Genetic factors in childhood cancer. Associations between tumors in childhood and adulthood, and prevalence of germline TP53 mutations

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2012

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Associations between tumors in childhood and adulthood, and prevalence of germline *TP53* mutations

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2012

ISBN 978-91-86871-86-4

ISSN 1652-8220
Lund University, Faculty of Medicine Doctoral Dissertation Series 2012:24

Cover illustration and illustrations in Figures 2 and 4 by Emma Larsson
Printed in Sweden by Media-Tryck, Lund University
Lund 2012

To my family

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Included papers

This thesis is based on the following papers:

I. H. Olsson, **S. Magnusson,** A. Bladström. Lower breast cancer survival in mothers of children with a malignancy: a national study. British Journal of Cancer, 2008; 98(11): 1876-1878

II. **S. Magnusson,** Å. Borg, U. Kristoffersson, M. Nilbert T. Wiebe, H. Olsson. Higher occurrence of childhood cancer in families with germline mutations in *BRCA2*, MMR and *CDKN2A* genes. Familial Cancer, 2008; 7: 331-337

III. **S. Magnusson,** T. Wiebe, U. Kristoffersson, H. Jernström, H. Olsson. Increased incidence of childhood, prostate and breast cancer in relatives to childhood cancer patients. Familial Cancer, 2011 (*Accepted*)

IV. **S. Magnusson,** D. Gisselsson-Nord, T. Wiebe, U. Kristoffersson, H. Olsson. Prevalence of germline *TP53* mutations and history of Li-Fraumeni syndrome in families with childhood adrenocortical tumors, choroid plexus tumors and rhabdomyosarcoma – a population-based survey over 50 years. (*In revision*)

Abbreviations

ACT	Adrenocortical tumors
ALK	Anaplastic lymphoma kinase
BRCA1	Breast cancer associated gene 1
BRCA2	Breast cancer associated gene 2
CDKN2A	Cyclin-dependent kinase inhibitor 2A
cDNA	Complementary DNA
CI	Confidence interval
CPT	Choroid plexus tumor
ddNTP	Dideoxy nucleoside triphosphates
DNA	Deoxyribonucleic acid
FANCD1	Fanconi anemia, complementation group D1
HBOC	Hereditary breast and ovarian cancer
HER-2	Human epidermal growth factor receptor 2
HNPCC	Hereditary non-polyposis colorectal cancer
IARC	International Agency for Research on Cancer
ICD	International classification of disease
IGF	Insulin-like growth factor
IGF-1	Insulin-like growth factor-1
LCCG-study	Lund childhood cancer genetic-study
LFL	Li-Fraumeni-like syndrome
LFS	Li-Fraumeni syndrome
MLH1	Human MutL homologues 1
MLPA	Multiplex ligation-dependent probe amplification
MMR	Mismatch repair
mRNA	Messenger ribonucleic acid
MSH2/3/6	Human MutS homologues 2/3/6
MSI	Microsatellite instability
NF1	Neurofibromin

OR	Odds ratio
PCR	Polymerase chain reaction
PMS1/2	Human post meiotic segregation homolog 1/2
p53	Tumor protein 53
RB1	Retinoblastoma gene
RMS	Rhabdomyosarcoma
SIR	Standard incidence ratio
TP53	Tumor protein 53 gene
WAGR-syndrome	Wilms' tumor aniridia genitourinary anomalies mental retardation syndrome

Abstract

The etiology of childhood cancer is largely unknown. Approximately 1-10% of all childhood tumors are associated with known cancer predisposition syndromes. However, the contribution may be underestimated due to the failure to detect patients with genetic susceptibility for cancer when relying on known family pattern and anomalies. Growing evidence indicates that patients with genetic susceptibility to cancer may be at higher than normal risk for therapy related cancers. Increased knowledge regarding the importance of hereditary factors in the development of childhood tumors may improve the medical care of such patients by identifying those in need of more individualized treatment. In this thesis, genetic factors, familial cancers, and their associations with childhood cancer have been studied. The general aim was to investigate the importance of hereditary factors in the etiology of childhood cancer and to evaluate possible associations between childhood and adult cancers.

In paper I, a national registry-based cohort of parous women with breast cancer was used to study whether the occurrence of childhood cancer in children affects the survival of mothers with breast cancer. Women who had a child with childhood cancer were found to have shorter survival compared to other parous patients with breast cancer, suggesting that hereditary factors may affect prognosis.

In paper II, the occurrence of childhood cancer in families with hereditary adult cancer syndromes was investigated. Families with *BRCA2*-associated hereditary breast and ovarian cancer, mismatch repair gene-associated hereditary non-polyposis colorectal cancer and *CDKN2A*-associated malignant melanoma were found to have a higher occurrence of childhood cancer compared to the general population. No increased occurrence of childhood cancer was found in families with *BRCA1*-associated breast and ovarian cancers.

In paper III, the incidence of childhood and adult cancer was evaluated in the extended families of patients with childhood cancer, and the frequency of germline *TP53* mutations in families with multiple childhood tumors was investigated. The relatives of patients with childhood cancer were found to have an increased incidence of childhood and adult cancers, particularly of the breast and prostate. Breast and prostate cancers were observed at earlier than average ages. No germline *TP53* mutations were found in families with multiple childhood tumors, which exclude *TP53* mutations as a contributor to the observed excess of childhood tumors.

In paper IV, a population-based material was used to confirm the prevalence of germline *TP53* mutations in children with adrenocortical tumors, choroid plexus tumors and early childhood rhabdomyosarcomas and investigate whether these may be early manifestations of Li-Fraumeni syndrome (LFS). Germline *TP53* mutations were found in few children with adrenocortical tumors and rhabdomyosarcomas. No mutations were found in children with choroid plexus tumors. Furthermore, neither the family history nor the observed tumor spectra in the relatives of most children with these rare tumors were suggestive of LFS. These data suggest that most children, particularly those with choroid plexus tumors or rhabdomyosarcomas, do not present early manifestations of LFS. Nevertheless, an increased cancer incidence, particularly for certain adult tumors, was found in the relatives of children with choroid plexus tumors and rhabdomyosarcomas, which suggests that other syndromes or predisposing factors may exist.

In summary, this thesis adds new data suggesting that hereditary factors play a role in the development of childhood tumors. In addition, these factors may also increase the risk for adult tumors, modify the onset age of common adult tumors, and affect breast cancer prognosis. Our findings further support the need for future studies regarding the importance of genetic susceptibility to childhood cancer, particularly in families with multiple childhood tumors. Also the associations between tumors of childhood and adulthood in the same family should be further studied.

Introduction

In Sweden, approximately 300 children and adolescents are diagnosed with cancer yearly.[1] Although, three of four patients will survive their disease, cancer is the second leading cause of death in children.[2] The survival rates have improved remarkably over the past decades due to highly specific diagnostic procedures and the introduction and continuous improvement of multimodal and risk adapted treatment strategies.[3-5] However, in recent years, such advances seem to have reached a plateau. Approximately 1 in 700 individuals ranging from 25-35 years of age is a childhood cancer survivor, resulting in a total number of 6 000-7 000 survivors in Sweden today.[6] A large proportion of these will be affected by late complications associated with their disease and treatment including an increased risk for second primary tumors.[7]

The etiology of childhood cancer is largely unknown. Although, most childhood cancers are thought to be sporadic or multifactorial, genetic susceptibility has been estimated to account for up to 10% of all cases.[8] However, the genetic contribution may be underestimated due to under-recognition when relying on known familial patterns and anomalies or the under-reporting of family history.[9,10] Family cancer history is dynamic, and this is important to consider when evaluating the genetic contribution, particularly in young children. Although tumors appear to be sporadic at the time of diagnosis, they may become recognized as familial when parents and siblings grow older.

Increased knowledge regarding the importance of hereditary factors in the development of childhood tumors is needed. Family history of cancer has been associated with increased risk for second primary tumors, particularly in cancer patients with young age of onset, which may suggest underlying genetic susceptibility to cancer.[11,12] Growing evidence indicates that patients with genetic predisposition to cancer may be at higher than normal risk for therapy related cancers.[13] Identifying patients with childhood cancer and survivors with underlying genetic susceptibility has multiple clinical benefits including potential cancer treatment modification, increased surveillance for subsequent malignancies, and the identification of at risk-relatives.[13,14] Accurate risk assessment and the implementation of an appropriate surveillance program may allow earlier diagnosis and treatment of cancers resulting in reduced morbidity and mortality and optimizing the chance of finding a cure.[15-17] A possible association between childhood and adult tumors should therefore be

investigated to improve the care of children with cancer and their families. Furthermore, family history and hereditary genetic factors are known cancer risk factors, but current knowledge of their role as prognostic factors for manifested tumors is limited. Increased knowledge regarding whether hereditary factors are of prognostic significance is needed to improve prognosis prediction, which may have the potential to guide treatment decisions.

In this thesis, genetic factors, familial cancers, and their associations with childhood cancer have been studied to investigate the importance of hereditary factors in the etiology of childhood cancer and to evaluate possible associations between childhood and adult tumors.

Background

Tumor development

Tumor development is a multistep process that is characterized by the accumulation of genetic alterations, clonal selection, and expansion. In this process, normal cells progressively transform into cancer cells through the acquisition of some essential properties that enable tumor growth and metastatic dissemination. These common traits, designated by Hanahan and Weinberg as the six hallmarks of cancer, include (1) self-sufficiency in growth signals, (2) insensitivity to anti-growth signals, (3) ability to evade apoptosis, (4) limitless replicative potential, (5) sustained angiogenesis, and (6) tissue invasion and metastasis capability.[18] Recently, two enabling hallmarks, including genomic instability and tumor promoting inflammation, were suggested to be crucial for the acquisition of the six hallmarks.[19]

The genetic alterations accumulated during tumorigenesis include mutations of single base pairs in the DNA sequence such as substitutions, deletions, duplications or rearrangements. The alterations may also involve larger chromosomal regions including copy number variations with gains and losses or larger rearrangements such as translocations and inversions. The genetic alterations can be inherited as germline mutations, but may also result from spontaneous mutations during cell division or may be induced by exposure to carcinogens or ionizing radiation. Genetic alterations may affect genes involved in growth regulation, such as tumor suppressor genes and proto-oncogenes, or genes involved in DNA repair.[20] Tumor suppressor genes normally function as negative regulators of cell proliferation due to regulation of cell cycle progression, DNA-repair and apoptosis. Proto-oncogenes are involved in regulation of cell growth and cell proliferation by providing positive growth signals. Inactivation of tumor suppressor genes or activation of oncogenes (the altered form of the normal proto-oncogene) may result in deregulation of cell proliferation and apoptosis, and may promote tumor development. In contrast, DNA stability genes are not immediately involved in the regulation of cell proliferation. Instead, their encoded proteins are implicated in maintaining genomic stability by regulating and maintaining DNA repair pathways. However, impaired function of DNA stability genes contributes to higher mutation rate and genomic instability, enabling tumor development.

In most hereditary cancer syndromes, inherited germline mutations in one allele of a tumor suppressor gene or DNA-repair gene predispose to cancer.[20] As long as there is one intact allele, the gene product may be sufficient to perform normal function. However, the subsequent inactivation of the second allele, according to Knudson's "two-hit" hypothesis may result in the initiation of tumor development.[21] Knudson's "two-hit" hypothesis was initially proposed in studies of retinoblastoma[22] but was later shown to be applicable to other hereditary cancers. Although some pediatric tumors, such as retinoblastoma, may be exceptional in requiring so few mutational events, more than two mutational events are required for the development of most adult tumors.[23]

Childhood cancer

Incidence and tumor patterns

In Sweden, the annual tumor incidence in children under 15 years is estimated to be 16.3/100 000 children. Generally, boys of any age are found to be more affected by childhood cancer than girls.[1]

Childhood cancer is not one disease entity but rather a spectrum of different malignancies. Whereas most adult tumors are carcinomas, childhood and adolescent tumors are more histologically diverse. Based on morphology, they are classified into 12 main diagnostic subgroups (Figure 1).[24] The largest groups are leukemia, tumors of the central nervous system, and lymphomas, which account for roughly two-thirds of all childhood tumors. Of the remaining diagnoses, embryonal tumors (i.e., neuroblastomas, retinoblastomas, nephroblastomas, embryonal rhabdomyosarcomas, and germ cell tumors) and other sarcomas constitutes the major tumors and account for more than one-fourth of all malignancies in childhood while carcinomas are rare.[1]

Cancer incidence and tumor patterns differ by age with the highest incidence found among young children.[25] During infancy, there is a predominance of embryonal tumors, while leukemia together with embryonal tumors and non-Hodgkin's lymphoma predominate in early childhood. In adolescence, there is an increase in lymphomas, bone and soft tissue sarcomas, gonadal germ cell tumors, and various carcinomas including thyroid and malignant melanoma. Tumors of the central nervous system are uniformly distributed during childhood and adolescence.[1,25]

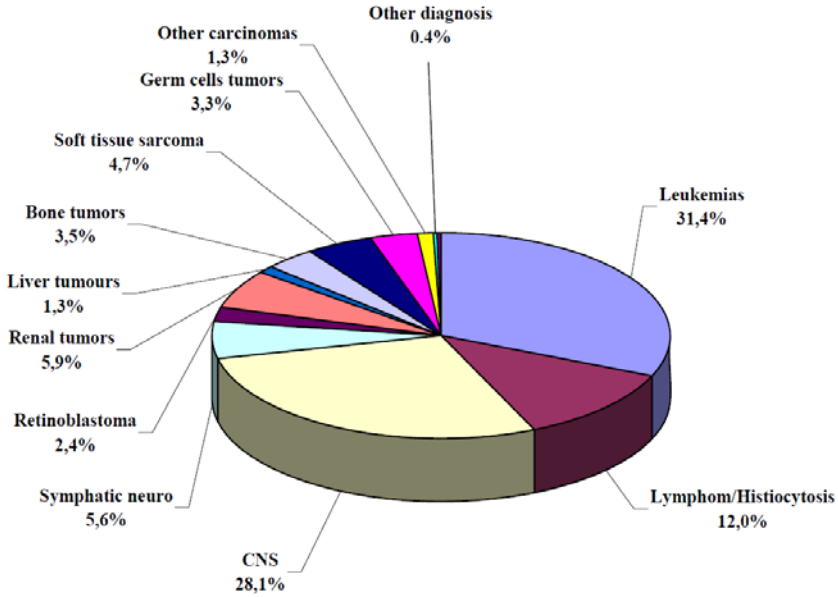

Figure 1. Distribution of childhood malignancies in Sweden diagnosed <15 years of age. Reprinted with permission from Göran Gustafsson; Childhood Cancer Incidence and Survival in Sweden 1984-2005, Report 2007 from the Swedish Childhood Cancer Registry.[1]

Etiology and risk factors

The etiology of childhood tumors remains unclear. Genetic susceptibility and ionizing radiation are well known risk factors, but are thought to account for only 5-10% of all tumors in children.[8,26,27]

Considering the window of carcinogenesis, childhood and adult tumors may be associated with different risk factors (Figure 2). While cancers in adults result from a multistep process and often progress over many years or decades, childhood tumors generally have a much shorter carcinogenic process. This may indicate that childhood tumors may require fewer events to progress and that the mechanisms underlying their initiation may be different.[28] Compared to adult tumors, postnatal environmental factors seem to have a minor etiological role considering the short latency period between possible exposures to clinical disease onset.[29] Instead, prenatal factors including genetic factors and exogenous exposure *in utero* are thought to play a more significant role, particularly for cancers that occur in young children.[30,31] Many cancers are thought to result from genetic aberrations early in the developmental process, which may reflect the histological appearance of embryonic tumors, which resemble that of tissue in the developing embryo and fetus.[28] These genetic

aberrations could be inherited or occur *de novo* in germline and thus be constitutional. However, most genetic aberrations are acquired and arise in somatic cells during cell division or by exposure to exogenous factors. In contrast to genetic aberrations that occur in germline, these cannot be passed on to the next generation.

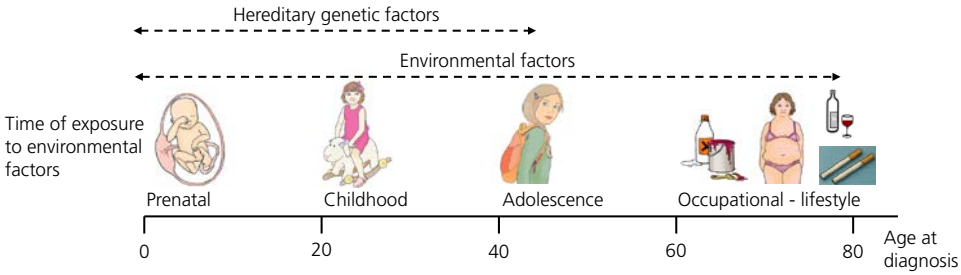

Figure 2. Environmental and hereditary genetic factors and the role of temporal association of exposure in relation to age at diagnosis. The cause of cancer in childhood is associated with inheritance of genetic susceptibility to cancer or to exposure to environmental factors early in life, probably mainly *in utero*. Tumors with late onset in life show an association with occupational life and lifestyle. Adapted from Håkan Olsson, 1996, Tumörsjukdomar, Studentlitteratur, Lund.

Environmental exposures

Only few environmental exposures are known risk factors for childhood cancer. *In utero* exposure to ionizing radiation and the formerly used diethylstilbestrol (a synthetic non-steroidal estrogen) are demonstrated to increase the risk for leukemia and clear-cell adenocarcinoma of the vagina, respectively.[32-34] In addition, therapeutic irradiation and infections by Epstein-Barr virus have been associated with increased risk for bone cancer and lymphoma, respectively.[35,36] Several other environmental exposures such as infections, electromagnetic fields, pesticides, *in utero* exposure to endocrine disruptors, parental occupational exposures, paternal smoking and, maternal consumption of cured meat and food containing DNA topoisomerase inhibitors have been suggested as risk factors. However, so far results have been inconsistent or limited.[26,37-40]

Birth associated characteristics

Birth characteristics such as increased birth weight and accelerated fetal growth have been associated with an increased risk for a variety of childhood tumors.[41-45] Insulin-like growth factors (IGFs) are important regulators of fetal growth, and imbalances in the IGF system have been suggested as a plausible mechanism behind these observations; however, the mechanisms are unclear.[46,47] Other factors such as advanced parental age and birth order have also been associated with an increased risk for childhood cancer, but the results are inconsistent.[48-51]

Genetic conditions and congenital anomalies

Genetic conditions are established risk factors for childhood cancer. Several genetic conditions are known to predispose for childhood cancer including hereditary syndromes and congenital disorders such as Down's and Beckwith-Wiedemann syndromes.[17] Some of the most well characterized hereditary syndromes involving childhood tumors include retinoblastoma, neurofibromatosis type 1, and Li-Fraumeni syndrome (LFS).[52]

Congenital anomalies have also been associated with childhood cancer. Several studies have reported an excess of congenital anomalies, such as those of the ribs and nervous and urinary systems, in patients with childhood cancer.[53-56] In addition, after excluding patients with known genetic conditions that are associated with congenital anomalies, associations between congenital anomalies and childhood cancer seemed to exist.[53,55] These observations suggests that constitutional genetic defects, possibly in genes involved in embryogenesis, may play an important role in childhood tumor development.[57]

Genetic predisposition and familial cancer risk

The proportion of patients with a clear genetic predisposition to cancer is small, accounting for up to 10% of all childhood cancers.[8,52] The fraction of childhood cases due to genetic predisposition varies widely between tumor types. The highest fractions are found in adrenocortical carcinoma (50-80%), optic gliomas (45%), and retinoblastoma (40%), while many other tumors fall in the range of 1-10%.[8,58-60] Children genetically predisposed to tumors, such as retinoblastoma, are more often afflicted with bilateral tumors and diagnosed at a younger age compared to children with sporadic disease.[17]

Mechanisms of genetic predisposition to childhood cancer

Genetic predisposition implies a genetic alteration that has been inherited from a parent or has occurred *de novo* in the gametocytes before fertilization. Different mechanisms may give rise to a genetic predisposition to childhood cancer including chromosomal abnormalities (abnormal number or structural alterations), imprinting errors, and Mendelian inheritance of gene mutations.[58] A number of the congenital syndromes associated with increased risk for childhood cancer are associated with chromosomal abnormalities and imprinting errors such as Down's syndrome (trisomy 21), WAGR-syndrome (11p13 deletions)[61], and Beckwith-Wiedemann syndrome (rearrangements in 11p15 and imprinting errors of genes in this region)[62]. Most of the identified inherited cancer predisposition syndromes are associated with a Mendelian inheritance of single-gene mutations.

The majority of the dominantly inherited childhood cancer predisposition syndromes, such as retinoblastoma, neurofibromatosis, and LFS, are caused by mutations within tumor suppressor genes including *RB1*, *NF1*, and *TP53*, respectively.[52] In this case, one allele is mutated and thereby inactivated in germline, while the other allele is somatically inactivated, which initiates tumor development. In contrast, in recessive inheritance syndromes, both alleles are mutated in the germline. Overall, a minority of known cancer predisposition syndromes are caused by recessive inheritance. However, several syndromes associated with childhood cancer predisposition, such as Ataxia-Telangiectasia and Fanconi anemia, are recessively inherited and commonly caused by mutations within DNA-stability genes.[63,64]

Familial risk

Numerous studies have evaluated the risk for cancer in the parents[11,65-67], siblings[11,67,68] and offspring[69-71] of patients with childhood cancer to evaluate the importance of hereditary predisposition. Generally, neither parents nor offspring were found to be at increased risk, except when known cancer predisposition syndromes were recognized. For siblings, the results are more inconsistent. Two population-based registry studies found no increased sibling risk other than when known cancer predisposition syndromes occurred.[67,68] However, siblings of childhood cancer survivors, particularly for survivors of hematological malignancies, were found to have an increased risk, which could not be explained by known predisposition syndromes.[11]

The occurrence of childhood cancer in siblings of patients with childhood cancer has been previously addressed in a number of population-based[68,72-74] and hospital-based studies.[75,76] Siblings of children with cancer were found to have an approximate two-fold increased risk for cancer during childhood and adolescence, but most of the risk could be attributed to known hereditary cancer syndromes.[68,72] However, Draper *et al.* found that siblings seemed to have increased risk, even when families with known cancer predisposition syndromes were excluded.[73] Studies of the concordance pattern and cancer risk in twins have suggested that twin concordance is mainly restricted to monozygotic twins and leukemia.[77-80] Transmission of leukemic cells through common placental circulation *in utero*, rather than genetic predisposition has been proposed as the mechanism for this observation.[81]

Although cancer incidence in close relatives has been well studied, there is a paucity of data on cancer incidence among the wider families of children with cancers. A few studies have considered risk in second degree relatives of children with leukemia,[82-85] lymphoma[84,86] and brain tumors[87,88]. Second degree relatives of children with hematological malignancies were found to have an increased risk, although some studies failed to show significant results.[82-85] In general, no statistically significant

increased cancer risk was found in second degree relatives of children with brain tumors.[87,88]

Is genetic susceptibility for childhood cancer underestimated?

In 1986, the *RB1* gene became the first hereditary cancer gene to be identified.[89] This was followed by the identification of several highly penetrant genes associated with childhood cancer such as *NF1* in patients with neurofibromatosis type 1[90] and *TP53* in families with LFS[91]. Despite multiple efforts, few highly penetrant childhood cancer susceptibility genes, such as the *ALK* gene underlying familial neuroblastoma,[92] have been identified in recent years. Instead, as previously suggested by Birch, it is more likely that other genetic mechanisms such as the inheritance of common allelic variation in susceptibility genes with low to moderate penetrance, which possibly modifies the response to environmental factors, are of higher importance in the development of childhood tumors.[93]

The first evidence that genes associated with dominantly inherited adult cancer syndromes may predispose to childhood cancer in a recessive manner was reported in the late 1990s.[94,95] Conversely, genes associated with recessively inherited childhood cancer predisposition syndromes, such as Ataxia-Telangiectasia and Fanconi anemia, were found to confer a modest increased risk for adult cancer in monoallelic (i.e., heterozygous) mutation carriers.[96] As previously suggested, it is likely that additional genes will be identified with different phenotypes in monoallelic and biallelic (i.e., homozygous or compound heterozygous) mutation carriers.[97] This may contribute to the increasing knowledge of the possible genetic associations in families with both childhood and adult tumors. Further epidemiological studies might reveal new potential tumor associations, which may aid in the identification of new candidate genes or pathways associated with adult and childhood tumors.

Even though most familial cancer clustering involving childhood cancer are suggested to be associated with known cancer predisposition syndromes, the underlying genetic susceptibility remains to be identified for some families.[9,98] Recent data indicate that it is likely that the genetic susceptibility to childhood cancer is underestimated due to under-recognition of predisposing mutations. Due to failure to detect mutations in a large proportion of patients highly likely to carry cancer susceptibility mutations, it was suggested that there may be a number of novel cancer susceptibility genes that remain to be identified.[9]

Hereditary adult cancer syndromes and their role in childhood cancer.

BRCA1/2 and mismatch repair protein in relation to DNA-repair

The BRCA1 and BRCA2 proteins are essential for the repair of DNA double strand breaks by homologous recombination.[99] The BRCA proteins are also involved in the Fanconi anemia pathway because one of the Fanconi anemia genes (*FANCD1*) was identified as *BRCA2*.[100] The Fanconi pathway is suggested to primarily coordinate a complex mechanism involving components from different DNA repair pathways to repair DNA interstrand crosslinks.[101]

The mismatch repair (MMR) system recognizes and repairs DNA errors that have occurred due to DNA polymerase slippage during DNA replication and recombination. The mismatches that are repaired include single base-pair substitutions, insertions and deletions.[102] The MMR process is highly conserved from prokaryotes to eukaryotes, and the human homologues of the MMR proteins include MSH2, MSH6, MSH3, MLH1, PMS2, and PMS1. These proteins interact in the repair process by forming complexes where MutSα (MSH2-MSH6), MutSβ (MSH2-MSH3), and MutLα (MLH1-PMS2) are the major actors.[103] Impaired MMR results in a characteristic pattern of somatic insertions and deletions in repetitive sequences. This pattern is called microsatellite instability (MSI) and is often found in tumors associated with MMR defects.[104,105]

Impaired DNA-repair pathway function results in genomic instability, which may enable tumor development. Heterozygous germline mutations in the *BRCA1/2* and MMR genes are associated with a high risk of adult-onset cancer.[99,106] In recent years it has been evident that biallelic mutations in some of these genes such as *BRCA2* and the MMR genes are associated with distinct clinical features including childhood cancer predisposition.[94,95,100]

Hereditary predisposition to common adult cancers

Heterozygous *BRCA1* and *BRCA2* germline mutations at chromosomes 17q21 and 13q12-13, respectively, are the major cause of hereditary breast and ovarian cancer (HBOC).[99,107,108] *BRCA1/2* mutation carriers have a 50-80% lifetime risk for developing breast cancer, which is usually with an earlier age of onset compared with

the general population.[99,109] The penetrance is lower for carrier of *BRCA2* mutations than for those with *BRCA1* mutations.[109] In addition, *BRCA1* mutation carriers have a 40-50% lifetime risk for ovarian cancer, while the risk for *BRCA2* mutation carriers is 20-30%.[110] *BRCA2* mutations are also associated with increased risk for other tumors including prostate, pancreas, gastric, malignant melanoma, and male breast cancer.[111] The overall prevalence of *BRCA1/2* mutation carriers have been estimated to be from 1/400 to 1/800, but vary between different ethnicities due to founder mutations especially among Ashkenazi Jews and Icelanders.[112,113]

Hereditary non-polyposis colorectal cancer (HNPCC), which is also known as Lynch syndrome, is caused by heterozygous germline mutations that primarily occur in *MLH1* at chromosome 3p, *MSH2* at chromosome 2p, and *MSH6* at chromosome 2p16.[106,114-117] Mutations are also rarely found in *PMS2* at chromosome 7p22, and they are associated with a lower penetrance compared to the other MMR genes.[118-120] Mutation carriers have a 60-80% lifetime risk of colorectal cancer and a 40-60% risk for endometrial cancer, usually with an earlier onset age compared with the general population.[121,122] Increased risk is also found for a variety of other extracolonic cancers including ovary, stomach, pancreas, biliary tract, urinary tract, small bowel, brain, and skin cancer.[122] HNPCC is estimated to affect between 1/2000 and 1/600 individuals in the general population.[123]

Families with a family history that is suggestive of cancer for either HBOC or HNPCC are offered genetic counseling. National and European guidelines have been defined to identify families or patients with HBOC who should be considered for *BRCA1* and *BRCA2* genetic screening.[124-126] Corresponding international clinical criteria have been defined for HNPCC (The Amsterdam criteria and Bethesda guidelines).[127-129] After identification of a mutation in the family, presymptomatic testing can be offered to family members. The main goal of genetic counseling and genetic testing is to reduce the cancer morbidity and mortality through risk reducing strategies such as surveillance or preventive interventions.[124-126,130] There is no evidence of clinical benefit to start surveillance before early adulthood for HBOC and HNPCC. To protect autonomy, presymptomatic testing is therefore generally not offered before age 18.[131]

Childhood cancer and biallelic mutations in the *BRCA2* and MMR genes

Biallelic *BRCA2* mutations have been found to cause childhood cancer in the context of Fanconi anemia.[100,132] Fanconi anemia is a rare autosomal recessive chromosomal instability disorder characterized by congenital abnormalities, short stature, bone marrow failure, hypersensitivity to DNA-crosslinking agents, and a predisposition to

leukemia and other solid tumors.[133] *BRCA2*-associated Fanconi anemia has been associated with a severe phenotype and a strong cancer predisposition at an early age.[134,135] Increased risk of early-onset leukemia (acute myeloid leukemia) and a variety of solid tumors such as brain tumors (medulloblastoma and glioblastoma) Wilms' tumor, and neuroblastoma have been reported.[132,134-138]

Biallelic mutations in one of the MMR genes have been found to cause a disorder characterized by development of childhood cancer and colorectal cancer in adolescents or young adults.[139,140] Brain tumors (astrocytomas, glioblastomas, and medulloblastomas) and hematological malignances including lymphomas and leukemia were the main childhood cancers found in individuals with biallelic MMR gene mutations. Characteristically, most of these patients were also found to show dermatological features of neurofibromatosis, mainly café au lait spots.[140] Today, approximately 100 individuals with biallelic MMR gene mutations have been reported in the literature.[139-143] In most of these patients, a family history of HNPCC or consanguineous marriage was observed. In contrast with HNPCC, where *MLH1* and *MSH2* mutations are predominating, a predominance of biallelic *PMS2* mutations occur in the reported cases.[140,144]

The vast majority of published studies of individuals with biallelic mutations in the *BRCA2* and MMR genes are case reports. Knowledge of the occurrence of childhood cancer in families with HBOC and HNPCC is limited. A previous study that evaluated the contribution of familial *BRCA1/2* mutations to childhood cancer found no evidence of increased childhood cancer risk in families with *BRCA1* and *BRCA2* mutations.[145] No study has evaluated the occurrence of childhood tumors in families with HNPCC. Epidemiological studies investigating the role of childhood cancer in families with common adult cancer syndromes are generally missing. Increased knowledge may improve genetic counseling and the management of families.

Li-Fraumeni syndrome

In 1969, Li and Fraumeni reported an aggregation of childhood sarcomas and other early-onset tumors in the relatives of children treated for rhabdomyosarcoma, suggesting a new familial syndrome, which was later denoted LFS after the two physicians who first described it.[146] LFS is a rare autosomal dominant inherited disorder, which predisposes a wide spectrum of childhood and adult tumors and increased risk for multiple primary tumors. This syndrome is commonly associated with germline mutations in the *TP53* gene.[147]

Clinical features

The most common cancers in LFS are soft tissue sarcoma, osteosarcoma, pre-menopausal breast cancer, brain tumors and adrenocortical carcinomas, which account for approximately 80% of all LFS-related tumors.[60,148] Other cancers including early-onset melanoma, pancreas, lung, gastric, colon, prostate, ovarian, and hematopoietic malignancies have also been found in excess in some families.[148-154] The lifetime risk for individuals with LFS is high for any cancer, and it is estimated to be 73% in males and nearly 100% in females, primarily due to the high breast cancer incidence.[155] The risks are particularly high in younger ages, and 15% of individuals with LFS will develop a malignancy before age 15, with similar risks found between the sexes. At 45 years of age, the risks are estimated to be 27% and 82% for males and females, respectively.[155] Individuals with LFS are also at increased risk for multiple primary tumors with the highest risk found in childhood cancer survivors.[156]

Clinical criteria have been defined for LFS diagnosis and identifying individuals that should be considered for *TP53* mutation screening (Table 1).[157] Since the initial definition, families have been found with incomplete LFS clinical features and several definitions for Li-Fraumeni-like (LFL) syndromes have been described.[158-161]

Table I. Established clinical criteria for Li-Fraumeni syndrome

Classification scheme	Description
Classic LFS [157]	- A proband with a sarcoma diagnosed before age 45 years **and** - A first-degree relative with any cancer before age 45 years **and** - A first- or second-degree relative with any cancer before age 45 years or a sarcoma at any age
Birch [158]	- A proband with any childhood cancer or sarcoma, brain tumor, or adrenocortical carcinoma diagnosed before age 45 years **and** - A first- or second-degree relative with a typical LFS cancer (sarcoma, breast cancer, brain tumor, adrenocortical carcinoma, or leukemia) at any age **and** - A first- or second-degree relative with any cancer before age 60 years
Eeles [159]	- Two first- or second degree relatives with LFS related malignancies at any age

Chompret [160,161]	- A proband who has
	  - A tumor belonging to the LFS tumor spectrum (soft tissue sarcoma, osteosarcoma, pre-menopausal breast cancer, brain tumor, adrenocortical carcinoma, leukemia, or bronchoalveolar cancer) before age 46 years **and**
	  - At least one first- or second-degree relative with an LFS tumor (except breast cancer if the proband has breast cancer) before age 56 years or with multiple tumors; **or**
	- A proband with multiple tumors (except multiple breast tumors), two of which belong to the LFS tumor spectrum and the first of which occurred before age 46 years; **or**
	- A proband who is diagnosed with adrenocortical carcinoma or choroid plexus tumor, irrespectively of family history

Abbreviation: LFS, Li-Fraumeni syndrome

The tumor suppressor gene *TP53*

The *TP53* gene encodes a transcription factor, p53, which controls an integrated network of antiproliferative programs.[162] In response to different stress-inducing signals including DNA damage, oncogene activation or hypoxia, p53 activates a variety of antiproliferative pathways such as apoptosis, cell cycle arrest and senescence.

TP53 inactivation is one of the most common genetic alterations in sporadic cancer.[163]

Using a candidate gene approach, germline *TP53* mutations were found to be the underlying cause of LFS in 1990.[91] Germline *TP53* mutations are found in 70-80% of families, meeting the classic criteria for LFS, while the majority of families meeting the less stringent criteria do not harbor detectable germline *TP53* mutations.[160,164,165] Although several other genes involved in the *TP53* pathway have been considered as LFS candidate genes, no other gene has been identified thus far.[147,166-169]

The majority (72.8%) of identified germline *TP53* mutations are missense mutations (International Association for Research on Cancer (IARC), *TP53* database R15, November 2010, www-p53.iarc.fr).[170] Mutations are found throughout the coding sequence of the gene, although approximately 70% occur within exons 5 to 8, encoding the DNA-binding domain (Figure 3).[153,171] Larger deletions have also been reported in families with LFS.[172] Founder mutations are rare; however, a germline *TP53* mutation (R337H) has been found to be unusually common in Southeast Brazil.[173,174] The population frequency of germline *TP53* mutations is not clearly known, but current data suggest that germline *TP53* mutations occur at a rate of 1 in 5 000 individuals with a relatively high frequency (7-20%) of *de novo* mutations.[175,176]

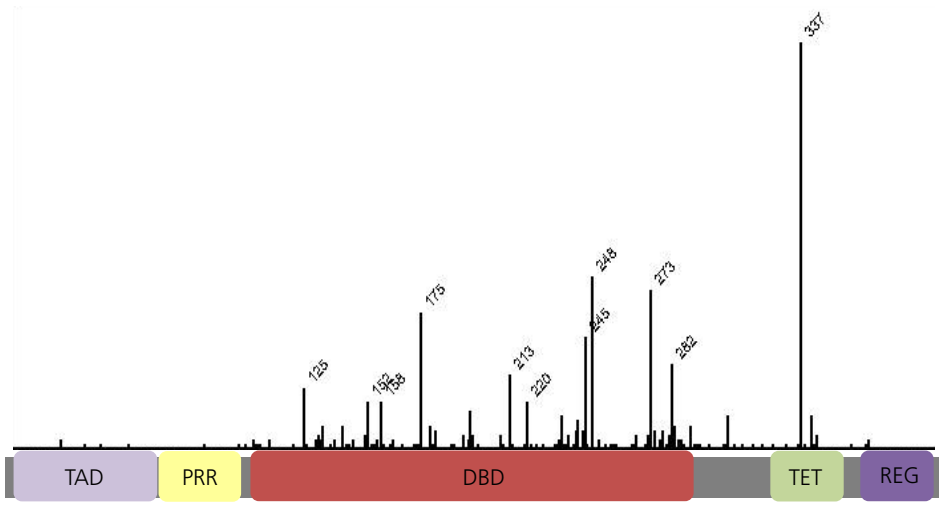

Figure 3. Schematic illustration of the TP53 protein and relative frequency of germline *TP53* mutations by codon. Most mutations are found in the DNA binding domain. The Brazilian founder mutation in exon 10 in the tetramerization domain constitutes the most frequent mutation. TAD = transactivation domain; PRR = proline-rich region; DBD = DNA binding domain; TET = tetramerization domain; REG = regulatory domain. Adapted from the IARC database (R15, November 2010)[170]

Clinical management and surveillance strategies

Management of families with germline *TP53* mutations is difficult due to the different sites and types of cancer associated with LFS and the variability in the age of onset. Except for breast cancer surveillance and prophylactic mastectomy, there is no consensus regarding the surveillance of other tumor types. Because evidence of clinical benefit for *TP53* mutation testing is limited, presymptomatic testing for germline *TP53* mutations has been debated. Efforts to determine surveillance strategies have been performed. Several strategies have been suggested, including the use of fluorodeoxyglucose F18-positron emission tomography and computed tomography scanning for the early detection of cancers in *TP53* mutation carriers.[177] However, concerns about radiation exposure were expressed, which may hamper the use of this screening method. Recently, a comprehensive surveillance protocol was reported that included biochemical and image studies such as magnetic resonance imaging to detect asymptomatic cancers in *TP53* mutation carriers.[178] Although this was a small study, indications of potential survival advantages were observed. This may lend support to incorporating the genetic screening of at-risk patients and families in clinical care. However, larger prospective studies are needed to evaluate the long-term outcome of patients undergoing surveillance.

A high sensitivity for developing radiation-induced secondary tumors has been reported in *TP53* mutation carriers.[179-182] Consequently, it has been suggested that the use of radiation therapy should be avoided or used with adjusted doses whenever possible to reduce the risk for secondary tumors.[164,183,184] Otherwise, manifested LFS-related tumors are treated according to routine management with the exception of breast cancer, for which mastectomy is recommended. After completing therapy, patients should be followed closely to identify any secondary tumors.[184]

High frequency of *TP53* mutations within certain childhood tumors

The frequency of germline *TP53* mutations in patients with apparently sporadic LFS-associated tumors has been extensively studied. Children with adrenocortical carcinoma are found to have a remarkably high frequency of germline *TP53* mutations, which is estimated to be at 50-80%, regardless of family history.[59,60,173,185] Choroid plexus tumors are another rare tumor that has been associated with LFS.[186] Choroid plexus tumors are intraventricular neoplasms of epithelial origin, which are classified into three distinct subgroups: choroid plexus papilloma, atypical choroid plexus papilloma and choroid plexus carcinoma.[187] In a series of patients with choroid plexus tumors referred for clinical mutation screening, all patients (8/8) were found to

be a mutation carrier regardless of family history.[60] Subsequently, patients with adrenocortical carcinomas and choroid plexus tumors were suggested to be considered for *TP53* mutation screening irrespective of family history.[160,161] Recently, after the initiation of study IV in this thesis, it was demonstrated that patients with choroid plexus carcinoma who did not meet the clinical criteria for LFS and patients with choroid plexus papilloma had a low likelihood of harboring *TP53* germline mutations.[188,189] Furthermore, these studies estimated the prevalence of germline *TP53* mutations to be 36-50% in patients with choroid plexus carcinoma.[188,189]

Rhabdomyosarcoma is the most common soft tissue tumor in childhood.[190] Although, rhabdomyosarcoma is commonly found in families with LFS, an excess of germline *TP53* mutations has also been found in children with apparently sporadic rhabdomyosarcoma. In a series of sporadic cases of patients with childhood rhabdomyosarcoma, 9% (3/33) were found to be carriers of a germline *TP53* mutation.[191] All of the identified mutations occurred in patients that were diagnosed before three years of age. In a series of survivors with childhood soft tissue sarcoma, 6.6% (7/107) harbored a germline *TP53* mutation.[192]

According to the latest versions of the clinical criteria for LFS, children with adrenocortical carcinomas and choroid plexus tumors should be considered for *TP53* mutation screening regardless of family history.[160,161] Although not yet included in the established clinical criteria, it was recently suggested that patients with rhabdomyosarcoma before the age of five years should also be considered for testing regardless of family history.[153] Knowledge of the *TP53* mutation status may be beneficial in the clinical management of patients. However, the identification of a *TP53* mutation in a patient with an apparently sporadic childhood tumor may also have consequences for the family. Although tumors appear to be sporadic at the time of diagnosis, they may eventually become familial when the parents and siblings grow older. Improved knowledge of whether childhood adrenocortical tumors, choroid plexus tumors and rhabdomyosarcoma may be early manifestations of LFS may be helpful in the counseling of patients and family members. Most studies regarding the prevalence of *TP53* mutations in children with adrenocortical carcinoma, choroid plexus tumors and rhabdomyosarcoma are based on hospital series of patients or cohorts of patients referred for clinical *TP53* mutation testing. To validate previous findings, sequential analysis of a population-based series of patients with these rare tumors unselected for family history should be performed.

Biological and prognostic factors in familial tumors

Family history and hereditary genetic factors are known cancer risk factors. However, current knowledge about their role as prognostic factors for manifested tumors is limited. Recently, familial concordance in cancer survival was reported, suggesting a possible heritable basis of cancer outcome within families.[193]

It has been previously hypothesized that hereditary factors may affect the tumor biology and prognosis of breast cancer.[194,195] This hypothesis suggests that the age at the onset of disease may partially reflect the time of tumor initiation and differentiation of the cell of origin. Tumors diagnosed at a young age most likely have been initiated early in life in undifferentiated tissue. In addition, these tumors are, to a higher extent, most likely associated with germline mutations in the cell cycle regulatory genes, resulting in syndromes with high penetrance and broad tumor spectra, such as the association between germline *TP53* mutations and LFS. Thus, breast cancers diagnosed at a young age were suggested to be less differentiated and have low estrogen and progesterone receptor expression, which are characteristics associated with poor prognosis.[195,196]

The above mentioned hypothesis was based on knowledge and clinical experience mainly from breast tumors associated with germline *BRCA1/2* mutations. Breast cancers associated with germline *BRCA1* mutations are generally considered to have characteristics associated with poor survival including low differentiation and an estrogen and progesterone receptor negative status.[197,198] Compared with patients with sporadic breast cancer, *BRCA1*-associated breast cancers have been suggested to be associated with lower survival, although the results are inconsistent.[197,199,200] In addition, somatic *TP53* mutations have frequently been observed in *BRCA1*-associated tumors.[201] The presence of somatic *TP53* mutations has been associated with poorer prognosis for a variety of cancers, particularly breast cancer.[202,203] Thus, it is possible that germline *TP53* mutations are associated with poor prognosis; however, the data are currently limited.

Aims

The general aims of this thesis were to investigate the importance of genetic factors in the etiology of childhood cancer and to evaluate possible associations between childhood and adult cancers. The specific aims were as follows to:

- To study whether the occurrence of childhood cancer in children affects the survival of mothers with breast cancer because an association may imply that shared genetic factors affect survival after breast cancer. *(Study I)*
- To investigate the occurrence of childhood cancer in families with *BRCA1*/2-associated HBOC, MMR-associated HNPCC and, *CDKN2A*-associated familial malignant melanoma. *(Study II)*
- To evaluate the incidence of childhood and adult cancers in the extended families of patients with childhood cancer and investigate the frequency of germline *TP53* mutations in families with multiple childhood tumors. *(Study III)*
- In population-based material, to confirm the prevalence of germline *TP53* mutations in children with adrenocortical tumors, choroid plexus tumors, and early childhood rhabdomyosarcomas and investigate whether these may be early manifestations of LFS. *(Study IV)*

Materials and Methods

Lund childhood cancer genetic study (LCCG-study)

When this thesis study was initiated in 2007, it became evident that access to biological material for studying constitutional genetic factors in patients with childhood cancer was limited. To enable such studies, the Lund Childhood Cancer Genetic study (LCCG-study), whose primary aim is to enable studies regarding hereditary genetic factors associations with childhood cancer was initiated. The study comprises a retrospective and prospective inclusion of patients. The LCCG-study has been approved by the Regional Ethics Review Board in Lund (no. 2008/233, 2010/231, 2011/33).

The medical care of patients with childhood cancer is centralized to six pediatric oncologic centers in Sweden. The Children´s Hospital at Skåne University Hospital, Lund, is a referral center for medical care for all patients with childhood cancer in the South Swedish Health Care Region, which comprises approximately 1.8 million inhabitants. After completed therapy, patients are followed at the outpatient clinic until 18 years of age. Thereafter, they are referred to the Late Effect Clinic at the Skåne Oncology Clinic, Lund, Skåne University Hospital for further follow-up of late complications. The centralized management of patients with childhood cancer enables a population-based recruitment of patients.

Study design

An overview of the study design of the LCCG-study is illustrated in Figure 4. The recruitment of patients for the LCCG-study began in September 2008. Patients with a newly diagnosed cancers and survivors visiting the outpatient clinics are invited to participate in the study. For inclusion in the study, participants have to be diagnosed with any malignancy (International classification of disease (ICD) 7th revisions: 140-209) at 18 years or younger. Patients within a year of their date of diagnosis are included in the prospective arm of the study.

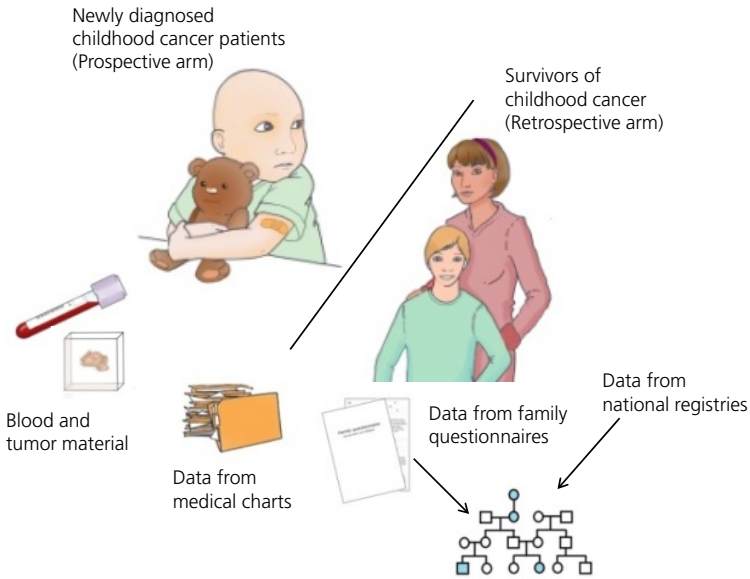

Figure 4. Overview of study design of the Lund Childhood Cancer Genetic Study.

Written informed consent is obtained from parents if the patients are younger than 18 years of age, and it is also obtained from patients 15 years or older. Consent is given to collect a blood sample (blood cells, plasma, and serum) for molecular biological analyses and to use clinical data from medical charts and tumor specimens that are stored in clinical setting in the Department of Pathology.

Patients and their parents are also requested to complete a standardized self-reporting questionnaire that includes name, date of birth, date of death, and history of cancer in their first to third degree blood relatives (parents, siblings, children, nephews/nieces, grandparents, uncle/aunts and cousins). In addition, a question regarding cancer in more distant relatives is included. Information about the specific types of cancer and diagnosis date or age at diagnosis for each relative with cancer is obtained. In the case of an incomplete questionnaire, supplementary information about the family structure is collected using the Swedish Population Registry, and pedigrees are constructed. The Swedish Population Registry is used to confirm and identify the relatives' personal identification numbers to enable linkage to national registries including the Total Population Registry, the Swedish Cancer Register, the Cause of Death Registry, the Swedish National Inpatient Register, and the Regional Outpatient Register. Regular updates will be performed because family history is a dynamic process as illustrated in Figure 5.

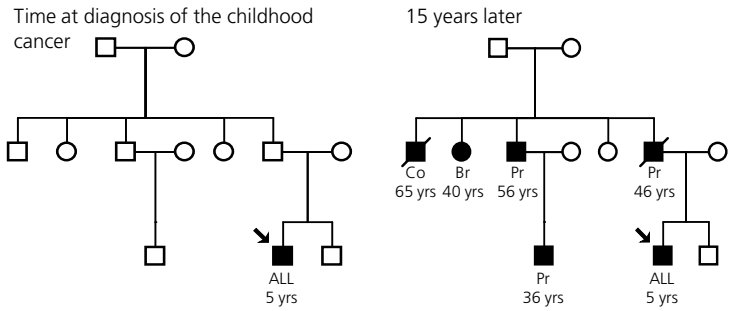

Figure 5. A pedigree at two time points demonstrating that family history is a dynamic process. ALL = acute lymphatic leukemia; Pr = prostate cancer; Br = breast cancer; Co = colon cancer.

Present status of LCCG-study

A total of 532 patients diagnosed in 1970 and later were invited to take part in the study between the beginning of patient recruitment in September 2008 and December 2011. In total, 354 of 405 retrospectively invited patients and 126 of 127 prospectively invited patients were included. Thus, there was a greater loss of patients in the retrospective arm. Altogether, a total of 480 patients were included. Of these patients, 361 had returned the family questionnaire by December 2011. In addition, two patients diagnosed before 1970 were included because they fulfilled the criteria for study IV, where all patients with adrenocortical tumors, choroid plexus tumors, and rhabdomyosarcomas diagnosed between 1958 and 2008 were included.

A first linkage to the Total Population Registry and the Swedish Cancer Register was performed during the fall of 2010. A cohort including patients enrolled during the first year who had returned the questionnaire by October 2009 and their relatives who had a Swedish personal identification number were followed until the first event of migration, death or 31 December 2008. This cohort constituted the study population in study III.

Material

Study I

The Swedish Cancer Register, Swedish Total Population Registry and Fertility Register were used to identify all parous women diagnosed with breast cancer (n=75 035) between 1961 and 1999. Only primary tumors were considered and no information about recurrence or the development of secondary tumors was available. All women were followed from breast cancer diagnosis until the first event of emigration, death or 31 December 2001. The personal identification number and different registries including the Swedish Total Population Registry, the Fertility Register, and national censuses kept by Statistics Sweden were used to identify the children of these women. Children were linked to the Swedish Cancer Register for the identification of any diagnosis of childhood cancer including sarcomas, brain tumors, lymphomas, acute lymphatic leukemia's or myeloid leukemia's diagnosed at 20 years or younger. The number of patients included in the study is summarized in Figure 6.

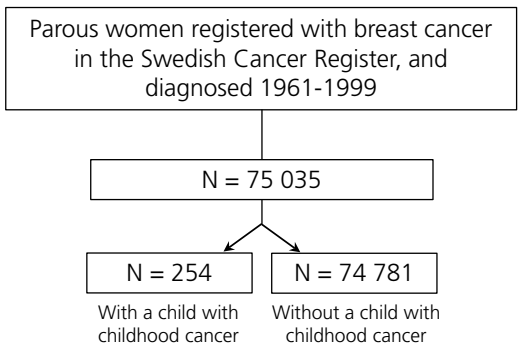

Figure 6. Overview of the number of parous women included in Study I.

Study II

The Oncogenetic clinic at the Skåne University Hospital in Lund has offered genetic counseling to the South Swedish Health Care region since 1993, resulting in a population-based recruitment of families.[204] Families identified with HBOC- or HNPCC-associated mutations within the *BRCA1*, *BRCA2* or MMR (*MLH1*, *MSH2* or *MSH6*) genes (n=172) during the period between 1993 to June 2006 were included. In addition, families with *CDKN2A*-associatied familial malignant melanomas (n=15) identified through malignant melanoma research studies at the Department of Oncology until June 2006 were included. All *CDKN2A*-associated families were carriers of the Swedish founder mutation Ins113Arg.[205,206] Pedigrees based upon questionnaire and counseling data were reviewed to evaluate the occurrence of childhood tumors diagnosed ≤18 years of age. Genetic testing for familial mutations was not performed in any of the children with cancer. A reference group consisting of two different population-based samples was used to compare the prevalence of childhood tumors between families with hereditary syndromes and the general population. The reference group consisted of one cohort of families that included residents of a parish (Heliga Trefaldighets församling, Kristianstad) in which cases of childhood tumors were identified by the records of the Swedish Cancer Register and the Southern Swedish Regional Tumor Registry. The other group consisted of individuals previously used as controls in a case-control study regarding non-Hodgkin's lymphoma, where information was derived from standardized questionnaires.[207] The number of families with adult hereditary cancer syndromes and controls included in the study is summarized in Figure 7.

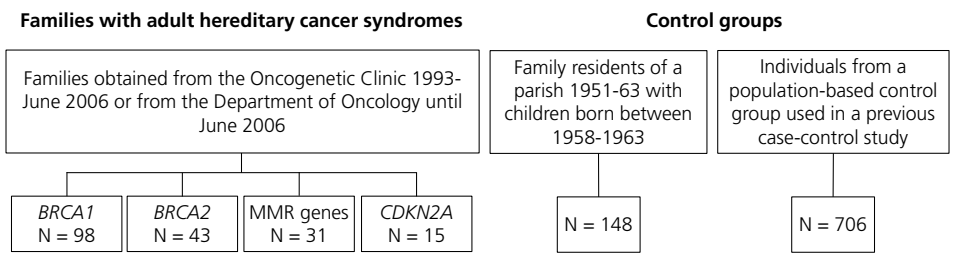

Figure 7. Overview of the number of families with adult hereditary cancer syndromes and the two control groups included in Study II.

Study III

Patients recruited to the LCCG-study between September 2008 and August 2009 and for which the family questionnaire was received by October 2009 were included in the study. After the exclusion of patients that reported to be the relative of another patient, 194 patients remained in the analysis. In the descriptive portion of the study, relatives were included regardless of relative degree and whether they were a Swedish citizen. Analyses were performed to assess the incidence of childhood and adult cancers in the relatives. In these analyses, only first to third degree relatives who had been linked to the Total Population Registry and the Swedish Cancer Register were included. Mutation screening for *TP53* mutations was performed in genomic DNA from index patients in families with multiple cases of childhood tumors. The study design and stepwise inclusion of patients and relatives are summarized in Figure 8.

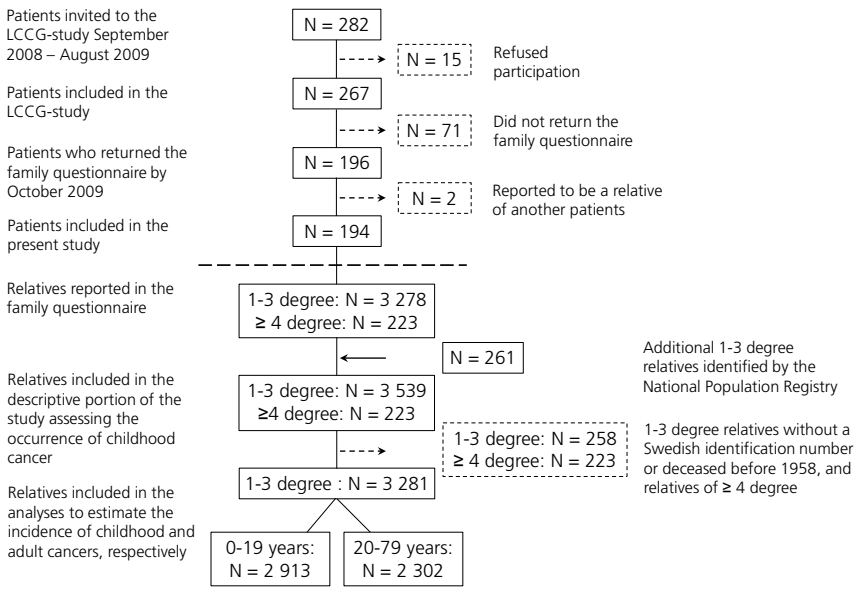

Figure 8. The upper part of the flowchart shows the stepwise inclusion (solid lines) and exclusion (broken lines) of patients with childhood cancer in Study III. The lower part of the flowchart shows the number of relatives included in and excluded from analyses.

Study IV

Using the Southern Swedish Regional Tumor Registry, all patients with adrenocortical tumors or choroid plexus tumors diagnosed ≤18 years of age and all patients with rhabdomyosarcomas diagnosed ≤5 years of age during the period between1958 and 2008 were identified. After excluding misclassified patients, three patients with adrenocortical tumors, seven patients with choroid plexus tumors and, 29 patients with rhabdomyosarcomas were included. Family pedigrees were constructed from registry data. In the case of incomplete registry-expansion, questionnaire data were used as a complement when available. First to third degree relatives (identified by registry data) were included in the analyses to estimate cancer incidence in the families of each histologic type of childhood tumors. Analyses were performed for total cancer incidence (0-79 years of age) and early-onset cancer (0-49 years of age). Patients that remained alive (n=29) were invited to participate in the LCCG-study. In total, 26 patients agreed to participate and were screened for germline *TP53* mutations. The study design and number of included patients and relatives in the different analyses is summarized in Figure 9.

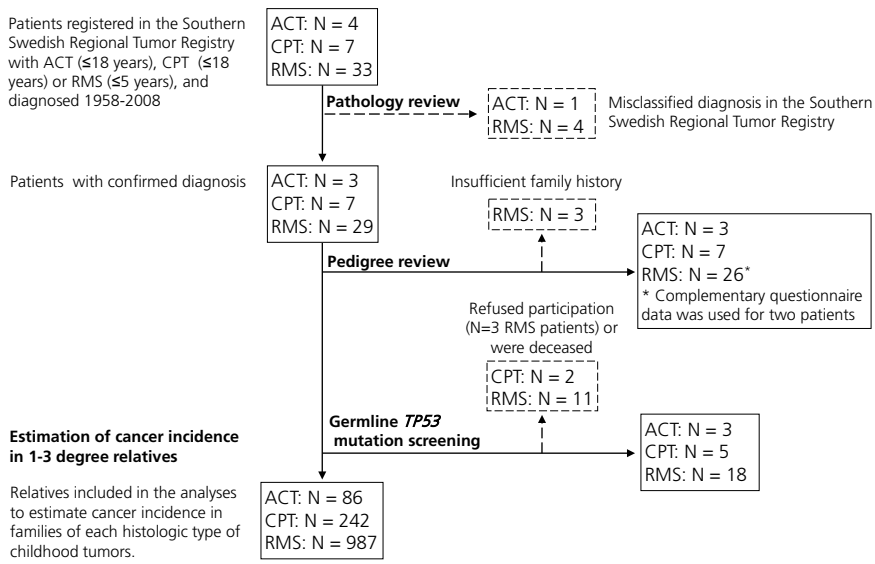

Figure 9. The flowchart shows the number of patients or relatives included in (solid lines) and excluded (broken lines) from analyses in Study IV. ACT = adrenocortical tumor; CPT = choroid plexus tumor; RMS = rhabdomyosarcoma.

All studies were approved by the Regional Ethics Review Board in Lund.

Methods

National Registries used in this thesis

The Swedish National Population Registry

The population registration in Sweden dates back to the beginning of the 17th century when the church and the parishes were responsible for the census. Since 1 July 1991, the Swedish Tax Agency (Skatteverket) has the responsibility for population registration in Sweden. All persons registered in Sweden are given a personal identification number for identification. The identification number provides information regarding the date of birth (six digits), a birth number (three digits), and a control digit. The registry contains information including name, residence, place of birth, family relationships, any immigration to or emigration from Sweden, death, and place of burial.[208] Statistics Sweden provides the Total Population Register, which is an extract from the Swedish Population Registry, which dates back to 1968.[209]

The fertility register

The fertility register (Föddaregistret), which is kept by Statistics Sweden, includes all of the births in Sweden between 1961 and 1997, and it contains the identification numbers of both the mother and child.[210]

The Swedish Cancer Register

The Swedish Cancer Register was established in 1958. Due to the mandatory independent double reporting of all cancer diagnoses by the responsible clinician and pathologist, the registry is estimated to contain 96% of all cancers diagnosed in Sweden.[211] The registry is divided into six regional cancer registries, which are associated with the regional Oncologic center in each medical region of Sweden, where the registration, coding, major quality control, and corrections are performed. The regional registry in the South Health Care Region is referred to as the Southern Swedish Regional Tumor Registry. Annually, the regional registries send information from the newly registered cases to the Swedish Cancer Register. All malignant tumors and carcinoid tumors, all tumors (including benign tumors) of the central nervous system, endocrine gland (excluding benign tumors of the thyroid), and some premalignant lesions must be reported. If a patient has multiple primary tumors, each tumor is separately registered. The registry includes three types of information; patient data (i.e., personal identification number, sex, age, and, place of residence), medical data (i.e., site of tumor, histologic type, stage according to the appropriate staging system, basis of diagnosis, diagnosis date, the reporting hospital and

department, the reporting pathology/cytology department and, identification number of specimen) and follow-up data (i.e., date of death, cause of death and, date of migration).[212]

The Cause of Death Register

The Cause of Death Register includes all deceased individuals who were registered in Sweden at the time of death. The registry was established in 1961 and is updated annually. Additionally, a historical registry exists, covering the years 1952-1960.[213]

TP53 mutation screening

The *TP53* gene is located at chromosome 17p13 and consists of 11 exons, and exons 2 to 11 are coding.[184] Screening for germline *TP53* mutations was performed using direct sequencing and Multiplex ligation-dependent probe amplification (MLPA).

DNA extraction

Genomic DNA was extracted from peripheral lymphocytes using the Quick Gene DNA whole blood kit (FUJIFILM Corporation, Life Science Products Division, Akasaka, Japan) according to manufacturers' protocol.

Direct sequencing

Mutation screening by direct sequencing was performed to allow for the detection of point mutations, small deletions, duplications and, insertions. The principle of the method is that single-stranded DNA molecules that differ in length by just one nucleotide can be separated from one another by polyacrylamide gel electrophoresis.[214] During the enzymatic synthesis of the complementary DNA strand, which mimics the natural process of DNA replication, dideoxy nucleoside triphosphates (ddNTP) are randomly incorporated and terminate the elongation process, resulting in chains ending at specific positions. By labeling each of the four ddNTP chain terminators with different fluorescent dyes that emit light at different wavelengths, the DNA sequences are visualized in a chromatogram after capillary gel electrophoresis.

By direct sequencing, the entire *TP53* coding region (exons 2-11) and splice junctions were analyzed in both directions. Using genomic DNA, the coding exons were amplified by polymerase chain reaction (PCR). The sequencing reaction was performed on purified PCR products, using the BigDye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) according to manufacturer's protocol. The fragments were analyzed by capillary electrophoresis in

an automated sequencer (3130xl Genetic Analyzer; Applied Biosystems, Foster City, CA, USA). The resulting sequence chromatograms were analyzed using the Sequencher™ 4.5 software (Gene Codes Corporation, Ann Arbor, MI, USA).

Multiplex ligation-dependent probe amplification

MLPA is a method for detecting larger genomic alterations including deletions and duplications. The principle of this method is the amplification of MLPA probes that hybridize to target sequences. To become amplified, the probe pairs that bind to adjacent target sequences must be joined by a ligation reaction. The sequences are then simultaneously amplified with the use of only one primer pair, resulting in a mixture of amplification products. The amplification products of each MLPA probe has a unique length and can be analyzed by capillary electrophoresis. The resulting peak pattern is compared to that of reference samples to enable the detection of any deletions or duplications of genomic regions of interest.[215]

MLPA analysis was performed on genomic DNA using the SALSA MLPA kit P056-A2 *TP53* (MRC Holland, Amsterdam, The Netherlands) according to the manufacturer's protocol. The amplification products were separated and visualized using capillary electrophoresis (3130xl Genetic Analyzer; Applied Biosystems, Foster City, CA, USA). The chromatograms were analyzed using the GeneMarker 1.6 software (SoftGenetics LLC®, State College, PA, USA).

Statistical analyses

The statistical software programs PASW 17.0 and SPSS 19.0 were used for statistical analyses. For incidence analyses, the *Syd*CAP version 1.0 software and the OpenEpi version 2.3.1 software[216] were used.

Overall survival was compared between patients with breast cancer that had children with or without childhood tumors using Cox proportional hazards model. Kaplan-Meier survival curves were used to illustrate differences in survival (Study I).

Fisher's exact test was used to compare proportional differences in dichotomous variables (Studies II and III), while the Mann–Whitney U test was used to compare continuous variable differences (Study III). The Fisher's exact test was chosen instead of Chi-squared test because of the small sample size. The Mann-Whitney U test was chosen because of the skewed, and hence non-normal, distributions. Logistic regression was used to calculate the odds ratios (OR) and 95% confidence intervals (CI) for dichotomous variables such as childhood cancer in families with hereditary syndromes and for cancers in relatives that were identified by questionnaire and registry reported data (Studies II and III). McNemar's test was used to evaluate the

concordance of cancer events in relatives between questionnaire and registry reported data (Study III).

In studies III and IV, the risk assessment for tumors in the relatives of patients with childhood cancer was performed by calculating standardized incidence ratios (SIR) by dividing the observed number of cancers with the corresponding expected number. Relatives were followed from 1 January 1958 or date of birth, until the first malignancy, migration, death (when they were censored) or until the 31 December 2008, last date of follow-up. All cancer diagnoses were coded according to ICD-7, and only diagnoses registered in the Swedish Cancer Register were included in the analyses. Cervical tumors were excluded from all analyses, except in the estimation of childhood cancers in relatives of patients with childhood cancer in study III. Cancer incidence within the Southern Health Care Region was used as reference. By stratifying age, sex and calendar year, the expected number of cancers in the general population was calculated. SIR was estimated for total cancer and by specific tumor site. Fisher's exact test was used to calculate the 95% CI and *P*-values.

Two-sided *P*-values are presented in the studies of this thesis. A significance level of 5% was used in all analyses. Although a large number of statistical analyses were performed, mainly in the risk assessment for cancer in relatives, no correction for multiple testing by the Bonferroni correction method was performed because the studies were considered to be hypothesis generating. The Bonferroni correction method may be used to reduce the risk for false positive results when there is no *a priori* hypothesis; however, it is too stringent in many cases and increases the risk for false negative findings.

Methodological considerations

The strengths and limitations for each study are summarized in Table 2. Because childhood cancer and hereditary cancer is rare, access to large study materials is limited, which results in small sized studies with statistical uncertainty. This is important to be aware of when interpreting the results, and confirmation in independent cohorts is warranted.

Table 2. Methodological considerations regarding strengths and limitations

	Strengths	Limitations
Studie I	– National registry based material. – Analyses were adjusted for number of children and time since last childbirth, which are factors known to affect prognosis.	– Overall survival was assessed instead of breast cancer specific survival. – Information regarding therapeutic treatments and tumor characteristics related to prognosis were absent, which is why we were unable to adjust for these factors in the analysis.
Study II	– Population based material assessed at a single National Health Service Oncogenetic clinic serving the Southern Health Care Region. – Registry-based expansion of the opposite parental line to evaluate the possibility of inheritance of biallelic mutations. – Populations-based control groups.	– Limited number of mutation carrying families. – Children were not tested for the familial mutation.
Study III	– Population-based material, where all of the children were treated at a single Pediatric Clinic. – Extended pedigrees. – Cohort was linked to the Swedish Cancer Register for estimation of cancer incidence in relatives. – *TP53* germline mutation screening of patients with a relative affected with childhood cancer.	– Small sample size. – Only 75% of patients had returned the family questionnaire. The family history of the non-responders is unknown and may influence our results. – Survivor bias may occur.
Studie IV	– Population-based material covering 50 years. – Registry-based pedigree expansion and linkage to the Swedish Cancer Register for identification of cancer diagnoses. – *TP53* germline mutation screening was performed for 26 of the 29 patients that remained alive and thus could be tested.	– Small sample size of children. – Survivor bias could occur in the estimation of germline *TP53* mutation frequency. – All tumors were not considered for pathology re-examination – Long time period, which is why the criteria for diagnostic classification may vary over time.

In Sweden, the medical care of patients with childhood cancer and oncogenetic counseling are centralized in six different centers, which allows for the possibility of population-based studies, which is an advantage. In addition, Swedish national registries such as the Total Population Registry and the Swedish Cancer Register enable population-based registry studies and registry-based pedigree expansion. A comparison of cancer diagnoses in relatives that were reported by patients and Swedish Cancer Register data showed that patients and their families failed to report all cancers in relatives. In addition, patients were almost twice more prone to report relatives with cancer than relatives without cancer.[217] This strengthens the importance of expanding families through registries to obtain a more accurate estimate of cancer incidence in the families. Studies II – IV are based on regional population-based material. Using a national study design would have resulted in a larger study population; however, extended registry-based pedigree expansion is very expensive and time-consuming, which would make it difficult to perform in large patient series. Furthermore, it may be easier to recruit study participants on a regional basis through physicians and nurses directly involved in the care of the patients.

It is important to consider that the family history is a dynamic process and thus changes over time as family members grow older (Figure 5). This may result in an underestimation of adult tumor associations in children newly diagnosed with cancer. Continued follow-up of the cohorts, allowing the relatives to grow older, is important to be able to fully assess the implications of hereditary factors.

The age definition for what we considered to be childhood cancer varied between the studies. This variation was because the studies were designed at different time periods. The analytic tools also enforced methodological limitations, for example, *SydCAP* uses 5-year intervals in the expected cancer incidence estimates.

Results and Discussion

Study I

Family history and hereditary genetic factors are known cancer risk factors. However, knowledge of their role as prognostic factors for manifested tumors is limited. Using childhood tumors as a surrogate measure for hereditary factors such as LFS-associated TP53 mutations, the survival was compared between women with breast cancer with and without having a child with childhood cancer.

Population-based registry data were used to identify 75 035 parous women with breast cancer, and 254 of these women had a child with a childhood cancer. Those women who had a child with cancer were found to have a shorter survival compared to other parous women. The differences in survival were present in younger and older patients, but occurred earlier and were more pronounced in younger patients (Figure 10). Having a child with sarcoma or myeloid leukemia was associated with a particularly poor prognosis; however, this is based on few observations and should be interpreted with caution.

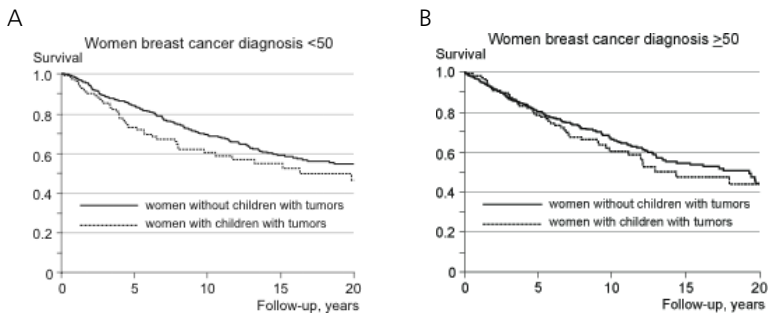

Figure 10. Kaplan-Meier survival curves demonstrating differences in overall survival for patients with breast cancer that had children with or without childhood cancer in (A) patients diagnosed <50 years of age and (B) patients diagnosed ≥50 years of age.

It has been previously hypothesized that hereditary genetic factors including germline *TP53* mutations may affect tumor biology and prognosis.[194,195] The occurrence of genetic factors, such as *TP53* mutations, could be considered a plausible explanation for the observed shorter survival in women who had children with childhood cancer,

at least for a small subpopulation of these women. Breast cancer is numerically the most common cancer in LFS and is usually diagnosed at an early age.[148,149] Several of the included childhood tumors including sarcoma, brain tumors, and leukemia belong to the tumor spectra associated with LFS, although the association between leukemia and LFS is inconsistent.[149,157,176] Thus, considering the age at diagnosis and type of childhood tumors, women diagnosed at an early age or having a child with sarcoma most likely have a higher likelihood of being associated with germline *TP53* mutations.

Because of the registry-based study design, information regarding some prognostic factors, such as tumor stage, receptor status, and therapeutic treatments, were unavailable. Therefore, we were unable to evaluate if the shorter survival for women having a child with cancer was associated with specific tumor characteristics. Studies of the tumor characteristics in carriers of germline *TP53* mutations are limited. Hereditary *TP53*-associated breast cancers have previously been hypothesized to originate from early progenitor cells, resulting in poorly differentiated tumors with a negative estrogen and progesterone receptor status, and thus they are associated with a shorter survival.[195] In contrast, a high prevalence of estrogen and/or progesterone receptor positive tumors was recently reported in carriers of germline *TP53* mutations.[218] However, breast tumors arising in *TP53* mutation carriers were also associated with human epidermal growth factor receptor 2 (HER2)-amplifications, which may have prognostic implications.[218,219] HER2 is a growth factor receptor that is found on the cell membrane, and it is overexpressed in 15% of all breast cancers due to gene amplification.[220] Sporadic *TP53* mutations have been identified in HER2 positive breast tumors and were associated with poor prognosis.[221] Therefore, considering the suggested association between germline *TP53* mutations and HER2 positive breast cancers, this may support the hypothesis that germline *TP53* mutation-associated breast cancer may be associated with poor prognosis.

With the exception of hereditary genetic factors, other causes may be considered as a possible explanation for the observed survival differences. Being a parent to a child with cancer is associated with psychological stress, which may lead to increased mortality by direct stress effects or by lifestyle changes.[222] However, current knowledge suggests no increased mortality in parents of patients with childhood cancer; therefore, this idea is probably of minor importance to our findings.[66,223,224] Furthermore, shared environmental factors that may possibly contribute to increased cancer incidence and increased mortality risk cannot be excluded.

Although further studies are needed to study tumor characteristics and possibly associations with germline *TP53* mutations among women having a child with cancer, our findings suggests that hereditary factors could affect tumor prognosis.

Study II

Monoallelic germline mutations in the BRCA1/2, MMR and CDKN2A genes are associated with hereditary cancer syndromes predisposing to adult-onset tumors. Although biallelic mutations in genes such as BRCA2 and MMR genes have been found to cause childhood cancer, knowledge of the occurrence of childhood cancer in families with cancer syndromes primarily associated with adult-onset tumors is limited. Improved knowledge regarding the occurrence of childhood tumors in families with hereditary adult cancer predisposition syndromes could have implications in the clinical management of families with these syndromes.

Using the pedigrees of families with *BRCA1*/*2*, MMR or *CDKN2A* mutations obtained from the Oncogenetic clinic and the Department of Oncology, the occurrence and risk for childhood cancer for each syndrome was studied in relation to the general population. We found an increased occurrence of childhood tumors in families with *BRCA2*-associated HBOC, MMR gene-associated HNPCC, and *CDKN2A*-associated familial malignant melanoma (Figure 11). The risk was found to be highest for families with *CDKN2A* and MMR gene mutations and slightly elevated in families with *BRCA2* mutations, while families with *BRCA1* mutations were found to have a risk that was similar to the general population.

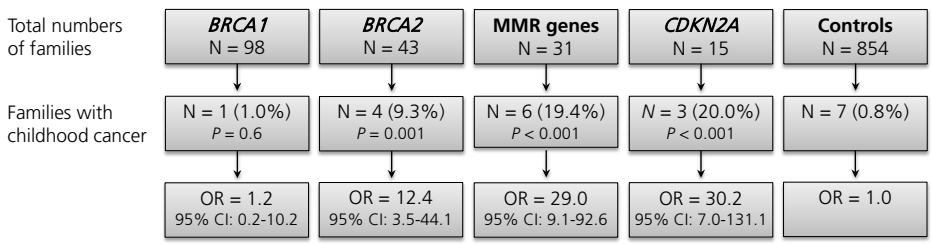

Figure 11. Occurrence of and odds ratio (OR) for childhood cancers in families with *BRCA1/2*-associated HBOC, MMR gene-associated HNPCC, and *CDKN2A*-associated familial malignant melanoma. The occurrence of childhood cancer was similar for the two control groups used (Parish-based control group: 1/148, 0.7%; control group from a previously case-control study: 6/706, 0.8%), therefore these were merged into one control group.

Growing evidence indicates that biallelic germline mutations in adult cancer-associated genes such as *BRCA2* and MMR genes may cause childhood cancer.[137,139-142] However, we found no family history evidence to support the inheritance of biallelic mutations for childhood tumors in families with *BRCA2*, MMR genes or *CDKN2A* mutations. This may suggest that other mechanisms could explain our

findings. Nevertheless, the occurrence of hypomorphic alleles with reduced penetrance in the heterozygous state in the apparently unaffected branch of the family could not be excluded.[140,225] Hypothetically, inactivation of the wild-type allele by epigenetic changes could be considered another possible mechanism that functionally resembles the inheritance of biallelic mutations.[226] Furthermore, another possibility is that a familial mutation by itself, or maybe in association with modifying genes or environmental factors, may predispose a monoallelic carrier to childhood tumors. However, this hypothesis needs to be proven in future studies.

Childhood brain tumors were found in all affected families with MMR gene mutations. Childhood brain tumors have frequently been reported in children with biallelic MMR gene mutations[142,143,227,228] and in families with Turcot syndrome,[229-231] which is associated with germline MMR-gene mutations. These data may suggest that impaired MMR-function could possibly be associated with childhood brain tumorigenesis.

Knowledge of the occurrence of childhood tumors in families with HBOC, HNPCC and familial malignant melanoma is limited. The prevalence of childhood tumors in families with *BRCA1/2*-associated HBOC was previously assessed in a study by Brooks *et al.* in which no evidence of increased childhood cancer risk in families with *BRCA1* or *BRCA2* mutations was found.[145] However, they used hereditary breast cancer families that tested negative for *BRCA1/2* mutations as a comparison group, which may have influenced their results by the occurrence of other cancer-predisposing genes in these families. To our knowledge, this is the first study considering the childhood tumor risk in families with *BRCA1/2*-associated HBOC, MMR gene-associated HNPCC, and *CDKN2A*-associated familial malignant melanoma in relation to a reference group that represents the general population.

Although we found an increased childhood tumor risk in families with germline mutations in *BRCA2*, MMR, and *CDKN2A* genes, the occurrence of childhood tumors within these families is a relatively rare event. Considering the rarity of childhood tumors and the small study population, our data do not suggest a benefit in screening for childhood tumors in families with these syndromes. Thus, with the current knowledge, our findings may not have implications for the clinical management of families with *BRCA2*, MMR, and *CDKN2A* gene mutations for which surveillance should be in accordance with current guidelines.[124-126,130,232,233]

However, the occurrence of childhood cancer may constitute the first indication of the occurrence of familial predisposition, suggesting that pediatricians and clinical geneticists should be aware of the suggested associations between childhood cancer and adult cancer predisposition syndromes. Strategies for identifying patients with childhood cancer to be considered for MMR gene testing were recently suggested.[141]

The identification of potentially predisposed children may have clinical benefits for the patient, including the potential modification of treatment and surveillance strategies and the identification of at-risk relatives, which would facilitate introducing them to the appropriate surveillance programs. This was recently demonstrated in a report by Durno *et al.* in which the clinical benefits of surveillance of a kindred with biallelic MMR gene mutation carriers was reported.[234]

Study III

Most of the increased cancer risk in the relatives of patients with childhood cancer could be explained by known hereditary factors; however, the genetic susceptibility may be underestimated. Although the cancer risk in first degree relatives is well studied, data on the cancer incidence in extended families of patients with childhood cancer are limited.

In this study, we assessed the occurrence of childhood and adult cancers in the extended families of 194 patients with childhood cancer included in the LCCG-study. Our main finding was that the relatives of patients with childhood cancer were found to have an increased incidence of childhood cancer and certain adult cancers as well.

Considering the occurrence of childhood cancer, 21 of 194 patients were found to have at least one relative with a childhood tumor (Figure 12). Overall, first to third degree relatives had a significant two-fold increased incidence of childhood tumors. The highest incidences were observed in second and third degree relatives, although the results obtained from subgroup analyses were not statistically significant.

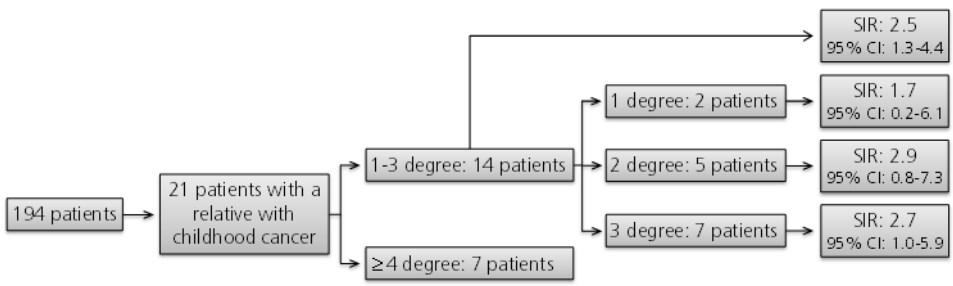

Figure 12. Occurrence and incidence of childhood cancer in relatives of childhood cancer patients. In the estimation of standard incidence ratios (SIR), one second degree and one third degree relative without a Swedish identification number was excluded. The incidence of childhood cancer in relatives was estimated for first to third degree relatives combined, and for each relative degree separately.

Previous studies assessing the occurrence of childhood tumors in siblings of patients with childhood cancer showed similar results, suggesting an approximate two-fold increased risk for childhood and adolescent cancers in siblings, but in most cases, the increased risk seemed to be attributed to known hereditary cancer syndromes.[68,72-74] We found no evidence suggesting that known hereditary syndromes could explain the observed incidence of childhood tumors in most of our families with the exception of two index patients, one of whom had Down's syndrome and one of whom had neurofibromatosis type 1. No germline *TP53* mutations were found in patients who had a relative with childhood cancer. Most of the affected relatives were of more distant relationships than first and second degree relatives, which may suggest that it is unlikely that highly penetrant genes account for the increased incidence of childhood tumors. Genes with a recessive inheritance pattern may contribute to the susceptibility of childhood cancer in these families. Other possible mechanisms may be the dominant inheritance of common allelic variants of susceptibility genes with low to moderate penetrance, possibly modifying the response to environmental factors.[93] This needs to be further addressed in future studies.

Furthermore, we found an increased incidence of adult cancers in the relatives of patients with childhood cancer, mainly in first (SIR: 2.2, 95% CI: 1.2-3.5) and second degree relatives (SIR: 1.4, 95% CI: 1.2-1.7). In particular, an increased incidence of breast (SIR: 1.7, 95% CI: 1.2-2.4) and prostate cancers (SIR: 2.7, 95% CI: 1.9-3.8) was observed in first to third degree relatives. Increased breast cancer incidence was observed in first and second degree relatives, while an increase in prostate cancer was observed in second degree relatives. Breast and prostate cancers were diagnosed at an earlier than average age. In contrast to our findings, most previous studies have not found increased risk for adult tumors in parents[11,65-67] or siblings,[67,68] except when known hereditary cancer predisposition syndromes were present. Increased breast cancer risk in mothers and sisters may be partially, though not fully, explained by known syndromes, as previously reported.[65,68] In this study, all breast cancers observed in first degree relatives occurred in mothers who had a low median age at diagnosis (47.5 years). However, we have no evidence that suggests that the mothers belong to families with *BRCA1/2*-associated HBOC, which otherwise could have explained the earlier onset. With the exception of families with multiple childhood tumors or mothers with breast cancer, we have not examined the occurrence of known cancer predisposition syndromes, which could partially explain the difference in the observed risks. The observed cancer incidence in second degree relatives is similar to previous findings.[82-84]

A recent study by Plon *et al.* indicated that the genetic susceptibility for childhood cancer may be underestimated.[9] Our findings lend additional support to the hypothesis that familial factors may play a role in the etiology of childhood tumors.

The observed increased risk for childhood tumors and earlier than average age of onset of common adult cancer in the relatives of patients with childhood cancer may suggest shared pathways that both increase the risk for childhood cancer and modify the common adult tumor onset age. Identifying pathways or candidate genes, such as the IGF-1 pathway, which may confer an increased risk for childhood and adult tumors may reveal novel potential treatment targets.

Our results suggest that every tenth patient with childhood cancer had a relative affected by cancer in childhood or adolescence. Presently, the clinical importance of our findings is limited and does not suggest genetic counseling for families with multiple childhood tumors unless indications of known hereditary syndromes occur. Although single families with multiple childhood tumors may be due to chance, the high incidence of childhood tumors in the relatives of patients with childhood cancer in this study seems unlikely to be caused only by chance. Studying families with multiple childhood tumors may be a valuable approach to improving our understanding of the etiology of childhood tumors and identifying candidate genes or pathways that confer increased risk for childhood cancer.

Study IV

Regardless of family history, a high frequency of germline TP53 mutations have been found among children diagnosed with adrenocortical tumors, choroid plexus tumors and rhabdomyosarcomas. Identification of a germline TP53 mutation could have clinical implications in the management of the patient and may also have consequences for the family. Improved knowledge of whether these rare childhood tumors may be early manifestations of LFS may be helpful in the genetic counseling of patients and family members.

A population-based series of three children with adrenocortical tumors (one adenoma and two carcinomas), seven children with choroid plexus tumors (five papillomas and two carcinomas), and 29 children with rhabdomyosarcomas diagnosed between 1958 and 2008 were identified in the Southern Swedish Regional Tumor Registry for evaluation of the prevalence of germline *TP53* mutations and LFS history. Mutation screening of *TP53* was successfully performed for all patients with adrenocortical tumors, five patients with choroid plexus tumors and 18 patients with rhabdomyosarcomas. In total, two novel *TP53* mutations were identified: one in a patient with adrenocortical carcinoma and one in a patient with rhabdomyosarcoma (Figure 13). The observed *TP53* mutation frequency in children with adrenocortical carcinomas (1/2) and rhabdomyosarcomas (1/18) is similar to previous reports.[59,191,192]

No *TP53* mutations were identified in the five tested patients with choroid plexus tumors, of which four had a papilloma, which is in line with previous findings.[188,189]

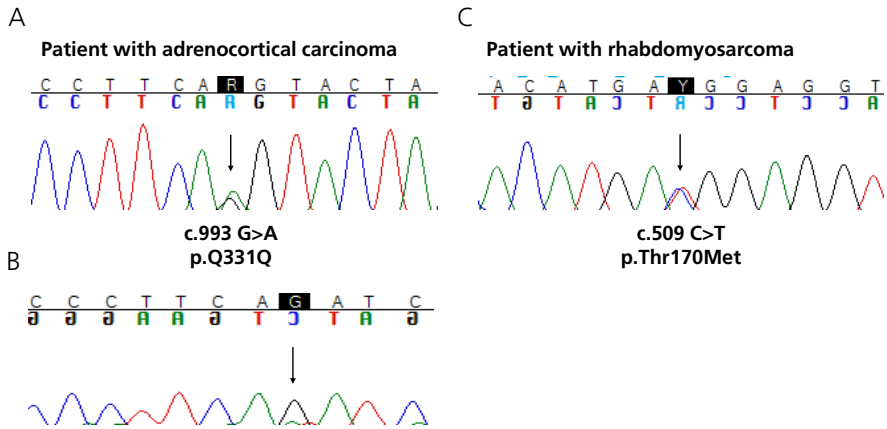

Figure 13. Chromatograms demonstrating the identified germline *TP53* mutations. (A) A silent mutation, with predicted effect on splicing due to its location at the last position of exon 9, was identified in genomic DNA in a patient with adrenocortical carcinoma. (B) Sequencing of cDNA revealed expression of only the wild type allele indicating that the mutation results in an unstable mRNA transcript degraded by nonsense-mediated decay. (C) A missense mutation in exon 5 was identified in genomic DNA in a patient with rhabdomyosarcoma. This substitution results in a change of a highly conserved threonine at codon 170 to a methionine, which is predicted to be functionally disruptive.

In considering the family history of patients identified as germline *TP53* mutation carriers, the child with an adrenocortical carcinoma had a family history corresponding to Chompret[160] and Eeles[159] criteria due to a breast cancer diagnosis in the mother. The mutation was confirmed to be inherited because the mother tested positive for the identified mutation. The patient with rhabdomyosarcoma had no family history corresponding to any of the clinical LFS criteria. The absence of a LFS-associated family history could possibly be due to a *de novo* mutation, which has been found to be relatively common in LFS.[176] Although primarily observed for mutations associated with adrenocortical carcinomas, evidence exists that certain *TP53* alleles confer relatively low penetrance, which may be another possible explanation for the weak family history.[148,173,185] Unfortunately, because the parents were not available for testing, we were unable to investigate the possibility of a *de novo* mutation or the occurrence of a low penetrance allele. In summary, our findings may support previous suggestions that children with adrenocortical carcinoma and young children with rhabdomyosarcomas may be considered for *TP53* mutation screening regardless of family history.[153,160]

Identifying patients harboring a germline *TP53* mutation may have important clinical benefits, which supports suggestions that children with adrenocortical tumors, choroid plexus tumors and rhabdomyosarcomas should be considered for *TP53* screening regardless of family history. In addition to the genetic susceptibility for multiple primary tumors, growing evidence indicates an increased risk of radiation-induced second primary tumors in individuals with germline *TP53* mutations.[156,181,182] Knowledge of the mutation status has the potential to improve the clinical management of patients by modifying treatment strategies to reduce the risk of second primary tumors and also by optimizing the surveillance of the patients in relation to the risk organs.[164,183,184] It has also been suggested that the occurrence of LFS and *TP53* mutations may be used as indicators of clinical outcome in patients with choroid plexus carcinoma, but the data are conflicting.[188,189] However, the identification of *TP53* germline mutations may also have implications for the families. Because evidence of clinical surveillance benefits is limited, presymptomatic testing for LFS has been debated. One of the main concerns regarding offering genetic testing for LFS is its potentially adverse psychological impact. However, recent studies may be reassuring; high-risk family members considering *TP53* testing were found to function psychologically well, and an unfavorable test result was in general not found to cause adverse psychological effects.[235,236] In addition, a comprehensive surveillance program with potential clinical benefits was recently reported, which may lend support to the future counseling and management of families with LFS.[178] Both the psychological consequences and the clinical benefit of the suggested surveillance strategy need further evaluation. Genetic counseling is important and should always be undertaken prior clinical *TP53* testing. Even though individuals with LFS were found to cope well, a substantial minority of individuals were found to exhibit clinically relevant levels of distress, which suggests that genetic counseling also should include assessments tools to identify those individuals in need of professional psychosocial support.[236] Childhood predictive testing in LFS has been demonstrated to be satisfactory in some cases but should only be carefully undertaken on a case by case basis.[237]

Although a proportion of patients with adrenocortical tumors and rhabdomyosarcomas were found to be carriers of germline *TP53* mutations, our results suggest that rhabdomyosarcoma and choroid plexus tumors are not generally considered early manifestations of LFS. In line with mutation screening, few patients had a family history corresponding to the clinical LFS criteria. Furthermore, except for a trend towards an excess of early-onset breast cancer in relatives of patients with choroid plexus tumors, no increased incidence of any LFS-associated tumors, including breast cancer, sarcoma, or brain tumors, was found. The absence of breast cancer in close relatives of rhabdomyosarcoma patients is in contrast with previous

findings.[192,238,239] However, the increased risks found in these studies were suggested to be mainly attributed to LFS, which seems to occur only in a few patients in this cohort. Nevertheless, families of patients with choroid plexus tumors and rhabdomyosarcomas were found to have an increased cancer incidence (Figure 14).

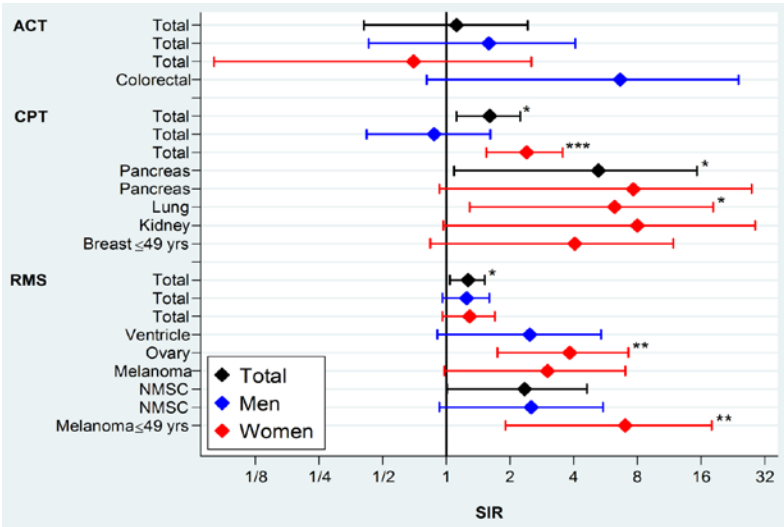

Figure 14. Forest plot of standardized incidence ratios (SIR) for cancers in first to third degree relatives of children with adrenocortical tumors (ACT), choroid plexus tumors (CPT) and rhabdomyosarcomas (RMS). Results for total cancer incidence, in total and stratified by sex, are presented for each histologic type of childhood tumors. In addition, specific tumor types with increased incidence with a *p*-value <0.1and at least two observations are presented. The estimates include tumors diagnosed between age 0 and 79 years, unless otherwise stated. * $p<0.05$; ** $p<0.01$; *** $p\leq0.001$

In particular, an excess of pancreatic and female lung cancer in families with choroid plexus tumors was noted, while the relatives of patients with rhabdomyosarcoma had an excess of ovarian cancers and female early-onset melanomas. Although most of these tumors have been found in excess in some families with LFS,[148-150,152-154] the absence of closely associated LFS tumors and identifiable *TP53* mutations in most of these families may suggest the possibility of the occurrence of other genetic or environmental factors, which may explain the increased risks. However, when the analysis was stratified on germline *TP53* mutation status, the increased incidence for early-onset melanoma was restricted to the relatives of patients with positive or unknown mutation status. Future studies are needed to address the observed associations with particular adult tumors and to elucidate whether the increased incidence of early-onset melanoma is associated with *TP53* mutations.

Conclusions and future aspects

Study I

In this registry-based study, women with breast cancer who had a child with childhood cancer had a significant shorter overall survival than other parous patients with breast cancer. The difference was more pronounced in women with early-onset breast cancer.

- It would be interesting to elucidate whether hereditary genetic factors, such as germline *TP53* mutations, may affect breast cancer prognosis.
- Furthermore, it would be useful to examine whether the difference in survival can be explained by differences in breast tumor characteristics between patients whose children have had or have not had childhood cancers.

Study II

The occurrence of childhood cancer was higher in families with germline mutations in the *BRCA2*, MMR or *CDKN2A* genes compared with the general population. No increased risk of childhood cancer was found in families with *BRCA1* mutations. Furthermore, there was no evidence that supports that the inheritance of biallelic mutations would have caused the childhood tumors in these families.

- It remains to be proven whether the affected children are actually carriers of the respective familial mutation.
- Further studies may elucidate other mechanisms besides biallelic mutations that are involved in the predisposition to childhood cancer in families with hereditary adult cancer syndromes.
- It would be of interest to study the potential role of the *BRCA2*, MMR and *CDKN2A* genes in the development of childhood cancer.

Study III

Increased incidence of childhood and adult tumors was found in the extended families of patients with childhood cancer. In particular, an excess of breast and prostate cancers were found, and these patients had an earlier than average age of onset. Therefore, familial factors may play a role in the etiology of childhood tumors and modify the age of onset of common adult tumors. Germline *TP53* mutations were not found to contribute to the increased incidence of childhood tumors because no *TP53* mutations were found.

- Further studies may elucidate candidate genes or pathways that confer an increased risk for childhood cancer and modify the age of onset for common adult tumors.
- Studying extended families with multiple childhood tumors may be a valuable approach to improving our knowledge of the importance of genes and the environment in the etiology of childhood cancer.

Study IV

Our findings confirm that germline *TP53* mutations may be found in some children with adrenocortical tumors and rhabdomyosarcomas, regardless of family history. These data support the suggestion that children with these malignancies may be considered for *TP53* mutation screening. No *TP53* mutations were found in children with choroid plexus tumors. Taking into account both the mutation status and the family history of cancer our data suggests that most children with these rare tumors, particularly those with choroid plexus tumors and rhabdomyosarcomas, would not be considered early manifestations of LFS. Nevertheless, an increased incidence of cancer, particularly of certain adult tumors that do not belong to the closely associated LFS tumors, was found in the relatives of children with choroid plexus tumors and rhabdomyosarcomas. This suggests that other syndromes or predisposing factors may exist.

- Further studies may investigate the occurrence of possible underlying syndromes or predisposing factors responsible for the increased risk of certain adult tumors in relatives of children with choroid plexus tumors and rhabdomyosarcomas.

In summary, this thesis adds new data suggesting that genetic susceptibility plays a role in the development of childhood tumors. In addition, these factors may also increase the adult tumor risk, modify the age of onset of common adult tumors, and affect breast cancer prognosis. Our findings further support the need for future studies regarding the importance of genetic susceptibility to childhood cancer, particularly in families with multiple childhood tumors. Also the associations between tumors of childhood and adulthood in the same family should be further studied.

In future studies, whole exome sequencing or whole genome sequencing may be useful approaches to study families with remarkable family histories of cancer and may reveal new genes or pathways that may be implicated in the development of childhood and adult cancers. The identification of such genes or pathways may reveal novel potential treatment targets. The occurrence of hereditary factors may be of prognostic importance and needs to be addressed in further studies. Increased knowledge regarding the importance of genetic susceptibility in childhood cancer may improve the medical care of patients by identifying those who are possibly in need of more individualized treatment.

Given the recent increasing knowledge of epigenetics in disease,[240,241] epigenetic factors may play a role in the genetic susceptibility to childhood cancer. Imprinting errors in children with familial Beckwith-Wiedemann syndrome have been observed, which may give rise to unusual inheritance patterns due to a sex-related mode of inheritance.[242-244] Epigenetic factors may be involved in the mechanism behind the observations of multiple childhood tumors in families without a strong family history (study III). Further studies in this area may elucidate a potential role for constitutional epigenetic changes in the predisposition to childhood cancer.

The LCCG-study will provide valuable material in the future that will enable studies of genetic susceptibility and childhood cancer to improve our knowledge of the importance of genetic factors in childhood cancer development. The prospective inclusion of patients will enable the design of prospective studies to examine prognosis and survival in association with hereditary factors. To further enhance the value of this study, we plan to begin the collection of DNA from the parents of the children included in this study. Access to DNA from both children and their parents will enable studies regarding the role of *de novo* mutations, polymorphic variance, low penetrance genes and epigenetic changes, all of which may play important roles in the etiology of childhood cancer.

Populärvetenskaplig sammanfattning

Varje år insjuknar omkring 300 barn i cancer. Förbättrade behandlingsmetoder under de senaste decennierna har lett till att mer än tre av fyra barn idag faktiskt botas från sin sjukdom. Tyvärr drabbas en stor andel av de som överlever sin cancer av så kallade sena komplikationer till följd av den tuffa behandling de har utsatts för. Bland annat har man sett en livslång ökad risk för att drabbas av nya cancersjukdomar. Orsaken till att barn får cancer är i de allra flesta fall okänt, men upp till 10 % av barncancerfallen kan förklaras av kända ärftliga orsaker. Det finns dock studier som tyder på att betydelsen av ärftliga faktorer kan vara underskattad. När barnet insjuknar i sin cancer finns i de flesta fall ingen tidigare cancer i familjen som talar för att det finns någon ärftlig bakomliggande orsak. I en del familjer förändras denna bild med tiden och en barntumör som till en början verkade vara sporadisk kan visa sig vara familjär när föräldrar och syskon blir äldre. Det har visat sig att patienter med en ärftlig benägenhet för cancer har en särskilt hög risk att drabbas av nya tumörer till följd av tidigare cancerbehandling. Ökad kunskap om ärftliga faktorers betydelse för uppkomsten av barncancer skulle kunna förbättra omhändertagandet av patienter och deras familjer genom att identifiera de patienter som är i behov av mer individualiserade behandlingsstrategier.

De fyra studier som ingår i den här avhandlingen har undersökt ärftliga faktorers betydelse vid barntumörer och eventuella samband mellan barn- och vuxentumörer i samma familj. Det har sedan länge varit känt att ärftliga faktorer ökar risken för att drabbas av cancer. Däremot vet vi betydligt mindre om hur ärftliga orsaker påverkar sjukdomsförloppet. I studie I studerades överlevnaden för bröstcancerpatienter i relation till om deras barn har haft barncancer, där barncancer användes som en markör för förekomst av ärftliga faktorer. De mödrar som har haft ett barn med cancer hade en sämre överlevnad jämfört med de andra bröstcancersjuka mödrarna. Detta tyder på att ärftliga faktorer kan ha en betydelse för patienters sjukdomsförlopp.

I studie II granskades förekomsten av barntumörer i familjer med ärftliga vuxencancersyndrom, inkluderat ärftlig bröst- och äggstockscancer, familjär malignt melanom, samt ett syndrom som orsakar ärftlig tjocktarmscancer och som kallas hereditär icke polypös kolorektalcancer (HNPCC). Familjer med HNPCC och familjär malignt melanom uppvisade en högre förekomst av barntumörer jämfört med den generella befolkningen. Ärftlig bröst- och äggstockscancer orsakas av ärftliga genetiska förändringar (mutationer) i bröstcancergen 1 och 2 (*BRCA1* och *BRCA2*).

En ökad förekomst av barntumörer identifierades hos familjer med mutationer i *BRCA2* men inte i familjer med mutationer i *BRCA1*.

I studie III undersöktes risken för barn- och vuxencancer hos släktingar till barncancerpatienter. En ökad risk för både barn- och vuxentumörer påvisades. I var tionde familj fanns ytterligare ett fall av barncancer, ofta hos mer avlägsna släktingar. Den ökade förekomsten av barntumörer kan inte förklaras av sedan tidigare kända ärftliga orsaker. Vuxna släktingar hade en ökad risk för bröst- och prostatacancer, och var yngre vid insjuknande än den genomsnittliga i befolkningen.

Studie IV handlar om ett ovanligt ärftligt tillstånd som kallas Li-Fraumeni syndrom, vilket orsakas av mutationer i genen *TP53*. Individer med detta syndrom har en mycket hög risk för att drabbas av ett flertal olika cancrar, ofta i mycket ung ålder. Man har också sett att dessa individer är känsliga för strålning och att många efter strålbehandling för en cancer med tiden drabbas av en ny tumör i det behandlade området. I familjer med Li-Fraumeni syndrom finns det oftast en stark familjehistoria av cancer hos nära släktingar, dock har man i tidigare studier sett att barn med vissa typer av tumörer ibland kan vara bärare av en mutation i *TP53* utan att det finns en sjukdomsbild i familjen som talar för det. I studie IV undersöktes det vidare hur vanligt förekommande det är med medfödda mutationer i *TP53* hos barncancer-patienter med binjurebarkstumörer, plexus-tumörer (en ovanlig typ av hjärntumör) och rabdomyosarkom (tumör som utvecklas från muskelceller). Medfödda *TP53* mutationer kunde identifieras hos enstaka patienter med binjurebarkstumörer och rabdomyosarkom. Detta talar för att man skulle kunna överväga att testa barn med dessa tumörformer för *TP53* mutationer, för att kunna anpassa behandlingen och minska risken för nya terapirelaterade tumörer. I de allra flesta fall kunde barn-tumörerna inte kopplas till förekomst av medfödda *TP53* mutationer. En ökad förekomst av cancer påvisades dock i familjer till patienter med plexus-tumörer och rhabdomyosarkom, vilket tyder på att eventuellt andra ärftliga faktorer kan förekomma i familjerna.

Sammanfattningsvis tillför den här avhandlingen ytterligare stöd för att ärftliga faktorer kan spela en roll för uppkomsten av barntumörer. Framtida forskning bör särskilt inriktas på att studera sambandet barn- och vuxentumörer i samma familj och familjer med multipla barntumörer.

Acknowledgements

The work in this thesis was performed at the Department of Oncology, Lund University. I am truly grateful to everyone that kindly contributed during this journey, making this work possible as well as making these years enjoyable and memorable. I would especially like to thank:

My supervisor *Håkan Olsson,* for accepting me as a PhD student and for enthusiastic and inspiring support during these years. You have always been there showing kindness, thoughtfulness, and encouragement whenever needed. Thank you for introducing me into the scientific world. It has been a pleasure to work with you.

My co-supervisors; *Thomas Wiebe*, *Ulf Kristoffersson* and *Helena Jernström,* It have been a pleasure to work with you. *Thomas*, for your enthusiastic support, encouragement, and sharing your vast knowledge of childhood cancer. *Ulf*, for your accuracy, encouragement, and giving me insight to your huge knowledge of genetics. *Helena*, for your kind support and for sharing your excellent writing skills.

Mef Nilbert, *Bo Baldetorp*, and *Mårten Fernö* for running the department and creating a stimulating and enjoyable atmosphere. *Bo Baldetorp* for your optimism and thoughtfulness.

All my co-authors and collaborators, especially, *Åke Borg* and *Mef Nilbert* for valuable discussions and productive collaborations. *David Gisselsson Nord*, for your enthusiasm, fruitful collaborations, and for sharing your tremendous knowledge of pathology and genetics of childhood cancer. I really appreciated taking part in your journal clubs! *Jenny*, *Linda*, *Gisela*, *Daniel*, *Charles*, and *Ingrid*, thank you for stimulating and educational discussions regarding childhood cancer.

Pär-Ola Bendahl and *Philippe Wagner* for your generous help and for patiently trying to get me to understand statistics. *Ingrid Mårtensson*, *Karin Henriksson*, *Daniel Filipazzi-Zackrisson*, and *Bo Midberg* for always being so helpful.

All co-workers involved in the LCCG-study, especially *Jessica*, *Ingrid*, *Charlotte*, *Karin*, *Viveka*, *Agneta*, *Marie*, and *Anna* for patient recruitment and blood sampling. Without your enthusiasms and invaluable efforts this had not been possible to perform. *Sol-Britt*, for assistance with the blood samples. *Mercedes*, for providing us with an excellent database in INCA. *Ingemar*, for patient detective work searching for old patient charts. *Lars Hjorth*, *Eva Cavallin-Ståhl*, *Christian Moëll*, and *Anna Sällfors-*

Holmqvist, for giving me an insight in the clinical work with childhood cancer patients and survivors.

All former and present members at the Department of Oncology for creating a pleasant and stimulating atmosphere. Especially, *Anita* and *Elisabeth*, for your never ending support, help, and encouragement. I really appreciate your thoughtfulness. *Ulla*, *Gunilla*, *Tessan*, *Steina* and *Ellinor* for always helping me in the lab. *Eva* and *Mats* for your generously support, help, and fruitful discussions. *Anders*, for guidance in how to manage and link large files in Excel. You saved me many long working days! *Susanne A* for your incredible ability to solve whatever administrative problem that may occur and for being the kindhearted, warm, and helpful person you are.

My former and present room-mates, *Erika*, *Emma*, *Maria Hi*, *Carolina*, and *Emelie* for many laughs, support, and encouragement whenever needed, and for good friendship.

My fellow PhD colleagues for interesting and fruitful scientific discussions, especially *Maria He*, *Sophie*, *Katrin*, *Carina* and, *Anna E* for never ending support and thoughtfulness and for being not only colleagues but also really dear friends!

All my dear friends, especially, *Anna* and *Emma* for being such wonderful friends always being there for me, and *Emma*, for drawing the illustrations to this thesis.

My Lundahl/Ersbrand family, for letting me be a part of your wonderful family, and for endless support and encouragement. My dear 'sisters', *Karin* and *Hanna*, what would I be without you?

My beloved family, for endless love and support and for always believing in me. My *dad*, for always being there for me, I am truly grateful for that. I honestly don't know what I would have done without you during these years. My *mum*, I really wish you still were here with us. My *brother*, for being the best brother in the world.

My thoughts and gratitude also go to all patients and their families who generously participated in this work.

The studies of this thesis were supported by grants from the Swedish Childhood Cancer Foundation, Swedish Cancer Society, South Swedish Health Care Region (Region Skåne) and Lund Hospital Fund. I also gratefully acknowledge travel grants from the John and Augusta Persson Foundation and the Childhood Cancer Foundation.

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