From THE INSTITUTE OF ENVIRONMENTAL MEDICINE
Karolinska Institutet, Stockholm, Sweden

GENES AND BRAIN TUMORS

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Karolinska Institutet

Stockholm 2018

Published by Karolinska Institutet.
Printed by Eprint AB 2017

ISBN 978-91-7676-871-6

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THESIS FOR DOCTORAL DEGREE (Ph.D.)

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To the memory of my beloved grandparents

“Education isn’t for getting a job.

It’s about developing yourself as a human being.”

Liz Berry

ABSTRACT

Although brain tumors are rare and combined with other nervous system tumors account for ~ 2% of all cancers, they are the second most common type of pediatric cancer. The etiology of brain tumors, known as multifactorial traits, is poorly understood. In this thesis we aimed at identifying genetic risk factors for pediatric brain tumors by investigating the association between adult glioma susceptibility loci and risk of pediatric brain tumors.

Phacomatoses are a series of rare genetic syndromes that predispose individuals to development of nervous system tumors. The etiology of de novo occurrence of phacomatoses is also largely unknown. It is hypothesized that de novo phacomatoses and nervous system tumors might share common risk factors. Therefore, in this thesis, we also assessed the association between parental age and risk of de novo phacomatoses in offspring.

Study I is a systematic review and meta-analysis of published studies investigating the association between germ-line single nucleotide polymorphisms (SNPs) of DNA repair genes and glioma risk. In total, 105 SNPs in 42 DNA repair genes were identified of which 10 SNPs in 7 DNA repair genes were evaluated in at least 4 studies and therefore were included in our meta-analysis. Based on the findings of this study we can conclude that low-penetrance susceptibility loci for glioma are located on *ERCC1*, *ERCC2 (XPD),* and *XRCC1* while variations in DNA repair genes *MGMT* and *PARP1*might protect against glioma risk.

Studies II and III are based on the CEFALO study which is a population-based multicenter case-control study of children and adolescents diagnosed with intracranial central nervous system tumors aged 7-19 years at diagnosis. In total, saliva DNA from 245 cases and 489 controls was included in these two studies. In **Study II** saliva DNA was genotyped for 29 SNPs identified by genome-wide association studies (GWAS) on adult glioma. The findings of this study indicate that the adult glioma GWAS susceptibility loci at 5p15.33 (*TERT*), 8q24.21 (*CCDC26*), 9p21.3 (*CDKN2A-CDKN2B*), and 20q13.33 (*RTEL1*) are also associated with pediatric brain tumor risk. In **Study III** saliva DNA was genotyped for 68 SNPs identified by candidate-gene association studies of adult glioma related to DNA repair, cell cycle, metabolism, and inflammation pathways. In total, 63 SNPs were satisfactorily genotyped. This study provides evidence that of the investigated genetic variations, variants in *EGFR*, *ERCC1*, *CHAF1A*, *XRCC1*, *EME1*, *ATM*, *GLTSCR1*, and *XRCC4,* belonging to DNA repair and cell cycle pathways, known to be associated with adult glioma, are also associated with pediatric brain tumors risk. The findings of **Studies II and III** together indicate that adult and pediatric brain tumors probably have some genetic risk factors in common.

Study IV is a nested case-control study within the Swedish population. By using the Patient register, 4625 phacomatosis cases were identified and further classified as familial or non-familial. Ten controls per case were randomly selected from the eligible population. Analyses were performed for neurofibromatosis alone and other phacomatoses combined. This study

indicates that advanced paternal age increases the risk of de novo occurrence of phacomatoses in offspring with the most pronounced effects on neurofibromatosis.

This thesis provides evidence that adult and pediatric brain tumors probably have common genetic risk factors and might share similar etiological pathways. Moreover, this thesis provides evidence of an increased risk of de novo neurofibromatosis by increasing paternal age, suggesting an increasing rate of de novo mutations in the *NF1* and *NF2* genes in older fathers' sperm.

LIST OF SCIENTIFIC PAPERS

This thesis is based on the following articles/manuscripts which will be referred to in the text by their Roman numerals.

I. **Adel Fahmideh M**, Schwartzbaum J, Frumento P, Feychting M.
Association between DNA repair gene polymorphisms and risk of glioma: a systematic review and meta-analysis.
Neuro Oncol. 2014 Jun;16(6):807-14.

II. **Adel Fahmideh M**, Lavebratt C, Schüz J, Röösli M, Tynes T, Grotzer MA, Johansen C, Kuehni CE, Lannering B, Prochazka M, Schmidt LS, Feychting M.
CCDC26, CDKN2BAS, RTEL1 and TERT polymorphisms in pediatric brain tumor susceptibility.
Carcinogenesis. 2015 Aug;36(8):876-82.

III. **Adel Fahmideh M**, Lavebratt C, Schüz J, Röösli M, Tynes T, Grotzer MA, Johansen C, Kuehni CE, Lannering B, Prochazka M, Schmidt LS, Feychting M.
Common genetic variations in cell cycle and DNA repair pathways associated with pediatric brain tumor susceptibility.
Oncotarget. 2016 Sep 27;7(39):63640-63650.

IV. **Adel Fahmideh M**, Tettamanti G, Lavebratt C, Talbäck M, Mathiesen T, Lannering B, Johnson K.J., Feychting M.
Parental age and risk of genetic syndromes predisposing to nervous system tumors: nested case-control study.
Submitted

CONTENTS

LIST OF ABBREVIATIONS

A	Adenine
AD	Autosomal dominant
AICDA	Activation induced cytidine deaminase
ALDH3A1	Aldehyde dehydrogenase 3 family member A1
ALOX5	Arachidonate 5-lipoxygenase
APC	APC, WNT signaling pathway regulator
AR	Autosomal recessive
AT	Ataxia telangiectasia
AT/RTs	Atypical teratoid/rhabdoid tumors
ATM	ATM serine/threonine kinase
ATRX	ATRX, chromatin remodeler
BCHE	Butyrylcholinesterase
BRAF	B-Raf proto-oncogene, serine/threonine kinase
C	Cytosine
CA	Canada
CASP14	Caspase 14
CASP8	Caspase 8
CAT	Catalase
CCDC26	CCDC26 long non-coding RNA
CCND1	Cyclin D1
CCNH	Cyclin H
CDK4	Cyclin dependent kinase 4
CDKN2A	Cyclin dependent kinase inhibitor 2A
CDKN2B	Cyclin dependent kinase inhibitor 2B
CHAF1A	Chromatin assembly factor 1 subunit A
CHEK2	Checkpoint kinase 2
Chr	Chromosome
CI	Confidence interval
CNS PNETs	Central nervous system primitive neuroectodermal tumors
COX2	Cytochrome c oxidase subunit II

CREBBP	CREB binding protein
CT	Computed tomography
CTNNBI	Catenin beta like 1
CYP1A1	Cytochrome P450 family 1 subfamily A member 1
CYP2E1	Cytochrome P450 family 2 subfamily E member 1
DNA	Deoxyribonucleic acid
DOM	Dominant model
EFEMP1	EGF containing fibulin extracellular matrix protein 1
EGFR	Epidermal growth factor receptor
EME1	Essential meiotic structure-specific endonuclease 1
ERBB2	Erb-b2 receptor tyrosine kinase 2
ERCC1	ERCC excision repair 1, endonuclease non-catalytic subunit
ERCC2	ERCC excision repair 2, TFIIH core complex helicase
ETMR	Embryonal tumors with multilayered rosettes
FMO1	Flavin containing monooxygenase 1
FMO3	Flavin containing monooxygenase 3
G	Guanine
GDP	Guanosine diphosphate
Gliogene	Glioma International Consortium
GLTSCR1	Glioma tumor suppressor candidate region gene 1
GRS	Genetic risk score
GSTM1	Glutathione S-transferase mu 1
GSTM3	Glutathione S-transferase mu 3
GSTP1	Glutathione S-transferase pi 1
GSTT1	Glutathione S-transferase theta 1
GTP	Guanosine triphosphate
GWAS	Genome-wide association studies
hMLH1	Human mutL homolog 1
hMSH2	Human mutS homolog 2
hMSH6	Human MSH6 protein
HWE	Hardy-Weinberg equilibrium

ICCC	International Classification of Childhood Cancer
ICCC-3	Third edition of International Classification of Childhood Cancer
ICD-10	Tenth revision of the International Classification of Diseases
ICD-7	Seventh revision of the International Classification of Diseases
ICD-8	Eighth revision of the International Classification of Diseases
ICD-9	Ninth revision of the International Classification of Diseases
ICD-O	International Classification of Diseases for Oncology
ICD-O-3	Third edition of International Classification of Diseases for Oncology
IDH1	Isocitrate dehydrogenase (NADP(+)) 1, cytosolic
IDH2	Isocitrate dehydrogenase (NADP(+)) 2, mitochondrial
IGF1R	Insulin like growth factor 1 receptor
IL-10	Interleukin 10
IL-13	Interleukin 13
IL-4	Interleukin 4
IL-4RA	Interleukin 4 receptor
IL-6	Interleukin 6
IL-8	Interleukin 8
KIF18B	Kinesin family member 18B
KRAS	KRAS proto-oncogene, GTPase
LD	Linkage disequilibrium
LISA	Longitudinal Integration Database for Health Insurance and Labour Market Studies
Log	Logarithm
LTL	Leukocyte telomere length
MALDI-TOF	Matrixassisted laser desorption/ionization-time-of-flight
MDM2	MDM2 proto-oncogene
MGMT	O-6-methylguanine-DNA methyltransferase
MRI	Magnetic resonance imaging
MTHFR	Methylenetetrahydrofolate reductase
MTRR	5-methyltetrahydrofolate-homocysteine methyltransferase reductase
MYC	MYC proto-oncogene, bHLH transcription factor

MYCN	MYCN proto-oncogene, bHLH transcription factor
MYO19	Myosin XIX
NC	North Carolina
NF1	Neurofibromatosis type 1
NF2	Neurofibromatosis type 2
NOS1	Nitric oxide synthase 1
OGG1	8-oxoguanine DNA glycosylase
OMIM	Online Mendelian Inheritance in Man
ON	Ontario
OR	Odds ratio
$p14^{ARF}$	p14ARF protein
$p16^{INK4A}$	Cyclin dependent kinase inhibitor 2A
PARP1	Poly (ADP-ribose) polymerase 1
PBT	Pediatric brain tumor
PHLDB1	Pleckstrin homology like domain family B member 1
PMS2	PMS1 homolog 2, mismatch repair system component
PON1	Paraoxonase 1
POT1	Protection of telomeres 1
PRKAR1A	Protein kinase cAMP-dependent type I regulatory subunit alpha
PRMT8	Protein arginine methyltransferase 8
PTCH1	Patched 1
PTCH2	Patched 2
PTEN	Phosphatase and tensin homolog
PTGS2	Prostaglandin-endoperoxide synthase 2
RB1	RB transcriptional corepressor 1
REC	Recessive model
RPA3	Replication protein A3
RR	Risk ratio
RTEL1	Regulator of telomere elongation helicase 1
RUNDC1	RUN domain containing 1
SELP	Selectin P

SLC7A7	Solute carrier family 7 member 7
SMARCB1	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily b, member 1
SNPs	Single nucleotide polymorphisms
SOD1	Superoxide dismutase 1
SOD2	Superoxide dismutase 2
SOD3	Superoxide dismutase 3
SOX5	SRY-box 5
SPAG9	Sperm associated antigen 9
SPOP	Speckle type BTB/POZ protein
STKY1	Serine threonine tyrosine kinase 1
SUFU	SUFU negative regulator of hedgehog signaling
T	Thymine
TERC	Telomerase RNA component
TERT	Telomerase reverse transcriptase
TP53	Tumor protein p53
Trend	Cochran-Armitage trend test of additivity
TSC	Tuberous sclerosis complex
TSC1	Tuberous sclerosis 1
TSC2	Tuberous sclerosis 2
U.S.	United States of America
VEGFR2	Kinase insert domain receptor
VHL	von Hippel-Lindau tumor suppressor
VHL	von Hippel-Lindau syndrome
WHO	World Health Organization
XPD	ERCC excision repair 2, TFIIH core complex helicase
XRCC1	X-ray repair cross complementing 1
XRCC4	X-ray repair cross complementing 4
XRCC5	X-ray repair cross complementing 5

1 INTRODUCTION

Brain tumors are rare and combined with other nervous system tumors account for ~ 2% of all cancers [1]; however, they are the most common pediatric solid tumors and represent a substantial burden in terms of morbidity and mortality in children. The etiology of brain tumors is largely unknown. The only established risk factors for brain tumors are exposure to ionizing radiation and rare inherited single gene disorders that all together cause a minority of cases [2]. Brain tumors are considered as multifactorial traits resulting from the alterations in various genes and their interactions with multiple environmental factors. Despite the extensive research, a small proportion of genetic variants contributing to the genetic architecture of brain tumors are detected of which the majority are attributable to adult brain tumors and the knowledge on the genetic risk factors of pediatric brain tumors remained limited.

Furthermore, the etiology of de novo phacomatoses, a series of rare genetic disorders known as to be nervous system tumors risk factors [3], is poorly understood. It is hypothesized that de novo phacomatoses and nervous system tumors might have common risk factors which provides a starting point of considering the potential mediator role of de novo phacomatoses in the observed associations for nervous system tumors.

This thesis aimed at identifying genetic variations of importance for pediatric brain tumor susceptibility and investigating whether adult and pediatric brain tumors share common genetic risk factors and have similar genetic architecture. Moreover, the association between parental age and risk of de novo phacomatoses predisposing to nervous system tumors in offspring was investigated in this thesis.

2 BACKGROUND

2.1 BRAIN TUMORS

2.1.1 Clinical Features and Histological Classification

A brain tumor forms when abnormal cells grow within the brain. Headache, nausea, vomiting, drowsiness, seizures, and visual abnormalities are the most common symptoms of brain tumors [4]. Brain tumor diagnosis is usually based on magnetic resonance imaging (MRI); if no MRI is available, which rarely happens, computed tomography (CT) scanning can also be used as a first diagnostic procedure [4]. Brain tumors are either primary, originating from the brain tissue, or metastatic tumors originating from a distant site. Brain tumors are heterogeneous in pathology, histopathology, mortality, and molecular features. Brain neoplasms are subclassified based on the International Classification of Diseases for Oncology (ICD-O) which has been initiated in 1976 and is a coding system for both the site (topography) and the histology (morphology) of neoplasms, that are usually obtained from a pathology report. The third edition of ICD-O (ICD-O-3) is its latest version which has been available since 2000. The first revision of ICD-O-3 (ICD-O-3.1) with some new or modified codes and terms was published in 2013. However, it has been established that the classification of childhood cancer should be based on the morphology of the tumor rather than the emphasis on primary site of origin [5]. Therefore, in 1996, the International Classification of Childhood Cancer (ICCC) was released. The third edition of ICCC (ICCC-3) which is the latest version, is based on ICD-O-3 and was published in 2005 [5]. Brain tumors can be further classified into several subtypes based on their morphology, genetic alterations, location, age distribution, biologic behavior, and clinical outcome according to the World Health Organization (WHO) classification of tumors of the central nervous system [6].

Adult brain tumors are classified into four major histologic categories including tumors of neuroepithelial tissue (gliomas), tumors of meninges, tumors of sellar region, and germ cell tumors [7]. Of these, gliomas are the most frequent type of primary brain tumors accounting for more than 70% of these tumors. Gliomas are further subclassified based on their predominant cell type of which glioblastoma is the most common and aggressive subtype with the mean age at onset of 53 years [4,7].

Pediatric brain tumors are categorized into glial tumors and nonglial (neuronal) tumors which arise from glial cells and neurons, respectively. Of glial tumors, gliomas are the majority of brain tumors and include astrocytomas, oligodendrogliomas, and ependymomas which share similar morphologies with the different lineages of glial cells: astrocytes, oligodendrocytes, and ependymal cells, respectively [8]. The most frequent histological type of pediatric brain tumors is astrocytoma which accounts for approximately 52% of the tumors and the age-specific incidence rates has two peaks; at age 5 and 13 years. Ependymoma and other gliomas account for 15% and 9% of pediatric brain tumors, respectively [2].

Neuronal tumors are dominated by embryonal tumors including medulloblastoma, which is the most frequent malignant brain tumor in children [8] , as well as atypical teratoid/rhabdoid tumors (AT/RTs) and other central nervous system primitive neuroectodermal tumors (CNS PNETs) [9]. PNET and medulloblastoma are considered as the second most common types of pediatric brain tumors with a frequency of about 21% [2]. Medulloblastoma and CNS PNET are often histologically indistinguishable, and were previously separated by their different anatomical sites. However, today we know they are two biologically different entities. Recently, the CNS PNET term has been removed from the diagnostic lexicon of WHO and reclassified as embryonal tumors with multilayered rosettes (ETMR), ependymoblastoma, and medulloepithelioma [6].

For brain tumors, localization also plays an important role, especially in overall prognosis of the patients [8].

2.1.2 Descriptive Epidemiology

The incidence of brain tumors differs by gender, age, race and ethnicity, and geography. The overall incidence rates of pediatric brain tumors in Sweden (1984-2005), Europe (1988-1997) and the United States (2005-2009) was 4.2 [10], 2.9 [11], and 5.0 [12] per 100,000 children up to 15 years of age, respectively. Pediatric brain tumors are slightly more common among boys than girls [10]. Brain tumors are relatively more common in adults than children; for example in the United States (2005-2009), the incidence in adults (20+ years) was 26.8/100,000 [12].

The brain tumor incidence has increased over time especially in a 10-year period from the mid-1980s to 1990s in which most of this increase is probably due to improved diagnostic imaging such as CT and MRI scans, refinements in classifications of specific tumor histologies, and improvements in medical care system and treatment of older patients. The incidence rate has been relatively stable since the 1990s [7].

Changes in classifications of tumor histologies also influenced the overall incidence of individual histologic types. In addition, the biologic variations of brain tumors with age lead to different incidence rates of specific histologic types in children compared to adults [7]. In children, glioma is the most common subtype [13], whereas in adults the incidence rates differ by gender. In men, glioma is the most common subtype, while in women the incidence rate for meningioma in Sweden is slightly higher than for glioma. The incidence rate for meningioma in men is considerably lower than in women (National Board of Health and Welfare, online database http://www.socialstyrelsen.se/statistik/statistikdatabas/cancer). This pattern varies between countries and is largely dependent on the completeness of tumor registration, especially with regard to benign tumors like meningioma. Meningioma is extremely rare in children.

Survival of brain tumor patients varies greatly and depends on various parameters including histologic type, genetic alterations, age at diagnosis, proliferation indices, tumor location, and extent of resection [14]. Despite these factors, adult patients diagnosed with WHO grade II and grade III brain tumors survive for more than 5 years and 2-3 years, respectively. The

prognosis for glioblastoma patients is poor and less than one third survive longer than 1 year [14]. In general, survival is relatively lower for patients in older ages; i.e. older than 65 years of age, and prognosis is slightly better in women than in men [7].

The survival rate for pediatric brain tumors overall at 10 years follow-up in Sweden was 72% (1984-2005) [10]; however, the prognosis varies considerably between tumor subtypes and is still poor for malignant brain tumors. Moreover, the prognosis is poorer among young children compared with older children, which is partly due to the fact that young children cannot be treated as intensively as older children [10]. Currently, the primary predicting factor for brain tumor survival is histologic type; however, molecular classification of brain tumors identified different tumor progression within specific histological types. Therefore, recent studies have focused on identifying molecular prognostic factors for brain tumor subtypes [15].

2.1.3 Heritability and Genetic Susceptibility

Genetic susceptibility is an inherited increase in the likelihood of developing a disease or trait resulting from specific inherited genetic variations that can have high or low impact on the development of a disease. When a single defective gene has a large effect on occurrence of a disease (a so-called high-penetrance gene), this genetic disorder is called a monogenic disease. The effect of this single gene on the risk of the disease is the same in all families [16]. Such disorders are rare and follow the Mendelian rules of inheritance. Comprehensive information of such human genes and genetic disorders can be obtained from Online Mendelian Inheritance in Man (OMIM™) (www.ncbi.nlm.nih.gov/omim) [17]. However, in most diseases variations in more than one gene result in a genetic predisposition to the clinical phenotype. Such complex diseases in which each genetic variation is often neither sufficient nor necessary and influences the disease phenotype to a small extent are called polygenic diseases. Moreover, environment and lifestyle play major roles in complex disease susceptibility [16,18]. While large population-based studies confer the significance and the relative impact of certain genes on the clinical phenotype of a disease, the effect of these genes could be completely different in different families. In addition, a rare genetic variation that might have a large effect on the disease susceptibility in one family, would have a very small and non-significant impact on the population level due to its rarity in the general population [18] (Figure 1). Therefore, it is important to take into consideration that although each genetic variation might only slightly increase a person's disease susceptibility, harboring variations in several different genes combined may significantly increase the disease risk. Hence, assessing the polygenic risk scores to investigate the combined effect of several different genetic variations underlying susceptibility to complex diseases and applying the risk prediction models are valuable.

Several approaches are used to study genetic factors that contribute to the development of a disease or trait including linkage studies, candidate-gene association studies and genome-wide association studies (GWAS) [16,19]. Linkage studies are performed in families to identify strongly associated and independently replicated loci. The linkage approach has been

successfully employed in identifying high-penetrance genes involved in monogenic diseases. Subsequently, this approach has been used in identifying the rare susceptibility loci underlying complex diseases [19]. The genetic association studies investigate the association between mostly single nucleotide polymorphisms (SNPs) and a specific phenotype or disease. In the candidate-gene association studies one or few SNPs in or near a gene are investigated based on *a priori* knowledge of the function of a particular gene and its possible role in the disease of interest. In GWAS, thousands of SNPs across the whole genome are investigated in relation to a specific trait or disease without *a priori* hypothesis. GWAS identify common genetic variations with a small impact on the risk of disease (low to moderate penetrant susceptibility genes) [19].

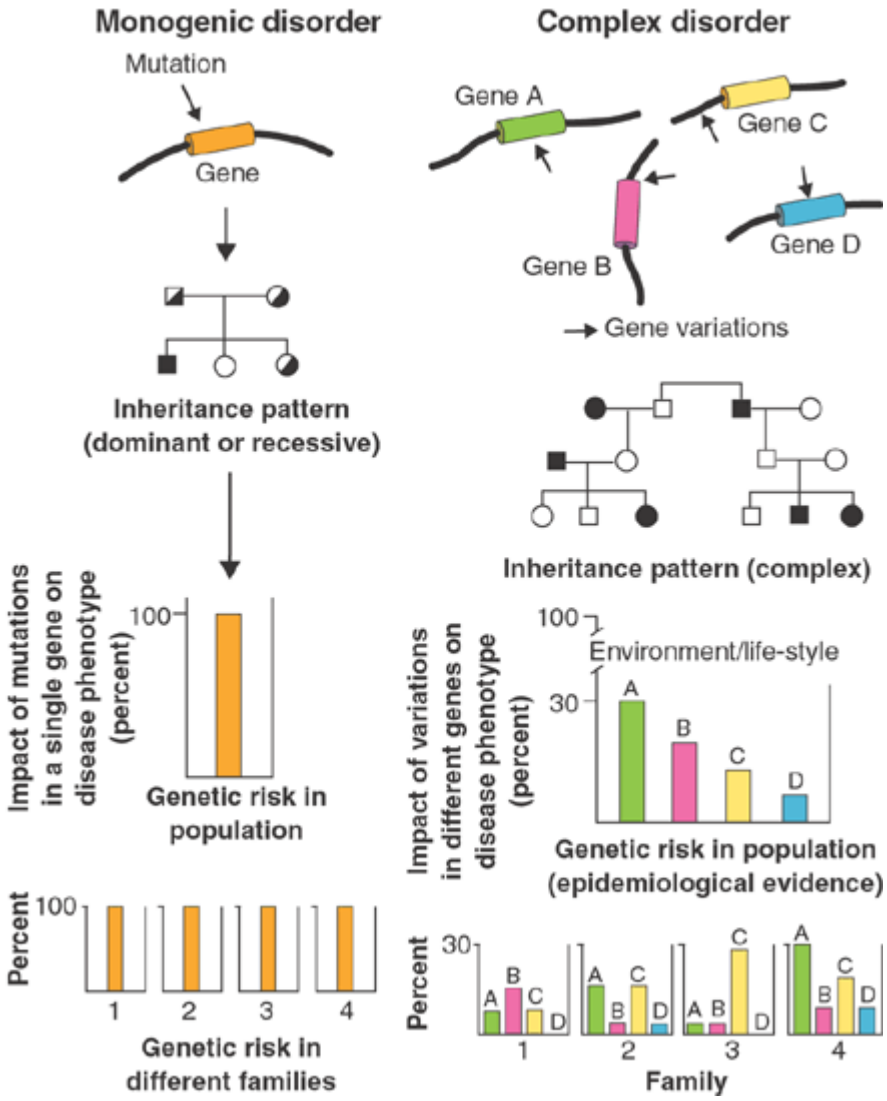

Figure 1. Effects of genetic risk factors on monogenic and complex disorders. *From Peltonen L, McKusick VA. Genomics and medicine. Dissecting human disease in the postgenomic era. Science, 2001* [18] *. Reprinted with permission from AAAS.*

Brain tumors are considered as multifactorial traits resulting from the alterations in various genes, where each has a very small impact, and the interactions between them in concert with multiple environmental factors. Very few and small studies have evaluated the effects of heritability in brain tumors using quantitative genetic analyses [20,21]. Estimates have shown that roughly 12% [21] of the risk of brain tumorigenesis is attributable to the genetic constitution, however the results are not consistent among studies. A small proportion of the estimated heritability is explained by genetic variations involved in familial brain tumors as well as in several genetic syndromes including Li-Fraumeni and Turcot's syndromes, neurofibromatosis type 1 and 2, von Hippel-Lindau disease, Gorlin syndrome and tuberous sclerosis (Table 1). The rest of the genetic constitution is attributed to the genetic variations which are already identified as susceptibility loci by genetic association studies, and also likely the genetic variants which are not located in these identified regions and did not reach the statistical significance level in genetic association studies and therefore remained undetected [22,23] as well as the mutations that potentially will be identified by the whole genome sequencing.

Table 1. Genetic syndromes predisposing to nervous system tumors

Syndrome (OMIM) [a]	Gene	Locus	Inheritance
Neurofibromatosis type 1 (162200)	*NF1*	17q11.2	AD[b]
Neurofibromatosis type 2 (101000)	*NF2*	22q12.2	AD
Schwannomatosis (162091)	*NF2/SMARCB1/?*	22q12.2/22q11.23/?	AD
Tuberous sclerosis complex (191100)	*TSC1/TSC2*	9q34.13/16p13.3	AD
Li-Fraumeni (151623)	*TP53/CHEK2*	17p13.1	AD
Gorlin (Basal cell nevus syndrome) (109400)	*PTCH1/PTCH2/SUFU*	9q22.32/1p34.1/10q24.32	AD
Turcot (276300)	*APC/ hMLH1/PMS2/ hMSH2/hMSH6*	5q22.2/3p22.2/7p22.1/2p 21-p16/2p16.3	AR[c]
Cowden (158350)	*PTEN*	10q23.31	AD
Rubinstein-Taybi (180849)	*CREBBP*	16p13.3	AD
Multiple hamartoma (601728)	*PMS2/PTEN*	7p22.2/10q23.31	AD
Ataxia telangiectasia (208900)	*ATM*	11q22.3	AR

von Hippel-Lindau (193300)	*VHL/CCND1*	3p25.3/11q13.3	AD
Carney complex (160980)	*PRKAR1A*	17q24.2	AD
Melanoma-astrocytoma syndrome (155755)	*CDKN2A*	9p21.3	AD
Hereditary retinoblastoma (180200)	*RB1*	13q14.2	AD

a: OMIM= Online Mendelian Inheritance in Man; **b:** AD=Autosomal dominant; **c:** AR=Autosomal recessive

2.1.4 Single Nucleotide Polymorphisms

Genetics is a field of biology that studies heredity, genes, and genetic variations in living organisms. The genome is a long sequence of DNA containing the hereditary information of every living organism. In humans, the genome is physically divided into 22 pairs of autosomal chromosomes, one pair of allosomes (XX in women and XY in men), and the mitochondrial genome [24]. Functionally, the genome is composed of genes, the sequences of DNA that produce functional products or regulate the genes' expression. The DNA sequence is made up of four nucleotides cytosine (C), guanine (G), adenine (A), and thymine (T) [24]. The whole human genome consists of ~3.3×10^9 base pairs of DNA and is 99.9% identical between individuals. The main cause of diversity and variation among individuals is mutations that can occur at different levels including across the whole genome, within a gene, or at a single nucleotide site that is the smallest mutation (point mutation) [24]. Mutation is a rare event and a mutation that occurs with the frequency of more than 1% in the population is called polymorphism. In a single nucleotide polymorphism (SNP), a specific base position can be occupied by two or sometimes several possible nucleotide variations (also called alleles). The frequency of alleles could differ between patients and non-patients in the study population and therefore the variant could be associated with a disease. The human genome contains several million SNPs that are located in both coding and non-coding regions. The SNP is considered functional if it sits in a coding region and alters the gene function and gene product or if it is located in a regulatory region affecting the gene expression. Roughly, 3-5% of the SNPs are functional [25].

The human Genome Project [26], the SNP Consortium [27], and the International HapMap Project [25] genotyped ~10 million SNPs in a limited set of global samples and established linkage disequilibrium (LD) patterns. Later, the 1000 Genome Project applied the whole-genome sequencing to the DNA from 2504 individuals and characterized 84.7 million SNPs [28]. By putting the knowledge of these SNPs together and using the established methods, the genome-wide association studies, which are hypothesis-free, are developed and could identify several novel susceptibility loci associated with different human complex diseases.

2.1.5 Genetic Risk Factors in Pediatric Brain Tumors

Very few and generally small genetic association studies have been conducted on brain tumors in children and adolescents, therefore, the importance of genetic variations in the etiology of pediatric brain tumors is largely unknown. Searles Nielsen and colleagues examined the association between common variations in genes involved in metabolism pathways including *PON1*, *BCHE*, *FMO1*, *FMO3*, *ALDH3A1*, and *GSTT1*, and risk of pediatric brain tumors among 201 cases and 285 controls, ≤ 10 years of age, born in California or Washington State; but no statistically significant associations were detected [29]. Salnikova *et al.* assessed the effects of genetic polymorphisms in *CYP1A1*, *GSTM1*, *GSTT1*, *GSTP1*, *GSTM3*, *XRCC1*, *XPD*, *OGG1*, *NOS1* and *MTHFR* on risk of pediatric brain tumors [30]. The study was conducted on 172 patients and 183 unmatched controls. They found that the risk of malignant brain tumors in children was associated with variants in *CYP1A1*, *NOS1*, and *XPD*. This study also showed an association between development of malignant brain tumors and deletion of *GSTT1* variants and even a higher risk associated with a double deletion of variants in *GSTM1-GSTT1*[30].

One study on 70 cases and 140 controls of Korean children under the age of 10 found that polymorphisms in *AICDA* and *CASP14* predispose to brain tumors in this population. These genes are known to be related to apoptosis and cell cycle control [31]. Sirachainan *et al.* investigated the association between polymorphisms in the genes encoding enzymes of the folate pathway and brain tumor susceptibility in Thai children [32]. The study was performed on 73 children, under the age of 18 years, with different types of central nervous system tumors and on 205 controls matched on age and sex. The results indicated an association between *MTHFR* polymorphisms and risk of embryonal brain tumors suggesting that folate metabolism might be important in the pathogenesis of some brain tumor subtypes in Thai children [32]. Greenop and colleagues also assessed the associations between variations in folate pathway genes and risk of pediatric brain tumors [33]. The study was a population-based case-control study performed on 321 cases and 552 controls in Australia. They found weak evidence that *MTRR* 66GG could confer protection against pediatric brain tumors particularly in combination with pre-pregnancy folic acid supplementation of the mother [33].

Yilmaz *et al.* investigated whether common variants in Vitamin D receptor gene are associated with pediatric brain tumor susceptibility [34]. No evidence of association between these genetic variations and risk of pediatric brain tumors was observed in this study which was based on 32 cases and 40 controls of Indian children [34].

More studies are required to confirm the results of these studies in larger sample sizes, explore the mechanisms through which these genetic variants affect the risk of cancer, and to examine potentially relevant gene-environment interactions underlying risk of pediatric brain tumors.

Although pediatric and adult brain tumors are different with regard to pathology and molecular features (Table 2), they share some similarities including common environmental

risk factors [7], similar somatic mutation patterns for tumors within specific histological types [35-37], and most importantly, similar cells of origin [38]. These similarities provide an important starting point for genetic risk factor identification of pediatric brain tumors, which might be similar to those involved in adult brain tumorigenesis. Pediatric brain tumors are rare, and the lack of large enough materials prevents sufficiently powered GWAS analyses. Therefore, knowledge about genetic risk factors for adult brain tumors may guide hypothesis based analyses of genetic risk factors for pediatric brain tumors.

2.1.6 Genetic Risk Factors in Adult Glioma

In the last few years, a considerable number of large genetic association studies have been conducted on adult brain tumors. GWAS have identified a number of susceptibility loci for adult glioma located at 5p15.33 (*TERT*), 3q26.2 (*TERC*), 8q24.21 (*CCDC26*), 9p21.3 (*CDKN2A-CDKN2B*), 20q13.33 (*RTEL1*), 11q23.3 (*PHLDB1*), and 7p11.2 (*EGFR*) [39-44]. GWAS have also shown that the susceptibility loci specifically associated with glioblastoma subtype are located at 1p31.3, 11q14.1, 16p13.3, 16q12.1, 22q13.1, and 12q23.33 while non-glioblastoma susceptibility loci are located at 1q32.1, 1q44, 2q33.3, 3p14.1, 10q24.33, 10q25.2, 11q21, 11q23.2, 12q21.2, 14q12, 15q24.2, and 16p13.3, indicating distinct genetic susceptibility for glioblastoma and non-glioblastoma subtypes [45,46]. Moreover, several candidate-gene association studies performed on adult brain tumors reported some common variations, consistently associated with adult glioma.

The majority of candidate-gene association studies performed on adult brain tumors have focused on four pathways including cell cycle, DNA repair, inflammation, and metabolism to identify genetic risk factors for adult brain tumors [47-53]. It has been suggested that genetic polymorphisms in these four main pathways play an important role in brain carcinogenesis [54].

DNA repair pathway genes restore genomic integrity by repairing DNA damage, which is considered to be an important mechanism in the development of brain tumors. Therefore, inherited variations in components of DNA repair pathway may lead to its functional deficiency and induce carcinogenesis [55]. Thus, extensive epidemiological studies have been performed to investigate the association between SNPs of several DNA repair-related genes and risk of developing brain tumors. It has been reported that common variants in DNA repair genes *ERCC1*, *ERCC2*, *XRCC1*, *XRCC4*, *XRCC5*, *RPA3*, *PARP1*, and *MGMT* are associated with adult glioma susceptibility [47,56-58].

If the products of the DNA repair pathway fail to repair damage, the cell accumulates excessive DNA damage that may induce activation of checkpoints to arrest the cell cycle, and/or cell apoptosis. Therefore, the failure in the function of cell cycle pathway can lead to proliferation and survival of cancer-prone cells and consequently induce tumorigenesis [55]. Dysregulation of the cell cycle pathway is a hallmark feature of most brain tumors and the association between hereditary variants in genes involved in this pathway and adult glioma has been investigated by several studies. It has been reported that adult glioma or specific adult glioma subtypes are significantly associated with variations in *CCND1*, *EFEMP1*,

CCNH, *MDM2*, *EGFR*, *ERBB2*, *VEGFR2*, *CASP8*, *SLC7A7*, and *IGF1R* genes [48,49,59-63]. However, because of insufficient population sizes, the statistical power of each of the studies is low and false positive and negative findings are likely to occur. The results of single studies indicating statistically significant associations are difficult to interpret and therefore replication studies are needed.

Some relatively consistent findings suggest that genetic variants in inflammation-related genes alter the risk of adult glioma. SNPs in *IL-4RA*, *IL-4*, *IL-6*, *IL-8*, *IL-10*, *IL-13*, and *COX2/PTGS2* have been found to be associated with glioma risk in the opposite direction of their association with asthma/ allergy risk [51,64-66]. Furthermore, SNPs in innate immune genes *SELP*, *ALOX5*, and *SOD1* were reported to be associated with risk of glioma [67]. In addition, a meta-analysis study indicated an inverse association between history of eczema, asthma, hay fever, or allergy and glioma [68]. However, it is unknown whether immunosuppressive gliomas inhibit allergies or whether allergies prevent glioma [54,69]. Hence, the effects of inflammation pathway variations on risk of glioma need to be clarified.

It has also been suggested that polymorphisms in genes involved in metabolism pathways are associated with risk of adult brain tumors. Rajaraman *et al.* reported that SNPs in *SOD2*, *SOD3*, and *CAT* genes mediating oxidative stress may influence brain tumor susceptibility [52]. Common variants in *GSTM3* [53], *MTHFR* and *MTRR* [70,71], *GSTP1*, and *CYP2E1* [72] are found to be associated with risk of adult glioma and these genes are involved in metabolisms of aromatic hydrocarbon, folate, hydrophobic and electrophilic compounds and xenobiotics, respectively. However, replication studies are needed to confirm the results of these studies in larger sample sizes.

Table 2. Main differences between adult and pediatric brain tumors

Characteristics	**Adult brain tumors**	**Pediatric brain tumors**
Most common histological subtype	Glioblastoma	Astrocytoma
Grade	High	Low
Location	Supratentorial	Cerebellum, brainstem, and optic pathway
Most common genetic mutations	1p/19q, *TP53*, *EGFR*, *KRAS*, *IDH1*, *IDH2*, *TERT*, *PTEN*, *ATRX*	6q, 17q, 7q34, *BRAF*, *CDKN2A*, *CDKN2B*, *MYC* or *MYCN*, *CTNNBI*, *TP53*
Most common epigenetic alterations	*MGMT* promoter methylation	-

2.1.7 Familial Brain Tumors

Family history of brain tumors and some rare genetic syndromes together are responsible for about 5% of all brain tumors [73]. A two-fold increased risk of glioma, which is the most common malignant primary brain tumor in adults, has consistently been reported for relatives of non-syndromic familial glioma patients [74,75]. Although different approaches have been used to identify susceptibility loci in the familial form of the disease, its genetic contribution is not well understood. Two segregation studies based on cohorts of families of glioma patients are available. These studies concluded that autosomal dominant and recessive modes of inheritance explain 1% and 2% of glioma cases, respectively. The multifactorial model and autosomal recessive gene were suggested to be the best explanation for the modes of inheritance [76,77]. A study by Paunu and colleagues [78] by using the genome-wide linkage analysis of 15 glioma families identified low-penetrance susceptibility loci on 15q. Using the candidate-gene approach, one study investigated the effects of some genes known to be altered in sporadic glioma on the familial form of the disease. Of 15 investigated families, an index case from one family showed a *p53* point mutation. A deletion in $p16^{INK4A}/p14^{ARF}$ region was also identified in an index case from another family. No evidence of germ-line mutations of *PTEN*, and *CDK4* was found in these families [79]. However, the results of these studies are inconclusive due to small sample size and thereby insufficient statistical power.

To understand the genetic background of familial glioma, the Genetic Epidemiology of Glioma International Consortium (Gliogene) was established to collect sufficient DNA samples to increase the statistical power [80]. In a study based on Gliogene, 46 U.S. families were investigated for multipoint linkage analyses using nonparametric (model-free) methods. The results of this study indicate a glioma susceptibility region at 17q12-21.32. The finding was replicated in 29 independent U.S. families. Additional susceptibility loci at 6p22.3, 12p13.33-12.1, 17q22-23.2, and 18q23 were also detected in first stage families [81]. To explore the genetic variations responsible for these linkage signals, the associations of 5122 SNPs mapping to these five regions were examined in 88 glioma cases with and 1100 cases without a family history of brain tumors. In the discovery phase, 12 SNPs were identified to be associated with family history of brain tumors. In the replication study including 84 familial and 903 sporadic cases, two of these 12 SNPs were confirmed; 12p13.33-12.1 *PRMT8* rs17780102 and 17q12-21.32 *SPOP* rs650461. In the combined study of the discovery and replication series, the strongest signals were found at 12p13.33-12.1 *PRMT8* rs17780102, *SOX5* rs7305773 and *STKY1* rs2418087, and 17q12-21.32 *SPOP* rs6504618 suggesting some potential target genes underlying gliomagenesis located on 12p13.33-12.1 and 17q12-q21.32 regions [82]. Further investigations were performed to identify candidate genes and variants for familial glioma on chromosome 17q using the next generation and Sanger sequencing. In a study of 21 very rare (< 0.1% frequency) non-synonymous candidate variants in 23 families with glioma, known to contribute the most into the linkage peaks based on the previous Gliogene studies, alteration in *MYO19* and *KIF18B* genes and rare variants *SPAG9* rs143491486 and *RUNDC1* rs61995866 were

identified [83]. Bainbridge *et al*. conducted the whole exome sequencing on genomic DNA of 90 cases of glioma from 55 glioma families. A variant was further investigated only if it was shared among affected individuals within a family. The variants that were rare, functional, and involved in genes known to be associated with cancer were prioritized. Using this approach, two novel functional variants in *POT1* were detected in two independent families (NM_015450:p.G95C, HG19:chr7:g.124503667C>A; NM_015450:p.E450X, HG19:chr7:g. 124481048C>A). Later, by sequencing *POT1* in a cohort of 264 glioma cases from 246 families, the third novel functional mutation in *POT1* was identified in the third independent family (NM_015450:p.D617Efs*8, HG19:chr7:g.124464068TTA>T). Roughly one third of the carriers developed glioma which indicates incomplete penetrance of the alleles. However, in the general population, mutations in this region of *POT1* are extremely rare (4/6000 individuals). Therefore, sequenced-based screening for *POT1* mutations can be beneficial to families with multiple gliomas [84].

The susceptibility loci identified by GWAS on adult glioma account for approximately 7-14% of the excess familial risk of glioma [41] and no evidence of a significant linkage peak was found for these GWAS hits in familial gliomas [81]. Thus, familial and sporadic glioma seems to have different predisposing risk factors and genetic contributions; however, additional large studies are required to discover multiple distinct etiologies for different forms of brain tumors.

2.1.8 Environmental Risk Factors

Since the majority of pediatric brain tumors occur at the very early ages of life, it has been suggested that prenatal and postnatal insults might be potential etiologic factors [2]. Potential associations between brain tumor risk and several environmental factors have been investigated, of which only exposure to therapeutic or high doses of ionizing radiation has a proven etiologic role [2,7]. The carcinogenic effects of ionizing radiation are even stronger in children who are more radiosensitive than adults and have a longer life expectancy to express the risk [85]. Some studies have shown that radiation therapy for early-onset childhood cancers is associated with development of brain tumors later in life [2,7]. Moreover, an association between maternal diagnostic radiation during pregnancy and an increased risk of brain tumors in offspring has been found [2]. Thus, the increasing use of diagnostic techniques such as computed tomography and positron emission tomography, which are associated with ionizing radiation, have raised health concerns. However, very few studies have been performed to evaluate the effects of postnatal diagnostic radiation on the risk of pediatric brain tumors [85-87].

In addition to ionizing radiation, many other environmental exposures have been investigated in relation to adult and pediatric brain tumor development including: N-nitroso compounds, pesticides, tobacco, electromagnetic fields, infectious agents, allergic conditions, trauma, parental occupational exposures, medications, vitamins, birth characteristics, parental age, and congenital anomalies. However, the results of these studies have yielded inconclusive, minimal, or no compelling evidence of an etiologic role for brain tumors. The failure in

finding consistent and statistically significant associations for the listed potential risk factors could be due to insufficient statistical power, low sensitivity and specificity of risk factors, invalid or imprecise exposure measurements, disease heterogeneity, and absence of true associations [2,7,13].

2.2 GENETIC SYNDROMES

2.2.1 Phacomatosis

The phacomatoses represent a series of genetic disorders characterized by multiple hamartomas of the central and peripheral nervous system, eye, skin, and viscera. Phacomatoses include several distinct disorders of which some conditions predispose individuals to development of nervous system tumors (Table 1) [3]. Neurofibromatosis type 1 and type 2 (NF1 and NF2) and tuberous sclerosis complex (TSC) are the most common types of phacomatoses [3,88].

2.2.2 Clinical Features

2.2.2.1 Neurofibromatosis type 1

Neurofibromatosis type 1 (NF1) which is known as von Recklinghausen disease is the most common type of phacomatoses occurring in 1 in 3000 individuals. It is an autosomal dominant disorder caused by mutations or deletion of the *NF1*gene located at chromosome 17q11.2 [3]. Approximately 90% of *NF1* mutations are point mutations and about 50% of NF1 patients are de novo with no family history of NF1 [88]. *NF1* gene functions as a tumor suppressor gene encoding a protein called neurofibromin. Neurofibromin negatively regulates Ras protein by the conversion of Ras-GTP to Ras-GDP. Therefore, *NF1* mutations results in uncontrolled cell proliferation and tumor formation [89,90]. The hallmark of NF1 is neurofibroma which is a benign tumor of the peripheral nerves. Roughly, 80% to 90% of neurofibromas are localized neurofibromas that are not present in children before age 6 but begin to appear in the preadolescent years and increase in size with age. In contrast, plexiform neurofibromas compose 10% to 20% of neurofibromas and are congenital [89,91]. Two or more neurofibromas or one plexiform neurofibroma constitutes one of the diagnostic criteria. Another presenting sign of NF1 is the occurrence of multiple cafe´-au-lait spots, found in 99% of patients, noticed in the early months of life. Six or more cafe´-au-lait spots, at least 5 mm in prepubertal individual or at least 15 mm after puberty is the second criterion. Other diagnostic criteria constitute skin freckling, optic nerve glioma, skeletal abnormalities, and Lisch nodules (iris hamartoma) [89,90].

2.2.2.2 Neurofibromatosis type 2

Neurofibromatosis type 2 (NF2) which is also inherited in an autosomal dominant manner, occurs due to mutations of the *NF2* gene at 22q12.2, with the prevalence of 1 in 60000 individuals. Almost 50% of NF2 cases are sporadic representing de novo mutations [3,88]. *NF2* encodes a protein called merlin or schwannomin which is a cytoskeletal protein and

functions as a tumor suppressor. NF2 is characterized by occurrence of bilateral vestibular schwannomas, although it might occur asynchronously. Other tumors associated with NF2 are meningioma, ependymoma, and rarely neurofibroma. The only non-tumor manifestation of NF2 is cataract; either posterior subcapsular cataract or cortical wedge opacity. NF2 constitutes approximately 5% of the neurofibromatosis [89,90].

2.2.2.3 Schwannomatosis

Schwannomatosis is the newest distinct form of neurofibromatosis, accounting for only a very small proportion of the cases with neurofibromatosis. It is inherited as an autosomal dominant disorder, although the majority of cases are sporadic. No high-penetrance gene has been identified for schwannomatosis [90]. Somatic mutations of *NF2* have been observed in the tumors of schwannomatosis patients; however, the germ-line mutations located on chromosome 22 associated with schwannomatosis are on a distinct locus from *NF2*. Therefore, the genetic mechanisms underlying schwannomatosis remained unclear. Schwannomatosis cases are identified by multiple schwannomas, excluding vestibular schwannomas. Affected individuals do not develop other nervous system tumors or other clinical manifestations [90].

2.2.2.4 Tuberous sclerosis complex

Tuberous sclerosis complex (TSC) is also an autosomal dominant syndrome and results from mutations in *TSC1* (9q34.13) or *TSC2* (16p13.3) with the prevalence of 1 in 10000. Roughly 65-85% of TSC cases are de novo with higher incidence of point mutations in *TSC2* than in *TSC1* (65% and 10%, respectively) [3]. *TSC2* encodes a protein referred to as tuberin while *TSC1* encodes hamartin. Tuberin and hamartin are involved in the control of cell division by forming a protein complex [90]. TSC is a multisystem disorder affecting the skin, heart, eye, kidneys, lungs, nervous system, and other tissues. TSC is characterized by neurological problems, particularly occurrence of seizures and developmental impairment. Some diagnostic criteria include retinal hamartomas, renal angiomyolipomas, giant cell astrocytoma, subependymal nodules, forehead plaque, and cardiac rhabdomyoma. Imaging including magnetic resonance, computed tomography, and ultrasound is required for full clinical evaluation [89,90].

2.2.2.5 von Hippel-Lindau syndrome

von Hippel-Lindau syndrome (VHL) is an autosomal dominant trait with the estimated prevalence of 1 in 60000. *VHL* gene is involved in VHL occurrence and its mutations result in activation of hypoxia-induced genes and consequently formation of vascular tumors. The major features of VHL are hemangioblastomas, mostly located on cerebellum, spinal cord, and retina; renal cysts; cysts in the pancreas or epididymus; and endolymphatic sac tumors [89,90].

2.2.2.6 Ataxia telangiectasia

Ataxia telangiectasia (AT) is a very rare autosomal recessive disorder caused by *ATM* gene mutations which is located on 11q22.3 and encodes ATM protein. ATM is involved in DNA repair pathway and is important in cell cycle control. AT is characterized by ocular telangiectasia, elevated serum level of alphafetoprotein, chromosomal rearrangement, premature aging, endocrine disorders, and gait ataxia [89,92].

2.2.3 Descriptive Epidemiology

The following descriptive reports are based on a cohort of 3706 individuals diagnosed with a given type of phacomatoses during the period between 1 January 1968 and 31 December 2014 in Sweden (unpublished data). The phacomatosis patients were identified through Patient register by using ICD-8 (743.40, 759.86, 759.87), ICD-9 (237H, 759F, 334W, 237H, 759G), and ICD-10 (Q85.0, Q85.1, G11.3, Q85.8C, Q85.8W, Q85.9). Phacomatosis patients were later classified as familial and non-familial through the Multigeneration Register by identifying their first and second degree relatives. The identified relatives were further linked to Patient register to investigate whether they were diagnosed with any type of genetic syndromes. Ten free phacomatosis individuals with no family history of genetic syndromes were randomly selected from the general population matched to the phacomatosis patients by age, sex, and geographical region.

Of the 3706 identified phacomatosis patients 1310 (35.4%) were familial and 2396 (64.6%) were non-familial. Distribution of phacomatoses subtypes were 2760 (74.5%) neurofibromatosis, 544 (14.7%) tuberous sclerosis complex, 190 (5.1%) von Hippel-Lindau syndrome, 53 (1.4%) ataxia telangiectasia, and 159 (4.3%) other phacomatoses. 51.6% of phacomatosis patients were male evenly distributed among different subtypes.

Phacomatosis patients had a higher risk of developing malignant tumors (OR 29.54 [95% CI 24.98-34.94]), benign tumors (OR 18.17 [95% CI 16.59-19.91]) and cardiovascular diseases (OR 4.38 [95% CI 3.89-4.92]) compared to individuals without phacomatoses. We also performed the analyses stratified by gender and family history of phacomatoses. In general, the ORs for developing malignant and benign tumors as well as cardiovascular diseases were higher for male phacomatosis patients (OR 31.03 [95% CI 24.39-39.49]; OR 21.90 [95% CI 19.23-24.94]; OR 4.90 [95% CI 4.17-5.76], respectively) compared to females (OR 28.18 [95% CI 22.32-35.58]; OR 14.98 [95% CI 13.19-17.01]; OR 3.88 [95% CI 3.27-4.59], respectively). Moreover, the ORs for developing malignant tumors were slightly higher for familial phacomatosis patients (OR 33.30 [95% CI 25.03-44.32]) compared to non-familial (OR 27.66 [95% CI 22.48-34.02]).

2.2.4 Etiology

Phacomatoses are usually inherited, but many occur de novo, with unknown etiology. There are only very few and small epidemiological studies available that have investigated the association between perinatal factors and risk of phacomatoses. Ståhl and colleagues

evaluated the association between paternal history of cancer and risk of congenital abnormalities including phacomatosis in offspring. Their study is based on a Swedish and Danish cohort including 1,777,765 singleton children born alive in Denmark and Sweden of whom 340 individuals were identified as phacomatosis patients. Children with a paternal history of cancer had a non-significant elevated risk of phacomatosis in comparison to children without a paternal history of cancer (RR = 2.4, 95% CI = 0.9 to 6.5, $p = 0.078$) [93]. A few small studies have examined the effect of parental age on the risk of phacomatosis in offspring [94-102]. The majority of these studies include only neurofibromatosis cases and their results remain inconclusive due to insufficient statistical power. Thus, the etiology of de novo occurrence of phacomatoses is largely unknown.

3 AIM

3.1 OVERALL AIM

The overall aims of this thesis were to identify genetic risk factors for brain tumors, with the main focus on pediatric brain tumors, and to assess the effect of parental age on the risk of being born with a de novo genetic syndrome associated with developing nervous system tumors.

3.2 SPECIFIC AIM

Study I: To perform a systematic review and meta-analysis of published studies investigating the association between germ-line DNA repair gene SNPs and glioma risk.

Study II: To determine whether the SNPs identified by GWAS to be associated with risk of adult glioma are also associated with pediatric brain tumor risk.

Study III: To investigate whether the SNPs identified by candidate-gene association studies on adult brain tumors are also associated with the risk of brain tumors in children.

Study IV: To test the hypothesis that advanced parental age increases the risk of de novo phacomatoses predisposing to nervous system tumors in offspring.

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4 MATERIALS AND METHODS

4.1 STUDY I

4.1.1 Literature search, study characteristics, and data extraction

The PubMed database was searched (up to December 2012, which was the latest date possible when the study was conducted) using combinations of the keywords: “brain tumor”, “single nucleotide polymorphism”, “association”, “gene”, “risk”, “case control”, “susceptibility”, and “polymorphism”. All the published peer-reviewed articles investigating the association between DNA repair germ-line SNPs and risk of brain tumors were identified, of which the English-language literature containing glioma germ-line DNA samples and the necessary data for obtaining crude odds ratios (ORs) and confidence intervals (CIs) were considered eligible. Moreover, we evaluated whether the SNPs reported by GWAS to be associated with adult glioma risk are involved in DNA repair pathway to be included in our analyses. In addition, the references of the identified articles were manually investigated to include all the appropriate literature.

All the essential data were extracted from the eligible articles and were summarized. If overlapping data were used by several publications for a certain SNP, only the study covering the majority of samples was included in the meta-analysis. If at least 4 eligible studies were available for a certain SNP, the meta-analysis was performed for that SNP.

4.1.2 Statistical analysis

Hardy-Weinberg equilibrium (HWE) was calculated in the controls using the χ^2 goodness-of-fit test to assess the quality of the studies. Studies that deviated from HWE (p value < 0.05) were considered low-quality studies and meta-analysis was conducted with and without these studies to examine the robustness of the estimates. The reported meta-analysis was based on the crude ORs and CIs of the studies since the adjusted ORs and CIs were not comparable due to heterogeneous adjustment factors across studies. However, we also pooled the adjusted estimates to detect potential conflicts between the crude and adjusted results; no conflict was observed.

Data pooling was conducted under homozygote and heterozygote comparisons, as well as dominant and recessive models, using the fixed-effects model. If significant heterogeneity (p value < 0.1) was detected among studies, the obvious heterogeneous studies were omitted or the random-effects model was applied. RevMan software version 5.2 (Cochran Collaboration) was employed to create forest plots and funnel plots in order to compare ORs among studies and to identify publication bias, respectively. Egger’s test was applied to evaluate symmetry of the funnel plots [103]. The reference p values for an experiment-wide significance with the Bonferroni corrections were provided to evaluate the potential false-positive findings.

4.2 STUDIES II AND III

4.2.1 Study Design and Populations

Both study II (on SNPs identified by GWAS on adult glioma) and III (on SNPs related to DNA repair, cell cycle, metabolism, and inflammation pathways identified by candidate-gene association studies on adult glioma) used data from the CEFALO study.

The CEFALO Study

CEFALO is a population-based multicenter case-control study performed in Sweden, Norway, Denmark, and Switzerland originally initiated to assess the association between mobile phone use and pediatric brain tumors. At the time of the data collection, mobile phone use was very rare among pre-school children and therefore follow-up of tumor occurrence started when a child turned 7 years old. Eligible cases were children and adolescents diagnosed with intracranial central nervous system tumors during the study period and aged 7-19 years at the time of diagnosis. The study period varied slightly between study centers (Norway: from September 2004 to August 2008, Denmark: from January 2004 to April 2008, Switzerland: from May 2004 to May 2008, and Sweden: from April 2004 to August 2008). The patients should be diagnosed with intracranial central nervous system tumors with the ICD-O-3 code C71 to be eligible. Tumor types were defined according to ICCC-3 group III as following: ependymoma (IIIa) (9383, 9391-9393), astrocytoma (IIIb) (9384, 9400-9401, 9410-9411, 9420-9424, 9440-9442), intracranial embryonal tumors (IIIc) (9470-9474, 9480, 9502-9504, 9508), other gliomas (IIId) (9380-9382, 9430, 9444, 9450-9451, 9460), other specified intracranial neoplasms (IIIe) (8743, 9064, 9071, 9080, 9161, 9390, 9412-9413, 9492-9493, 9505-9507, 9560), unspecified intracranial neoplasm (IIIf) (8000-8005, 9990, 9999). Further, tumors were subclassified according to WHO classification of tumors of the central nervous system, fourth edition.

Case identification varied across the participating countries. In Sweden and Denmark, the unique personal identification numbers were used to identify the cases through population-based registries (National Cancer Registry, Childhood Cancer Registry, Pathology Registry, The National Patient Registry, and Swedish Regional Cancer Registries) as well as the medical reports from pediatric, oncology, and neurosurgery departments. In Switzerland, the Swiss Childhood Cancer Registry was used to identify the cases below 16 years of age at diagnosis while the cases 16 years and older at diagnosis were identified through neurosurgery clinics, departments of pathology and cantonal general cancer registries. Norwegian cases were identified from the Norwegian National Cancer Registry. All diagnoses were confirmed histologically or based on unequivocal diagnostic imaging. Date of diagnosis was established based on the medical records and was defined as the first diagnostic imaging date that led to the diagnosis. Neurofibromatosis or tuberous sclerosis patients were excluded.

Two controls per case were randomly selected from the general population matched to the case by age, sex and geographical region. In Sweden, Denmark, and Norway the

nationwide population registries were used to select the controls while in Switzerland, a two-stage random sampling procedure was used. At the beginning, a community with the same language region as each case was randomly selected and then the control was randomly selected from the corresponding communal population registry.

Data collection was performed by face to face interview with the child and at least one parent, preferably the mother. In Sweden, Denmark and Switzerland data collection began in June 2006 while data collection in Norway began in December 2007. All the cases for whom physicians authorized contact were interviewed within 5 years from date of diagnosis, and 63% were interviewed in the first 2 years of diagnosis. Interviews were performed with 352 (82%) cases and 646 (71%) controls. Cases and controls were informed about the aim of the study as investigating the risk factors for brain tumors and the questions were focused on the child's exposures early in life or during the fetal period and birth.

The saliva of cases and controls were collected by using the Oragene self-collection kit (DNA Genotek, Ottawa, ON, Canada), and the saliva DNA was extracted following the manufacturer's recommended protocol. PicoGreen (Invitrogen, Carlsbad, CA) was applied to quantitate the DNA yield. In total, saliva DNA from 286 case and 578 controls was extracted. Since the medulloblastoma cases are the subject of a separate study, they have been excluded from studies II and III. Overall, DNA from 245 cases and 489 controls was included in these studies. Over 70% of the included pediatric brain tumors in these studies were glioma and embryonal tumors constitute only 3% of brain tumor cases. Other specified intracranial neoplasms and unspecified intracranial neoplasm constitute 20% and 6% of the included subjects, respectively. Therefore, since pediatric gliomas were the majority of the brain tumor subtypes included in these studies, the term pediatric brain tumors refers to pediatric gliomas.

4.2.2 SNP selection and genotyping

We searched the PubMed database (up to December 2012) using combinations of the keywords "brain tumor", "single nucleotide polymorphism", "association", "gene", "risk", "case control", "susceptibility", and "polymorphism" to identify all the published GWAS and candidate-gene association studies of brain tumors. In total, 29 SNPs associated with risk of adult glioma detected by GWAS as well as 68 SNPs reported by pediatric brain tumor genetic association studies or at least two of the candidate-gene association studies of adult brain tumors as being statistically significant were selected for genotyping.

All the 29 GWAS SNPs and 63 SNPs from candidate-gene association studies were satisfactorily genotyped. Genotyping was conducted using the Sequenom iPlex Gold platform with matrixassisted laser desorption/ionization-time-of-flight (MALDI-TOF) mass spectrometry. The average success rate was 97% and the concordance rate for duplicate genotyping was 100%.

4.2.3 Statistical analysis

The χ^2 goodness-of-fit test was applied to the controls of all SNPs to evaluate whether the allele frequencies were consistent with HWE and $p < 0.001$ was considered statistically significant. The association between SNPs and pediatric brain tumor (PBT) susceptibility was assessed based on three models including the Cochran-Armitage trend test of additivity (trend), dominant (DOM), and recessive (REC) models using unconditional logistic regression model with adjustment for the matching variables (age, sex, and country). To compare the allelic frequencies of SNPs between cases and controls the allelic model was used by applying the χ^2 test. We conducted analyses for all pediatric brain tumors combined, and for astrocytoma alone. To evaluate the consistency of results across countries, country specific analyses were conducted.

To assess the linkage disequilibrium (LD) between SNPs, D´ was measured. Haploblocks were characterized according to the default LD block parameters in Haploview v4.2. . We conducted the haplotype analyses for the haploblocks harboring the SNPs that were associated with risk of PBTs. We further analyzed only the haplotypes with a frequency of > 1%. If the distribution of all the haplotypes in a haploblock was suggestively different between cases and controls ($p < 0.05$ for all PBTs; $p < 0.1$ for subgroup analyses), the effects of haplotypes were investigated. The reference p values for an experiment-wide significance with Bonferroni corrections were provided to consider the possibility of false positive findings. PLINK v1.07 [104] and SAS statistical software version 9.3 (SAS Institute, Cary, NC) were used for the procedures.

4.2.4 Ethics

Studies II and III have been approved by the national data protection boards and ethical committees in all the study centers, and written informed consent was obtained from all participants and/or their parents. In Sweden, the CEFALO study has been approved by the Regional Ethical Review Board in Stockholm (2005/1562-31/1).

4.3 STUDY IV

4.3.1 Study Design and Populations

This study is a register-based case-control study nested within the Swedish population. The individuals who were born and resident in Sweden at any time during the study period (from 1 January 1960 until 31 December 2010, and alive in 1987) with available information of parental age for both biological parents constitute the eligible population [105]. By using the unique personal identification number assigned to each Swedish resident and through linkage of the population to the Patient register, phacomatosis cases were identified. ICD-9 and ICD-10 were used to identify the eligible cases of neurofibromatosis (NF1, NF2, and schwannomatosis) (ICD-9: 237H; ICD-10: Q85.0), tuberous sclerosis complex (ICD-9: 759F; ICD-10: Q85.1), von Hippel-Lindau syndrome (ICD-9: 759G; ICD-10: Q85.8C), ataxia telangiectasia (ICD-9: 334W; ICD-10: G11.3), and other unspecified phacomatoses (ICD-9:

759; ICD-10: Q85) through the Patient register. The patients who were born in Sweden from 1960 and were still alive in 1987 when the ICD-9 was introduced were the included cases. Due to limitations in ICD coding system, it was not possible to specify the specific phacomatosis type for almost half of the cases. Among patients with neurofibromatosis, it was not possible to distinguish NF1, NF2 and schwannomatosis, but around 95% of neurofibromatosis is NF1 and most of the remaining cases are NF2, whereas schwannomatosis constitutes only a very small proportion of neurofibromatosis.

We further classified cases into familial and non-familial. First and second degree relatives of eligible individuals were identified through the Multigeneration Register [106]. Later, we linked the identified relatives to the Patient register to collect the information on occurrence of genetic syndromes in them. Since the earlier versions of ICD codes used in the Patient register are less precise, if any genetic syndrome was detected in a relative, the case was defined as familial to ensure that familial cases were not classified as non-familial. For the period 1964-1967, ICD-7 code 759, and for 1968-1986, ICD-8 codes 759, 743.40 and from 1987 onwards, the ICD-9 and ICD-10 codes mentioned above were used to define familial phacomatosis.

We randomly selected ten controls per case from the eligible population matched to the case on sex, birth year, and residence in Sweden at date of case detection [105]. Moreover, no genetic syndrome should have been diagnosed among first or second degree relatives of controls.

4.3.2 Exposure and covariates assessment

We considered parental education and parental country of birth as potential confounders. Registers available at Statistics Sweden were used to extract information about parental education. We used educational information from the census conducted in 1970, and yearly information from the Longitudinal Integration Database for Health Insurance and Labour Market Studies (LISA), available from 1990. The Multigeneration Register was used to collect information about parents' birth years and countries of birth.

4.3.3 Registers used in the study

The Patient Register

The Patient register which was initiated in 1964 includes information on diagnostic codes and surgical procedures for all in-hospital care in Sweden with a complete nationwide coverage since 1987, as well as outpatient specialist care data, available nationwide since 2001 [107].

The Multi-Generation Register

The Multigeneration Register contains the relationships between parents and their children for all individuals born in 1932 or later and resident in Sweden since 1960 [106].

The Total Population Register

The Register of the Total Population contains all Swedish residents since 1968 and their annual characteristics including age, sex, country of birth, marital status, immigration, emigration, and place of residence [105].

The Longitudinal Integration Database for Health Insurance and Labour Market Studies (Swedish acronym LISA)

LISA contains information on annual individual characteristics including highest level of formal education and personal disposable income for all Swedish residents since 1990.

4.3.4 Statistical analysis

We applied conditional logistic regression to assess the association between parental age and risk of non-familial and familial phacomatoses in offspring adjusted for parental education, parental country of birth, and mutually adjusted for maternal and paternal age. Analyses were performed for neurofibromatosis alone and all other phacomatoses combined. We defined the parental education as the highest household education level, and categorized it into low = elementary school; intermediate = high school or apprenticeship; high = university or postgraduate education. Parental country of birth was categorized into Nordic and non-Nordic countries; if both parents were born in a Nordic country we considered the parental country of birth as Nordic. In 1987 the Patient register became available nationwide and ICD-9 was introduced. Therefore, we conducted sensitivity analyses where the population was restricted to individuals born 1987 or later to reach a more complete case identification and thereby less misclassification of heritability. STATA statistical software version 13 (College Station, Texas, USA) and SAS statistical software version 9.3 (SAS Institute, Inc., Cary, NC, USA) were used to perform the analyses.

4.3.5 Ethics

The study was approved by the Ethical Review Board in Stockholm, Sweden (2011/634-31/4, 2016/27-32).

5 RESULTS

5.1 STUDY I

Of the 36 identified articles investigating the association between SNPs in DNA repair genes and brain tumor risk, 27 articles met our eligibility criteria and therefore were further investigated. In these studies, in total, 105 SNPs in 42 DNA repair genes were analyzed of which 10 SNPs in 7 DNA repair genes were evaluated in at least 4 studies and therefore were included in our study.

An elevated risk of glioma was observed to be associated with the A allele of *ERCC1* rs3212986 (OR_{REC} 1.35 [95% CI 1.08-1.68]), C allele of *ERCC2* (*XPD*) rs13181 (OR_{DOM} 1.18 [95% CI 1.06-1.31]), and the A allele of *XRCC1* rs25487 (OR_{DOM} 1.14 [95% CI 1.02-1.28]). One study deviated from HWE for *XRCC1* rs25487; in a sensitivity analysis where this study was excluded, the finding remained significant under the dominant model.

The T allele of *MGMT* rs12917 (OR_{DOM} 0.84 [95% CI 0.73-0.96]), and C allele of *PARP1* rs1136410 (OR_{DOM} 0.78 [95% CI 0.68-0.89]), were found to be associated with decreased risk of glioma. The conclusion of association between *PARP1* rs1136410 and glioma risk remained intact after sensitivity analysis.

No publication bias was observed by the funnel plots drawn for *XRCC1* rs25487 and *ERCC2* rs13181, and no evidence of funnel plots' asymmetry was found by Egger's test (P_{Egger} = 0.93 and P_{Egger} = 0.88, respectively).

5.2 STUDY II

The 29 SNPs identified from GWAS on adult glioma were investigated in this study. All SNPs were successfully genotyped and were consistent with HWE. Minor alleles of SNPs rs4977756, rs1412829, rs2157719, rs1063192 in *CDKN2BAS* were associated with increased risk of PBTs under the dominant model (OR_{DOM} 1.45 [95% CI 1.03-2.06]; OR_{DOM} 1.45 [95% CI 1.02-2.05]; OR_{DOM} 1.53 [95% CI 1.08-2.19]; OR_{DOM} 1.53 [95% CI 1.07-2.19], respectively). A decreased risk of PBTs associated with the A allele of *TERT* rs2736100 was observed (OR_{DOM} 0.66 [95% CI 0.46-0.93]).

The stratified analyses identified some genetic variants specifically associated with the two investigated histological subtypes. The minor alleles of *RTEL1* rs6089953, rs6010620, rs2297440 were associated with a decreased risk of astrocytoma (OR_{DOM} 0.64 [95% CI 0.43-0.96]; OR_{DOM} 0.66 [95% CI 0.44-0.99]; OR_{DOM} 0.64 [95% CI 0.41-0.98], respectively) while an increased risk of this subtype associated with the C allele of *RTEL1* rs4809324 was detected (OR_{DOM} 1.54 [95% CI 0.98-2.39]). Moreover, SNPs *CCDC26* rs10464870 and rs891835 were associated with increased risk of non-astrocytoma tumor subtypes (OR_{DOM} 1.70 [95% CI 1.11-2.60]; OR_{DOM} 1.59 [95% CI 1.04-2.44], respectively).

Two haploblocks harboring the SNPs that were associated with risk of PBTs with strong LD ($D' \geq 0.95$) were detected (*CDKN2BAS* (rs1412829, rs2157719, and rs1063192), and

RTEL1 (rs6089953, rs6010620, rs2297440, and rs4809324)). The haplotype analyses showed that by increasing the number of risk alleles in *CDKN2BAS* and *RTEL1* SNPs in each haploblock, the risk of PBTs was increased.

After applying the Bonferroni correction, none of the observed associations remained significant (the reference *p* value is 0.0004 for an experiment-wide significance level of 0.05, and 0.0009 for a significance level of 0.10)

Genetic risk scores (new analyses, not included in the papers)

Genetic risk score (GRS) is a composite score derived from the alleles which are associated with a trait at a certain threshold and their effect sizes in a discovery sample. Then, an independent target sample is used to generate the GRS for each individual based on the risk alleles weighted by the effect size (e.g. log OR) obtained from the discovery sample. Later, the regression model is applied to assess the association between the GRS and the phenotype in the target sample adjusting for covariates. Polygenic risk scores are employed to demonstrate the polygenic architecture underlying complex genetic disorders [108].

The current study aimed to examine whether the genetic risk scores for adult brain tumors are associated with risk of pediatric brain tumors by estimating the additive impact of the known adult glioma susceptibility loci on the variance of pediatric brain tumors. We generated the GRSs for adult brain tumors based on the alleles and associated effect sizes derived from a previously published GWAS [41] based on the meta-analysis of two GWAS including 1878 cases and 3670 controls and also three additional independent replication series including 2545 cases and 2953 controls. Quality control procedures and the GWAS results were described in details in the original paper [41]. The CEFALO study was employed as the target sample. The genotyping and the quality control procedures of the CEFALO study are described in pages 33 and 34. We selected the discovery sample for these analyses based on its similarity with the target sample for ethnicity, as well as its large sample size and its overlap with the majority of SNPs genotyped in the CEFALO study.

If the SNPs were associated with the adult brain tumor risk at the genome-wide significance level in the combined results of the discovery sample, they were selected for the GRS analyses. For SNPs which were in LD ($r^2 \geq 0.1$), only the SNP with the strongest association estimate was included in the GRS calculation. The GRSs were calculated based on 8 SNPs (rs6010620, rs2736100, rs4977756, rs1412829, rs1063192, rs4295627, rs10464870, rs498872) and corresponding to the number of the risk alleles weighted by the logarithm of the ORs across this set of SNPs. PLINK was used to perform the analyses [104].

The GRS was then standardized. The logistic regression model was applied to evaluate whether the standardized GRS is associated with the risk of pediatric brain tumors adjusted for age, sex and country as covariates (full model). To measure the variance explained by the effect of GRS, Nagelkerke pseudo-R^2 was calculated as the difference of R^2 in the full

model compared to the reduced model including the covariates but not the GRS. The analyses were conducted using R (http://www.R-project.org).

The results indicated that the standardized genetic risk scores for adult brain tumors were associated with increased risk of pediatric brain tumors (OR 1.23 [95% CI 1.04-1.46], p = 0.014). Moreover, 1.1% of the variance in pediatric brain tumors can be explained by the effect of the genetic risk scores (R^2= 0.011). The estimated SNP-heritability on the liability scale was 0.008.

5.3 STUDY III

Of the 68 SNPs identified from candidate-gene studies related to DNA repair, cell cycle, metabolism, and inflammation pathways, 63 SNPs were successfully genotyped, of which 3 SNPs (rs4444903, rs9288516, and rs61754966) deviated from HWE and were therefore excluded from the analyses.

The minor alleles of *EGFR* rs730437 and rs11506105 were associated with a decreased risk of PBTs (OR_{DOM} 0.59 [95% CI 0.42-0.83]; OR_{DOM} 0.71 [95% CI 0.51-0.98], respectively), while the A allele of rs3212986 in *ERCC1*was associated with an increased risk of PBTs (OR_{DOM} 1.53 [95% CI 1.11-2.09]).

The stratified analyses suggested some variations associated with different histological subtypes. Variants in *CHAF1A* and *XRCC1* were associated with a decreased risk of astrocytoma subtype while non-astrocytoma tumor subtype was associated with SNPs in *EGFR*, *EME1*, *ATM*, *GLTSCR1*, and *XRCC4*. All the identified variants belong to either DNA repair pathway or cell cycle pathway.

When the Bonferroni correction was applied, none of the reported associations remained significant (the Bonferroni corrected reference p value was 0.0002 for an experiment-wide significance level of 0.05, and 0.0004 for a significance level of 0.10).

5.4 STUDY IV

In this study 4625 phacomatoses cases and 46250 matched controls were identified. Among cases, 2089 were diagnosed with neurofibromatosis of whom 61% were non-familial and 2536 were identified as other subtypes combined, of whom 87% were non-familial.

In non-familial neurofibromatosis, the estimated OR for offspring of fathers aged 35-39 years compared with 25-29 years was 1.43 [95% CI 1.16-1.74]. The OR increased to 1.74 [95% CI 1.38-2.19] in offspring of fathers aged ≥ 40 years. We could not detect any association between advanced paternal age and risk of familial neurofibromatosis. No association between advanced maternal age and risk of neurofibromatosis in offspring was found.

In other non-familial phacomatoses, the estimated OR for offspring of fathers aged ≥ 40 compared with 25-29 years was 1.23 [95% CI 1.01-1.50]. A non-consistent increased risk associated with young maternal age was also observed; the OR was estimated at 1.21 [95%

CI 1.06-1.37] for offspring of mothers aged < 25 years. No association between advanced paternal or maternal age and risk of other familial phacomatoses was observed.

The results of the sensitivity analyses restricted to subjects born 1987 or later were similar to the main analyses results, but more pronounced, potentially due to a more complete case identification and a more correct distinction between familial and non-familial cases.

6 DISCUSSION

6.1 MAIN FINDINGS AND IMPLICATIONS

6.1.1 Study I

The findings of this study indicate that variations in DNA repair genes *MGMT* and *PARP1*might protect against adult glioma risk while low-penetrance susceptibility loci for adult glioma are located on *ERCC1*, *ERCC2 (XPD)*, and *XRCC1*.

DNA repair is a main pathway involved in maintaining genomic stability and therefore defects in its genes play an important role in brain tumorigenesis. A considerable number of small studies are available on the associations between several DNA repair gene SNPs and risk of adult glioma. However, the results of these studies were inconclusive due to low statistical power and thus provided a starting point for performing a meta-analysis.

Of the 105 SNPs in 42 DNA repair genes investigated by 36 studies, we could conclude that adult glioma low-penetrance susceptibility loci are located at19q13.3. It has been shown that the somatic chromosome arms 1p/19q codeletion is a common event in glioma tumors and is associated with chemotherapeutic response and overall survival of glioma patients and thereby is used as a prognostic marker [109]. A study by Eckel-Passow and colleagues [110] identified five principal groups of glioma based on the three tumor markers including 1p/19q codeletion and some studies have investigated the association between these groups and glioma germ-line variants identified by GWAS [110,111]. The studies showed that the molecular groups were associated with specific germ-line variants. However, the correlation between germ-line variants at 19q and glioma somatic 1p/19q codeletion is unknown. Germ-line variations at 19q might potentially lead to accumulation of excessive mutations relevant to gliomagenesis including 19q deletion in glial cells and therefore might be used as early detection markers for glioma. Thus, more studies are required to investigate the correlation between 19q germ-line variants and 19q somatic deletion as well as other glioma somatic markers.

Our meta-analysis also provides evidence that *MGMT* and *PARP1*variants are adult glioma protective factors. *MGMT* promoter methylation is known as glioma prognostic and predictive biomarker [112-115]. Some studies found associations between germ-line *MGMT* promoter variations (rs16906252), somatic *MGMT* promoter methylation, and glioma patient survival [116,117]. However, no evidence of a direct association between *MGMT* rs12917 and *MGMT* methylation has been observed. A study by Zawlik *et al.*[118] showed a significant positive correlation between *MGMT* rs12917 and rs16906252 in glioblastomas. However, the study did not detect a perfect linkage disequilibrium between these SNPs and therefore further investigations of this observed association are needed.

It has been shown that higher tumor levels of DNA repair enzymes including PARP1 confers resistance to glioma adjuvant therapy and is associated with poor survival. PARP-1 deficiency in the cells results in increased apoptosis and thereby prevents the survival of

cancer prone cells. PARP1 inhibition is a potential therapeutic strategy for glioma [119,120]. Functional studies are needed to explore the mechanisms behind the protective effects of *PARP-1* rs1136410 on glioma risk and its potential correlation with PARP-1 deficiency.

6.1.2 Study II

This study provides evidence that the adult glioma susceptibility loci at 5p15.33 (*TERT*), 8q24.21 (*CCDC26*), 9p21.3 (*CDKN2A-CDKN2B*), and 20q13.33 (*RTEL1*) identified by GWAS are also associated with pediatric brain tumor risk suggesting similar etiological pathways between adult and pediatric brain tumors.

The susceptibility loci identified in this study are involved in the cell cycle pathway. Aberrations in the cell cycle pathway result in genomic instability and thereby accelerate tumorigenesis. Functional deficiency of cell cycle genes leads to deregulated cell proliferation and suppressed cell death and therefore may cause tumor progression [121]. The majority of the identified variants in this study are related to telomere and telomerase and their dysregulations induce carcinogenesis [122].

There is evidence of correlations between alterations in telomere length and telomerase activity and adult and pediatric brain tumors [123-126]. Studies have shown that somatic mutations of the *TERT* promoter are common events in adult glioma tumors [127] and harboring somatic *TERT* promoter mutations and long telomere length are associated with poor clinical outcomes and resistance to radiotherapy [128]. Although somatic *TERT* alterations are rarer in pediatric glioma tumors compared to adult gliomas, they are still promising prognostic biomarkers for pediatric brain tumors [124,129-131]. Walsh and colleagues [43] have investigated the relationships between the identified susceptibility loci for adult glioma and leukocyte telomere length (LTL) and found that variants near *TERT* were associated with increased risk of glioma and longer LTL while variants near *RTEL1* were associated with increased risk of glioma and shorter LTL. However, the most significant *RTEL1* SNPs associated with glioma risk were not in LD with the most significant *RTEL1* SNPs associated with LTL indicating different causal alleles affecting these two phenotypes. Moreover, no association was observed between other susceptibility loci and LTL suggesting that aberrations in telomere dynamics is only one of the multiple mechanisms contributing to glioma risk [43]. More functional studies are needed to study how these susceptibility SNPs influence the genes they reside in; for example to explore the correlations between these SNPs and the DNA methylation patterns and expression status of their resident and nearby genes (in tumor tissues and body fluids) as well as their potential influence on telomere length and to investigate whether a combination of these biomarkers with some other identified markers could potentially be employed as a panel of biomarkers for early detection of brain tumors. The findings of study II also indicate that genetic risk profiles of pediatric brain tumors differ by histology. This evidence is in line with the results of some recent GWAS on adult brain tumors that identified specific susceptibility loci for different histological subtypes [44,45]. Moreover, studies have shown that different molecular groups of glioma are associated with specific germ-line variants [110,111]. However, further investigations are required to explore

how different germ-line variations trigger specific genetic and epigenetic alterations during embryonic cell differentiation and/or later in life that leads to different tumor type progression.

Furthermore, larger quantitative genetic studies are needed to obtain more accurate estimates of the proportion of the risk of brain tumorigenesis which is attributable to the genetic constitution. To date, to our knowledge, two quantitative genetic studies have evaluated the contribution of inherited genetic factors to the development of brain tumors. However, the results of these studies are inconsistent and both studies were underpowered [20,21]. Therefore, the effects of heritability on the susceptibility of brain tumors remain unclear.

A study by Sampson *et al*. assessed the array-based heritability of thirteen different cancer types, including also glioma on the liability scale (for glioma $h^2 = 0.046$). Moreover, they have assessed the heritability of glioma based on the known glioma susceptibility loci (rs1412829, rs2157719, rs2736100, rs2853676, rs4295627, rs4809324, rs4977756, rs498872, rs6010620, rs891835) ($h^2 = 0.017$) [22].

Kinnersley *et al*. also investigated the proportion of glioma heritability attributable to known glioma susceptibility loci (rs2736100, rs11979158, rs2252586, rs4295627, rs4977756, rs498872, rs6010620) as well as the SNPs that did not reach the genome-wide significance level. The estimated genetic variance of glioma explained by all SNPs and the seven risk loci was 25% and 1.6%, respectively [23]. The difference observed between the estimated array-based heritability by Sampson *et al*. ($h^2 = 0.046$) [22] and Kinnersley *et al*. ($h^2 = 0.25$) [23] can partly be explained by their quality control processes and the qualified included SNPs as well as the estimated disease prevalence which was used to transform the estimated heritability to the liability scale (0.68% in Sampson *et al*. versus 0.24% in Kinnersley *et al*.). The estimated disease prevalence of 0.68% used in Sampson *et al*. is considered as brain and other nervous system tumors prevalence rather than glioma prevalence.

Using genetic risk score analysis we estimated the additive impact of the known adult glioma susceptibility loci identified by GWAS on the genetic variance of pediatric brain tumors. We determined that 0.8% of the variance in the pediatric brain tumors can be explained by the effect of the genetic risk scores containing eight known adult glioma susceptibility loci (rs6010620, rs2736100, rs4977756, rs1412829, rs1063192, rs4295627, rs10464870, rs498872; identified at the time of performing the studies included in this thesis). This provides evidence that the heritable risks of adult and pediatric brain tumors are in-part attributable to some common genetic variants. However, more polygenic analyses based on large sample sizes and big genotyping data for both adult and pediatric brain tumors are required to determine the similarity of genetic architecture in adult and pediatric brain tumors.

By using segregation analysis based on pedigree analyses (in families of glioma patients), two studies determined the values of 0.68 [77] and 0.66 [76] for polygenic heritability of glioma. The estimated polygenic heritability based on segregation analysis also reflects rare and uncommon genetic variants which are not detectable by currently available genome-wide SNP chips. However, these high-risk causing variants do not solely explain a high proportion of genetic variance; rather common variants with small effect sizes probably contribute to the genetic architecture of brain tumors in a large proportion. Therefore, new larger GWAS using higher-density SNP chips are needed to capture a higher proportion of genetic variants attributable to the genetic variance of brain tumors [23].

6.1.3 Study III

The findings of this study indicate that of the investigated genetic variations in DNA repair, cell cycles, inflammation, and metabolism pathways known to be associated with risk of adult glioma, the variants belonging to DNA repair and cell cycle pathways were also associated with risk of pediatric brain tumors.

The DNA repair low-penetrance susceptibility loci for pediatric brain tumors identified in this study are located at chromosome 19 which is consistent with chromosomal location of DNA repair low-penetrance susceptibility loci for adult glioma identified by our meta-analysis study [47]. This finding emphasizes the potential important role of chromosome 19 in susceptibility of brain tumors. The genetic variations located on chromosome 19 associated with brain tumor risk could either be rare high-risk mutations or common low risk variants that could not be captured by GWAS.

Although none of the *EGFR* variants identified by GWAS on adult glioma were associated with pediatric brain tumor risk in our samples, we detected three other variants in *EGFR* associated with adult glioma reported by candidate-gene studies to be associated with PBT risk. The difference in detected alleles in *EGFR* for adult and pediatric brain tumors could be due to insufficient statistical power of our study or different causal alleles within *EGFR* affecting these two phenotypes.

Genetic and epigenetic alterations in cell cycle and DNA repair pathway genes are potential diagnostic and prognostic biomarkers for pediatric brain tumors [9,132-136]. A study by Korshunov and colleagues [137] identified three molecular subtypes of pediatric glioblastoma based on their genomic and epigenetic signatures of which one group is characterized by *EGFR* amplification. Homozygous deletions of *CDKN2A/B* and loss of Chr10q were also common events in this molecular subgroup of glioblastoma [137]. Moreover, *CDKN2A/B* deletion is associated with a worse outcome in pediatric low grade gliomas [138].

In our study, we did not observe any associations between variants involved in inflammation and metabolism pathways and risk of pediatric brain tumors. This could be due to low statistical power of our study and/or small effect sizes of these common variants. Consistently, there was no genetic variation from these two pathways among the significant hits reported by GWAS on adult glioma.

By putting the results of study II and III together, we can conclude that the majority of the identified adult glioma susceptibility loci are also associated with pediatric brain tumor risk and by increasing the sample size, the remaining unassociated SNPs could potentially also reach the statistical significance level. This pronounces the possible similarity between adult and pediatric brain tumors in genetic architecture although some caution in the conclusions is necessary because of the low statistical power of our analyses of pediatric brain tumors.

6.1.4 Study IV

This study provides evidence that advanced paternal age is associated with de novo occurrence of phacomatoses predisposing to nervous system tumors with the most pronounced effects on neurofibromatosis suggesting an increasing rate of new mutations in the *NF1* and *NF2* genes in spermatozoa of older fathers.

The fact that we observed a consistent increased risk estimate of non-familial phacomatoses in offspring with increasing paternal age, but not with maternal age, could be explained by higher de novo mutation rate in the male germ-line compared to the female germ-line due to more cell divisions and consequently more DNA replications in spermatogenesis than in oogenesis [139].

Advanced paternal age has been identified as a risk factor of numerous diseases in offspring [140] including psychiatric disorders [141,142] such as schizophrenia [143,144], autism [145,146], and impaired neurocognitive outcomes [147] as well as several types of cancer [140,148-151] including pediatric brain tumors [13,149,151]. Our finding of an association between advanced paternal age and increased risk of non-familial neurofibromatosis is consistent with the results of the previously largest study [95].

Some studies have investigated the mechanisms through which advanced paternal age might influence the susceptibility of different diseases. Unryn and colleagues have shown that advanced paternal age was correlated with longer telomeres [152]. They also found that paternal age was significantly associated with increased telomere length in both male and female offspring while maternal age did not influence the telomere length in offspring [152]. These findings were replicated by Eisenberg *et al.* [153]. Moreover, there is evidence that in addition to point mutations and copy number variations, epigenetic alterations occurring during spermatogenesis play an important role in the effect of advanced paternal age on the disease susceptibility in offspring [154]. A study by Jenkins *et al*., using the DNA methylation array approach, determined age-associated aberrations in DNA methylation of sperm. Interestingly, a portion of the genes correlated with the altered methylation regions are known to be associated with the risk of schizophrenia and bipolar disorder [155]. Moreover, animal studies could show that offspring of older male mice inherited the DNA methylation alterations observed in the paternal sperm. These offspring showed similar methylation patterns and transcriptional dysregulations of the altered region associated genes in their brains. Noteworthly, these dysregulated genes were correlated with autism and schizophrenia [156].

Despite extensive research, a large proportion of the mechanisms underlying such associations remain unclear and there are more questions than answers. Speaking about advanced paternal age and risk of phacomatoses in offspring, to date and to our knowledge, no relevant functional study is available and the mechanisms that drive this process are unclear. Furthermore, it is interesting to explore whether the observed association between advanced paternal age and increased risk of pediatric brain tumors [13] are driven and mediated by phacomatoses predisposing to nervous system tumors; work that is currently ongoing in our research group.

6.2 METHODOLOGICAL CONCERNS

6.2.1 Study I

Statistical power

In this study, we included SNPs in the meta-analysis if they had been investigated in at least four studies. Combining published data from several studies is a way to increase statistical power. However, there is a possibility that the total sample size of two available studies might be larger than the total sample size of four available studies; thus, statistical power was not the only reason for limiting analyses to SNPs where at least four studies were available. It is also important to have evidence from a larger number of studies, as consistent evidence from several studies that are using slightly different study designs and are conducted in different countries makes overall findings stronger. By putting these issues together, considering at least four available studies as inclusion criterion is meaningful and will provide a sufficient sample size in most cases. One must also consider if overlapping samples were used in this series of publications with large sample sizes, in which overlapping is often the case.

Publication bias

The extent and influence of publication bias is a major concern in meta-analysis studies. In the current study, to assess the influence of publication bias on the reported estimates, funnel plots were created. In the presence of publication bias the plot will be asymmetrical while if there is no such bias the plot will be symmetrical. However, in order to decrease the error in evaluating the symmetry of funnel plots by simple visual examination, Egger's test was applied to statistically detect the asymmetry of the plots [103]. Although, no evidence of funnel plots' asymmetry and publication bias was detected in our analyses based on the results of the Egger's test, publication bias is a phenomenon that always exists [157,158]. However, the potential influence of publication bias on our effect estimates was likely minor as it was not detectable by statistical tests.

Confounding

In this study, we reported the crude ORs and 95% CIs. This is due to the fact that although most of the studies included in the meta-analysis provided the adjusted ORs and 95% CIs, the included covariates in the models were not consistent across studies and thereby their

adjusted findings were not comparable. However, we also pooled the adjusted reported results to investigate the importance of potential confounding factors; we did not observe any conflicts with the corresponding crude results indicating the small impact of confounders on the effect estimates.

6.2.2 Studies II and III

Statistical power

Statistical power and a sufficient population size are always main concerns in epidemiological studies, especially when studying rare determinants of a rare disease. In the CEFALO study, limited statistical power is an issue even though the study includes the hitherto largest series of pediatric brain tumor cases for which DNA samples are available. The CEFALO study is based on data from four countries including Sweden, Denmark, Norway, and Switzerland. However, considering the rarity of pediatric brain tumors, large universal collaborations are required to collect large enough sample sizes and reach sufficient statistical power. With the sample size available in the CEFALO study, a candidate-gene approach was the only possible basis for the genetic association analyses, i.e. a hypothesis-based approach when selecting SNPs for analyses, rather than conducting an exploratory analysis, such as in a genome-wide search for associations.

The limited statistical power of the CEFALO study might have led to undetected associations between rare genetic variants with small effect sizes and pediatric brain tumors. There is also a possibility that some of the observed associations are chance findings, despite a priori defined hypotheses. To further investigate the potential influence of random variation, CEFALO country specific analyses were performed to check the consistency of results across countries.

Misclassification of disease

All diagnoses of brain tumors in CEFALO were confirmed histologically or based on unequivocal diagnostic imaging which is considered very accurate. However, misclassification of disease among different histological subtypes might occur due to random errors, but this is believed to be very rare and cannot be considered as a source of bias in our study. In the CEFALO study, there is the possibility that we have not been able to identify all cases with brain tumors; however, because brain tumors in children are very rare, this issue would likely only affect the statistical power and the precision of the study results.

Assessment of genetic data

Although genotyping errors cannot be omitted, they are expected to be non-differential. CEFALO samples were genotyped by the staff blinded to sample status. The average success rate was 97% and the concordance rate for duplicate genotyping was 100% which is considered quite high.

Selection bias

In the CEFALO study the participation rate was high among both cases (82%) and controls (71%). Studies II and III are based on the subset of CEFALO subjects who provided saliva samples. There is no reason to believe that willingness to donate a saliva sample is related to the genotypes studied. However, it is likely that children diagnosed with the most aggressive brain tumors and suffering from severe symptoms were less willing to donate a saliva sample, either because they were too ill to participate at all, or had greater difficulties accruing a sufficient amount of saliva, which results in the lack of power to identify SNPs specifically associated with these tumor subtypes. Moreover, medulloblastoma cases were not included in studies II and III.

Confounding

A confounder should be associated with both outcome and exposure, but should not be an effect of the exposure. The CEFALO study, is a case-control study matched on age, sex, and country; however, studies II and III were based on a subset of CEFALO subjects who provided saliva samples. Therefore, in these studies, we broke the matching to not lose incomplete case-control sets and thereby reduce statistical power considerably. Thus, data were analyzed using unconditional logistic regression, with adjustment for the matching factors. Age is not associated with germ-line variations, and therefore is not considered a potential confounder, but as it was one of the matching variables, age was controlled in the analysis. Sex and country, as a proxy for ethnicity, were potential confounders in these studies. However, adjusted and corresponding crude estimates provided similar results, which indicate that the adjusted confounding factors had a small effect. The impact of unknown confounders cannot be excluded.

Strengths

A case-control study is the most cost-effective design for investigating a rare disease which would require exposure assessment for a large number of subjects compared with a corresponding cohort study design. A close collaboration with the clinics treating patients with pediatric brain tumors ensured a complete and rapid case ascertainment, and coverage was further improved by extraction of new cases from the population based regional cancer registers. Moreover, controls were randomly selected from the general population at the time when cases were identified (incidence density sampling) as the best way of selecting the individuals at risk from the study base reflecting the exposure frequency of the study base.

6.2.3 Study IV

Misclassification of disease

In this study, cases of phacomatoses were identified through linkage of the population to the Patient register and by using ICD-9 and ICD-10 codes. However, for almost 44% of cases we could not specify the phacomatosis type due to limitation in the ICD coding system that has

assigned very limited numbers of codes to several different types of genetic syndromes. Moreover, misclassification of familial cases as non-familial cases is a concern in our study that might lead to dilution of effect estimates of paternal age on non-familial cases. However, for 70% of the eligible participants we could identify all the four grandparents and thereby all the first and second degree relatives and only for 8% of the eligible participants none of the grandparents could be detected. In addition, our conservative approach in defining familial cases as individuals with relatives diagnosed with any genetic syndrome would reduce the information bias resulting from limitation in ICD coding system.

Missing data

Eligible participants in this study were individuals with information available on parental age for both biological parents. Only 1% of the individuals born in Sweden during the study period did not have complete information on parental age. No missing data was detected for parental country of birth and only less than 0.5% of the subjects had missing data on parental education, and were omitted from the analyses.

Confounding

Ethnicity and its related genetic susceptibility factors potentially influence the prevalence of various diseases including genetic syndromes [159,160]. In this study, we adjusted for parental country of birth since it can be considered a proxy for ethnicity, which can be associated with parental age and also with a different genetic predisposition to phacomatoses. Parental occupation and parental socioeconomic status are also potential risk factors for several diseases in offspring [161-164]. In the present study, we have used the parental education as a proxy for parental socioeconomic status since socioeconomic status is associated with higher age at first birth and education also often delays childbirth. Lower socioeconomic status may be associated with other risk factors for phacomatoses (smoking, alcohol, occupational exposure to chemicals), although little is known about the etiology of phacomatoses. Paternal and maternal age are known to be highly correlated. In this study, we adjusted the estimates for these potential confounders and the difference between adjusted and corresponding crude results suggests a small impact of confounding. There might be some unknown confounders and their effects cannot be excluded.

Strengths

The present study is the hitherto largest investigation of de novo phacomatoses, and as it is a large population based study using several registries, it provided the unique possibility of investigating a rare disease with sufficient statistical power. Furthermore, using reliable registers to determine the family history of the investigated disease in the study subjects, in order to classify them as familial and non-familial cases, remarkably reduced the potential selection and information bias. Moreover, in this study, we could investigate both paternal and maternal age. As expected, both paternal and maternal age were not associated with familial phacomatoses. These findings add credibility to the results for de novo

phacomatoses, as familial and non-familial cases were identified using the same methods and potential biases would not be expected to affect only the results regarding de novo cases.

7 CONCLUSIONS AND FUTURE PERSPECTIVE

Based on the findings of the studies included in this thesis we can conclude that variants in *TERT*, *CCDC26*, *CDKN2A-CDKN2B*, and *RTEL1* identified by GWAS on adult glioma are also associated with pediatric brain tumor risk. Although none of the known GWAS susceptibility loci in *EGFR* were associated with risk of pediatric brain tumors, three other variants in *EGFR* were found to be associated with pediatric brain tumors indicating its important role in the etiology of brain tumors possibly through different causal alleles for pediatric and adult brain tumors. Moreover, variants in chromosome 19, and more specifically SNPs in DNA repair genes located at 19q, were observed to be associated with pediatric and adult brain tumor risk. Furthermore, adult glioma known susceptibility loci explain 0.8% of genetic variance of pediatric brain tumors. Taken these together, this thesis provides evidence that adult and pediatric brain tumors probably have some genetic risk factors in common. This finding can help in enhanced etiological understanding of brain tumors that in the future may lead to prevention and improved treatment of brain tumors.

Despite extensive research, a large proportion of variants contributing to the genetic architecture of brain tumors remain undetected. To be able to detect the remaining rare high-risk mutations and common low risk variants, large universal collaborative studies are required to provide large enough sample sizes to reach the sufficient power. Moreover, in order to capture a higher proportion of genetic variants, higher-density SNP chips and next generation sequencing technologies are essential. It is clinically relevant from a risk prediction and treatment perspective to explore also the similarity of genetic architecture between adult and pediatric brain tumors.

Furthermore, very little is known about the functional relevance of the identified genetic variants and their role in brain tumor etiology. Therefore, large well-designed studies planned by multidisciplinary teams are necessary to investigate how these variations affect the genes they reside in; for instance assessing their influence on the DNA methylation and expression patterns of their resident and nearby genes as well as their potential correlations with telomerase activity and telomere length. It is also clinically relevant to evaluate whether a combination of these biomarkers with some other identified markers can serve as a panel of early detection biomarkers in the body fluids for brain tumors. This thesis also provides evidence that advanced paternal age increases the risk of de novo phacomatoses predisposing to nervous system tumors in offspring. The risk estimates were more pronounced for neurofibromatosis suggesting an increasing rate of de novo mutations in the *NF1* and *NF2* genes in older fathers' sperm. However, whether or not the de novo mutation rate in older father's sperm is particularly high in *NF1* and *NF2* remains to be elucidated, including also underlying mechanisms. Furthermore, investigating the potential mediator role of phacomatoses predisposing to nervous system tumors in the observed association between advanced paternal age and increased risk of pediatric brain tumors is valuable.

8 SAMMANFATTNING PÅ SVENSKA

Hjärntumörer är sällsynta i befolkningen; i kombination med andra tumörer i nervsystemet, utgör de ca 2% av alla cancerformer. De är dock den näst vanligaste typen av tumörer hos barn. Etiologin är sannolikt multifaktoriell, men trots omfattande forskning är endast ett fåtal riskfaktorer kända. Syftet med denna avhandling var att identifiera genetiska riskfaktorer för pediatriska hjärntumörer genom att undersöka om genetiska variationer som är associerade med risk för gliom hos vuxna, också kan påverka risken för pediatriska hjärntumörer.

Fakomatoser är en serie sällsynta genetiska syndrom som predisponerar individer för utveckling av tumörer i nervsystemet. Etiologin till de novo fakomatoser, dvs fakomatoser utan känd familjehistorik, är till stor del okänd. Det finns en hypotes att de novo fakomatoser och tumörer i nervsystemet kan ha gemensamma riskfaktorer. I avhandlingen ingår därför också en studie av sambandet mellan föräldrarnas ålder vid barnets födelse och risken för de novo fakomatoser hos barnet.

Studie I är en systematisk granskning och metaanalys av publicerade studier som undersökt associationen mellan nedärvda genetiska varianter, s.k. single nucleotide polymorphisms (SNPs) i DNA-reparationsgener och risken för gliom. Totalt identifierades 105 SNPs i 42 DNA-reparationsgener, varav 10 SNPs i 7 DNA-reparationsgener utvärderades i minst 4 studier och inkluderades därför i metaanalysen. Baserat på resultaten från denna studie kan vi dra slutsatsen att genetiska varianter associerade med en ökad risk för gliom finns i *ERCC1*, *ERCC2* (*XPD*) och *XRCC1*, medan variationer i DNA-reparationsgenerna *MGMT* och *PARP1* är förenat med en reducerad risk för gliom.

Studierna II och III är baserade på CEFALO-studien som är en populationsbaserad, multicenter fall-kontrollstudie av barn och ungdomar som diagnostiserats med intrakraniala centrala nervsystemstumörer i åldern 7-19 år vid diagnos. Totalt inkluderades DNA från saliv för 245 fall och 489 kontroller i dessa två studier. I **studie II** genotypades DNA från saliv för 29 SNPs som identifierades genom s.k. genome-wide association studies, GWAS, av gliom hos vuxna. Resultaten av denna studie indikerar att loci som identifierats i GWAS-studier som riskvarianter för gliom hos vuxna vid 5p15.33 (*TERT*), 8q24.21 (*CCDC26*), 9p21.3 (*CDKN2A-CDKN2B*) och 20q13.33 (*RTEL1*), också är associerade med pediatrisk hjärntumörrisk. I **studie III** genotypades 68 SNPs som är relaterade till DNA-reparation, cellcykeln, metabolism och inflammation. Dessa identifierades från associationsstudier av gliom hos vuxna med kandidatgen approach. Totalt genotypades 63 SNPs tillfredsställande. Denna studie visar att av de undersökta genetiska variationerna, så är varianter i *EGFR*, *ERCC1*, *CHAF1A*, *XRCC1*, *EME1*, *ATM*, *GLTSCR1* och *XRCC4*, relaterade till DNA-reparation och cellcykeln, och kända för att vara associerade med gliom hos vuxna, också förknippade med risk för pediatriska hjärntumörer. Sammantaget visar resultaten från **studie II och III** att hjärntumörer hos vuxna och barn troligen har delvis gemensamma genetiska riskfaktorer.

Studie IV är en nested fall-kontrollstudie baserad på Sveriges befolkning under perioden 1960-2010. Genom att använda Patientregistret identifierades 4625 fall med fakomatos, som sedan klassificerades som familjär eller icke-familjär. Tio kontroller per fall valdes slumpmässigt från befolkningen. Analyserna gjordes för neurofibromatos separat, och för andra fakomatoser kombinerat. Denna studie indikerar att risken för de novo fakomatoser hos barn ökar med pappans ålder; starkast samband hittades för neurofibromatos.

Denna avhandling ger evidens för att hjärntumörer hos vuxna och hos barn sannolikt har vissa genetiska riskfaktorer gemensamt, och därför till viss del kan ha liknande etiologi. Vidare ger avhandlingen evidens för att risken för nya fall av neurofibromatos, utan tidigare familjehistorik, ökar med hög ålder hos pappan vid barnets födelse, vilket antyder en ökad förekomst av de novo mutationer i *NF1* och *NF2* generna i sperma från äldre fäder.

9 ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to all the people who have contributed to making my doctoral education an exciting and joyful journey. In particular, I wish to acknowledge all the following people.

First of all, I would like to thank the CEFALO participants and their parents for their contributions to the study and for making my doctoral thesis possible.

Maria Feychting, my main supervisor. I am very grateful to you for giving me the opportunity to do my Ph.D. studies in genetic epidemiology with you, which was a great adventure. Thank you for your invaluable help and support all along the way, for keeping your door always open to me and being available literally at any time, for your trust and for giving me all the freedom that I needed, but also keeping me on track and for providing me the best education possible.

Catharina Lavebratt, my co-supervisor. Thank you for your excellent supervision, for sharing your profound knowledge in genetics and your always valuable comments on my work. I am very grateful to you for all the support along the way, your guidance, your kindness, and for being always promptly available for me when I needed you.

Birgitta Lannering, my co-supervisor. Thank you for sharing your excellent clinical expertise on brain tumors and your always valuable comments on the manuscripts. I am grateful to you for guiding me to think critically about my research and its clinical implications.

Giorgio Tettamanti, my co-supervisor and my friend. I am very thankful to you for unconditionally giving me your time, for sharing your vast expertise in statistics, epidemiology, and programming, and for always patiently answering my epidemiological questions. And thank you especially for your excellent assistance with my last study which made it smoother.

Per Hall, my mentor. Thank you for sparing your time and for your scientific advices and for being available for a meeting whenever I asked you.

Anders Ahlbom and **Ulla Stenius**, former and present chairmen of the Institute of Environmental Medicine. Thank you for providing a stimulating and fruitful research environment.

Zahra Golabkesh (Yasi), my favorite colleague and friend. Thank you for your unconditional support during these years, your invaluable help, your kindness, and for listening me nagging about life when it is tough.

Parisa Gol Mohammadi (Paris), my forever friend. Thank you for all the beautiful moments, chats, laughs, funs, nags, and for your encouragement and support. I am thankful to you for standing by my side when times get hard and simply for your great friendship.

Hanna Mogensen, my office mate and friend. I am thankful to you for your kindness, your support, your encouragement and for your genuine friendship. I am deeply grateful to you for all the time that you spent helping me with translating Swedish texts and especially for your invaluable help with the Swedish abstract of my doctoral thesis.

Hannah Brooke, my office mate and friend. Thank you for being an incredible colleague, such a positive, inspiring, and caring person and a true friend. I am grateful to you for being always up for helping me when I needed.

Rebecka Hjort, thank you for being such a nice and kind person, for all the nice talks, and for your invaluable help with my Swedish citizenship.

Mats Talbäck, I am grateful to you for your assistance with my registry based projects, for your advice and your patience.

I would like to express my gratitude to **Karin Fremling**, **David Pettersson**, and **Korinna Karampampa**, my former office mates for such a warm welcome when I joined the unit of epidemiology. Karin, I will never forget your invaluable support and your kindness when I started my work at the unit.

I would like to thank all the members of the unit of epidemiology for creating such a friendly and nice work atmosphere.

I am grateful to all my co-authors, for sharing your deep knowledge and for your professional contributions to our papers.

I am thankful to all of my friends in Sweden; special thanks to **Alireza Azimi**, **Soudabeh Rad Pour**, **Pedram Kharaziha**, and **Mohsen Besharat Pour**, for all the quality time we spent together and for making my doctoral studies enjoyable.

I would like to thank **Catharina Larsson** who welcomed me to the fantastic field of genetics and epigenetics at Karolinska Institutet and **Monica Nistér** who introduced me to the amazing field of brain tumors.

Last but not least, my deepest heartfelt gratitude belongs to my family who has supported me unconditionally during my life time, by all means. Thus, my special thanks belong to:

Ashraf, my aunt, my role model who taught me to believe in myself and has always inspired me.

Taban and **Akbar**, my parents, whom I always adore. How grateful I am to you for your endless support and love can hardly be expressed in words. You have always been there for me and inspired me; without you this would not have been possible.

Ghazal and **Maziyar**, my sister and brother, for your unconditional love and support. I am extremely lucky to have you in my life.

Akbar and **Kobra**, my beloved late grandparents. Your passing away within four months of each other was the biggest loss in my life. I wish you were here to see this day. Thank you for loving me unconditionally.

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