sample sizes are needed as well. Thus, this method is poorly suited for rare diseases. However, despite childhood cancer being a rare disease, studies have shown reliable results using WGS, locating variants associated with ALL (217-219), neuroblastoma (220-222), WT (223), osteosarcoma (224), and Ewing sarcoma (225).

It is likely that most high penetrance variations have already been identified, as they have the highest likelihood to yield a significantly increased risk for the development of childhood cancer. With the very stringent p-value used during WGS, it is likely that low penetrance variants are being excluded. Therefore, the estimate of total heritability, that is, the proportion of the risk due to genetic variation, will be negatively biased. A British study from 2012 on pre-B ALL

pooled all SNVs identified by WGS and estimated that 24% of the total variation in pre-B ALL risk is accounted for by common genetic variation (14), which is five times higher than what can be explained by known germline variants (12). It is clear that the biology of the cell is driven by the simultaneous expression of multiple genes acting in unison. To further study genes involved in the genesis of cancer, we may have to study how several genes act together, rather than focusing on the effects of single-gene events.

Aims

In this thesis project, we studied families within the childhood cancer population

in the Southern health care region of Sweden in order to:

- describe the epidemiology of pediatric cancer patients and their extended family regarding cancer incidence, cancer diagnosis, sex, age at onset, outcome, and degree of relation;

- determine the frequency of genetic aberrations in the study populations and compare it with recent studies of similar populations; and

- reveal potential novel predisposition factors for childhood cancer with regard to family characteristics.

Materials and methods

Lund Childhood Cancer Genetic study cohort

The Lund Childhood Cancer Genetic (LCCG) study was initiated in 2008 with the aim to examine potential genetic predispositions in families with reported cases of childhood cancer and investigate the risk of adult and childhood cancer among relatives of children with cancer. The study is ongoing. All children with malignancies diagnosed, treated, and followed at the Pediatric Oncology and Hematology ward, as well as childhood cancer survivors visiting the Late Effect Clinic at Skåne University Hospital in Lund, Sweden, are invited to participate.

The eligibility criteria for patient inclusion are: (a) diagnosis before 19 years of age of a malignancy with codes 140–209 according to the International Classification of Diseases, seventh revision (ICD-7) and (b) diagnosis after January 1st, 1970. Blood samples from patients and parents are collected. The questionnaire is explained and handed out by the study nurse/physician, and returned by mail.

The Swedish National Population Register enables the identification of individuals through their unique personal number, as well as the degree of relation and vital status of relatives of pediatric cancer patients. All relatives are subsequently matched to the Swedish Cancer Register to identify cancer diagnoses among relatives.

Study I

The 543 children with cancer included in the ongoing LCCG study as of October 2014 were included in this study. Through the Swedish National Population Register and the Swedish Cancer Register, all childhood cancer diagnoses in their families were identified and/or confirmed. All relatives up to the third degree were

included. As childhood cancer cases were reported in relatives of a higher degree, the pedigrees were expanded to establish the degree of relationship with the study patient. Childhood cancer patients in the same family up to the fifth degree of relation were considered. The other children with cancer included in the LCCG study were used as a reference group.

Study II

As of December 31st, 2015, a total of 757 children with cancer had been enrolled in the LCCG study. Altogether 16 430 relatives up to the third degree were identified through the National Population Register. Of these, 250 (1.5%) were excluded due to invalid personal numbers, the majority because of emigration or death having occurred too long previously for records to be available. Finally, a further 43 relatives (0.3%) were excluded as they had died before 1958, when the National Cancer Register was initiated. This resulted in 16 137 relatives up to the third degree whose identity could be confirmed, totaling 606 558 person years at risk.

Study III

All 797 children in the LCCG study who had provided blood samples before December 31st, 2016, were included in this study. All were under the age of 18 at diagnosis and the most prevalent cancers were leukemia and CNS tumors. Compared to the distribution of pediatric cancers in the general Swedish population, the LCCG cohort with available blood samples contained a lower proportion of CNS tumors (19% vs. 28%), germ cell tumors, RBs, and carcinomas, and higher proportions of lymphomas (17% vs. 12%) and bone tumors.

At least two replicate sequencing libraries were prepared and sequenced for each of the 797 DNA samples. Less than 90% of the assay target region was covered by 30 high quality aligned reads in seven samples. These seven samples were therefore excluded from further analysis. In the remaining 790 samples, we performed targeted sequencing of the 22 genes with pathogenic and likely pathogenic variants reported by Zhang et al. (12).

Registers

Swedish National Population Register

Since 1991, the Swedish Tax Agency (Skatteverket) has been responsible for the Population Registry in Sweden. Since 1947, all residents in Sweden have been allocated a personal identification number. The number is based on date of birth (first six digits), a birth number (three digits), and a control number (last digit, introduced in 1967). The identification number is permanent and unique to the individual. The register contains the person's name, place of residence, place of birth, family relations, marital status, migration status, and place of burial.

Swedish Cancer Register

The Swedish Cancer Registry was established in 1958. In Sweden, it is mandatory to report all cancer diagnoses confirmed both by the clinician and the pathologist, which in many cases results in (an intended) double reporting of newly diagnosed cancers. It is estimated that 96% of all cancers in Sweden are registered in the Swedish Cancer Register. The registry is divided into six sections, correlating to the six medical regions in Sweden and their oncology centers. The regional centers provide their information of newly diagnosed cancers to the Swedish Cancer Registry.
The following diagnoses are mandatory to report: all malignant tumors, all carcinoid tumors, all tumors of the CNS, all endocrine gland tumors (excluding benign tumors of the thyroid gland), and some specific premalignant diagnoses.

Cause of Death Register

The Swedish Cause of Death Register includes all deceased persons who were registered in Sweden at their time of death. It includes time, place, and cause of death. The register was established in 1961.

DNA extraction and sequencing

DNA was extracted from blood samples using the Qiagen QIAmp DNA Maxi Kit. Using primers specific for the 22 previously defined genes, DNA libraries were prepared with the Fluidigm Juno targeted DNA sequencing library preparation system (see Study III). For every sample, a minimum of two libraries were prepared to allow for quality control. The libraries were then sequenced using the Illumina HihSeq 2500 system and the generated genes were aligned to the GRCh37 reference genome. Comparing the genes from the sample with the reference genome allowed identification of variants. These variants were then classified for pathogenicity according to the American College of Medical Genetics and Genomics (ACMG) and American College of Pathology (AMP) framework for variant classification, in consultation with experts in Clinical Genetics and Oncology at Lund University and the University of Amsterdam, the Netherlands. Identified pathogenic and likely pathogenic variants were validated using Sanger sequencing.

Statistical analysis

For statistical analyses within the cohort, SPSS 22.0 was used (IBM, Armonk, NY, USA). For comparison of continuous variables, such as age at diagnosis and time since diagnosis, Student's t-test was applied. For cohort comparison regarding diagnosis distribution, Fisher's exact test was used. Results were adjusted for gender, date of birth, age, and degree of relation. P-values were two-sided and a significance level of $p < 0.05$ was used.

For calculation of cancer risk over time, the Poisson distribution was applied. Cancer diagnoses were coded according to the ICD-7. The number of person years at risk was calculated as the difference between the date of birth, or January 1st, 1958 (in cases where individuals were born before January 1st, 1958), and date of death/emigration or end of follow-up on December 31st, 2015. Person years at risk were stratified by age, sex, and calendar year, and multiplied by year-, age-, and sex-specific rates of cancer types obtained from the Swedish Cancer Register, to yield the expected rates of each cancer type. Standardized incidence ratios (SIRs), observed/expected ratios, 95% confidence intervals (CIs), and p-values were computed. Correction for false discovery rates (FDRs) and Bonferroni correction was applied to account for multiple testing.

Results and Discussion

Study I

The aim of Study I was to investigate the characteristics of childhood cancer cases within the same family, and to study patterns indicating a hereditary component.

Forty-seven out of the 543 study patients in the LCCG study at the time of the study had a relative with childhood cancer, which resulted in 528 families with childhood cancer. Six study patients were related to another study patient who was already included in the study. Consequently, 41/528 (7.8%) families had more than one case of childhood cancer and 47/534 (8.8%) of all children had a relative with childhood cancer. When restricting inclusion to relatives up to third degree, 23/528 (4.4%) families had more than one case of childhood cancer.

When comparing diagnosis distribution in families with multiple childhood cancer cases to families with only one case, we found no significant difference in diagnostic distribution. There was also no difference in age at onset between these two patient cohorts.

In the 41 families with more than one case of childhood cancer, 86 children had cancer. The patient who was included first in the LCCG study was defined as "study patient," and the childhood cancer patients in the same family were defined as "relatives." Therefore, we had 41 study patients and 45 relatives.

When comparing the diagnosis of the relatives to that of the study patient we saw a correlation in the type of diagnosis. Sixty-nine percent (9/13) of the study patients with leukemia had a relative with childhood leukemia and 60% (6/10) of the study patients with a CNS tumor had a relative with a CNS tumor. This was more than twice as much as would be expected regarding diagnosis distribution of the cohort compared to the general population.

We observed a predominance of girls with cancer among those who had a relative with childhood cancer, and this was especially prominent for children with leukemia (Table 2).

Table 2. Characteristics of index persons with childhood leukemia and relatives in families with multiple cases of childhood cancer

Index patient				Relative of index patient			
No	Diagnosis	Gender	Age	Diagnosis	Gender	Age	Degree of relative
1	ALL	m	4.3	ALL	m	4.2	5
				ALL	f	3.9	5
2	ALL	f	2.1	ALL	f	5.6	5
				ALL	f	7.5	5
3	ALL	f	6.0	ALL	f	14.1	3
4	ALL	f	4.4	ALL	f	5.3	1
5	ALL	f	2.3	ALL	f	1.3	4
6	ALL	f	3.2	Leukemia	f	13.0	2
7	ALL	f	8.1	AML	m	17.7	2
8	ALL	f	3.4	HL	m	10.5	4
9	ALL	f	2.1	Ganglioglioma	m	7.0	1
10	ALL	f	8.6	Ewing sarcoma	f	8.2	5
11	ALL	f	1.6	RB	f	2.1	3
12	AML	f	12.4	ALL	f	4.5	4
13	AML	f	2.3	AML	m	0.3	4

Abbreviations: ALL = acute lymphocytic leukemia; AML = acute myeloid leukemia; RB = retinoblastoma; HL = Hodgkin lymphoma

The gender difference was especially prominent among leukemia patients, where 77% (23/30) of the children with cancer were female, and for CNS tumors, where 65% (15/23) were female.

Out of all childhood cancer cases in families with more than one case of childhood cancer, regardless of diagnosis, 62% were female. This percentage is significantly higher than that in families with only one case of childhood cancer (40%, 197/487), also when compared to the general public where on average 46% of childhood cancer patients are female.

When redoing our calculations during 2020 for the extended cohort in Study II, the results remained significant, but to a lesser degree, both for gender distribution and for matching childhood cancer diagnosis in the same family.

The increased probability of having two children with cancer diagnoses in families with more than one case of childhood cancer indicates that there might be a hereditary component specific for these types of cancers. The predominance of girls in these specific families (families with CNS tumors and leukemia) indicates that this hereditary component could be linked to gender.

This is a novel finding and has, to our knowledge, not been reported before. It is well known that childhood cancer risk differs between the sexes (1, 44). However, a sex-linked inherited childhood cancer predisposition has not previously been studied. Larger cohorts would be needed to verify these findings, but it could lay the ground for targeted WGS for members of these specific families.

Study II

In Study II, we aimed to investigate the risk for childhood and adult cancer among relatives of children with cancer. This has previously been done; however, very few authors have included relatives up to the third degree (202). With a cohort including 757 families, we also aimed to investigate the difference in cancer risk depending on gender and diagnosis of the child with cancer.

The results showed that children up to the third degree of relation had a 48% higher risk for cancer compared to the general population, which is less than reported by some previous studies (200, 202) but in line with the most recent (201). We believe our estimated risk to be close to the true increased risk.

For adult cancer among relatives up to third degree, there was a moderate but significantly increased cancer risk of 7%. First-degree relatives had the highest risk, with 22%; third-degree relatives had a 10% increased risk while second-degree relatives showed no increase in risk (Table 3).

The increased cancer risk for first-degree relatives seems mainly to affect female relatives, which is in accordance with previous studies (199, 201, 203). By contrast, for third-degree relatives, we found a significantly increased risk for male, but not female, relatives.

Table 3. Standard incidence ratios (SIRs) for cancer risk in relatives of children with cancer, by degree of relation.

Degree of relation	Gender of relative					
	Male		Female		Total	
	SIR	p	SIR	p	SIR	p
First degree	1.17	0.106	1.27	**0.020**	1.22	**0.009**
Second degree	1.06	0.079	0.97	0.293	1.02	0.268
Third degree	1.14	**<0.001**	1.05	0.112	1.10	**<0.001**

Abbreviations: SIR = Standardized insidence ration

The increased risk for adult relatives mainly concerned cancer of the gastrointestinal tract and lung cancer, with a 24% and 21% increased risk, respectively. Men and women showed an increased risk for different cancer diagnoses.

Relatives of both girls and boys with cancer showed an increased risk for adult cancer. For relatives of girls with cancer, the risk was only increased for male relatives. Among relatives of boys with cancer, both male and female relatives showed an increased risk, but this was only significant for male relatives (Table 4).

Table 4. Standard incidence ratios (SIR) for cancer risk in relatives of children with cancer, by gender of the child with cancer.

Gender of child	Gender of relative					
	Male		Female		Total	
	SIR	p	SIR	p	SIR	p
Girl	1.14	**0.002**	0.99	0.401	1.22.06	**0.026**
Boy	1.08	**0.023**	1.06	0.058	1.07	**0.006**

Abbreviations: SIR = Standardized insidence ration

Finally, we studied whether cancer risk in adult relatives varied by the cancer diagnosis of the child. Relatives of children with CNS tumors and lymphomas showed an overall increased risk; male relatives of children with leukemia had the highest significantly increased risk (Table 5).

Table 5. Standard incidence ratio (SIR) for cancer risk among relatives of children with cancer, by diagnosis of the children.

Diagnosis of child	Gender of relative					
	Male		Female		Total	
	SIR	*p*	SIR	*p*	SIR	*p*
Leukemia	1.15	**0.002**	0.96	0.239	1.06	0.061
CNS tumor	1.10	0.065	1.12	**0.029**	1.11	**0.008**
Lymphoma	1.13	0.066	1.07	0.212	1.10	**0.049**
Other	1.20	0.051	1.03	0.402	1.11	0.086

Abbreviations: SIR = Standardized insidence ration; CNS = central nervous system.

The increased cancer risk among relatives was also calculated for each specific type of adult cancer. Generally, an increased risk was seen mainly for cancers of the gastrointestinal tract. However, these findings are only hypothesis-forming and need to be verified in an independent study.

To investigate whether the increased cancer risk was caused by known pathogenic variants we excluded the patients who had tested positive for such variants in our parallel, then ongoing Study III, and observed that the increased cancer risk among

relatives remained unchanged. Therefore, the increased cancer risk is more likely explained by unknown pathogenetic variants or, even more likely, a combination of multiple low penetrance genes.

With this study, we confirmed previous notions that the increased cancer risk for adult relatives of children with cancer reaches as far as the third degree of relation. We also confirmed a generally increased risk for childhood cancer among relatives of children with cancer.

In addition, we observed a difference in the pattern of adult cancer diagnoses among relatives depending on the gender of the related child with cancer. If the child with cancer is a girl this increases the cancer risk for adult male relatives. Childhood leukemia is associated with increased cancer risk for adult male, but not female, relatives. These findings strengthen the hypothesis from our first study where we identified families with multiple cases of childhood leukemia as potential targets for genetic studies.

Study III

We aimed to investigate the prevalence of cancer predisposing germline variants in the childhood cancer population in the Southern health care region of Sweden, and to validate results from two recent publications on WGS in a mainly pediatric US and German population. In a study from the US by Zhang et al., including 1120 childhood and young adult cancer patients (12), WGS and WES showed that 8.5% of the cohort had a genetic variant that was likely pathogenic. A similar, German study, by Gröbner et al., with a cohort of 914 childhood cancer patients and young adults with cancer, showed a cancer prevalence of 7.6% (13).

We used targeted sequencing on the same 22 predisposition genes identified in the US study for 790 childhood cancer patients included in the cohort. When analyzing these 22 genes we found pathogenic germline variants in 3.8% of the cases of the cohort. Likely pathogenic mutations in the NF1 gene were the most frequent, followed by TP53 and BRCA2 (Figure 6).
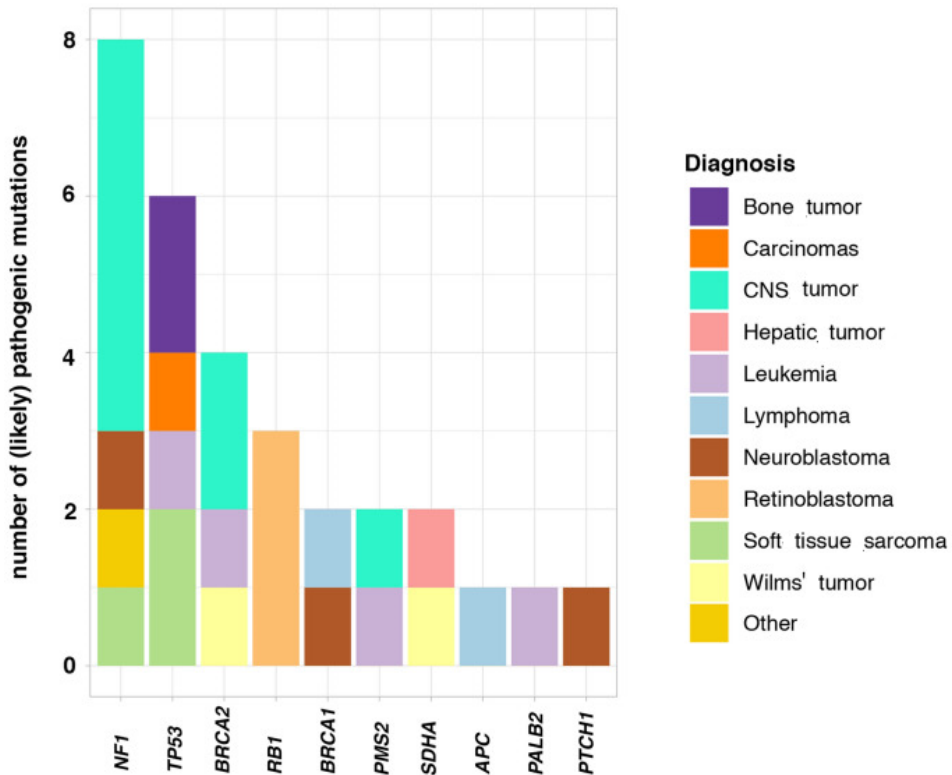
Figure 6. Distribution of germline pathogenic and likely pathogenic variants in patients with different pediatric diagnoses in the Lund Childhood Cancer Genetic (LCCG) cohort.

The childhood cancer diagnosis with highest prevalence of germline mutations was RB, with 60% of the cases carrying a germline variant. This was followed by neuroblastoma, with 8%, and soft tissue sarcoma and CNS tumors, which both showed 5% prevalence. Leukemia had a prevalence of 1.5%. The 3.8% prevalence of predisposing genetic variants is significantly less than that reported in previous studies (12, 13), due to the different diagnosis distributions of the study cohorts.

The Swedish cohort was underrepresented regarding CNS tumors, known to be caused by relatively high prevalence of pathogenic variants, and had a minor overrepresentation of leukemia cases, with a relatively low prevalence of pathogenic variants. The US study had an overrepresentation of leukemia, and also the rare hypodiploid leukemia and adrenocortical tumors (ACTs). In the German study there was an overrepresentation of CNS tumors. Therefore, CNS tumors,

hypodiploid leukemias and ACTs, diagnoses which are often caused by germline pathogenic variants (132), increased the total prevalence for these cohorts.

The two previous studies were not population-based and included patients above 18 years of age, and the diagnosis distribution were less representative of the pediatric cancer population compared to our study. We therefore compared the prevalence of pathogenic variants for each type of childhood cancer separately. When doing this, there was no statistical difference between the studies.

When comparing the prevalence of pathogenic variants for each individual gene, we found a significant difference regarding TP53 mutations only, a prevalence of 0.6%, compared to 4.3% in Zhang et al.'s cohort (12) and 2.6% in Gröbner et al.'s cohort (13). This could partly be explained by a more stringent definition of "pathogenic" and "likely pathogenic" in our study compared to Zhang et al. (12). When reclassifying their results according to our criteria, we redefined six out of 48 TP53 variants as being of uncertain significance. However, even when excluding these six variants, a significant difference remained. Information needed for reclassification of variants in the study by Gröbner et al. was lacking (13).

Both the US and the German cohort had a high representation of ACTs, which is very likely to have been caused by pathogenic variants in the TP53 gene. When excluding this diagnosis, no statistical difference was found in prevalence of TP53 mutations.

To match the study design from the US study, we investigated the prevalence of a family history of cancer (defined as cancer in a first- or second-degree relative) between children with a germline mutation and children without. We found that 46% of patients with a germline mutation had a first-degree relative with cancer, compared to 28% of the patients without a germline mutation. When including up to second-degree relatives, the percentage of those who had a family history of cancer increased to 96% and 82%, respectively. There was no significant difference between the groups, which is in accordance with the findings of the US study (12).

We reported a pathogenic germline variant in 3.8% of the childhood cancer cases. This is likely an underestimate of the real percentage in the general population. We focused on the 22 genes identified by Zhang et al. (12), thus we excluded other potential variants that by chance were not included in their cohorts. To avoid this bias, WGS would be required. Moreover, we had a slightly skewed diagnosis distribution, with an overrepresentation of leukemia, which has a relatively low prevalence of germline variants compared to CNS tumors and other diagnoses. When correcting our diagnosis distribution to that of the Swedish Childhood Cancer Registry we obtained a prevalence of 4.9%, which is probably closer to the true figure.

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With the overrepresentation of rare diagnoses in the US study, there is a risk of overestimation of the real prevalence of pathogenic germline variants, as the authors themselves have acknowledged (12). Removing these diagnoses decreases their estimate of their germline mutation rate to 5.6%, which is more likely closer to the true value. Moreover, the German study had a very skewed diagnosis distribution, with a large underrepresentation of leukemia, which is likely to result in an overestimate of the germline mutation rate in the general population (13), when they corrected for this they got an estimated rate of 6%.

To date, there is unfortunately no large population-based study on prevalence of pathogenic germline variants in childhood cancer, as neither Zhang et al. (12) nor Gröbner et al. (13) had a population-based cohort. Given the possibility to perform WGS, the LCCG cohort could be included in such research in the future. In addition, as the LCCG cohort also could include sequencing of parenteral blood samples, we could determine the proportion of inherited and de-novo mutations in future studies.

We observed no significant difference in family history of cancer between patients with and patients without an identified pathogenic germline variant. This is not surprising as it is not likely that inherited cancer predispositions are restricted to inherited single-gene variants. It is possible that they are caused by accumulation of multiple low penetrance variants which will not be detected by our methods.

Strengths and limitations

The strengths and limitations of each study are summarized in Table 6.

When conducting research on rare diseases, a small cohort size will limit conclusive results. Through continuous inclusion of patients since 2008, the LCCG study has grown to a respectable size, comparable to cohorts from similar studies (12, 13), and outsizing some (11). However, the results presented in this thesis were frequently limited due to weak statistical significance. This was especially clear when examining subgroups of the patient cohort regarding variables known to be rare.

As previously mentioned, Sweden is divided into six different geographic health care regions. The studies from the LCCG study presented in this thesis are based on patients treated and followed for pediatric cancer in the Southern health care region, Thus the cohort is population-based, with consideration given to the likelihood that patients of different ages, gender, and types of cancer may be more or less inclined to join the study. This has allowed us to generalize our findings to an extent that previous studies of the same size have not.

Through national population registers, valid data on family relations can be retrieved without being dependent on information reported by the patient or parents. Even if reported information was often correct, on multiple occasions it was shown to be different from the national registry. This was also shown in a previous study based on the LCCG cohort (202), where self-reporting of cancer missed 15% of all identified cancer cases.

The comprehensive national registries allowed us to extend our studies by including information on relatives up to the third degree of relation, something that is deemed the "golden standard" for medical genetics, genetic counseling, and research settings (226). It is a great advantage to include more distant relatives. This can enable us to identify low penetrance variants or those with atypical inheritance patterns such as imprinting errors.

At the time of the study, WGS was not yet possible to perform due to limited economic resources and timing. Instead, we selected a panel of 22 genes reported to be pathogenic in a large young cancer patient cohort (12). This has limited us

greatly as we have been unable to identify any genes outside of those. The rarity of these pathogenic variants makes it very unlikely that all variants present in the national childhood cancer population can be identified in a cohort of approximately 1000 patients. Therefore, by limiting the study to the genes identified by Zhang et al. (12), there is likely an underestimated prevalence of germline mutations among childhood cancer patients in our cohort.

Table 6. Strengths and limitations of Studies I–III.

	Strengths	Limitations
Study I	• Confirmed diagnoses through national registers and patient files • Confirmed degree of relation through national registers • Known predisposing hereditary syndromes in patient files • Population-based cohort; all children treated at the same clinic • The majority of childhood cancer diagnoses classified by microscopic diagnosis	• Cohort size limited statistical significance for less common childhood cancer diagnoses • Risk of unknown relatives with childhood cancer being excluded • Older diagnoses lacked specified typing, e.g., leukemia instead of ALL/AML • Potential survivor bias due to patient inclusion from the Late Effect Clinic
Study II	• Inclusion of family members up to the third degree of relation • National register-based family cohort • Confirmed diagnoses and identities of all patients and relatives • Population-based cohort; all children treated at the same clinic • Pathogenic germline variants tested for the majority of the cohort • Estimation of cancer incidence in relatives compared to national Cancer Register data	• Statistical comparison based on estimated numbers instead of a control cohort • Underrepresentation of CNS tumors compared to the general population • Potential survivor bias due to patient inclusion from the Late Effect Clinic
Study III	• Population-based study cohort • Family history of cancer up to the third degree of relation available • Stringent definition of likely pathogenic variant	• Targeted sequencing instead of WGS • Limited number of pathogenic variants analyzed • Potential survivor bias due to patient inclusion from the Late Effect Clinic

ALL = acute lymphocytic leukemia; AML = acute myeloid leukemia; CNS = central nervous system; WGS = whole genome sequencing.

Conclusions and future aspects

Study I

We investigated the childhood cancer distribution and basic characteristics of 41 families with more than one pediatric cancer case compared to 487 families with only one. We found that 8.8% of all children with cancer were related to another child with cancer, and that these children often had the same diagnosis in cases with leukemia and childhood CNS tumors. Had we had a larger cohort, it is possible that more rare pediatric cancer diagnoses would show similar patterns. We also observed that there was a predominance of girls with cancer in families with multiple childhood cancer cases. This may indicate a hereditary gender-specific risk factor in these families. These results remained when analyzing the larger study cohort (Study II).

- Families with multiple cases of leukemia or CNS tumors are interesting targets for WGS with focus on potential gender-specific alterations.

- A larger and independent cohort is required to confirm whether girls are at higher risk for familial cancer.

Study II

In this study of familial cancer risk in the LCCG cohort of 757 families compared to the general population, we conclude that adult relatives of children with cancer up to the third degree of relation have an at average increased cancer risk of 7%. Among distant relatives, the increased risk was more prominent for men. The gender and diagnosis of the child with cancer affected the risk of cancer among their relatives. Known germline mutations could not explain the increased cancer

risk among relatives, and the increased risk could be due to hereditary factors not yet identified.

- Extended family trees are useful to identify patterns of heredity that are not explained by high penetrance, dominant inherited pathogenic germline variants.

- A larger and/or independent cohort would be needed to confirm the observed difference in cancer risk for relatives of children with different types of cancer.

- Relatives had a higher increased risk of cancers of the gastrointestinal tract compared to other diagnoses. These results need to be verified in an independent cohort, as our study of specific diagnoses was hypothesis forming.

Study III

In this study of targeted germline sequencing of 790 children with cancer included in the LCCG cohort, we conclude that 3.8% of the children had a pathogenic germline variant. This estimate is lower than in the general population due to the limited selection of genes studied. The prevalence of pathogenic variants for each type of childhood cancer was in line with previous studies. However, a larger cohort would be needed to identify any true underlying differences in prevalence between the studies

Family history of cancer was more common in patients with identified pathogenic variants, although this did not reach statistical significance.

- In future studies, WGS would provide an exact estimate of the prevalence of pathogenic variations in the study cohort, which would provide the first data from a population-based study.

- Further studies with inclusion of sequencing of samples also from parents of children with cancer would help to distinguish between inherited and de novo mutations as well as analyze and compare the family history of cancer in these two sub-cohorts.

- Sequencing of parents' samples could reveal inheritance of benign single mutations from both parents, affecting the same cancer-causing pathway.

In conclusion, this study supports the role of genetics in the etiology of childhood cancer. It also supports a connection between the heredity of adult and childhood cancer, and that relatives of children with cancer have an inherited risk for both childhood and adult cancer. The currently known pathogenic germline variants that cause cancer can only explain a minority of pediatric cancer cases as they are present in only a fraction of the childhood cancer cases.

There is indeed an increased cancer risk for family members of children with cancer, a risk that is unlikely to be explained by shared environment as it reaches relatives up to the third degree. Likewise, known cancer syndromes and pathogenic variants do not explain the increased risk as the risk remains when families with pathogenic germline genetic aberrations are excluded.

Interestingly, leukemia, the diagnosis with the highest female predominance in families with multiple childhood cancer cases was also associated with the highest probability to have a matching diagnosis within the family (Study I). In addition, in these families we found the highest significantly increased risk for adult cancer among male relatives (no increased risk for female relatives) (Study II). These patterns could indicate underlying genetic aberrations. However, leukemia showed the lowest prevalence of pathogenic genetic variants (Study III). If these patterns in families of children with leukemia indeed are due to hereditary factors, then it is likely that these patients have genetic aberrations predisposing for childhood cancer that have yet not been identified.

Traditionally, we have studied single pathogenic variants and how they correlate with an increased cancer risk. However, it is rare that mutations in single genes create a phenotype; instead, phenotypes are created as a result of multiple genes acting in concert. Thus, the methods currently available may not be sufficient to identify the hereditary background to childhood cancer. By studying the accumulative effect of multiple germline mutations, we may reveal new information.

To further explore familial cancer, WSG of patients and parents included in the LCCG study is planned. With knowledge of which parent carries a cancer predisposing genetic variation, more precise estimates of the impact of family history of cancer can potentially be made. For families with multiple cases of childhood cancer, potential shared variants can be studied. Furthermore, with parallel analysis of blood samples from parents of children with cancer, we may be able to identify genetic variants that alone do not lead to cancer but might, when both are inherited by the child, affect cancer-promoting pathways.

Populärvetenskaplig sammanfattning

Barncancer är en ovanlig sjukdom som drabbar cirka 350 barn under 18 års ålder varje år i Sverige. Det är en skrämmande diagnos för många föräldrar och har genom historien varit associerad med en hög risk för död. Som tur är har det senaste halvseklet visat en drastisk förbättring i överlevnad. Tack vare utveckling av behandlingsmetoder, såsom cellgifter och strålbehandling, samt internationella samarbeten och standardiserade behandlingsprotokoll som möjliggör utvärdering, och en förbättring av behandling, så överlever fler barn med cancer än någonsin förr.

Det verkar dock som om vi behöver nya metoder för att hjälpa de barn som inte svarar tillräckligt bra på konventionell behandling. De senaste decennierna har forskningen börjat rikta in sig på kompletterande behandling som är mer *riktad* jämfört med mer konventionell behandling. Det innebär t.ex. läkemedel som riktar sig mot specifika protein som cancercellerna är beroende av.

För att förbättra behandlingen mot barncancer fokuserar vissa forskare på genetiken i cancerceller och i friska celler hos personer med cancer. Dels för att kunna identifiera vilka gener, och deras motsvarande protein, som kan vara måltavlor för nya behandlingar, men även för att identifiera medfödda variationer i genomet som kan innebära en ökad cancerrisk. Genom att hitta förändringar i generna som medför en ökad risk för cancer kan man ibland via regelbundna kontroller påvisa cancer i ett tidigare skede och på så sätt förbättra behandlingsresultat och överlevnad.

Mitt doktorandarbete har fokuserat på att undersöka släkter med kända fall av barncancer för att försöka identifiera de som verkar ha genetiskt ökad risk för att utveckla barn- och vuxencancer. Vi har tittat på hur släkter med flera fall av barncancer skiljer sig från släkter med ett fall av cancer, hur risken för cancer ser ut bland släktingar till barn med cancer och hur den genetiska bilden ser ut för barn med cancer. För att göra detta använde vi oss av LCCG (Lund Childhood Cancer Genetic) studien, som är en pågående studie sedan 2008 som inkluderar barn som behandlas för barncancer på Lunds universitetssjukhus. Familjer som går med i studien tillåter att vi släktforskar samt förälder och det cancerdrabbade barnet lämnar ett blodprov som kan användas för genetiska studier. Med hjälp av nationella populationsregistret och cancerregistret kan vi räkna ut om det finns en

ökad risk för cancer hos släktingar till barn med cancer. Blodprover på barnen använde vi för att göra en typ av DNA-analys på 22 specifika gener som vi vet ofta är avvikande hos barn med cancer.

I de släkter som hade flera fall av barncancer såg vi att det var mycket vanligare att flickor fick cancer än pojkar, när vi jämförde med släkter med ett fall av barncancer. Vi såg också att barn med cancer som var släkt ofta hade matchande cancer diagnos. Det här var tydligast för barn med blodcancer, leukemi, men sågs även för barn med hjärncancer.

Vi fann en ökad cancerrisk för släktingar till barn med cancer, en ökning med 22% för förstagradssläktingar och en ökning med 7% om man inkluderar alla upp till tredjegradssläktingar. Vi såg att risken skiljde sig mellan kvinnliga och manliga släktingar och att den varierade beroende på vilken diagnos och kön barnet med cancer hade. Det var störst ökning i risk för cancer i mag-tarmsystemet. Den ökade risken bland släktingar förblev den samma även när vi exkluderade alla släkter som visade sig ha en sannolik cancerorsakande variation i de gener som vi testade.

De 22 gener som vi kontrollerade för ärftliga variationer som kan orsaka barncancer fann vi att 3,8% av alla barncancerfall bar på en variation som sannolikt bidragit till utvecklingen av cancer. Detta varierade mycket mellan olika typer av barncancer vilket man också har sett i tidigare studier. När vi jämförde resultaten med tidigare studier skiljde sig därför den totala andelen, men inte när vi jämförde varje barncancerdiagnos för sig. När vi korrigerar för hur vanliga olika typer av barncancer är i Sverige uppskattar vi att 4,9 % av alla barncancerpatienter i Sverige har en av dessa genetiska avvikelser som orsak till cancern.

Våra resultat tyder på att det finns ärftliga aspekter inom barncancer som inte kan förklaras med de genetiska förändringar som redan är kända. Då en ökad cancerrisk för släktingar kvarstod även när vi exkluderar de vanligaste kända genetiska förändringarna är det sannolikt att det finns andra genetiska variationer som vi ännu inte känner till, som i alla fall delvis förklarar den ökade cancerrisken. Framför allt släkter med flickor med leukemi är intressanta för vidare genetisk forskning. Flickor med leukemi var ofta släkt med andra flickor med leukemi vilket tyder på att det kan finnas en ärftlig faktor involverad, men barn med leukemi hade en av de lägsta andelarna genetiska variationer. Det är möjligt att det gömmer sig okända genetiska förändringar i dessa släkter som förklarar bilden. Med så kallad helgenomssekvensering och genom att jämföra föräldrars genetiska bild med den genetiska bilden från deras cancerdrabbade barn hade det varit möjligt att identifiera sådana nya genetiska förändringar som skulle kunna hjälpa oss vidare i vår förståelse om barncancer, dess uppkomst och eventuellt utveckla nya metoder för att förbättra behandlingen av barncancer.

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Study I

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Predominance of girls with cancer in families with multiple childhood cancer cases

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Abstract

Background: Recent studies indicate that one of four childhood cancers can be attributed to hereditary genetic abnormalities.

Methods: The Lund Childhood Cancer Genetic study includes newly diagnosed childhood cancer patients as well as childhood cancer survivors visiting the Department of Pediatrics or the Late Effect Clinic at Skåne University Hospital, Lund, Sweden. Questionnaires regarding family history of cancer and blood samples were provided. Reported data were validated and extended by use of the Swedish Population- and Cancer Registries. Demographics in families with one case of childhood cancer (FAM1) were investigated and compared to families with multiple cases of childhood cancer (FAM > 1) as well as to childhood cancer in the general population.

Results: Forty-one out of 528 families (7.8%) had more than one case of childhood cancer. In 23 families the affected children were relatives up to a 3rd degree (4.4%). In FAM > 1, 69.2% of the children with leukemia and 60% of those with tumors in the central nervous system (CNS) had a childhood relative with matching diagnosis, both significantly higher than expected. Significantly more female than male patients were observed in FAM > 1 compared to FAM1. This female predominance was most striking in childhood leukemia (77% female) and also, yet to a lesser extent, in CNS tumors (68% female).

Conclusions: We conclude that the high proportion of children with leukemia or CNS tumors in FAM > 1 having a childhood relative with the same diagnosis suggests a hereditary background. Moreover, we report a female predominance in childhood leukemia and childhood CNS tumors in FAM > 1, which may indicate a hereditary gender-specific risk factor in these families.

Keywords: Pediatric cancer, Familial cancer predisposition, Hereditary cancer syndrome, Genetic cancer susceptibility

Background

The increased cancer risk amongst relatives of childhood cancer patients has been reported in several studies over the past decades. Although the reports are varying, they generally show an increased risk for cancer for both siblings [1–3] and parents [2–8]. In 1991, genetic conditions could explain 3.07% of childhood cancer cases but

that number increased to 4.2% when data from family history was included [9]. More recent studies estimate heredity to account for 29% of childhood cancer cases [10, 11]. Several studies confirm that earlier onset cancers have a hereditary component [3, 4, 12–14], which is more pronounced if the child has a central nervous system (CNS) tumor [4–7]. In addition, women in families with childhood cancer have an increased cancer incidence, especially with regard to breast cancer [2, 4–7, 12]. A recent genome sequencing study of pediatric cancer cases showed that 8.5% had germline mutations in known cancer predisposition genes yet no obvious association to familial history of cancer up to second degree was

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seen [15]. Furthermore, many of the studies mentioned above showed that even when excluding already known familial syndromes an increased risk of cancer remained [1, 2, 4, 5, 12].

Multiple primary cancers are common in hereditary syndromes such as familial Wilms tumor and heritable retinoblastoma, where multifocal and bilateral tumors in paired organs are often observed [16–18]. Down syndrome is associated with an increased risk of acute leukemia during childhood with a ten- to twenty-fold higher risk than in the general population [19, 20]. The number of rare pediatric cancer syndromes is increasing and the importance of early cancer surveillance strategies to reduce morbidity and mortality is extensively debated in the pediatric oncology community [21, 22]. Clinically evident hereditary syndromes have, to a large extent, already been defined and genetically explained, however, subclinical syndromes and/or hereditary genetic aberrations may yet to be discovered. Taking this into consideration, studies using high-throughput genetic techniques characterizing subclinical genetic predisposition to childhood cancers are of great importance.

In the current study, we present work from the Lund Childhood Cancer Genetic (LCCG) study, aimed at investigating possible genetic predispositions in families with reported cases of childhood cancer. In the LCCG-study, data on family history of cancer and blood samples are collected from childhood cancer patients and childhood cancer survivors in the southern healthcare region of Sweden. The overall aim of this on-going study is to characterize cancer predisposing aberrations and/or associations through in depth germ-line analyses with correlation to detailed and verified family history of cancer. With focus on families with multiple cases of childhood cancer (FAM > 1), we describe characteristics in terms of age at diagnosis, sex, diagnoses and diagnosis distribution. Here we identify families with potential previously undiscovered hereditary syndromes, laying ground for future genetic analyses, which could shed light on novel hereditary factors.

Methods

The Lund Childhood Cancer Genetic (LCCG) study was initiated in 2008. Children with malignancies diagnosed, treated and followed at the Pediatric Oncology and Hematology ward, as well as childhood cancer survivors visiting the Late Effect Clinic at the Department of Oncology, Skåne University Hospital in Lund, Sweden are offered to participate.

The eligibility criteria for patient inclusion from the Pediatric Department are 1) diagnosis before 19 years of age with a malignancy with codes 140–209 according to the International classification of diseases 7th edition (ICD-7) *and* from the Late Effect Clinic 2) diagnosis

since 1 January 1970. Blood samples from both the patients and parents are collected. Patients and parents are requested to complete a standardized self-reported questionnaire, querying for name, date of birth and the national identification number, history of cancer amongst first, second, and third degree relatives. Information regarding specific type of cancer and date of/age at diagnosis inclusive outcome (if fatal, date of death) for each relative with a history of cancer is obtained. In addition, questions about cancer in more distant relatives are included. The questionnaire is explained and handed out by the study nurse/physician and returned by mail. Although all types of cancers (including adult) were reported, only childhood cancers were included and examined in the current study.

Pedigrees were created for every family with the patient included in the LCCG study as *study patient*, using the program Progeny 9 (Progeny Software, LLC).

The Population Registry in Sweden was used to 1) collect and/or validate the identification of all patient's relatives living in Sweden and to 2) extend pedigrees to include all relatives of the chosen degree and thereby supplement data lacking from questionnaires. All participants were crosschecked with the Population Registry for data on vital status, and the Swedish Cancer Registry to confirm reported cancer diagnosis or to identify any potential relatives with unreported cancer diagnoses. For relatives living abroad, only questionnaire-based information was available.

Pathology reports and patient charts were reviewed for study patients and any relative with a childhood cancer in order to validate the diagnosis and to check for possible hereditary syndromes and other cancer predisposing factors. Eight childhood cancer cases in relatives could not be verified microscopically due to diagnosis either before 1970 or outside of Sweden. Three patients were diagnosed with unspecified leukemia. Due to unspecified diagnoses and the relatively small cohort, all leukemia diagnoses inclusive ALL and acute myeloid leukemia (AML) were grouped together for subsequent analyses. We further investigated the cytogenetic subtype and risk group of the acute lymphatic leukemia (ALL) cases in the study.

Following data collection of all childhood cancer cases, FAM > 1 were identified for characterization in terms of age at diagnosis, sex, diagnosis and diagnosis distribution. All relatives to the third degree were included. When childhood cancer cases in relatives of a higher degree were reported, the pedigree was expanded to ensure correct degree of relationship with the study patient.

The NORDCAN database of all childhood cancer patients from all Nordic countries (1970–2013) [23] was used for demographic comparisons with childhood cancer in the general population. This database lacked information regarding age at diagnosis, therefore we used

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the data of age at diagnosis from a recent epidemiological review of European childhood cancer databases for comparative statistics [24].

Statistical analysis

The statistical software SPSS 22.0 was used for statistical analyses. Pearson's chi-squared test was used to compare categorical data (gender and diagnosis) between childhood cancer affected individuals in families with one case of childhood cancer (FAM1) and FAM > 1. For categorical data with smaller samples ($n < 5$) Fisher's exact test was used. Student T test was used to compare mean age at diagnosis. All analyses were two-sided and p-values <0.05 were considered significant. False discovery rate (FDR) correction was applied to account for multiple testing between patient groups (comparisons limited to patient groups with $n > 5$) regarding diagnostic distribution and gender (Fig. 1 and Table 3).

Results

As of October 2014, 534/679 (78.6%) patients in the study had returned the questionnaires with 31 (4.6%) patients actively declining participation. Approximately half of the study patients were included from the Pediatrics Department and half from Late Effect Clinic.

Forty-seven study patients in the LCCG-study, 8.8% of 534 (95% CI: 6.4–11.2%), had a relative with childhood cancer. Six study patients were related to another study patient that was already included in the study. These were classified as relatives in subsequent analyses. Accordingly, 41 study patients, 7.8% of 528 (95% CI: 5.5–10.0%) had a relative diagnosed with childhood cancer. In four families there were three cases of childhood cancer. The 41 families with more than one childhood cancer (FAM > 1) with a total of 86 children are described in Table 1. Two of these families had relatives of the 6th

degree with a childhood cancer diagnosis, while the remaining 39 families had relatives up to the 5th degree (Table 1). In 23 of these families (4.4%, 95% CI: 2.6–6.1%) the study patient had a 1st to 3rd degree relative with a childhood cancer diagnosis.

When comparing the diagnosis distribution in the LCCG-study with the diagnosis distribution of childhood cancer in the Nordic countries, we observed a higher percentage of leukemia in the LCCG-study, 32% versus 24% (Fig. 1a). Furthermore, the percentage of CNS tumors was lower than that of the general childhood cancer population, 21% versus 26%, respectively. However, neither of these observations were significant after correction for multiple testing. There was no significant difference between the diagnosis distribution of FAM > 1 and the diagnosis distribution in FAM1 (Fig. 1b).

High proportion of multiple leukemia and CNS tumors in families with multiple childhood cancers

The 41 FAM > 1 were grouped according to the type of diagnosis of the study patient (Table 1). Out of the 13 study patients with leukemia, nine cases had a relative with childhood leukemia (69.2%) (Table 1), which is significantly higher portion than that of childhood cancer in the general population ($p = 0.001$). Two of these families had three cases of childhood ALL. Among patients with CNS tumors, six of the ten study patients (60%) had a relative with a childhood CNS tumor, which is also a higher portion than that of the general population ($p = 0.025$). Two families (Family 13 and 14) shared the same relative with a high-grade glioma, however, the two study patients were not related. In subgroup 3 (lymphomas) we found no relative with a childhood lymphoma. Of the four children with neuroblastoma (study patient or relative), three had a relative with a childhood CNS tumor. Four families had cases of both childhood lymphoma and childhood
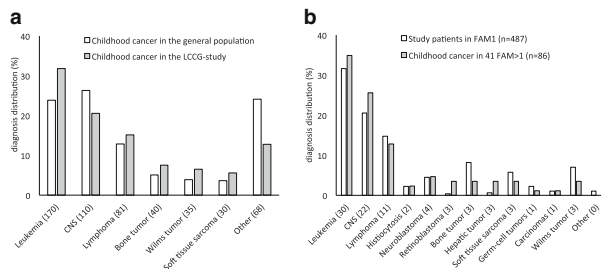
Fig. 1 Diagnosis distribution of childhood cancer cases in **a** Lund Childhood Cancer Genetic (LCCG) study (grey)($n = 534$) compared to the childhood cancer population in the Nordic countries [23] (white). The value in brackets represents the number of study patients in the LCCG-study with corresponding diagnosis. **b** Families with multiple childhood cancers (FAM > 1, $n = 86$ in 41 families) (grey) compared to families with one childhood cancer case (FAM1, $n = 487$) (white) in the LCCG-study. The value in brackets represents the number of study patients and relatives with corresponding diagnosis in FAM > 1. The higher number of leukemia cases and lower number CNS cases in the LCCG cohort compared to the general population were not significant after correction for multiple testing

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Table 1 Characteristics of study patients and corresponding relatives in families with more than one childhood cancer (FAM > 1)

Study patient			Relative of study patient				
Nr	Diagnosis	Gender	Age (years)	Diagnosis	Gender	Age (years)	Degree of relative
Leukemia							
1	ALL	m	4.3	ALL	m	4.2	5
				ALL	f	3.9	5
2	ALL	f	2.1	ALL	f	5.6	5
				ALL	f	7.5	5
3	ALL	f	6.0	ALL	f	14.1	3
4	ALL	f	4.4	ALL	f	5.3	1
5	ALL	f	2.3	ALL	f	1.3	4
6	ALL	f	3.2	Leukemia[a]	f	13.0	2
7	ALL	f	8.1	AML	m	17.7	2
8	ALL	f	3.4	Hodgkin lymphoma	m	10.5	4
9	ALL	f	2.1	Ganglioglioma	m	7.0	1
10	ALL	f	8.6	Ewing sarcoma	f	8.2	5
11	ALL	f	1.6	Retinoblastoma	f	2.1	3
12	AML	f	12.4	ALL	f	4.5	4
13	AML	f	2.3	AML	m	0.3	4
CNS tumors							
14	Ependymoma	m	11.7	High grade glioma	f	5.1	3
15	Ependymoma	m	6.8	High grade glioma	f	5.1	4
16	Ependymoma	f	10.2	CNS tumor[a]	f	13.1	2
17	Astrocytoma	m	1.6	Neuroblastoma	f	0.9	1
18	Astrocytoma	f	16.0	CNS undefined	f	7.1	5
19	Astrocytoma	f	14.0	Hodgkins lymphoma	f	14.2	3
20	Optic tract glioma	f	4.6	Ependymoma	f	0.8	4
21	Optic tract glioma	m	2.2	Wilms tumor	f	1.6	6
22	Ganglioglioma	f	14.9	Astrocytoma	m	14.5	2
23	Adenoma hypophysis	f	12.8	Neuroblastoma	m	2.6	3
Lymphomas							
24	Hodgkin lymphoma	m	8.3	ALL	f	5.6	4
25	Hodgkin lymphoma	m	13.5	ALL	f		4
26	Hodgkin lymphoma	f	15.3	Wilms Tumor	f	13.2	3
				Ewing sarcoma	m	13.2	3
27	Hodgkin lymphoma	f	17.0	CNS tumor[a]	m	0.0	3
28	Burkitt lymphoma	m	14.5	Astrocytoma	f	0.9	5
29	Burkitt lymphoma	m	16.1	Rhabdomyosarcoma	m	9.1	2
30	Anaplastic large cell lymphoma	m	12.9	Hepatoblastoma	m	0.9	5
31	Diffuse large B-cell lymphoma	m	16.5	AML	m	8.0	3
Other diagnoses							
32	Hepatoblastoma	f	2.6	Hodgkin lymphoma	m	11.7	3
33	Langerhans cell histiocytosis	m	4.3	Langerhans cell histiocytosis	m	2.7	1
34	Wilms tumor	m	3.5	ALL	m	5.3	2
35	Neuroblastoma	f	0.3	CNS tumor[a]	f	9.8	6
36	Ganglioneuroblastoma	f	2.7	Osteosarcoma	f	19.0	2

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Table 1 Characteristics of study patients and corresponding relatives in families with more than one childhood cancer (FAM > 1) (Continued)

Study patient			Relative of study patient				
Nr	Diagnosis	Gender	Age (years)	Diagnosis	Gender	Age (years)	Degree of relative
37	Rhabdomyosarcoma	m	2.2	CNS tumor[a]	f	6.0	5
				Malign melanoma[a]	f	17.5	4
38	Rhabdomyosarcoma	m	4.4	Astrocytoma	f	3.9	5
39	Retinoblastoma	f	0.4	Retinoblastom	m	0.3	1
40	Hepatoblastoma	f	2.5	Leukemia[a]	m	<19[b]	4
41	Dysgerminoma	f	12.6	Leukemia[a]	f	5.0	4

Abbreviations: *ALL* acute lymphatic leukemia, *AML* acute myeloid leukemia, *CNS* central nervous system, *m* male, *f* female, *age* age at diagnosis
[a]microscopic diagnosis not confirmed
[b]exact age unknown

leukemia, of which three cases were Hodgkin's lymphomas. As for other cancer diagnoses, there were no observed diagnosis patterns (Table 1).

Age distribution in families with multiple cases of childhood cancer

There was no significant difference in mean age at diagnosis between a) study patients combined with their affected relatives in FAM > 1 (n = 86), b) study patients in FAM1 and c) study patients in the LCCG-study (Table 2). In addition, no differences were observed when comparing age at diagnosis between all LCCG-patients with the general childhood cancer population.

Female predominance in families with multiple childhood cancers

Significantly more female than male childhood cancer patients were observed in FAM > 1, female = 53 (61.6%) and male = 33 (38.4%), than in FAM1, female = 197 (40.5%) and male = 290 (59.5%)(p = 0.001)(Table 1). The greatest gender difference was found among childhood leukemia cases (relatives with childhood leukemia included) in FAM > 1, female = 23 (76.7%) and male = 7 (23.3%)(p < 0.001, FDR p = 0.004) (Tables 1 and 3).

Among all 82 female childhood leukemia cases in the LCCG-study, 15 had an additional case of childhood cancer (18.3%, 95% CI: 9.9–26.6%). This observation is significantly higher than the 7.1% risk observed in the

Table 2 Age at diagnosis of pediatric cancer in families with one - or multiple children with cancer

	Mean age at diagnosis					
	FAM > 1	FAM1		LCCG-study		Gen. Pop.
Diagnosis	years ± SD (n)	years ± SD (n)	p	years ± SD (n)	p	years [24]
All diagnoses	7.1 ± 5.2 (86)	7.2 ± 5.0 (487)	0.842	7.2 ± 5.1 (534)	0.997	
Leukemia	5.9 ± 4.2 (30)	5.8 ± 4.3 (154)	0.974	5.8 ± 4.2 (170)	0.941	5.0
CNS tumor	7.6 ± 5.1 (22)	8.4 ± 4.5 (100)	0.506	8.4 ± 4.6 (110)	0.449	7.0
Lymphoma	13.7 ± 2.7 (11)	11.0 ± 4.7 (72)	0.067	11.3 ± 4.6 (81)	0.101	10.8
Histiocytosis	3.5 + 1.2 (2)	4.3 ± 3.7 (11)	0.783	4.2 ± 3.5 (12)	0.770	–
Neuroblastoma	1.6 ± 1.2 (4)	2.1 ± 2.7 (22)	0.721	2.1 ± 2.7 (24)	0.740	1.3
Retinoblastoma	0.9 ± 1.0 (3)	1.4 ± 1.0 (2)	0.652	0.9 ± 0.8 (4)	0.935	1.3
Bone tumor	13.4 ± 5.4 (3)	10.8 ± 4.9 (40)	0.370	10.8 ± 4.9 (40)	0.370	11.6
Hepatic tumor	2.0 ± 1.0 (3)	0.7 ± 0.9 (3)	0.179	1.4 ± 1.1 (6)	0.429	1.1
Soft tissue sarcoma	5.3 ± 3.5 (3)	5.7 ± 4.2 (28)	0.863	5.5 ± 4.1 (30)	0.910	6.5
Germ-cell tumors	12.6 (1)	8.9 ± 6.8 (11)	0.611	9.2 ± 6.5 (12)	0.627	8.7
Carcinomas	6.0 (1)	9.2 ± 4.3 (5)	0.532	9.2 ± 4.3 (5)	0.532	111
Wilms' tumor	6.1 ± 6.2 (3)	3.6 ± 3.3 (34)	0.238	3.6 ± 3.2 (35)	0.231	3.3
Others	- (0)	- (5)	–	- (5)	–	–

Abbreviations: *FAM > 1* families with multiple cases of childhood cancer, *FAM1* families with one case of childhood cancer, *LCCG* Lund Childhood Cancer Genetic, *Gen.Pop* General childhood cancer population, *CNS* central nervous system

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Table 3 Gender distribution of pediatric cancer in families with one - or more children with cancer

Diagnosis	FAM > 1 (n)	FAM1 (n)	LCCG-study (n)	General population [23]
All diagnoses	**0.62**[a,b] (86)	1.47 (487)	1.36 (534)	1.2
Leukemia	**0.30**[a,b] (30)	1.30 (154)	1.07 (170)	1.2
CNS tumors	**0.47**[a] (22)	1.63 (100)	1.50 (110)	1.2
Lymphoma	2.67 (11)	1.67 (72)	1.79 (81)	1.6
Histiocytosis	males only (2)	1.75 (11)	2.00 (12)	–
Neuroblastoma	0.33 (4)	1.75 (22)	1.40 (24)	1.2
Retinoblastoma	0.33 (3)	1.00 (2)	1.00 (4)	1.1
Bone tumor	0.50 (3)	1.50 (40)	1.50 (40)	1.4
Hepatic tumor	0.50 (3)	2.00 (3)	1.00 (6)	1.4
Soft tissue sarcoma	males only (3)	2.50 (28)	2.75 (30)	1.2
Germ-cell tumors	females only (1)	0.83 (11)	0.71 (12)	–
Carcinomas	males only (1)	1.50 (5)	1.50 (5)	–
Wilms tumor	0.50 (3)	1.27 (34)	1.33 (35)	0.9
Others	- (0)	0.25 (5)	0.25 (5)	–

All units are representative of the male-to-female ratio. Significant observations are marked as bold
Abbreviations: FAM > 1 families with multiple cases of childhood cancer, *FAM1* families with one case of childhood cancer, *LCCG* Lund Childhood Cancer Genetic, *CNS* central nervous system, *FDR* false discovery rate
[a]*FDR adjusted p < 0.05 when compared to childhood cancer in FAM1*
[b]*FDR adjusted p < 0.05 when compared to childhood cancer in the general population*

rest of the LCCG-study (*p* = 0.004) (Table 1). In contrast, of the 88 males with leukemia in the study, only two had a relative with a childhood cancer.

A female predominance was also found among patients with childhood CNS tumors in FAM > 1 compared to FAM1; female = 15 (65.2%) versus male = 8 (34.8) (Tables 1 and 3; *p* = 0.010, FDR *p* = 0.020). Among the 44 families with a female case of CNS tumors in the LCCG-study, 13.6% (*n* = 6/44) had two cases of childhood cancer. In families where a male child had a CNS tumor, 7.6% (5/66) had an additional case of childhood cancer.

The male-to-female ratio in the LCCG-study was in line with that of the childhood cancer in the general population except for soft tissue sarcoma where the LCCG-study had a higher ratio (*p* = 0.033, FDR *p* = 0.297) (Table 3). This observation, however, was not significant after adjusting for multiple testing. Interestingly, the male-to-female ratio of all identified individuals in the pedigrees of FAM > 1 (including those without a childhood cancer diagnosis) was 1.04 (female: 667, male: 697), therefore not accounting for the observed female predominance.

Cytogenetic characteristics of ALL
Data regarding cytogenetic typing of older cases of ALL or patients with ALL diagnosed abroad could not be achieved. Cytogenetic data was available for 15/22 and 120/132 cases in FAM > 1 and FAM1, respectively. Furthermore, data on risk group was available in 16/22 and 128/132 cases in FAM > 1 and FAM1, respectively. There was no observed difference in the cytogenetic type of ALL between FAM1 and FAM > 1. However, in FAM > 1,

there were no cases of high- or very high-risk leukemia. In contrast, in FAM1 21.4% of ALL (*p* = 0.025) were defined as high- or very high risk.

Cancer predisposition syndromes in families with multiple childhood cancers
A cancer predisposition syndrome was present in four of the study patients in FAM > 1. Two patients with optic nerve glioma were diagnosed with neurofibromatosis type 1. One patient with ALL was diagnosed with Down's syndrome. Interestingly, one study patient with bilateral retinoblastoma tested negative for any known hereditary mutations in the *RB* gene. However, this patient had a first degree relative with bilateral retinoblastoma and therefore both cases were defined as being hereditary.

Discussion
Here, we show that in the LCCG population, 4.4% of childhood cancer patients have a relative with a childhood tumor amongst relatives to a 3rd degree, which is in line with previous studies [2, 3]. Interestingly, we further examined relatives to a 6th degree, which displayed a 7.8% incidence of multiple childhood cancers within a family, indicating that our study may detect heritable genetic factors of low penetrance. Both these percentages are higher than that observed in the general childhood cancer population, which supports the observations that relatives of childhood cancer patients have an increased risk of childhood cancer.

There was a difference between the percentage of leukemia cases in the full LCCG-study and in the general

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population. The higher proportion of patients with childhood leukemia in our cohort may be due to the inclusion of childhood cancer survivors, the majority of which are patients with leukemia. We found a lower proportion of CNS tumors in the LCCG-study compared to the general population, which could be due to lower participation rates of these patients compared to patients with other tumors (Table 1).

The majority of study patients with a childhood leukemia or CNS tumor in FAM > 1 had a relative with a matching diagnosis. This is a higher proportion than expected when compared to the general childhood cancer population. Our study is in line with previous studies that have observed a generally increased risk for cancer in relatives of children with leukemia or CNS tumors [3–5, 7, 25].

Curtin et al. 2013 [3] recently described an increased risk of childhood cancer amongst relatives of patients with childhood leukemia, however the types of diagnoses amongst relatives were not specified. The high ratio of ALL among relatives of children with leukemia in our study suggests that ALL might be responsible for a part of the increased risk showed by Curtin et al. Interestingly, no ALL case in FAM > 1 were classified as high risk, and we can speculate as to whether the different ALL risk types are related to specific genetic predispositions.

The high probability of matching diagnoses in relatives of children with childhood leukemia or CNS tumors suggests that there might be a higher degree of heredity in these diseases compared to other childhood cancer types. This is further supported by two studies on siblings with childhood cancer from 1977 and 1996, where Draper et al. observed that the number of sibling pairs where both had the same childhood cancer diagnosis was especially high for siblings with leukemia and CNS tumors [25]. However, the portion of siblings with matched diagnoses was much lower than siblings with different diagnoses. In contrast, our present study showed that a majority of the leukemia and CNS tumor cases in FAM > 1 had a relative with a matching diagnosis. This discrepancy between studies is most likely attributed to the fact that our study included distant relatives while Draper et al. focused solely on siblings. With this in consideration, our study may shed new and broader light on the potential for hereditary factors in CNS tumors and leukemia.

Three of four patients with neuroblastoma had a relative with a childhood CNS tumor. Even though the group is too small to draw any conclusions it could be of interest to study the connection between neuroblastoma and CNS tumors in a larger study. To our knowledge no such observation has previously been made.

Patients with a childhood cancer diagnosis in FAM > 1 had a significantly higher proportion of females than those in FAM1. This disparity was only observed among families with childhood leukemia and CNS tumors (Table 3). It should also be noted that the male-to-female ratio of the entire LCCG-study as well as all individuals (patients and relatives) in FAM > 1 was greater than 1, and therefore does not account for the female predominance. Previous publications have shown that infants with leukemia are predominantly female [26, 27]. However, the mean onset age of childhood leukemia in FAM > 1 in the current study was five years of age (Table 2) and only one female FAM > 1 patient with childhood leukemia was younger than 2 years (Table 1). Interestingly, in a study of cancer heredity of 1st degree siblings the Swedish population, the six twins with ALL were all female [28]. A comprehensive review of all twin cases with ALL published in 2003 gives no data on gender [29]. Another study showed that daughters to mothers with multiple sclerosis (MS) had an increased risk for leukemia [30], however no cases of MS were identified among mothers of children with leukemia in FAM > 1.

Furthermore, in a study by Magnusson et al. published in 2011, a female predominance among childhood cancer patients in FAM > 1 was also observed, however these findings were considered coincidental due to the small size of their cohort [2]. As the cohort in the current study has been substantially expanded, we can strengthen the relevance of the observed female predominance in FAM > 1, specifically amongst leukemia and CNS tumors. In addition, the fact that we observe a higher risk for female patients with either leukemia or a CNS tumor to have a relative with a childhood cancer diagnosis (18.3% and 13.6%, respectively) could potentially indicate sex-dependent risk factors. Genetic analyses on females and males with childhood leukemia or CNS tumors in FAM > 1 are now in progress.

The comprehensive Swedish national registries allow us to extend the pedigrees to 3rd degree relatives, which has previously been done in a limited number of studies [2, 3]. As this study expands up to 6th degree it increases the chance of locating hereditary factors with a low penetrance, which may explain observed trends in our study that have previously been overlooked.

The fact that only 4.6% of all eligible individuals actively declined participation is encouraging. Adding those not returning the quite extensive questionnaire, despite intent of participation and providing blood samples, we here present a participation rate of 79%. This number suggests that our cohort is representative of the southern healthcare region in Sweden. The fact that we include both newly diagnosed patients and cancer survivors in the cohort might screw the results toward survivors' characteristics, but this should not influence the results presented here. The relative small number of families with more than one childhood cancer case is

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a limitation for results regarding smaller diagnostic groups.

Conclusions

Here we show that families with a case of childhood leukemia or CNS tumor have an increased risk of having a childhood relative with the same diagnosis. That this risk is higher if the patient is female could indicate gender-specific genetic factors responsible for a heritability of the disease. This study therefore serves to identify families suitable for further genetic analyses, which are currently underway.

Abbreviations
ALL: acute lymphatic leukemia; AML: acute myeloid leukemia; CNS: central nervous system; FAM > 1: families with multiple cases of childhood cancer; FAM1: families with one case of childhood cancer; FDR: false discovery rate; ICD-7: the International classification of diseases 7th edition; LCCG-study: Lund Childhood Cancer Genetic study

Availability of Data and Materials
Confidentiality of the datasets is protected in accordance with GCP and the Declaration of Helsinki. Data is available upon collaborative research. Please contact the corresponding author.

Authors' contributions
Study concept and design: HO, IØ, TW, LH. Acquisition of data: KJS, IØ. Statistical analysis and interpretation of the results: KJS, KVS, IØ, HO. Drafting of the manuscript: KJS, KVS, IØ. Critical revision of the manuscript: HO, KJS, KVS, IØ, TW, LH. All the authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate
The study was approved by the Regional Ethics Review Board, Lund University, Sweden (no. 2008/233, 2010/231 and 2011/33). Access to the Population Registry and Cancer Registry was approved for participants and relatives.

Consent for publication
Written informed consent for publication has been acquired from all 534 study patients and/or legal guardian.

Competing interests
The authors declare no conflict of interest. The funders of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit it for publication.

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Study II

CANCER EPIDEMIOLOGY, BIOMARKERS & PREVENTION | RESEARCH ARTICLE

Increased Cancer Risk in Families with Pediatric Cancer Is Associated with Gender, Age, Diagnosis, and Degree of Relation to the Child Ⓐ Ⓒ

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ABSTRACT

Background: Studies of cancer risk among relatives of children with cancer beyond parents and siblings are limited. We have investigated the cancer risk up to the third degree of relation in families with pediatric cancer to reveal patterns of inheritance.

Methods: A single-center cohort of 757 patients with pediatric cancer was linked to the Swedish National Population Register, resulting in 16,137 relatives up to the third degree of relation. All relatives were matched to the Swedish Cancer Register, and standard incidence ratios (SIR) were calculated to define relatives at risk.

Results: Children and adults up to the third degree of relation had increased cancer risk, with SIRs of 1.48 ($P = 0.01$) and 1.07 ($P < 0.01$), respectively. The SIRs for first- and third-degree adult relatives were 1.22 and 1.10, respectively, but no increased risk

was observed in second-degree relatives. Male relatives had a higher risk than females, especially when related to a girl and when the child had leukemia. The risk was mainly increased for lung, prostate, and gastrointestinal cancer. When excluding 29 families of children with known pathogenic germline variants, the increased risk remained.

Conclusions: Relatives to children with cancer up to third degree of relation have an increased cancer risk. Known pathogenic germline variants do not explain this increased risk.

Impact: The overall increased cancer risk among relatives of children with cancer in this population-based cohort strengthens the importance of surveillance programs for families with pediatric cancer.

Introduction

Previous studies have reported an increased risk of cancer among relatives of patients with pediatric cancer (1–12). However, studies including family members up to the third degree of relation are rare. Curtin and colleagues showed an approximate two-fold increased risk of pediatric cancer when including second-degree relatives, but they did not study adult cancer risk beyond first-degree relations (9). A previous study by our research group showed an increased risk of cancer among adult and pediatric relatives up to the third degree (8); however, the size of the cohort was limited.

A family history of cancer is considered a risk factor for most types of adult cancer (9, 13–16). Inherited pathogenic alterations causing cancer have been suggested to have a higher impact on pediatric cancer than on cancer in adults, which is partially attributed to children having a shorter period of environmental exposure (15). Younger age at onset of pediatric cancer correlates with a higher cancer risk among

adult relatives (17, 18), indicating that cancer at a young age is related to heredity and genetic irregularities.

The gender of relatives of children with cancer influences cancer risk. Female relatives have been reported to have a higher cancer risk than male relatives (10–12). However, no study has yet investigated whether the gender of the patient with pediatric cancer affects the cancer risk of relatives. In a study by Scheuner and colleagues of the prevalence of familial adult cancer, 14.6% of the study population had a two-fold increased risk of cancer, whereas 7.7% had a five- to seven-fold increased risk (19), indicating separate heredity patterns in family subsets. Similarly, a risk assessment by Knapke and colleagues revealed that 29% of families with cases of pediatric cancer were eligible for genetic counseling due to the increased risk of cancer among relatives (14), and the authors suggested that a high proportion of hereditary pathogenic variants remain unknown, and that novel hereditary syndromes have yet to be discovered. An estimated 10% of pediatric cancers are caused by inherited or sporadic germline pathogenic variants (20–26). The majority of highly penetrant clinically pathogenic variants have probably already been described, and novel approaches are therefore needed to detect other clinical syndromes with less obvious patterns.

The Lund Childhood Cancer Genetic (LCCG) study prospectively includes patients with a pediatric cancer diagnosis, and retrieves cancer diagnoses of relatives, in addition to blood samples of the child and parents. In an earlier study of the cohort, we observed that patients with pediatric cancer from the same family often had matching cancer diagnoses (27). Furthermore, we observed that families with more than one pediatric cancer case showed a higher prevalence of female patients with pediatric cancer than families with only one pediatric cancer case. We here investigated the cohort of 757 patients with pediatric cancer, and linked them to the comprehensive National Population and Cancer Registers to study the patterns of familial cancer up to third degree of relation.

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Materials and Methods

The ongoing LCCG study enrolls pediatric patients diagnosed with cancer below 18 years of age and treated at the Department of Pediatric Oncology, Skåne University Hospital in Lund, Sweden, covering a population of 1.9 million inhabitants. Blood samples from patients and parents are collected for germline analysis. Patients and parents complete a standardized self-reported questionnaire, providing a history of cancer among first-, second-, and third-degree relatives. Data on cancer type, date of/age at diagnosis, and outcome (if fatal, date of death) are obtained and recorded in pedigrees. All information from the questionnaires is verified and supplemented with data from national registers and medical records. Pathology reports for all patients with pediatric cancer included in the study were reviewed.

The Swedish National Population Register enables the identification of individuals through a unique personal number, as well as the degree of relation and vital status of relatives of patients with pediatric cancer. All relatives were subsequently matched to the Swedish Cancer Register to identify/confirm cancer diagnoses among relatives. As the national identification number was introduced in 1947, and the National Cancer Register was started in 1958, we decided not to include great-grandparents in the cohort as the identities and cancer diagnoses of these individuals could not be reliably confirmed.

Statistical methods

SPSS 22.0 was used for statistical analyses within the cohort. For comparison of continuous variables, such as age at diagnosis and time since diagnosis, Student t test was applied. For cohort comparison regarding diagnosis distribution, Fisher exact test was used. Results were adjusted for gender, date of birth, age, and degree of relation. P values were two-sided, and a significance level of $P < 0.05$ was used.

For calculation of cancer risk over time, the Poisson distribution was applied. Cancer diagnoses were coded according to ICD-7. The number of person years at risk was calculated as the difference between date of birth, or January 1, 1958 (in cases when the individuals were born before January 1, 1958), and date of death/emigration or end of follow-up on December 31, 2015. Person years at risk was stratified by age, sex, and calendar year, and multiplied by year-, age-, and sex-specific rates of cancer types obtained from the Swedish Cancer Register to yield the expected rates of each cancer type. Standardized incidence ratios (SIR), observed/expected ratios, 95% confidence intervals (CI), and P values were computed. P values were one-sided (as solely increased cancer risk was studied), and $P < 0.05$ was considered significant. To test for heterogeneity by sex, expected and observed values for male and female was compared using χ^2. P values were two-sided, and $P < 0.05$ was considered significant.

Cancer diagnoses among relatives were divided into 30 different diagnostic groups. Several groups were too small for statistical analysis. Thus, the results for some diagnoses should be considered as hypothesis generating as they have not been subjected to correction for multiple testing. All other results were adjusted by correction for FDRs to account for multiple testing. When analyzing cancer in relatives of children with different cancer diagnoses, they were divided in five groups; relatives to children with either (i) leukemia, (ii) central nervous system (CNS) tumors, (iii) lymphoma, (iv) sarcoma, or (v) other diagnoses. All intracranial tumors were defined as CNS tumors.

Results

By December 31, 2015, 757 children with cancer had been enrolled in the LCCG study. A total of 16,430 relatives up to the third degree were identified through the National Population Register. A total of 250 relatives (1.5%) were excluded because of invalid personal numbers, the majority of which were due to emigration or death having occurred too long ago for records to be available. Finally, a further 43 relatives (0.3%) were excluded as they had died before 1958, when the

Table 1. Characteristics of adults up to the third degree of relation to 757 patients with pediatric cancer.

Distribution of childhood cancer diagnoses, % (n)	Men		Women		All relatives	
	n (%)	Average age at cutoff (years)	n (%)	Average age at cutoff (years)	n (%)	Average age at cutoff (years)
Leukemia, 33.7% (255)	2,748 (50.7)	47.5	2,676 (49.3)	49.6	5,424	48.5
CNS tumor, 20.7% (157)[a]	1,721 (49.3)	49.0	1,767 (50.7)	49.4	3,488	49.2
Lymphoma, 13.5% (102)	1,084 (51.0)	48.6	1,041 (49.0)	50.0	2,125	49.3
Wilms tumor, 6.7% (51)	570 (50.6)	47.0	557 (49.4)	49.8	1,127	48.4
Soft-tissue sarcoma, 6.6% (50)	566 (50.9)	47.6	547 (49.1)	48.4	1,113	48.0
Bone tumor, 7.1% (54)[b]	501 (48.7)	48.6	528 (51.3)	48.2	1,029	48.4
Neuroblastoma, 4.0% (30)	279 (51.6)	47.5	262 (48.4)	48.8	541	48.2
Histiocytosis, 2.2% (17)	220 (52.8)	45.5	197 (47.2)	48.2	417	46.8
Germ-cell tumors, 1.8% (14)[a]	145 (52.0)	49.9	134 (48.0)	51.8	279	50.8
Hepatic tumor, 1.2% (9)	120 (52.6)	51.5	108 (47.4)	54.4	228	52.9
Retinoblastoma, 0.7% (5)	62 (54.9)	55.8	51 (45.1)	58.7	113	57.1
Carcinomas, 0.9% (7)[a]	61 (45.9)	45.7	72 (54.1)	45.4	133	45.5
Others, 0.8% (6)	53 (44.9)	44.0	65 (55.1)	52.2	118	48.5
Total, 100% (757)	8,130 (50.4)	48.1	8,006 (49.6)	49.5	16,136	48.8
Age at first diagnosis, years	66.5		62.9		64.8	
Cancer diagnoses, n	1,246		1,162		2,408	
Years at risk, years	297,923.4		308,634.2		606,557.5	
Cancer/1,000 years at risk, n	4.18		3.76		3.97	

[a]Significantly lower than the Swedish Childhood Cancer Register.
[b]Significantly higher than the Swedish Childhood Cancer Register.

National Cancer Register was set up, and the reported cancer diagnosis could not be confirmed. This resulted in 16,137 relatives up to the third degree whose identity could be confirmed, totaling 606,558 person years at risk. The characteristics of all adult relatives, divided according to gender and cancer diagnosis of the related child, are given in **Table 1**.

The distribution of diagnoses of the children included in the LCCG cohort was largely in line with that of the Swedish Childhood Cancer Register (**Table 1**, first column). However, an underrepresentation of CNS tumors, germ-cell tumors, and carcinomas was observed in addition to an overrepresentation of bone tumors. Seventy-four families (9.8%) had more than one pediatric cancer case in relatives up to the fifth degree of relation. When restricting the study up to third-degree relatives, 39 families (5.2%) had more than one case of pediatric cancer.

Adult cancer incidence in the LCCG cohort compared with the general population

We observed a significant increase in the risk of adult cancer among relatives of patients with pediatric cancer up to the third degree of relation (SIR 1.07, 95% CI: 1.03–1.11, $P < 0.01$, FDR $P < 0.01$), compared with the general population. Incidence ratios of cancers in adult relatives of children with cancer are presented in **Table 2**. When separated by gender, male relatives showed a higher risk of adult cancer than the general population (SIR 1.11, 95% CI: 1.05–1.17, $P < 0.01$, FDR $P = 0.01$). In contrast, female relatives up to the third degree showed no overall increased risk of cancer (SIR 1.03). The diagnoses associated with increased risk in adult relatives differed considerably between men and women, as can be seen from **Table 2**.

Childhood cancer incidence in relatives of patients with pediatric cancer

An increased risk of cancer was found in children up to third degree of relation to children with cancer (SIR 1.48, 95% CI: 1.05–2.02, $P = 0.01$, FDR $P = 0.03$), compared with the general population. The risk was significantly increased in girls, but not in boys: SIR 1.60 (95% CI: 0.98–2.48, $P = 0.03$) and SIR 1.37 (95% CI: 0.82–2.13, $P = 0.11$), respectively. However, the difference between the sexes was not significant ($P = 0.69$). As diagnoses of relatives were classified accord-

ing to ICD-7, comparison between specific pediatric cancer diagnoses was not possible.

Adult cancer risk in first- to third-degree relatives of patients with pediatric cancer

Adult cancer types associated with increased risk according to degree of relation and gender are given in **Table 3**. First-degree adult relatives showed the highest risk of cancer (SIR 1.22, 95% CI: 1.04–1.43, $P = 0.01$, FDR $P = 0.02$). Second-degree relatives showed no increased risk (SIR 1.02), while third-degree relatives did (SIR 1.10, 95% CI: 1.03–1.16, $P < 0.01$, FDR $P < 0.01$; **Table 3**).

Female first-degree relatives showed an overall increased risk of cancer (SIR 1.27, $P = 0.02$, 95% CI: 1.01–1.57, $P = 0.02$, FDR $P = 0.05$), while male first-degree relatives did not (SIR 1.17). Again, no increased risk was observed among second-degree relatives when separated by gender (men: SIR 1.06 and women: SIR 0.97). Among third-degree relatives, men showed a significantly increased risk of cancer at any location (SIR 1.14, 95% CI: 1.05–1.23, $P < 0.01$, FDR $P < 0.01$), whereas female relatives did not (SIR 1.05; **Table 3**). No overlap was observed in the types of cancer with increased risk between adult male and female relatives.

Adult cancer risk in relatives according to gender of the patient with pediatric cancer

The increase in cancer risk in first- to third-degree relatives of girls and boys with cancer was similar (SIR 1.06, $P = 0.03$, FDR $P = 0.05$ and SIR 1.07, $P < 0.01$, FDR $P < 0.01$, respectively; **Table 4**). Female relatives of girls with cancer showed no increased cancer risk (SIR 0.99), while male relatives did, (SIR 1.14, $P < 0.01$, FDR $P < 0.01$); however, there was no statistical significance when comparing the increased risk for men and women ($P = 0.11$). In contrast, male relatives of boys with cancer showed a higher risk, whereas women did not (SIR 1.08, $P = 0.02$, FDR $P = 0.05$, compared with SIR 1.06 $P = 0.06$, FDR $P = 0.10$), as can be seen from **Table 4**.

Adult cancer risk in relatives according to diagnostic group of the patient with pediatric cancer

The highest cancer risk was observed among relatives of children with CNS tumors (SIR 1.11, $P < 0.01$, FDR $P = 0.02$), followed by

Table 2. Incidence ratio of cancers in adult relatives of patients with pediatric cancer.

		Men		Women		SH	All relatives	
	n	SIR	95% CI	SIR	95% CI	*P*	SIR	95% CI
Any cancer	2,360	1.11[a]	1.05–1.17	1.03	0.97–1.09	0.23	1.07[a]	1.03–1.11
Prostate	335	1.11[a]	0.99–1.23	—	—	—	1.11[a]	0.99–1.23
Breast	307	1.06	0.13–3.82	0.97	0.87–1.09	0.98	0.97	0.87–1.09
Lung	176	1.12	0.91–1.36	1.35[a]	1.07–1.69	0.41	1.21[a]	1.04–1.40
Pharynx	16	1.66[a]	0.91–2.78	0.61	0.07–2.20	—	1.36	0.78–2.22
Esophagus	28	1.56[a]	0.95–2.40	1.69	0.73–3.34	0.95	1.59[a]	1.06–2.30
Stomach	80	1.12	0.82–1.48	1.33[b]	0.91–1.88	0.60	1.19[b]	0.95–1.49
Colon	184	1.04	0.82–1.30	1.31[a]	1.07–1.59	0.29	1.18[a]	1.01–1.36
Bile duct/gallbladder	33	1.88[a]	1.08–3.06	1.14	0.66–1.83	0.41	1.41[a]	0.97–1.98
Pancreas	63	1.26	0.87–1.76	1.18	0.79–1.69	0.83	1.22[b]	0.94–1.56
Kidney	69	0.94	0.65–1.32	1.57[a]	1.10–2.17	0.16	1.19[b]	0.93–1.51
Urinary tract	111	1.18[b]	0.95–1.45	0.72	0.44–1.11	0.13	1.06	0.87–1.28
Hodgkin	18	1.73[a]	0.90–3.03	1.20	0.44–2.62	0.64	1.51[b]	0.90–2.39

Note: Prostate, breast, and lung cancer and cancer types with at least one P value <0.1 are presented.
Abbreviation: SH, sex heterogeneity.
[a]$P < 0.05$.
[b]$P < 0.1$.

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Table 3. Incidence ratio of cancer in adult relatives of patients with pediatric cancer.

Degree of relation	Relatives (n)		n	Men		Women		SH	All relatives	
				SIR	95% CI	SIR	95% CI	P	SIR	95% CI
1st degree	2,138	Any cancer	154	1.17	0.91–1.48	1.27[a]	1.01–1.57	0.71	1.22[a]	1.04–1.43
2nd degree	4,990	Any cancer	1,019	1.06[b]	0.98–1.16	0.97	0.89–1.07	0.32	1.02	0.96–1.08
3rd degree	6,525	Any cancer	1,187	1.14[a]	1.05–1.23	1.05	0.97–1.14	0.36	1.10[a]	1.03–1.16
1st degree										
Median age 37 years		Prostate	16	1.00	0.57–1.63	—	—		1.00	0.57–1.63
		Breast	28	0.00	0.00–36.0	1.18	0.79–1.71		1.18	0.78–1.70
		Lung	9	0.57	0.07–2.06	2.32[a]	0.93–4.77		1.38	0.63–2.62
		Mouth	4	4.18[a]	0.86–12.22	2.48	0.06–13.82		3.57[a]	0.97–9.14
		Pharynx	4	5.58[a]	1.52–14.28	0.00	0.00–15.06		4.16[a]	1.13–10.64
		Pancreas	3	0.00	0.00–3.41	3.82[a]	0.79–11.17		1.61	0.33–4.70
		Cervix	8	—	—	2.54[a]	1.10–5.01		2.54[a]	1.10–5.01
		Testis	6	2.12[b]	0.78–4.62	—	—		2.12[b]	0.78–4.62
		Melanoma	15	1.14	0.37–2.66	1.91[a]	0.92–3.52		1.56[b]	0.87–2.57
		CNS tumor	9	0.99	0.21–2.91	1.96[b]	0.72–4.27		1.48	0.68–2.81
2nd degree										
Median age 57 years		Prostate	164	1.13[b]	0.96–1.31	—	—		1.13[b]	0.96–1.31
		Breast	143	0.00	0.00–4.60	0.97	0.82–1.15		0.97	0.82–1.14
		Lung	77	1.14	0.84–1.52	1.08	0.73–1.53		1.12	0.88–1.39
		Esophagus	11	1.68[b]	0.81–3.09	0.52	0.01–2.90		1.40	0.70–2.50
3rd degree										
Median age 47 years		Prostate	155	1.1	0.93–1.29	—	—		1.1	0.93–1.29
		Breast	136	0.99	0.25–7.33	0.94	0.79–1.11		0.95	0.79–1.12
		Lung	90	1.15	0.86–1.50	1.55[a]	1.10–2.13		1.29[a]	1.04–1.58
		Mouth	12	1.38	0.51–3.01	1.78	0.65–3.87		1.56[b]	0.80–2.72
		Esophagus	15	1.44	0.66–2.72	2.27[b]	0.83–4.95		1.68[a]	0.94–2.78
		Stomach	50	1.11	0.74–1.59	1.39[b]	0.86–2.13		1.21	0.90–1.60
		Colon	111	0.97	0.68–1.34	1.70[a]	1.33–2.13		1.36[a]	1.12–1.64
		Bile duct/gallbladder	20	2.56[a]	1.32–4.47	0.88	0.38–1.74		1.45[b]	0.89–2.25
		Pancreas	37	1.52[a]	0.95–2.30	1.08	0.61–1.78		1.31[b]	0.92–1.80
		Kidney	37	0.86	0.48–1.41	1.76[a]	1.10–2.67		1.23	0.87–1.70
		Urinary tract	65	1.40[a]	1.05–1.83	0.80	0.41–1.40		1.23[b]	0.95–1.56
		Thyroid	13	2.37[a]	0.87–5.17	1.06	0.43–2.18		1.42	0.76–2.43
		Non-Hodgkin	38	1.53[a]	0.99–2.26	0.89	0.48–1.53		1.23	0.87–1.69
		Hodgkin	12	2.77[a]	1.27–5.25	1.24	0.25–3.61		2.11[a]	1.09–3.69

Note: Prostate, breast, and lung cancer and other cancer with at least one P value <0.1 are presented.
Abbreviation: SH, sex heterogeneity.
[a]P < 0.05.
[b]P < 0.1.

lymphoma (SIR 1.10, $P = 0.05$, FDR $P = 0.09$), leukemia (SIR 1.06, $P = 0.06$, FDR $P = 0.10$), "other" diagnoses, mainly Wilms tumor and neuroblastoma (SIR 1.05, $P = 0.15$, FDR $P = 0.19$), and finally sarcomas (SIR 1.01, $P = 0.43$, FDR $P = 0.43$). Only relatives of children with CNS tumors showed a significantly increased risk of cancer of any type (for details, see **Table 5**). Male relatives of patients with pediatric leukemia showed a significantly higher cancer risk than female relatives (SIR 1.15 and SIR 0.96, respectively, $P = 0.01$). Relatives to children with sarcoma showed a higher risk for men than women, although this was not statistically significant (SIR 1.10 and SIR 0.92, respectively, $P = 0.10$). A gender-dependent pattern was not seen among relatives of children with CNS tumors or lymphoma

Adult cancer risk of first-degree relatives according to age at diagnosis of pediatric cancer

First-degree relatives of children diagnosed before 5 years of age showed a higher risk of adult cancer than the general population (SIR 1.42, $P < 0.01$, FDR $P = 0.02$; **Table 6**). Male relatives exhibited an increased risk of adult cancer (cancer in the mouth/pharynx), whereas females did not. Relatives of children diagnosed after 5 years of age did not show any overall increased risk compared with the general population. No significant difference was found in the cancer risk of relatives of children diagnosed with cancer before or after 5 years of age, when the two groups were compared with each other, instead of with the general population (SIR 1.18, $P = 0.31$).

Adult cancer risk in families with one or more than one pediatric cancer

Seventy-four children in the LCCG cohort (9.8%) had a relative up to the fifth-degree with pediatric cancer. When comparing the cancer incidence in relatives up to third degree in families with one (FAM1) or more than one pediatric cancer (FAM>1), no significant difference was seen in the diagnosis distribution or age at onset of adult cancer (Supplementary Table S1). Adult relatives in FAM>1 showed no overall increase in cancer risk compared with the general population (SIR 1.00, $P = 0.49$). Adult relatives in FAM>1 showed no increase in risk when compared with relatives in FAM1.

Table 4. Incidence ratio of cancer in relatives of patients with pediatric cancer according to gender.

Gender of child		n	Men SIR	Men 95% CI	Women SIR	Women 95% CI	SH P	All relatives SIR	All relatives 95% CI
Girl	Any cancer	1,018	1.14[a]	1.05–1.24	0.99	0.90–1.08	0.11	1.06[a]	1.00–1.13
Boy	Any cancer	1,342	1.08[a]	1.00–1.17	1.06[b]	0.98–1.15	0.83	1.07[a]	1.02–1.13
Girl									
	Prostate	156	1.18[a]	1.00–1.38	—	—		1.18[a]	1.00–1.38
	Breast	122	1.21	0.03–6.77	0.89	0.74–1.06		0.89	0.74–1.07
	Lung	75	1.16	0.85–1.56	1.22	0.83–1.75		1.19[b]	0.93–1.49
	Mouth	9	0.72	0.15–2.09	2.07[b]	0.76–4.50		1.27	0.58–2.41
	Stomach	39	1.24	0.78–1.85	1.59[b]	0.91–2.59		1.36[a]	0.97–1.86
	Colon	84	1.03	0.71–1.43	1.47[a]	1.09–1.94		1.25[a]	1.00–1.55
	Bile duct/gallbladder	17	1.90[b]	0.76–3.92	1.59	0.76–2.93		1.71[a]	0.99–2.73
	Pancreas	26	1.54[b]	0.91–2.44	0.76	0.33–1.50		1.17	0.77–1.72
	Pharynx	7	1.86[b]	0.75–3.83	0.00	0.00–6.15		1.60	0.64–3.30
	Urinary tract	49	1.25[b]	0.90–1.69	0.59	0.24–1.22		1.08	0.80–1.43
	Leukemia	11	0.95	0.26–2.44	1.91	0.77–3.94		1.40	0.70–2.51
	CLL	15	1.69[b]	0.77–3.22	1.91	0.70–4.15		1.77[a]	0.99–2.93
Boy									
	Prostate	179	1.05	0.90–1.22	—	—		1.05	0.90–1.22
	Breast	185	0.94	0.02–5.22	1.04	0.89–1.20		1.03	0.89–1.20
	Lung	101	1.09	0.82–1.42	1.45[a]	1.06–1.94		1.23[a]	1.00–1.49
	Mouth	13	2.03[a]	1.01–3.64	0.51	0.06–1.85		1.40	0.74–2.39
	Esophagus	17	1.79[a]	0.95–3.06	1.47	0.40–3.76		1.70[a]	0.99–2.73
	Bile duct/gallbladder	16	1.87[b]	0.85–3.55	0.81	0.33–1.67		1.19	0.68–1.93
	Pancreas	37	1.05	0.60–1.70	1.49[b]	0.92–2.28		1.26[b]	0.89–1.74
	Testis	13	1.65[b]	0.88–2.82	—	—		1.65[b]	0.88–2.82
	Kidney	46	1.11	0.69–1.68	1.83[a]	1.17–2.73		1.40[a]	1.02–1.86
	CNS tumor	42	0.86	0.48–1.41	1.36[b]	0.89–1.97		1.12	0.81–1.52
	Hodgkin	10	1.80[b]	0.72–3.71	1.06	0.22–3.11		1.49	0.71–2.74

Note: Prostate, breast, and lung cancer and other with at least one P value <0.1 are presented.
Abbreviations: CLL, chronic lymphocytic leukemia; SH, sex heterogeneity.
[a]P < 0.05.
[b]P < 0.10.

Adult cancer risk in families with known pathogenic germline mutations

Twenty-nine of 728 patients with pediatric cancer from the LCCG cohort (4%) tested positive for a pathogenic germline variant in a parallel ongoing study. Targeted sequencing (Illumina HiSeq 2500) of 22 autosomal dominant predisposition genes analyzed in a study by Zhang and colleagues (25) revealed 29 carriers in 10 of these genes (NF1, TP53, BRCA2, RB1, BRCA1, PMS2, SDHA, APC, PALB2, and PTCH1, while none for ALK, ATM, CDH1, KRAS, MSH2, MSH2, MSH6, NF2, NRAS, RET, RUNX1, SDHAB, and VHL). Upon exclusion of these patients, and 29 patients in which no germline analysis had been performed (n = 58), no difference in the increased risk of cancer in adult relatives was observed, thus SIR 1.07 (P < 0.01) remained unchanged.

Discussion

We have shown that first-degree adult relatives of children with cancer had a 22% increased risk of cancer, and third-degree adult relatives had a 10% increased risk, compared with the general population. There was no increase in cancer risk among second-degree adult relatives. The increased risk in first-degree adult relatives is in line with reports from previous studies (6, 7, 9, 12). Heath and colleagues

reported a decreased risk in second-degree relatives (10), which is confirmed in this study but difficult to explain in combination with increased risk for third-degree relatives. Winther and colleagues reported that adult relatives had an increased cancer risk at younger age (6). In this study, second-degree relatives had a higher median age than first- and third-degree relatives (Table 3), which could partly explain why second-degree relatives had no increased risk of cancer when compared with the general population. Nevertheless, after correcting for age, second-degree relatives still showed no overall increased risk of adult cancer in this study. To further speculate, environmental factors might affect epigenetics and penetrance of inherited germline variants (28–30), and it may be that second-degree relatives were exposed to different lifestyle factors in their generation of time. The increased cancer risk in third-degree adult relatives is novel and intriguing. We attribute this finding mainly to the increased cancer risk among male relatives.

The risk of pediatric cancer in families with cases of pediatric cancer was increased by 48% in first-degree relatives, compared with the general population. This is lower than the two-fold increase reported previously (4, 9), but families with pediatric leukemia, the most frequent pediatric cancer diagnosis, showed an increased risk of 86% in this study. We observed an increased risk of cancer in the gastrointestinal (GI) tract, prostate, and lungs in adult relatives of

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Table 5. Incidence of cancer in adult relatives of patients with pediatric cancer.

Diagnosis of child		n	Men		Women		SH	All relatives	
			SIR	95% CI	SIR	95% CI	P	SIR	95% CI
Leukemia	Any cancer	779	1.15[a]	1.05–1.27	0.96	0.86–1.07	0.01[a]	1.06[b]	0.98–1.13
CNS	Any cancer	542	1.10[b]	0.97–1.24	1.12[a]	1.00–1.27	0.84	1.11[a]	1.02–1.21
Lymphoma	Any cancer	319	1.13[b]	0.96–1.31	1.07	0.91–1.26	0.75	1.10[a]	0.98–1.23
Sarcoma	Any cancer	290	1.10	0.94–1.28	0.92	0.77–1.09	0.10	1.01	0.90–1.13
Other	Any cancer	429	1.02	0.88–1.16	1.09	0.95–1.24	0.61	1.05	0.95–1.16
Leukemia	Prostate	124	1.24[a]	1.03–1.48	—	—		1.24[a]	1.03–1.48
	Breast	98	0.00	0.00–5.89	0.93	0.76–1.14		0.93	0.75–1.13
	Lung	60	1.25	0.88–1.72	1.23	0.78–1.84		1.24[b]	0.95–1.59
	Mouth	11	2.20[a]	0.88–4.52	1.74	0.48–4.46		2.01[a]	1.00–3.59
	Stomach	31	1.27	0.75–2.01	1.61[b]	0.86–2.76		1.40[a]	0.95–1.98
	Bile duct/gallbladder	11	2.14[b]	0.79–4.66	1.00	0.32–2.33		1.41	0.70–2.52
	Pleura	4	3.12[a]	0.85–8.00	0.00	0.00–13.2		2.57[b]	0.70–6.57
	Melanoma	39	1.24	0.73–1.95	1.34	0.83–2.04		1.29[b]	0.92–1.76
	Leukemia	8	1.57	0.51–3.66	1.05	0.22–3.06		1.32[b]	0.57–2.60
CNS tumor	Prostate	61	0.93	0.71–1.19	—	—		0.93	0.71–1.19
	Breast	89	2.40	0.06–13.4	1.26[a]	1.01–1.55		1.27[a]	1.02–1.56
	Lung	34	0.98	0.59–1.53	1.20	0.67–1.97		1.06	0.74–1.49
	Pharynx	6	2.73[a]	0.89–6.37	1.38	0.03–7.68		2.35[a]	0.86–5.11
	Esophagus	8	2.14[b]	0.79–4.66	1.89	0.23–6.84		2.07[a]	0.89–4.08
	Colon	49	0.78	0.42–1.34	2.01[a]	1.41–2.78		1.42[a]	1.05–1.87
	Pancreas	18	2.04[a]	1.05–3.56	1.08	0.40–2.35		1.57[a]	0.93–2.49
	Kidney	19	1.18	0.54–2.24	1.94[a]	0.93–3.57		1.49[b]	0.89–2.32
	Hodgkin	5	3.34[a]	1.08–7.78	0.00	0.00–3.38		1.93	0.63–4.50
	Leukemia	8	1.43	0.29–4.17	2.61[a]	0.85–6.09		1.99[b]	0.86–3.92
Lymphoma	Prostate	49	1.19	0.88–1.58	—	—		1.19	0.88–1.58
	Breast	32	4.01	0.10–22.3	0.76	0.52–1.08		0.78	0.54–1.11
	Lung	19	0.76	0.35–1.44	1.38	0.66–2.53		0.99	0.60–1.55
	Colon	30	1.47[b]	0.83–2.43	1.48[b]	0.83–2.45		1.48[a]	1.00–2.11
	Bile duct/gallbladder	7	4.39[a]	1.43–10.3	1.05	0.13–3.79		2.30[a]	0.92–4.74
	Ovary	12	—	—	1.86[a]	0.96–3.24		1.86[a]	0.96–3.24
	Testis	6	3.10[a]	1.14–6.74	—	—		3.10[a]	1.14–6.74
	Kidney	13	1.29	0.47–2.81	2.39[a]	0.96–4.92		1.71[a]	0.91–2.93
	CNS	17	1.67	0.67–3.45	2.17[a]	1.04–4.00		1.94[b]	1.13–3.10
	Myeloma	6	2.47[b]	0.80–5.77	0.66	0.02–3.66		1.69	0.62–3.68
Sarcoma	Prostate	42	1.07	0.77–1.44	—	—		1.07	0.77–1.44
	Breast	40	0.00	0.00–14.9	1.00	0.71–1.36		0.99	0.71–1.35
	Lung	24	1.36	0.78–2.21	1.12	0.49–2.21		1.27	0.81–1.89
	Skin	16	1.51[b]	0.83–2.54	0.29	0.04–1.06		0.99	0.57–1.61
Other	Prostate	59	1.05	0.80–1.36	—	—		1.05	0.80–1.36
	Breast	48	0.00	0.00–10.6	0.83	0.61–1.10		0.82	0.61–1.09
	Lung	39	1.15	0.69–1.8	1.9[a]	1.16–2.94		1.44[a]	1.03–1.98
	Colon	39	0.99	0.54–1.66	1.72[a]	1.11–2.53		1.36[a]	0.96–1.85
	Skin	2	1.26	0.75–2.00	1.38	0.73–2.36		1.31[b]	0.89–1.86
	Urinary tract	26	1.53[a]	0.96–2.32	0.79	0.21–2.02		1.34[a]	0.87–1.96
	Non-Hodgkin	17	1.19	0.52–2.35	1.7[b]	0.78–3.22		1.42	0.82–2.27
	Hodgkin	5	3.15[a]	0.86–8.07	1.1	0.03–6.14		2.3[b]	0.75–5.36

Note: Prostate, breast, and lung cancer and cancer types with at least one P value <0.1 are presented.
Abbreviation: SH, sex heterogeneity.
[a]P < 0.05.
[b]P < 0.1.

children with cancer (**Table 2**). The cancer risk in the GI tract was independent of the degree of relation, gender, or diagnosis of the child with cancer, but relatives of children with CNS tumors and leukemia were particularly at risk. Pathogenic germline variants in the *APC* gene has been associated with an increased risk for medulloblastoma in children as well as for colorectal cancer in adults (31), and future sequencing studies might reveal additional pathogenic variants behind these associations.

Pediatric rhabdomyosarcoma, CNS tumors, and skin cancer are associated with increased risk of breast cancer in adult relatives (8, 11, 12). We indeed observed an increase in the risk of breast cancer among female relatives of children with CNS tumors (SIR 1.27, *P* = 0.02; **Table 5**). Compared with relatives of children with any other cancer diagnosis, we observed a SIR of 1.49 and a three-fold higher risk in first-degree relatives to children with CNS tumors (SIR 3.34). The relatively low number of children with CNS tumors in the current

Table 6. Adult cancers in first-degree relatives of patients with pediatric cancer.

Age at diagnosis of child		*n*	Men		Women		SH	All relatives	
			SIR	95% CI	SIR	95% CI	*P*	SIR	95% CI
0–5 years of age	Any cancer	65	1.59[a]	1.10–2.22	1.27	0.86–1.80	0.56	1.42[a]	1.10–1.81
>5 years of age	Any cancer	89	0.94	0.66–1.30	1.27[b]	0.95–1.66	0.35	1.11	0.89–1.36
0–5 years of age									
	Prostate	6	1.14	0.42–2.48	—	—		1.14	0.42–2.48
	Breast	11	0.00	0.00–98.5	1.26	0.63–2.26		1.26	0.63–2.25
	Lung	5	0.86	0.02–4.77	3.76[a]	1.02–9.62		2.24[b]	0.73–5.23
	Mouth	3	7.82[a]	0.95–28.27	6.66	0.17–37.1		7.39[a]	1.52–21.6
	Pharynx	3	11.8[a]	2.43–34.50	0.00	0.00–40.2		8.68[a]	1.79–25.4
	Liver	2	3.91	0.10–21.81	9.27	0.23–51.7		5.50[b]	0.67–19.9
	Urinary tract	3	2.79[b]	0.58–8.16	0.00	0.00–11.0		2.13	0.44–6.22
	Melanoma	7	1.82	0.38–5.33	1.97	0.54–5.04		1.90[b]	0.77–3.92
	Non-Hodgkin	4	2.19	0.27–7.93	3.50	0.42–12.6		2.70[b]	0.73–6.91
>5 years of age									
	Prostate	10	0.94	0.45–1.73	—	—		0.94	0.45–1.73
	Breast	17	0.00	0.00–56.7	1.14	0.66–1.82		1.13	0.66–1.81
	Lung	4	0.43	0.01–2.38	1.53	0.32–4.48		0.93	0.25–2.38
	Esophagus	1	0.00	0.00–8.80	9.88[b]	0.25–55.1		1.92	0.05–10.7
	Pancreas	2	0.00	0.00–5.19	3.95[b]	0.48–14.3		1.64	0.20–5.93
	Cervix	7	—	—	3.71[a]	1.49–7.64		3.71[a]	1.49–7.64
	Testis	4	2.50[b]	0.68–6.41	—	—		2.50[b]	0.68–6.41
	CNS	7	1.08	0.13–3.91	2.66[a]	0.86–6.21		1.88[b]	0.75–3.87
	Hodgkin	3	2.14	0.05–11.92	5.48[b]	0.66–19.8		3.61[b]	0.74–10.5
	CLL	2	5.46[b]	0.66–19.72	0.00	0.00–22.3		3.76	0.46–13.6

Note: Prostate, breast, and lung cancer and cancer types with at least one *P* value <0.1 are presented.
Abbreviations: CLL, chronic lymphocytic leukemia; SH, sex heterogeneity.
[a]*P* < 0.05.
[b]*P* < 0.1.

cohort could explain the lack of overall increased breast cancer risk among first-degree relatives. Specific pathogenic germline variants are associated with increased risk for both pediatric CNS tumors and breast cancer, such as variants in *TP53, BRCA2, BRCA1,* and *APC* (31, 32). However, in the present cohort, these variants were identified in a minority of the patients with childhood cancer (*n* = 12). Hence, the increased risk of breast cancer in relatives of children with CNS tumors is more complex than current knowledge can explain.

When dividing the relatives according to gender, we found that first-degree female relatives had an increased risk of cancer of any kind, but this was not seen in second- or third-degree female relatives. An increased risk of cancer in first-degree female relatives has been reported previously (10–12), often related to the increased risk of breast cancer (11, 12). Studies reporting no increased risk still indicated a higher risk of cancer in women than in men (18, 33). A total of 55% of first-degree female relatives in the study cohort were mothers to pediatric cancer patients. Parous women have lower risk for cancer, especially for breast cancer (34, 35), which might affect the reported results in this study.

In this study, we found that the overall relative risk in first- to third-degree relatives was higher in men than in women (SIR 1.14 and 1.05, respectively); however, the difference was not significant. Moreover, we found that third-degree male relatives had a higher overall cancer risk than women (**Table 3**), although not statistically significant. This tendency could be due to the protective effect of female sex hormones, as discussed in the case of colorectal cancer (36). Another explanation could be that men and women are exposed to environmental factors to different degrees, or that one gender is more susceptible to them than the other.

We observed a difference between women and men regarding cancer types associated with increased risk. Women showed increased risks of colon, lung, and kidney cancer, while in men the risks of pharynx, esophagus, and biliary tract/gallbladder cancer and Hodgkin disease were increased (**Table 2**). Differences between the genders were also seen when subcategorizing according to degree of relation, gender of the child with cancer, and pediatric cancer diagnosis (**Tables 3–5**). These findings are intriguing, and indicate that there may be gender differences in cancer vulnerability in families with pediatric cancer.

The current data suggest that the risks of cancer in male and female relatives of patients with pediatric cancer differ depending on the characteristics of the child. Male relatives were found to have a higher risk of cancer if the patient with pediatric cancer was female and/or the child had leukemia, while female relatives did not. In a previous study, we showed that children with leukemia in families with more than one case of pediatric cancer were likely to be related to another child with leukemia (69%) and that 77% of all leukemia cases were girls (27). In this larger cohort, we confirm that 56% of patients with leukemia in FAM>1 had a relative with leukemia and that 69% of the patients with leukemia were girls. However, no general increase in cancer risk was found in adult women in these families compared with the general population. The observation that the gender of a child with cancer affects the risk of cancer in relatives has not been described previously, and these results will have to be independently validated.

The diagnostic group of the child did not affect the overall adult cancer risk among relatives. However, there was a clear gender difference in risk between relatives of children with leukemia; women showed no increased risk whereas men did (SIR 0.96 and 1.15, respectively, *P* = 0.01; **Table 5**). Relatives of children with sarcoma

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indicated a similar increased risk for men, while relatives of children with CNS tumors or lymphoma did not (**Table 5**). Families with cases of pediatric leukemia were the only ones to show an increased risk of prostate and lung cancer. Families with cases of pediatric CNS tumors were the only ones to show an increased risk of acute leukemia, breast cancer and pancreatic cancer. Furthermore, families with cases of pediatric lymphoma were the only ones to show an increased risk of ovarian or testicular cancer and CNS tumors. Relatives of children with sarcoma showed unexpectedly the lowest overall cancer risk, currently we cannot explain this observation.

No significant difference was observed in the cancer risk between first-degree relatives of children diagnosed with cancer before or after 5 years of age. Thus, we could not confirm the observations of Goldgar and colleagues or Friedman and colleagues (17, 18). However, first-degree relatives of children diagnosed before 5 years of age did show an increased risk of adult cancer when compared with the general population (**Table 6**), while relatives of children diagnosed later did not. The smaller cohort in our study could explain why we did not observe a significant difference when comparing the two groups.

We have previously observed differences in pediatric cancer characteristics in families with one (FAM1) or more (FAM>1) pediatric cancers (27), and used this here as a variable to study adult cancer in relatives of children with cancer. In the current cohort, 9.8% of the families were FAM>1, which is in accordance with our previous study, where we found a value of 8.8% (27). There was no differences between FAM>1 and FAM1 in the distribution of cancer diagnoses or age at onset for adult relatives (Supplementary Table S1). Adult relatives in FAM>1 showed no increased risk of adult cancer of any type up to the third degree of relation (SIR 1.00), compared with the general population, however, the FAM>1 group of 74 families might be too small to detect significant differences in cancer incidence.

In a parallel study, most of the children in this LCCG cohort were tested for 22 of the most common autosomal dominant germline mutations among pediatric cancers. When families to children with positive germline mutations and those not tested were excluded, an increased cancer risk remained, (SIR 1.07, 95% CI: 1.02–1.11, $P < 0.01$). Thus, known cancer predisposition germline mutations do not explain the increased risk of cancer among adult relatives of children with cancer. Future extended analysis with "Trio-Sequencing," i.e., germline sequencing of parents and child might identify combinational effect of inherited risk variants in the same signalling pathway in children with cancer (37).

In conclusion, relatives of children with cancer have an increased cancer risk when compared with the general population of Sweden, which cannot be explained by currently known cancer predisposition germline variants. Moreover, this risk extends to adults up to the third degree of relation, where mainly male relatives are at risk. The risk in adults was primarily increased for cancer of the GI tract, lungs, and prostate. Men and women showed distinct differences in the cancer types with increased risk. Furthermore, the gender of the child with cancer affected the cancer risk of male relatives, but not female relatives. The study strengthens the importance of surveillance programs for families with pediatric cancer.

Disclosure of Potential Conflicts of Interest

Authors' Contributions

K.-J. Stjernfelt: Data curation, formal analysis, funding acquisition, investigation, visualization, methodology, writing–original draft, project administration. **K. von Stedingk:** Resources, data curation, formal analysis, supervision, investigation, writing–review and editing. **T. Wiebe:** Conceptualization, supervision, validation, writing–review and editing. **L. Hjorth:** Conceptualization, resources, validation, writing–review and editing. **U. Kristoffersson:** Resources, validation, writing–review and editing. **M. Stenmark-Askmalm:** Validation, writing–review and editing. **H. Olsson:** Conceptualization, resources, supervision, funding acquisition, investigation, project administration, writing–review and editing. **I. Øra:** Conceptualization, resources, supervision, funding acquisition, validation, investigation, visualization, methodology, writing–original draft, project administration, writing–review and editing.

Acknowledgments

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Prevalence of germline pathogenic variants in 22 cancer susceptibility genes in Swedish pediatric cancer patients

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Up to 10% of pediatric cancer patients harbor pathogenic germline variants in one or more cancer susceptibility genes. A recent study from the US reported pathogenic variants in 22 out of 60 analyzed autosomal dominant cancer susceptibility genes, implicating 8.5% of pediatric cancer patients. Here we aimed to assess the prevalence of germline pathogenic variants in these 22 genes in a population-based Swedish cohort and to compare the results to those described in other populations. We found pathogenic variants in 10 of the 22 genes covering 3.8% of these patients. The prevalence of *TP53* mutations was significantly lower than described in previous studies, which can largely be attributed to differences in tumor diagnosis distributions across the three cohorts. Matched family history for relatives allowed assessment of familial cancer incidence, however, no significant difference in cancer incidence was found in families of children carrying pathogenic variants compared to those who did not.

Abbreviations
LCCG   Lund childhood cancer genetics
SCCR   Swedish childhood cancer register
NHL   Non-Hodgkin's lymphoma
ALL   Acute lymphoblastic leukemia
AML   Acute myeloid leukemia
ACT   Adrenocortical tumor
AF   Allele frequency
WGS   Whole genome sequencing

Pediatric cancer is linked to a number of inherited disorders including Li-Fraumeni syndrome, retinoblastoma and neurofibromatosis. However, these and other germline predisposition syndromes explain a small proportion of pediatric cancers, currently estimated to account for 10% of cases[1,2].

In 2008 we initiated the Lund Childhood Cancer Genetics (LCCG) study, the aim of which was to prospectively include all pediatric patients diagnosed with cancer in southern Sweden[3,4]. We have reported that approximately 5% of pediatric cancer patients in this population-based cohort have a pediatric relative with the same disease diagnosis within third-degree relations, most often found among patients with leukemia and CNS tumors. Furthermore, we observed a significant female predominance among familial pediatric leukemia and CNS cancer patients in families with more than one pediatric cancer case[4].

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In a study carried out by Zhang et al. in 2015, blood samples were collected from a cohort of 1120 pediatric and young adult cancer patients from the US and examined using WGS and/or exome sequencing. They identified pathogenic or likely pathogenic variants in 8.5% of their cohort[5]. The reported variants were detected in 21 of the 60 autosomal dominant cancer predisposition genes analyzed, the most frequently affected of which was *TP53*. A biallelic pathogenic variant was also found in *ATM*, although this gene was not investigated as an autosomal dominant cancer predisposition gene. Among the patients presenting with germline pathogenic mutations in cancer-associated genes, only 40% had a reported family history of cancer, which is not significantly higher than in those patients with no identifiable germline mutations. In another recent comprehensive analysis of 914 children and young adult cancer patients compiled from various sources, the majority of which were German, Gröbner et al. reported that approximately 6% of patients harbored a cancer predisposing germline variant[6].

In the present study, we performed targeted sequencing of the 22 genes with pathogenic and likely pathogenic variants reported by Zhang et al.[5] in 790 blood samples from the LCCG cohort of pediatric cancer patients. The aim was to estimate the prevalence of germline pathogenic variants in these genes in a population-based Swedish cohort. By doing so, we aim to compare the results to those in the studies by Zhang et al. and Gröbner et al., as well as elucidate potential differences in the prevalence of mutations in these predisposition genes in different populations.

Results

Patient cohort. Our study includes 790 pediatric cancer patients from the LCCG study[7] (referred to as the LCCG cohort). All were under the age of 18 years at diagnosis and the most prevalent cancers are leukemia and CNS tumors (33% and 19%, respectively) (Fig. 1, Table 2). Compared to the distribution of pediatric cancers in the general Swedish population (according to the Swedish Childhood Cancer Registry (SCCR) 2013 Report), the LCCG cohort contains lower proportions of CNS tumors (19% vs. 28%), germ-cell tumors, retinoblastoma and carcinomas, and higher proportions of lymphomas (17% vs. 12%), and bone tumors (Fig. 1, Supplementary Table S1). Greater differences were observed when comparing the distribution of diagnoses in our cohort to the two recent childhood cancer studies published by Zhang et al. in 2015 (referred to as the Zhang cohort) and Gröbner et al. in 2018 (referred to as the Gröbner cohort)[5,6] (Fig. 1, Supplementary Table S1). The Zhang cohort of US patients has a higher proportion of leukemia patients (53%), as well as a higher percentage of adrenocortical tumors (ACT, 3.5%), than in the LCCG cohort[8]. In Sweden, ACT accounts for a mere 1–2% of already rare childhood carcinomas, and the LCCG cohort contains only 1 ACT patient. (These differences will be discussed below in the context of the frequency of *TP53* mutations.) The study by Gröbner et al., which includes samples from multiple centers across Europe and the US, has a large proportion of CNS tumors (58%) and a low percentage of leukemia cases (13.5%). It should also be noted that both the Zhang and the Gröbner cohort contained a small proportion of young adults (Zhang; up 20 years of age, Gröbner; up to 25 years of age), while our LCCG cohort consisted exclusively of patients under 18 years of age at diagnosis.

Target enrichment and sequencing. At least two replicate sequencing libraries were prepared and sequenced for each of the 797 DNA samples (Supplementary Table S2). All samples passed our minimum base quality score requirement of 80% of bases of base quality 30 or higher. However, less than 90% of the assay target region was covered by 30 high-quality aligned reads in seven samples. These seven samples were therefore excluded from further analyses. In the remaining 790 samples, 94.6% of the target region was covered by 30 or more high-quality aligned reads, on average, and the mean sequence coverage was 1741 reads. Only 1.1% of the assay target region had no coverage, on average per sample (Supplementary Table S7).

Spectrum of genetic variation and detected variants. We identified 1429 genetic variants in the 22 targeted genes (Table 1 & Supplementary Tables S8 and S10). Of these, 416 were common variants (allele frequency (AF) in the Genome Aggregation Database (gnomAD ≥ 1%)), 563 were uncommon (AF < 1% and AF ≥ 0.01%), and 450 were rare (AF < 0.01%). Most of the variants in the coding region were missense (372; 72 common, 166 uncommon and 134 rare), but we also identified 9 frameshift deletions, 10 stop-gain variants, and 3 in-frame deletions. Another 53 variants were found within the splice region of an intron (the first 8 bases or the last 17 bases of an intron), and 6 of these affected the two canonical splice donor and acceptor bases adjacent to the exon border. The remaining variants were identified in UTR regions, in introns, or up- or downstream of the target genes (Supplementary Table S9).

Each individual carried, on average, 118 variant alleles in the targeted region, of which 96% were common (AF > 1%) and 2.2% were rare (AF < 0.01%). About one fifth of the individuals (168) carried one or more private variants not found in any other individual in this study or in gnomAD. Averaged over the assay target region covering 111 kilobases, the rate of variation was 1.00 per kilobase for single nucleotide variants (SNVs), and 0.07 per kilobase for non-SNVs, on average, per individual (Supplementary Table S9).

A clear majority of all variants were classified as benign or likely benign (73.8%). Pathogenic and likely pathogenic variants comprised 9 stop-gain variants, 6 frameshift variants, 5 missense variants and 5 variants affecting splicing (Supplementary Figure S3). Of these 25 pathogenic and likely pathogenic variants, 23 were rare variants (AF < 0.0001) and 2 were uncommon (AF AF < 1% and AF ≥ 0.01%) (Supplementary Tables S3 and S10). The two uncommon ones affect SDHA and PMS2-both genes in which pathogenic variants have low to moderate penetrance for their respective associated diagnoses in heterozygous carriers and pathogenic variants can have relatively high population prevalence[9–11]. These 25 variants were detected in a total of 30 patients, indicating that germline pathogenic variants were present in 3.8% of childhood cancers in the LCCG cohort (Supplementary Table S3). The remaining 349 variants (24.4%) were classified as being of uncertain significance (Supplementary Table S8). Among rare variants, 223 were classified as (likely) benign, 204 as of uncertain significance and 23
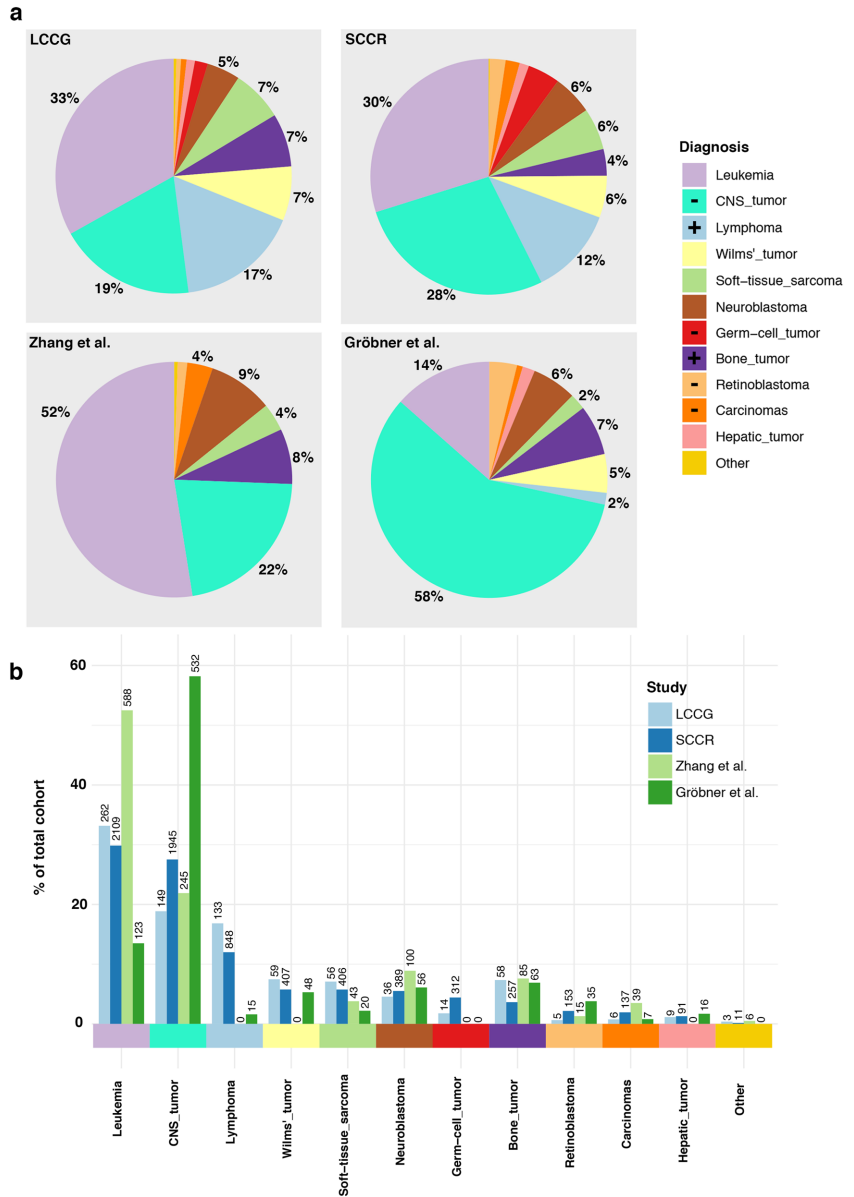
Figure 1. Pediatric cancer diagnosis distributions. (**a**) Distributions of the current LCCG cohort (n = 790; top left), the SCCR cohort (n = 7065; top right), the Zhang cohort (n = 1120; bottom left) and the Gröbner cohort (n = 914; bottom right). + and − indicate significant over- or under-representation of diagnoses in the LCCG cohort compared to the Swedish Childhood Cancer Registry (SCCR) cohort. Diagnosis percentage of each cohort for the largest diagnoses are displayed on each respective pie chart. (**b**) Distribution bar-plot of all cohorts divided according to diagnosis. Number of patients in each cohort diagnosis group is displayed above each bar. Comparative statistics (Fisher's Exact test) are provided in Supplementary Table S1.

ALK	CDH1	NF2	RB1	TP53
APC	KRAS	NRAS	RET	VHL
ATM[A]	MSH2	PALB2	RUNX1	
BRCA1	MSH6	PMS2	SDHA	
BRCA2	NF1	PTCH1	SDHB	

Table 1. List of sequenced autosomal dominant predisposition genes. [A]*ATM* found to be biallelic pathogenic variant by Zhang et al.

as pathogenic or likely pathogenic. The other two pathogenic variants were categorized as uncommon (Supplementary Table S8).

Pathogenic or likely pathogenic variants were detected in 10 of the 22 analyzed genes (Fig. 2, Table 2, Supplementary Table S3). *NF1* showed the highest prevalence (n = 8; 1% of cases); the majority of patients having CNS tumors. *TP53* had the second highest prevalence (n = 6; 0.76%), with patients showing a variety of different tumor types, including soft-tissue sarcomas, osteosarcoma, leukemia, and carcinoma (in this case an ACT). *BRCA2* pathogenic variants were found in 4 patients (0.5%), with diagnoses including CNS tumors, Wilms' tumor and leukemia. Pathogenic variants in *RB1* were detected in 3 patients, followed by *SDHA, BRCA1,* and *PMS2* each with 2 patients, while individual patients exhibited pathogenic or likely pathogenic variants in *APC, PALB2,* and *PTCH1.*

Prevalence of pathogenic variants by tumor type.

Retinoblastoma patients exhibited the highest prevalence of germline pathogenic or likely pathogenic variants; three out of five patients (60%) carrying variants in the disease-associated *RB1* gene (Fig. 2)[12]. Three out of 36 neuroblastoma patients (8%) carried germline pathogenic variants in *NF1, BRCA1,* and *PTCH1*. Germline pathogenic variants in *NF1* have been described previously in neuroblastoma patients[13,14]. Among 56 patients with soft-tissue sarcoma, we found three carrying pathogenic or likely pathogenic variants (5%), all in genes previously linked to this tumor type (*TP53*: 2 patients; *NF1*: 1 patient)[15–17]. Patients with CNS tumors harbored germline pathogenic or likely pathogenic variants in 8 of 149 cases (5%): five in *NF1*, two in *BRCA2* and one in *PMS2*; all genes previously reported in patients with CNS tumors[5,18–20]. Four of 268 patients (1.5%) with leukemia carried pathogenic or likely pathogenic variants in *TP53, BRCA2, PALB2,* and *PMS2*. Only *TP53* is associated with susceptibility to leukemia[21], although pathogenic variants in *BRCA2* and *PALB2* have been reported previously in leukemia patients. Two of the 59 patients (3%) with Wilms' tumor carried pathogenic variants in *BRCA2* and *SDHA*.

Two patients carrying pathogenic variants in *TP53* were found among 58 patients (3.4%) with bone tumors, which are associated with Li-Fraumeni syndrome[5,22]. Single patients with pathogenic variants in *TP53* and a likely pathogenic variant in *SDHA* were found among the seven carcinomas and nine hepatic tumors, respectively. The *TP53* variant was found in an ACT case, a tumor type associated with germline *TP53* pathogenic variants[5,17,20].

Comparison with previous studies.

In order to more accurately compare cohorts, we examined only variants that were screened for in all three studies. As our screening methods do not detect copy number variations (CNVs), CNV variants from the Zhang et al. and Gröbner et al. cohorts were excluded. We found a lower overall prevalence of pathogenic and likely pathogenic variants in the 22 screened genes in our LCCG cohort than in the US-based study by Zhang et al. (OR 2.2, FDR-adjusted *p*-value = 0.001) and in the multi-center study by Gröbner et al. (OR = 1.8, FDR-adjusted *p*-value = 0.028) (Supplementary Table S5). No significant difference was detected between the Zhang and Gröbner cohorts.

As shown above, the distribution of diagnoses differs substantially between the cohorts and this could influence both the distribution and prevalence of pathogenic variants in the analyzed genes. Examining the overall prevalence of pathogenic variants within each diagnosis subgroup we find no significant differences across the three cohorts (Fig. 3, Supplementary Table S6).

On an individual gene basis, the only difference between the LCCG cohort and the Zhang and Gröbner cohorts was the prevalence of *TP53* mutations, although this difference with the Gröbner cohort was not significant after FDR-adjustment of the *p*-values (LCCG cohort vs. Zhang cohort: OR = 5.8, FDR-adjusted *p*-value < 0.001; LCCG cohort vs. Gröbner cohort: OR = 3.5, FDR-adjusted *p*-value = 0.154). Again, no significant differences were found between the Zhang and Gröbner cohorts for any of the genes (Supplementary Table S5). Exclusion of *TP53* from the comparisons removed any statistical differences in aggregate prevalence for all the genes between the studies. It should be noted that the number of carriers of mutations in all genes other than *TP53* were low, and a much larger cohort size would be required to identify any true underlying differences in prevalence between the populations.

While the lower prevalence of *TP53* mutations in our study could be attributed to a true lower population burden, it could also be due to differences in mutation classification across studies. To determine whether such differences in criteria for classification of variant pathogenicity contributed to the observed differences in prevalence between the LCCG cohort and the Zhang cohort, we re-classified all pathogenic *TP53* mutations reported by Zhang et al. (information required for re-classification was not available for the Gröbner cohort). Six of the 22 missense variants reported by Zhang et al. as pathogenic were classified as being of uncertain significance according to our criteria, reducing the number of *TP53* carriers from 48 to 42. However, this only explained a
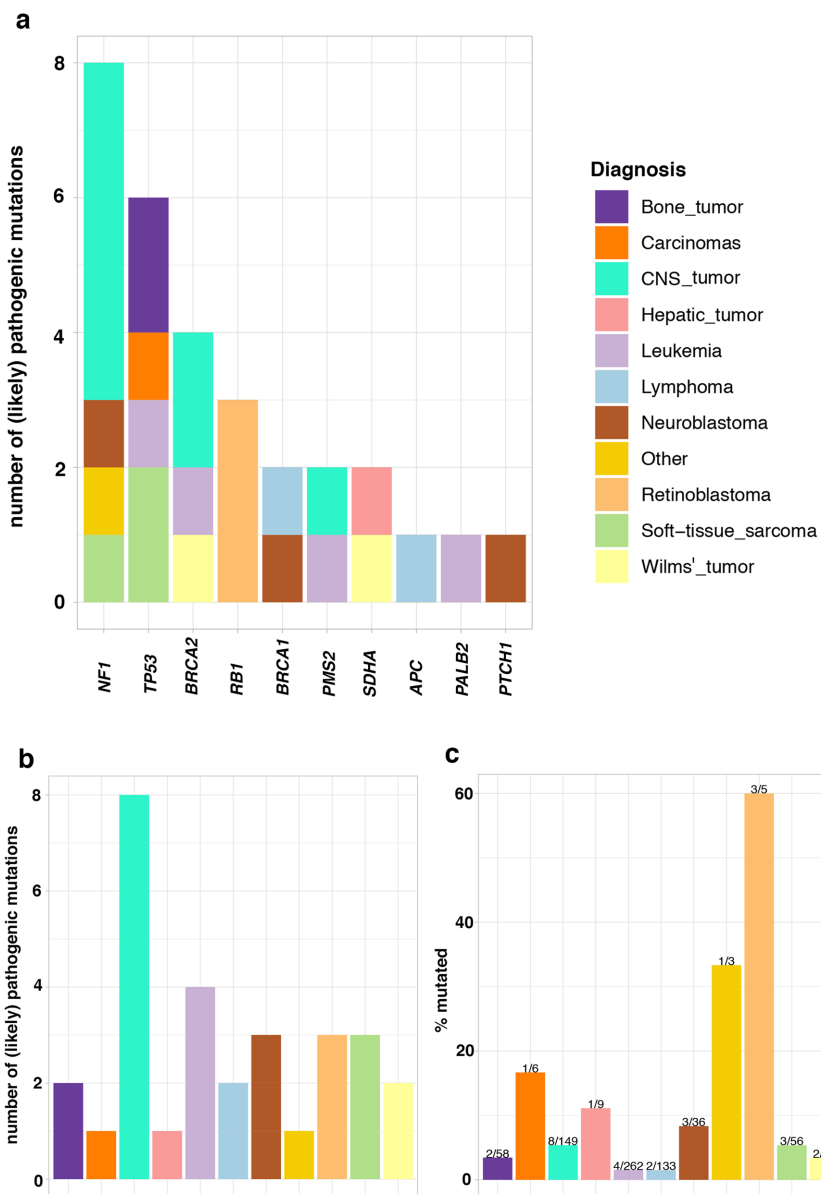
Figure 2. Distribution of germline pathogenic and likely pathogenic variants in patients with different pediatric diagnoses in the LCCG cohort. (**a**) Number of patients with (likely) pathogenic variants per gene. Colors indicate the diagnosis group of each patient in which the variant was detected. (**b**) Total number of patients carrying (likely) pathogenic variants per cancer diagnosis group for all genes summed. (**c**) Percentage of patients with (likely) pathogenic variant per cancer diagnosis group for all genes summed. The number of patients carrying (likely) pathogenic variants and the total number of patients in each diagnosis group is shown above the bars.

Group #	Diagnosis	nᴬ	Diagnosis Subgroup	nᴮ	nMutᶜ	Germline Mutations
1	Leukemia	262	ALL	219	2	*BRCA2, TP53*
			AML	37	2	*PMS2, PALB2*
			Other	6	0	na
2	CNS_tumor	149	Astrocytoma	61	4	*2 × BRCA2, PMS2, NF1*
			Ependyoma	14	0	na
			Medulloblastoma	25	0	na
			Ganglioma	6	0	na
			Craniopharyngioma	6	0	na
			Opticusglioma	9	3	*3 × NF1*
			Other_Brain_tumor	28	1	*NF1*
3 & 9	Lymphoma	133	NHL	56	2	*APC, BRCA1*
			Hodgkin's_lymphoma	58	0	na
			Histiocytosis	19	0	na
4	Wilms'_tumor	59	Wilms'_tumor	59	2	*BRCA2, SDHA*
5	Soft-tissue_Sarcoma	56	Non-rhabdomyosarcoma	16	1	TP53
			Rhabdomyosarcoma_Embryonal	30	1	TP53
			Rhabdomyosarcoma_Alveolar	7	0	na
			Rhabdomyosarcoma_Unspecified	3	1	NF1
6	Neuroblastoma	36	Neuroblastoma	36	3	*BRCA1, PTCH1, NF1*
7	Germ-cell_tumor	14	Germ-cell_tumor	14	0	na
8	Bone_tumor	58	Osteosarcoma	22	2	*2 × TP53*
			Ewing_sarcoma	36	0	na
10	Retinoblastoma	5	Retinoblastoma	5	3	*3 × RB1*
11	Carcinoma	6	ACT	1	1	TP53
			Other Carcinoma	5	0	*na*
12	Hepatic_tumor	9	Hepatoblastoma	9	1	*SDHA*
13	Other	3	Other	3	1	*NF1*

Table 2. Diagnosis distribution of LCCG cohort including subgroups with corresponding germline mutations. *ALL* acute lymphoblastic leukemia, *AML* acute myeloid leukemia, *NHL* non-Hodgkin's lymphoma. ᴬNumber of patients within each main diagnosis group. ᴮNumber of patients in each diagnosis subgroup. ᶜNumber of (likely) pathogenic mutations.

small proportion of the difference, and a significant difference in the prevalence of the *TP53* mutation remained between our cohort and that of Zhang et al. ($P < 0.0001$, OR = 5.08).

Both Zhang et al. and Gröbner et al. observed the highest prevalence of *TP53* mutations (69% and 50%, respectively) in ACTs, which accounted for 3.5% (n = 39) and 0.9% (n = 8) of their cohorts, respectively. Only one ACT was found in our cohort. Excluding ACTs from the Zhang et al. and Gröbner et al. cohorts removed the difference in the prevalence of *TP53* mutations in both studies after FDR-adjustment of the *p*-values (Supplementary Table S5).

Family history of cancer. In our total cohort of 790 patients, data on family history of cancer were available for 86% of the patients (n = 676/790). Overall, 28% (n = 190/676) of patients had a first-degree relative with a cancer diagnosis, and 83% (n = 560/676) had a cancer diagnosis in the family up to the second-degree of relation. We further divided the cohort into those with and without mutations in the examined cancer susceptibility genes. In patients with detected mutations, family history data were available for 80% (n = 24/30), of which 46% (n = 11) had a family history of cancer within first-degree relatives, and 96% (n = 23/24) within second-degree relatives. In patients without detected cancer susceptibility gene mutations, family history data were available for 86% (n = 652/760), of which only 27% (n = 179/652) had a first-degree relative with cancer and 82% (n = 537/652) within the second-degree. Neither the observed differences in first-degree relatives with cancer diagnoses nor second-degree relatives were significantly higher in patients with a detected mutation (Fisher's exact $P = 0.06$, OR = 2.23, and $P = 0.10$, OR = 4.92, respectively). This observation is also in line with the findings of Zhang et al. who reported no difference. It is notable that in the US study, Zhang et al. found a family history of cancer within the first-degree in 42% of patients without germline mutations, which is higher than the 27% observed in our cohort ($P = 0.054$, OR = 0.53). No significant difference was observed in the prevalence of germline mutations between genders (Fisher's exact $P = 0.71$, OR = 0.86).
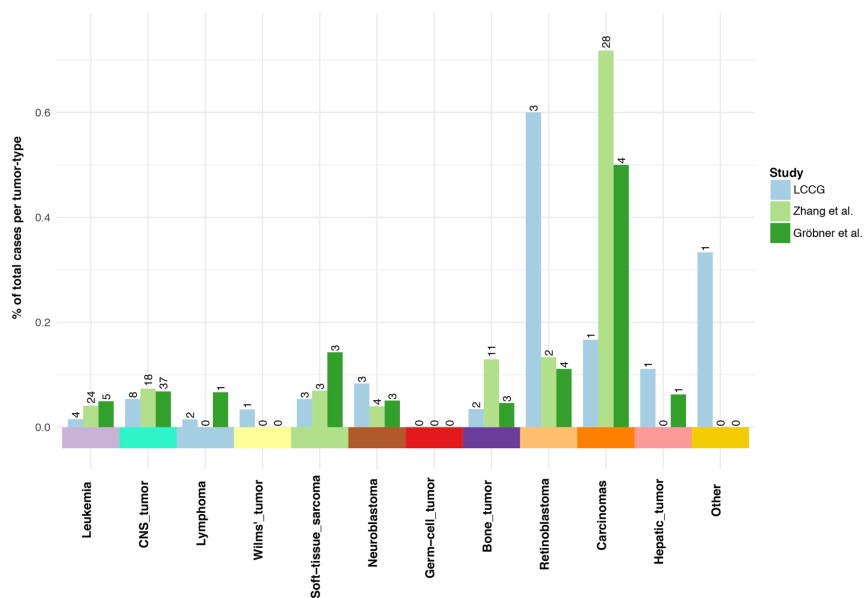
Figure 3. Mutation prevalence per tumor-type. Distribution bar-plot of LCCG, Zhang et al. and Gröbner et al. cohorts divided according to diagnosis. Number of (likely) pathogenic variants in each cohort diagnosis group is displayed above each bar. Comparative statistics are provided in Supplementary Table S6.

Discussion

We have performed targeted DNA sequencing of 22 previously described autosomal dominant cancer predisposition genes[5] in blood samples collected from 790 pediatric cancer patients diagnosed in southern Sweden. We found that 3.8% of patients in this cohort harbored germline pathogenic or likely pathogenic variants in one of the 22 cancer predisposition genes examined. This is lower than that reported in two recent studies of pediatric and young adult cancer patients, where pathogenic or likely pathogenic variants in these 22 genes were found in 6.7% (Zhang cohort) and 8.0% (Gröbner cohort) of cases (excluding 5 copy number variants because this type of variant is not detectable with our assay)[5,6]. On an individual gene basis, the only significant difference between the three cohorts was the prevalence of *TP53* mutations, and removing this gene from the comparison removed the significant difference in the aggregate prevalence of pathogenic variants between the cohorts. Both the Gröbner and the Zhang cohorts had a substantially higher proportion of ACTs than our cohort, which in both cases was associated with the highest rates of *TP53* mutations, ranging from 50 to 69%, respectively. Zhang et al. acknowledged the fact that their cohort included a greater-than-expected proportion of patients with ACTs and hypodiploid acute lymphoblastic leukemia. When these were excluded, the germline mutation rate was 5.6%, which is comparable to that in the study by Gröbner et al. Our cohort contained only one case of ACT, which, as may be expected, did indeed harbor a germline *TP53* mutation. Adjusting for discrepancies in ACT patients across all studies showed that it was a significant contributing factor to the discrepancy in *TP53* mutations across the three studies.

The comparison between our cohort and that of Zhang et al. is inherently biased because we chose to screen only the 22 genes in which Zhang et al. had found pathogenic variants, causing a regression towards the mean type of bias. The comparison is also biased if we only consider the prevalence of variants in these 22 genes, aggregate or individually, although the relative effect will be smaller for genes with a higher prevalence of pathogenic variants. Comparisons of our cohort with that of Gröbner et al. do not suffer from this bias and similar results were obtained.

The purpose of our study was to estimate the prevalence of germline pathogenic variants in 22 cancer susceptibility genes, previously described by Zhang et al., in Swedish pediatric cancer patients and to obtain insights into the contribution of genetic predisposition to childhood cancer. It is highly likely that there are indeed germline mutations in other genes not analyzed in this study, as well as epigenetic alterations underlying the different pediatric cancers, and that the percentage of familial pediatric tumors is higher than observed here. Considering that we were able to identify a prevalence of germline mutations among pediatric cancer patients that is comparable to those described in recent broader screening studies[5,6] in this limited analysis of 22 genes, suggests that these 22 genes harbor a substantial fraction of germline mutations in cancer susceptibility genes carried by pediatric cancer patients.

We found that the most commonly affected genes were *NF1*, *TP53*, the majority of which are seen in cancers associated with the predisposition syndromes neurofibromatosis and Li-Fraumeni syndrome, respectively. These cancers include CNS tumors such as optic glioma and astrocytoma resulting from *NF1* mutations, and osteosarcoma, soft-tissue sarcoma and ACT resulting from *TP53* mutations. We also found mutations in genes with no reported association to the diagnosis of the patient, such as *BRCA1* and *PTCH1* mutations in neuroblastoma, as well as *PMS2* mutations in leukemia. Incidental findings such as these are not uncommon when screening multiple cancer susceptibility genes and do not imply causation. Observed frequencies of these mutations are not inconsistent with those in the general population. For example, the frequency of *BRCA1* pathogenic variants in healthy non-Finnish European controls in gnomAD is 0.38% (81 of 21,384) compared to 0.25% in our cohort[23].

We did not observe any significantly higher incidence of cancer among relatives of patients with germline mutations in cancer predisposition genes. This is in line with the findings reported by Zhang et al. While no significant association was found in either study, a numerical difference was found in our study when comparing cancer diagnoses among relatives of patients with and without germline mutations: 28% vs. 42%. This may suggest that a trend may emerge in investigations on a larger number of patients and/or broader genetic analyses including more variants.

In addition to identifying germline mutations in the tumor-bearing patients, a study by Kuhlen et al. highlighted the importance of assessing the presence of heterozygous mutations in the parents affecting the germline of the children, a procedure they termed 'trio sequencing'[20,24]. This may help to identify mutations that could be candidates for familial surveillance with the aim of early detection and treatment. Implementation of surveillance has resulted in increased long-term survival of cancer patients from families with predisposition syndromes[25]. We currently have parent blood samples from a substantial number of patients presenting with germline mutations in our cohort, and trio sequencing studies are being planned together with larger-scale whole-genome sequencing approaches to examine genetic events that could have been overlooked in the highly focused analysis in the current study.

Materials and methods

Patients. The patients included in this study have been described previously[4]. In brief, the LCCG study enrolls pediatric cancer patients that are diagnosed and treated at the Skåne University Hospital in Sweden, including cancer survivors that are seen at the Late Effects Clinic. Patients are eligible for inclusion if diagnosed before the age of 18 years. The Swedish National Population Register was used to identify all relatives of patients up to the third-degree of relation. The Swedish Cancer Register was used to identify any cancer diagnoses of all relatives within the families of the patients up to the third-degree of relation.

Sequencing and variant classification. Sequencing libraries were prepared from germline DNA extracted from 790 blood samples from the childhood cancer patients using the Fluidigm Juno technique. The assayed genes included the 21 autosomal dominant cancer predisposition genes for which pathogenic- or likely pathogenic variants were detected by Zhang et al.[5], plus *ATM* (Table 1).

At least two libraries were prepared from all samples to maximize the sensitivity. Libraries were sequenced on an Illumina HiSeq 2500 system. The pathogenicity of the identified variants was determined according to ACMG-AMP (American College of Medical Genetics and Genomics—American College of Pathology) guidelines or ClinGen-approved gene-specific expert panel criteria, if available, in consultation with experts in clinical genetics and oncology at Lund University and the University of Amsterdam. Identified pathogenic variants were confirmed with Sanger sequencing and cross-referenced with patient clinical data and family history to identify associations with specific diagnoses as well as potential associations with increased familial cancer incidence. A detailed description of the sequencing and classification procedures is provided in Supplementary Methods, and the bioinformatic workflow is depicted in Supplementary Figure S1.

Statistical analyses. Statistical comparisons were carried out using R statistical language (Version 3.3.1). The prevalence of diagnoses and of detected pathogenic variants in the sequenced genes were compared between cohorts using Fisher's exact test and FDR-adjustments were applied to Fisher exact test p-values using p.adjust function from the stats (v3.1.1) R package with BH method[26]. For gene mutation prevalence comparisons, total cohort comparisons (including total cohort comparisons after removing TP53 mutations) were considered as one group of test for p-value adjustments, while all other individual gene test were considered a separate group of test. This also applies to mutation prevalence across different diagnoses, where total cohorts were considered one group and individual diagnoses analyses were considered a second group of tests. FDR-adjusted p-values (or p-values where applicable) < 0.05 were considered significant.

Ethical approval. The study was approved by the Regional Ethics Review Board, Lund University, Sweden (no. 2008/233, 2010/231 and 2011/33). Access to the Population. Registry and Cancer Registry was approved for participants and parents. Written informed consent was received from patients and/or legal guardians prior to inclusion in this study and all research was performed in accordance with relevant guidelines/regulations.

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Author contributions

I.Ø., H.O., K.J.S., U.K., M.S.A., T.W. and L.H. were involved in data collection. K.V.S., A.K., C.W. and J.K. were involved in sequencing, data analysis and interpretation. K.V.S., K.J.S., A.K. and I.Ø. were involved in the writing of the manuscript. K.V.S., K.J.S., U.K., M.S.A., T.W., L.H., H.O. and I.Ø. were involved in the study design.

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Competing interests

The authors declare no competing interests.

Additional information

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