KU LEUVEN

FACULTY OF MEDICINE

Genetic modulation of treatment-related neurocognitive impairment in pediatric cancer survivors – A systematic review

Master Thesis proposed to achieve the degree of master in medicine by

MADOE Aline

Unit: Faculty of Medicine

Departement: Pediatrics

Promotor: Prof. dr. UYTTEBROECK Anne
Mentor: SLEURS Charlotte

Leuven, 2016-2017

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COVER LETTER

Dear members of the editorial board,

I wish to submit my new manuscript 'Genetic modulation of cancer treatment-related neurocognitive impairment in pediatric cancer survivors – A systematic review' for publication in your esteemed journal.

Many childhood cancer survivors experience neurocognitive impairment, potentially induced by treatment. Deficits in core cognitive processes such as attention, memory, working memory, information processing speed and visual-motor integration frequently occur and can have a profound, life-long impact on daily functioning and quality of life. An interesting observation is the large degree of interpatient variability in neurocognitive outcomes, which has been related to demographic and treatment variables. However, these factors account for only a proportion of the observed variability, and emerging evidence indicates that several germline genetic variants may modulate the risk of developing treatment-related neurocognitive deficits. To the best of my knowledge, my review is the first to provide a systematic overview of the current literature in this promising research field. Moreover, I discuss several important limitations of the reviewed studies, and I present recommendations for future studies. I sincerely believe that the question asked in this review is among the priorities in childhood cancer survivor care, as knowledge on genetic risk factors could minimize adverse neurodevelopmental outcomes in pediatric cancer patients by enabling (1) prognostication of neurocognition, followed by risk-adapted, genotype-tailored treatment regimens and prophylactic interventions, and (2) identification of targets for rational interventions to protect the nervous system.

I declare no financial or other competing interests. The study was approved by the Ethics Committee of University Hospital Leuven, Belgium. The manuscript was not published, nor under editorial review for publication elsewhere.

I hope you to appreciate reading the manuscript and consider it for publication in your journal.

Yours sincerely,

Aline Madoe

ABSTRACT

The rise in childhood cancer survival rates has raised concerns about the long-term adverse effects of cancer treatment, including neurocognitive impairment. Neurocognitive deficits in core cognitive processes such as attention and processing speed are frequently observed and can have a profound, lifelong impact on children's everyday functioning and quality of life. Interestingly, large interpatient variability exists in cognitive outcomes. Emerging evidence indicates that such differences may be related to germline genetic variation. The aim of our review was to systematically summarize the current literature on the modulatory effects of genetic polymorphisms on cancer treatment-induced cognitive changes in childhood cancer survivors. The PubMed/Medline database was screened using a search strategy focused on four components: "cancer", "cancer treatment", "neurocognitive outcome" and "germline genetic variation". Seventeen studies meeting predefined eligibility criteria were analyzed, including sixteen candidate gene studies and one genome-wide association study. 38 polymorphisms in 15 genes across proposed pathophysiological pathways, including (1) neural plasticity and repair, (2) neuroinflammation and defenses against oxidative stress, (3) neurotransmission, and (4) folate metabolism pathway, were reported to be significantly associated with treatment-related neurocognitive dysfunction or neuroimaging abnormalities. However, study results were often discordant, possibly because of large study heterogeneity and several important methodological limitations. Further research, including large-scale, collaborative genome-wide association studies integrating both neurocognitive assessments and advanced neuroimaging techniques, is obviously required. Eventually, knowledge on genetic risk factors could be used in clinical practice to minimize adverse neurodevelopmental outcomes in pediatric cancer patients.

NEDERLANDSTALIGE SAMENVATTING

Naarmate de overlevingscijfers na behandeling voor kanker bij kinderen verbeteren, neemt ook de bezorgdheid toe aangaande mogelijke nevenwerkingen van behandeling op lange termijn. In het bijzonder worden regelmatig neurocognitieve stoornissen gezien, die kunnen interfereren met cognitieve kerntaken zoals aandacht en snelheid van uitvoering. Onvermijdelijk kan dit een belangrijke impact hebben op het alledaagse functioneren en de levenskwaliteit, niet enkel tijdens de kinderjaren maar ook in het latere leven. Weliswaar worden er onder behandelde kinderen grote verschillen gezien in cognitieve weerslag. Er is toenemende evidentie dat genetische variatie in de kiemlijn hierin een rol kan spelen. Het doel van ons onderzoek was om de recente literatuur aangaande de invloed van genetische polymorfismen op therapie-geïnduceerde cognitieve veranderingen bij curatief behandelde kinderen met kanker samen te vatten. We screenden de PubMed/Medline database met behulp van vier zoektermen: “cancer”, “cancer treatment”, “neurocognitive outcome” en “germline genetic variation”. Zeventien studies die voldeden aan de vooraf gedefinieerde criteria werden geanalyseerd, waaronder zestien kandidaat gen studies en één genoom wijde associatie studie. 38 polymorfismen in 15 genen met betrekking tot voorgestelde pathofysiologische processen (meer bepaald (1) neurale plasticiteit en herstel, (2) neuroinflammatie en verdediging tegen oxidatieve stress, (3) neurotransmissie en (4) folaatmetabolisme) bleken significante correlaties te vertonen met therapie-gerelateerde neurocognitieve stoornissen of afwijkingen bij beeldvorming van het centraal zenuwstelsel. Desondanks bleken de verschillende studieresultaten vaak discordant, mogelijk als gevolg van beduidende heterogeniteit tussen de studies en soms belangrijke methodologische beperkingen. Verder onderzoek, met inbegrip van grootschalige multicentrische genoom wijde associatie studies (GWAS) die zowel neurocognitieve beoordelingen als gevorderde beeldvormingstechnieken omvatten, is zonder twijfel nodig. Gehoopt kan worden dat een betere kennis van genetische risicofactoren in de toekomst klinisch nuttig zal zijn voor het beperken van neurologische ontwikkelingsstoornissen bij jonge kankerpatiënten.

INTRODUCTION

Major treatment advances have significantly improved the outlook for many children with cancer, with now close to 80% of them surviving five years or more[1]. This has raised concerns about the long-term adverse effects of cancer treatment and their impact on functional outcomes and quality of life, including neurocognitive development. Up to 40% of pediatric cancer survivors may experience long-term cognitive deficits potentially induced by treatment[2]. Therapeutic modalities most consistently associated with neurocognitive impairment include cranial irradiation, intensive systemic and intrathecal chemotherapy, and corticosteroids[3–7]. Given that most of the postnatal brain development occurs during childhood, children could be particularly vulnerable for treatment-induced neurotoxicity[8]. The cognitive domains most commonly affected include attention, memory, working memory, information processing speed and visual-motor integration[3,4,9]. As developmental demands increase with age, deficits in these core cognitive processes may affect academic performance, job success, social functioning and mental health over time[2]. Hence, neurocognitive impairment can have a profound, life-long impact on childhood cancer survivors' everyday functioning and quality of life.

Notwithstanding increasing research in this area, many questions remain regarding the effects of cancer treatment on the brain. Human imaging, animal and in vitro studies have supported a neurobiological basis for treatment-related neurocognitive impairment[10]. However, underlying pathophysiological mechanisms are still not fully elucidated, which limits the development of effective therapies to prevent or treat this potentially debilitating condition. An intriguing observation is the large degree of interpatient variability in cognitive outcomes. This has been related to demographic (e.g., female sex and young age) and treatment variables (e.g., cranial irradiation therapy, intrathecal or high-dose intravenous methotrexate)[9,11–17]. Nevertheless, these factors account for only a proportion of the observed variability. Therefore, a compelling need exists to identify other factors that could more precisely predict which children are most at risk for long-term neurocognitive impairment.

Emerging evidence indicates that several germline genetic variants may modulate the risk of developing treatment-related neurocognitive deficits[7,18]. For example, Krull et al. (2013) reported associations between measures of attention and genetic polymorphisms in apolipoprotein E (ApoE), methionine synthase (MS), monoamine oxidase A (MAO-A), and glutathione S-transferases (GST) in acute lymphoblastic leukemia (ALL) survivors treated with chemotherapy[19]. This research field may offer opportunities to personalize pediatric cancer treatment through identification of patients at risk, thereby enabling risk-adapted or genotype-tailored treatment regimens or prophylactic interventions. Moreover, it also holds promise for furthering our understanding of the etiology of treatment-related neurocognitive dysfunction, and that way, identifying potential targets for the development of rational interventions to protect the nervous system. Hence, elevated risk of long-term adverse effects on cognitive function could be minimized.

The purpose of this systematic review was to synthetize current evidence on the modulatory effects of germline genetic polymorphisms on cancer treatment-induced cognitive changes in children. Moreover, we discuss the methodological limitations of the reviewed studies and may as such present a more distinct lead for future research in this clinically relevant area. A special focus will be given to those genetic polymorphisms that could be used in the pediatric cancer setting to tailor therapy in order to attain the ultimate goal of pediatric cancer treatment today: not simply medical cure, but adapted cure preserving the survivors' long-term neurocognitive abilities and quality of life.

METHODS

Search strategy

This review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines[20]. A comprehensive literature search was performed in July 2017 using the PubMed/Medline database without restrictions concerning publication dates. The search strategy was based on four components: "cancer", "cancer treatment", "neurocognitive outcome" and "germline genetic variation". We did not narrow our search to studies in children since treatment-related cognitive dysfunction has been extensively studied in adult cancer populations. Similar pathways and genes may contribute to susceptibility to neurocognitive impairment in both children and adults[21]. We restricted our search to the treatment modalities most commonly implicated in neurotoxicity in the pediatric oncology setting, including radiotherapy, chemotherapeutic agents and corticosteroids. To provide a broad perspective on the issue, we also searched for studies investigating the occurrence of leukoencephalopathy as the outcome of interest. Although such structural changes often seem transient, they might interfere with normal brain maturation and development, leading to long-term neurocognitive deficits[22]. Detailed search terms are presented in Supplementary Table S1.

Study selection

Selection was conducted at three sequential levels: (1) first, potential citations were reviewed by their titles to exclude studies which were clearly not applicable to the key question of this review; (2) selected titles were reviewed by abstract, and, (3) if the abstract was eligible, by full text, to ensure that they fulfilled the inclusion/exclusion criteria. This systematic screening process was supplemented with a manual search of cited references from retrieved articles and other relevant papers.

Publications were deemed eligible for inclusion in this review if they met all of the following criteria: (1) original research studies in (2) treated (3) human cancer populations, (4) which investigated the modulating effects of germline genetic variation on (5) objective and/or subjective neurocognitive function, or on the occurrence of leukoencephalopathy. (6) To focus on the long-term effects of cancer treatment in the neurocognitive area, neurocognitive data must have been collected at least six months after treatment took place. We selected a minimum of six months post-(consolidation) treatment as the cut-off to exclude assessment of the (sub)acute, transitory effects of cancer and its treatment on cognition. (8) English publication language was requisite and (9) the full text had to be available. Studies were excluded if they were meta-analyses, reviews, commentaries, editorials, conference or workshop abstracts and case studies; if they were not related to defined outcomes of interest (e.g., focus on survival or peripheral neuropathy); if they investigated the neurocognitive adverse effects of novel, biologic agents or endocrine therapy; and if data on the modulatory effects of germline variants on outcome were omitted. Preclinical in vitro and animal studies were beyond the scope of this review paper. Studies that used mental status screening instruments such as the Mini Mental State Examination (MMSE) only were also excluded. These instruments have insufficient sensitivity to detect cancer treatment-related neurocognitive dysfunction and thus would lead to an underestimation of its incidence.

Data extraction

Data were extracted from included publications using a standardized data extraction form. Information was collected that pertained to geographic location, ethnicity, study design, participant demographics and clinical characteristics, genotyping technique, cognitive assessment tools and timing of assessment.

RESULTS

Study and patient characteristics

The literature search strategy and screening process is summarized in the flow chart presented in Figure 1. A total of seventeen studies involving 2036 participants with available genotype data were included in the current review. The characteristics of the included studies are presented in Table 1.

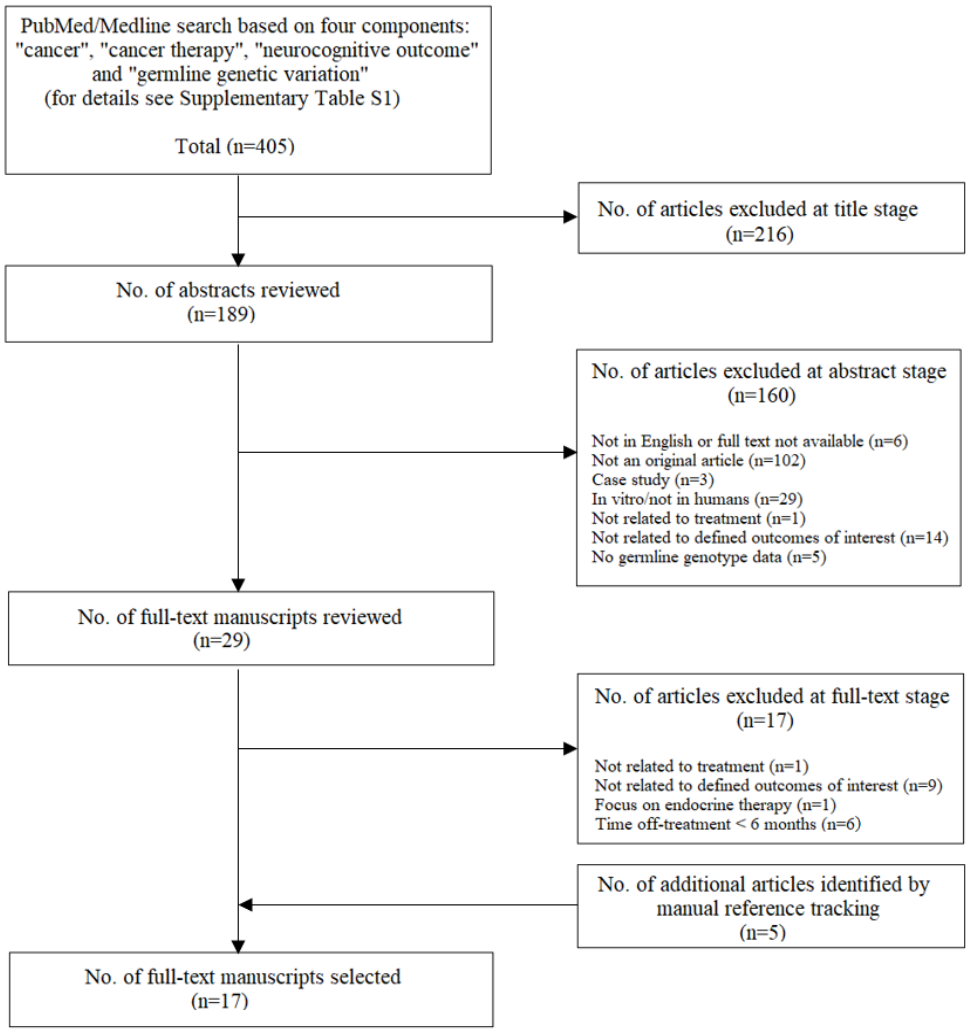

Figure 1. Flow diagram showing the selection process and criteria of the included studies.

In twelve articles a cross-sectional design was used[19,23–33] whereas the remaining five were longitudinal studies[34–38] with baseline assessments of neurocognitive function (n = 3)[35,36,38] or pre-treatment brain MRIs (n = 2)[34,37]. Three studies included a healthy control group[26,27,38].

Sample sizes were usually rather small, with only four studies including more than 200 cancer patients[24,28,29,31]. None of the studies mentioned an a priori sample size calculation. Nine studies had clearly defined criteria to exclude patients with a history of events that could negatively impact neurocognitive function (e.g., genetic or neurologic conditions, pre-existing neurocognitive or neuropsychological disorders, head injury, …)[19,23–26,29,30,32,38]. The remaining studies made no mention of such exclusion criteria.

Fourteen studies were conducted in North-America[19,23–32,35,36,38], two in Europe[34,37], and one in Asia[33]. Fourteen studies described the race/ethnicity of their participants[24–28,30–32,34–38] though the reporting of race/ethnicity data was inconsistent. For example, Krull et al. (2008) described their participants as Caucasian or not[24], while Cole et al. (2015) characterized their participants as White, Black or African-American, Asian, Hispanic and others[31]. Almost equal numbers of studies were conducted in pediatric (n = 10)[19,24,25,27,28,30,31,33,35,36] and adult (n = 7)[23,26,29,32,34,37,38] populations.

The most common types of cancer examined were breast cancer (n = 3)[23,38], ALL (n = 7)[19,24,25,28,31,33,35] and brain tumors (n = 7)[27,29,30,32,34,36,37]. Due to this variety, the investigated treatment regimens were very heterogeneous as well. In all studies, (a subgroup of) patients had received chemotherapy. Methotrexate (MTX) was part of the chemotherapy regimen in nine studies[19,24,25,28,31,33–35,37], of which six studies specifically focused on MTX-related neurotoxicity[24,25,33–35,37]. In seven studies, treatment included cranial radiation therapy for at least a subset of patients[27,29–32,35,36]. For more treatment details, we refer the reader to Table 1 and the study population sections of the respective studies.

Thirteen studies analyzed objective and/or subjective neurocognitive performance as the outcome of interest[19,23–27,29–32,35,36,38]. Objective assessments involved standard international neurocognitive function test batteries, including IQ testing, as well as computerized tasks assessing reaction times and processing speed. Subjective assessments included self-report questionnaires and caregiver ratings. In two out of these fourteen studies, brain MRI was evaluated as a complement to neurocognitive assessments[29,32]. The remaining four studies evaluated brain MRI only[28,33,34,37]. For the studies evaluating neurocognitive performance as the outcome of interest, timing of assessment varied considerably, ranging from an average of 6 months post-treatment to an average of 8.8 years post-treatment.

Thirteen studies used a single bio-specimen type of either blood (n = 8)[19,23–25,29,32,36,38], buccal cells (n = 2)[27,30], saliva (n = 1)[26] or bone marrow (n = 1)[31], while one study used blood as well as bone marrow specimens[33]. The remaining studies did not report the bio-specimen used for genotyping. Method of genotyping was universally reported. Seven studies used a single genotyping assay[23,24,26,33,36–38], while the remaining studies used multiple genotyping assays. The most commonly used assay techniques were PCR-RFLP (n = 9)[23,24,29,30,32,34,35,37,38], TaqMan genotyping assay (n = 5)[25–27,31,33] and GoldenGate genotyping assay (n = 3)[28,29,32]. In the only GWAS included in this review, genotyping was performed using Affymetrix 500K/6.0 array sets[28].

Treatment-related neurocognitive dysfunction and genotype

One GWAS was identified[28], and the remaining studies investigated specified candidate single nucleotide polymorphisms (SNPs). A total of 112 polymorphisms involving 46 genes were reported by the candidate gene studies. Six of these candidate gene studies investigated

polymorphisms in a single candidate gene[23,24,26,29,30,38]; the remainder included polymorphisms in multiple candidate genes. All these studies provided specific hypotheses justifying the selection of the gene(s) and variant(s). 38 polymorphisms in 15 of the investigated genes were reported to be significantly associated with treatment-related neurocognitive dysfunction, or leukoencephalopathy or white matter changes (WMC), by at least one study. These genes could be broadly divided into four categories based on their role in hypothesized molecular mechanisms underlying cancer treatment-induced central neurotoxicity, namely (1) neural plasticity and repair, (2) neuroinflammation and defenses against oxidative stress, (3) neurotransmission and (4) folate pathway metabolism. Here, the results of the studies will be presented and described by category and by gene or gene family. A summary of significant findings is presented in Table 2. For a detailed description of the proposed mechanisms underlying cancer-treatment related neurocognitive dysfunction, we refer the reader to published reviews on this specific topic[39,40]. The results of the only GWAS in this review will be discussed separately.

CANDIDATE GENE STUDIES

1. Neural plasticity and repair

Apolipoprotein E (ApoE) gene

ApoE is a complex glycoprotein involved in lipid metabolism that appears to play an important role in neuronal repair and plasticity after injury[41,42]. The ApoE gene is polymorphic and occurs in three major alleles (ApoE ε2, ε3 and ε4). These alleles vary in amino acids at positions 112 and 158: ε2 (cysteine/cysteine), ε3 (cysteine/arginine), and ε4 (arginine/arginine). About 25-30% of the population carries at least one ApoE ε4 allele[43].

Five studies examined the risk of treatment-related neurocognitive impairment associated with ApoE polymorphisms[19,23,29,38,44], of which four found significant associations.

Ahles et al. (2003) reported that long-term survivors of breast cancer and lymphoma treated with standard dose chemotherapy who carried at least one ε4 allele performed worse on visual memory and spatial ability, and tended to score lower on psychomotor functioning, compared with non-ε4-carriers[23]. Similar findings have been reported by Krull et al. (2013) in a large cohort of childhood ALL survivors treated with risk-adapted chemotherapy[19].

Increased parent-reported attention problems were identified in children carrying the ε4 allele. In a more recent study, Ahles et al. (2014) longitudinally investigated the relationship between post-treatment cognitive changes, ApoE genotype, and smoking in breast cancer patients[38]. They found that the detrimental effect of the ApoE ε4 genotype on post-treatment cognitive functioning was moderated by smoking history. Patients carrying the ε4 allele without a smoking history showed worse performance on processing speed and working memory compared to those with a smoking history and healthy controls. For processing speed, the deleterious effect of ApoE ε4 on non-smokers was more pronounced in patients treated with chemotherapy than in the no chemotherapy (primarily endocrine treatment) group. By contrast, for working memory, the ApoE ε4 by smoking interaction was observed in the no chemotherapy group only.

Comparable results have been reported by Correa et al. (2014) in a large cohort of adult brain cancer survivors, most of whom completed treatment with chemotherapy and/or cranial irradiation[29]. The authors found that patients with at least one ε4 allele performed worse on verbal learning and delayed recall, and slightly worse on executive functions, relative to ε4-negative patients. Smoking also seemed to attenuate some of the risk associated with the ε4 genotype. Patients with at least one ε4 allele and a history of smoking scored better on attention

and verbal learning, and notably, albeit nonsignificantly, better on delayed recall and verbal fluency than ε4 carriers who never smoked. Furthermore, the authors identified nine additional ApoE SNPs associated with attention, executive functioning, and/or memory. None of the SNPs provided a good fit for the white matter (WM) abnormality ratings on MRI.

Brain-derived neurotrophic factor (BDNF) gene

BDNF is the most widely distributed neurotrophin in the CNS, particularly in the prefrontal cortex and hippocampus. It has been associated with synaptic plasticity and remodeling, dendritic and axonal growth, induction of long-term potentiation, modulation of gene expression, and resilience to neuronal insults[45,46].

We identified two studies which assessed associations between BDNF polymorphisms and cancer-treatment related neurocognitive impairment[19,32].

Correa et al. (2016) evaluated neurocognitive outcome in a cohort of adult brain tumor survivors who had completed treatment with cranial radiotherapy and/or chemotherapy[32]. Three BDNF SNPs were related to memory (learning, delayed recall, recognition) performance, with variant allele carriers of SNPs rs10767664 and rs10835210 having lower scores, and variant allele carriers of SNP rs11030104 having higher scores. In addition, SNP rs2030324 was associated with executive functions, with variant allele carriers showing worse performance. There were no associations between SNP rs6265 (Val66Met) and any of the cognitive outcomes, a finding consistent with the results of Krull et al. (2013)[19]. Furthermore, none of the BDNF SNPs were significantly associated with WM abnormalities on MRI.

Dystrobrevin-binding protein 1 (DTNBP1) gene

DTNBP1 is widely expressed in regions of the brain that are critical to cognitive function, particularly the hippocampus and prefrontal cortex[47]. It influences glutaminergic, GABAergic, nicotinic, and dopaminergic neurotransmitter systems[48,49], and is involved in regulation of neuroplasticity[50].

Correa et al. found that DTNBP1 rs742106 was associated with recognition memory, with carriers of the variant alleles showing worse performance[32]. None of the DTNBP1 SNPs were significantly associated with WM abnormalities on MRI.

2. Neuroinflammation and defenses against oxidative stress

Gluthation-S-transferase (GST) gene family: GSTP1, GSTM1, GSTT1

GSTs are polymorphic enzymes which catalyze the detoxification of a variety of compounds, including cytotoxic agents (i.e., alkylating agents and platinum compounds) and their metabolites, as well as reactive oxygen species formed spontaneously or generated by chemotherapy and radiation treatment[51]. They are highly heterogeneous proteins with a broad distribution in normal human tissues, including the brain[52]. 42-60% and 13-26% of the white population have a homozygous deletion of respectively GSTM1 and GSTT1 (null genotype) and thus do not express the respective enzymes[53]. GSTP1 is characterized by two different SNPs resulting in amino acid substitutions that lead to reduced enzyme activity and affinity for electrophilic substrates (1404A>G, Ile105Val; 2294C>T, Ala114Val)[51,54].

Three of the four studies[19,27,36,44] investigating at least one member of the GST gene family reported significant associations.

Barahmani et al. (2009) longitudinally investigated the relationship between GSTM1 and GSTT1 null genotypes and IQ scores in a small cohort of pediatric medulloblastoma patients

treated with craniospinal radiation and systemic chemotherapy[36]. They found that patients with at least one null GSTM1 or GSTT1 genotype showed significant declines in full-scale, performance, and verbal IQ scores compared to those without null genotypes.

Complementary to this finding, Brackett et al. (2012) reported that childhood medulloblastoma survivors with the GSTM1 null genotype showed greater psychological distress, compared to those with the non-null genotypes[27]. However, in contrast to the findings of Barahmani et al. (2009), no significant associations between genetic polymorphisms and self-reported neurocognitive function emerged.

Krull et al. (2013) found that pediatric ALL survivors treated with risk-adapted chemotherapy who carried the GSTT1 null or GSTP1 polymorphisms demonstrated elevated rates of inattentiveness compared to the general population[19]. Moreover, children with the GSTT1 null genotype also showed increased reaction time variability.

Cole et al. (2015) reported that pediatric leukemia survivors with at least one GSTP1 T allele had lower estimated IQ and Digit Span scores than those who were homozygous for the wild-type C allele[44]. Nevertheless, this association with impairment was not significant after False Discovery Rate (FDR) correction for multiple hypothesis testing.

Endothelial nitric oxide synthase (NOS3) gene

Endothelial nitric oxide (NO) synthase catalyzes the formation of the vasodilator endothelial NO, a key mediator regulating vascular tone that also exhibits antioxidant properties. In the NOS3 gene, a G894T substitution results in a Glu298Asp replacement leading to decreased enzyme activity with less endothelial NO production, and consequently a diminished capacity for protection against oxidative stress[55].

The two studies examining the effect of NOS3 polymorphisms both reported significant associations[35,44].

In a longitudinal study, Krajinovic et al. (2005) found that NOS3 894T homozygosity was associated with a decline in IQ scores in ALL patients, but only in case of cranial irradiation[35]. Furthermore, the authors observed that the interaction between NOS3 894T homozygosity and cranial irradiation was more obvious in patients treated with the DFCI 95-01 protocol, which included prednisone (in contrast to dexamethasone in the DFCI 91-01 protocol). This finding suggests that the neurobehavioral toxicity associated with steroids might interact with chemotherapy and radiation treatment.

Cole et al. (2015) expanded on this finding in a larger cohort of childhood ALL survivors that included a subset of the patients from the study of Krajinovic et al.[44]. They confirmed the association between the NOS3 variant and decreased IQ, with the odds of IQ impairment being approximately five times higher for survivors homozygous for the 894T allele than for those without the polymorphism.

Solute carrier organic transporter 2A1 (SLCO2A1) gene

SLCO2A1, also known as the prostaglandin transporter, is involved in the uptake and clearance of prostaglandins in numerous tissues, including the brain. Prostaglandins are major lipid mediators that, among other functions, influence inflammatory responses within the CNS[56]. Since oxygen radicals are generated in the setting of chronic inflammation, functional polymorphisms affecting prostaglandin entry into the CNS possibly alter the balance between reactive oxygen species and protective mechanisms.

Cole et al. (2015) reported that pediatric ALL survivors carrying the variant G allele of SLCO2A1 rs762035 had lower mean estimated IQ and lower Digit Span scores than those who

were homozygous for the wild-type C allele[44]. In addition, the polymorphism was associated with risk for parental reporting of behavioral symptoms of inattention.

3. Neurotransmission

Catechol-O-methyltransferase (COMT) gene

COMT is an enzyme crucial for the metabolism of catecholamines, including dopamine. Its enzymatic activity is especially important for regulating dopamine levels in the prefrontal cortex. COMT contains a widely-studied SNP that leads to a substitution of valine by methionine on codon 158 (Val158Met). The Val allele is associated with higher enzymatic activity, leading to increased dopamine degradation and consequently lower dopamine availability in the prefrontal cortex. Dopamine is critically important for prefrontally-mediated cognitive functions[57–60]. Cancer survivors are at risk for decreased efficiency in prefrontal cortex networks, and they appear to require greater brain activation to maintain cognitive abilities[61,62]. Hence, individuals with higher dopamine degradation could display less resiliency against neurotoxicity as they have less prefrontal dopamine available to draw upon to compensate for the acquired brain changes.

We identified five studies which assessed the effect of COMT polymorphisms[19,26,30,32,44], of which four reported significant findings.

In a study by Small et al. (2011) including breast cancer survivors treated with chemotherapy and/or radiotherapy and a healthy control group, differences were found in favor of the COMT-Met homozygotes in tests of attention, verbal fluency, and motor speed, compared to COMT-Val carriers[63]. Additionally, COMT-Val carriers treated with chemotherapy performed worse on attention tasks compared to controls who were also Val-carriers, suggesting that COMT genotype may modify the presence of cognitive differences as a function of cancer treatment.

Correa et al. (2016) extended on this finding in a study of adult brain tumor survivors who had completed treatment with cranial radiotherapy or chemotherapy[32]. They reported that the COMT Val158Met polymorphism was associated with delayed recall performance, with Val homozygotes having lower scores relative to Met homozygotes. Moreover, they described ten additional COMT SNPs influencing attention, memory and executive functions. None of the COMT SNPs were significantly associated with WM abnormalities.

Conflicting results have been reported in pediatric studies. Howarth et al. (2014) examined the relationship between COMT genotype and working memory in pediatric brain tumor survivors treated with conformal radiotherapy[30]. Contrary to their a priori hypothesis that COMT-Val carriers would perform worse, the authors reported better working memory performance for those with the Met/Val genotype compared to Met homozygotes. This finding can be explained by assuming an inverted-U dose-response relationship between dopamine concentrations in the prefrontal cortex and cognitive performance[64], in which deficient or excessive levels of dopamine result in poorer performance. Furthermore, COMT polymorphisms were more strongly associated with verbal compared to visual working memory tasks.

Cole et al. (2015) observed a marginal association between the Val158Met polymorphism and parental reporting of both attention and hyperactivity in pediatric ALL survivors, with Met homozygotes performing worse than Val carriers[44]. The authors stated that the COMT gene may not only be associated with inactivation of catecholamine neurotransmitters, but also with susceptibility to oxidative stress. According to this hypothesis, decreased COMT enzyme activity may lead to less protection against oxygen radicals[65,66], and as such increase

susceptibility to treatment-related neurocognitive impairment. By contrast, Krull et al. (2013) did not find such an association in ALL survivors[19].

Monoamine oxidase A (MAO-A) gene

MAO-A is a mitochondrial enzyme critical for normal brain function which catalyzes the oxidative deamination of amines, such as dopamine, norepinephrine and serotonin[67]. Low enzyme activity has been associated with increased norepinephrine and overactivation of the sympathetic nervous system[68]. This process may result in increased anxiety and/or physiological stress, which have been associated with attention problems[69].

Two studies assessed the effect of MAO-A polymorphisms[19,44].

Krull et al. demonstrated increased reaction time variability in childhood ALL survivors with the MAO-A 1460T>C polymorphism[19]. By contrast, Cole et al. did not find this association[44].

4. Folate metabolism pathway

Methotrexate (MTX) is an antifolate drug used widely in pediatric oncology for treating both hematologic (e.g., ALL) and non-hematological (e.g., osteosarcoma) malignancies. It acts as a competitive analog of folic acid blocking dihydrofolate reductase (DHFR). This leads to decreased levels of 5,10-methylenetetrahydrofolate (5,10-MTHF), needed for nucleic acid synthesis and thus for cellular replication, and 5-methyltetrahydrofolate (5-MTHF), the primary form of circulating folate and co-substrate for the remethylation of homocysteine (Hcy) to methionine[70]. Prior studies have demonstrated reduced 5-MTHF levels and elevated Hcy in the cerebrospinal fluid after MTX therapy[71–73], particularly in patients with acute MTX-related neurotoxicity or leukoencephalopathy[74,75]. High Hcy levels have been proposed to underlie MTX-associated neurotoxicity, through induction of oxidative damage to neuronal tissue and vascular endothelium, and through further metabolism to excitotoxic glutamate analogs[73,76,77]. In addition, deficiency of the active form of methionine, S-adenosylmethionine (SAM), can cause demyelination of the CNS[78].

Methylenetetrahydrofolate reductase (MTHFR) gene

Methylenetetrahydrofolate reductase (MTHFR) catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the primary circulatory form of folate and the methyl donor needed for the remethylation of Hcy to methionine[79]. Two SNPs in the MTHFR gene, C677T and A1298C, which lead to Ala222Val and Ala429Glu amino acid substitutions in the catalytic and regulatory domains, respectively, result in reduced enzyme activity[80,81].

We identified seven studies investigating MTHFR polymorphisms[19,24,25,31,34,35,37], of which four reported significant results.

In two studies by Linnebank et al. (2005, 2009), MRI was performed on primary CNS lymphoma patients before and after treatment with MTX-based polychemotherapy[34,37]. In the first study, all patients received intraventricular treatment[37]. The authors found an association between the MTHFR 677C>T polymorphism and occurrence of white matter changes (WMC), albeit nonsignificant after correction for multiple testing. However, a combined risk haplotype, defined as presence of at least one of the genotypes MTHFR 677TT, MTR 2756AG/GG, or transcobalamin 2 776GG, was a highly significant risk factor for WMC, conferring a relative risk of 4.7.

In the second study, the sample of the previous study was expanded with patients treated without intraventricular drug administration. Occurrence of WMC was independently predicted

by the TT genotype of MTHFR 677C>T and the AA genotype of MTHFR 1298A>C. The observed association between MTX-induced WMC and the 677C>T variant is in accordance with the hypothesis on elevated homocysteine levels and reduced SAM levels as causes of MTX-induced neurotoxicity. By contrast, the described association between the A allele of 1298A>C and WMC contradicts this hypothesis. However, the MTHFR 1298A>C and 677C>T variants are in linkage disequilibrium, with the 677T allele being linked to the 1298A allele[82]. Therefore, it is possible that the 1298A allele is not directly associated with neurocognitive dysfunction but is a surrogate marker for the tightly linked polymorphism 677T that is in fact responsible for the association seen in this study.

Based on parental ratings and interviews, Krull et al. (2008) investigated if MTHFR polymorphisms could partially explain development of attention-deficit/hyperactivity disorder (ADHD) symptoms in a small cohort of pediatric ALL survivors[24]. They found that patients with genotypes related to lower folate levels, i.e. MTHFR 1298A>C and 677C>T, were more likely to show ADHD symptoms, especially the inattentive subtype. Contrary to the findings of Linnebank et al. (2009), the 1298A>C genotype appeared to be the predominant linkage, leading to a 7.4-fold increase in risk for ADHD diagnosis, compared to a 1.3-fold increase for the 677C>T genotype.

Kamdar et al. (2011) extended on these findings in and expanded sample of ALL survivors from the same center. They found that MTHFR 1298A>C carriers had a 3-fold increased risk of global cognitive impairment compared to non-carriers[25]. More specifically, patients carrying this variant showed worse executive function performance. In addition to examining the effect of individual folate pathway polymorphisms, the authors also calculated a composite folate pathway risk score. Survivors with six or more adverse alleles in the investigated folate pathway variants performed consistently worse on attention and processing speed tasks compared to those with less than six adverse alleles. The authors stated that individuals with several at-risk genotypes may have prominent variation in folate or homocysteine levels making them more susceptible to neurocognitive deficits in the setting of intermittently exaggerated folate depletion caused by MTX therapy.

Vitamin B12-dependent methionine synthase (MS) or 5-methyltetrahydrofolate-homocysteine S-methyltransferase (MTR) gene

Methionine synthase catalyzes the remethylation of homocysteine to methionine with methylcobalamin as a cofactor[79]. The MTR A2756G polymorphism, resulting in an Asp919Gly amino acid substitution, has been suggested to affect the secondary structure of the protein and therefore to have functional consequences[83,84]. However, the influence of this polymorphism on total homocysteine levels is still a matter of debate.

Three of the five studies examining the effect of this MTR polymorphism reported a significant association[19,25,34,35,37].

Linnebank et al. (2005) found that the MTR 2756A>G polymorphism was over-represented among PCNSL patients with WMC after MTX treatment, though significance did not remain after correction for multiple testing[37]. However, as previously mentioned, a combined risk haplotype defined as presence of at least one of the genotypes MTR 2756A>G, Tc2 776C>G, and MTHFR 677C>T, was a highly significant risk factor for WMC.

Kamdar et al. (2011) demonstrated that childhood ALL long-term survivors with the AA genotype of MTR2756A>G had a 3.8-fold increased risk of global cognitive impairment compared to those with the AG/GG genotypes[25]. Moreover, the AA genotype also appeared to be related specifically to deficits in focused attention and processing speed.

Discordant findings have been reported by Krull et al. (2013). They found that childhood ALL survivors with the MTR 2756AG/GG genotypes were more likely to show worse performance in attentiveness and response speed than those with the AA genotype[19].

Transcobalamin 2 (Tc2) gene

The Tc2 gene encodes transcobalamin 2, a plasma globulin that acts as the main transport protein of cobalamin (vitamin B12), which is necessary to remethylate methionine and SAM from homocysteine. The missense polymorphism Tc2 776C>G lowers the affinity of Tc2 to cobalamin and leads to reduced concentrations of blood Tc2-cobalamin complexes, thus reducing the biological availability of cobalamin for conversion of Hcy to methionine and SAM[85].

In two studies by Linnebank et al. (2005; 2009), the effect of the Tc2 776C>G polymorphism was investigated[34,37]. Both studies assessed associations between folate polymorphisms and the occurrence of WMC after treatment with MTX-based polychemotherapy in patients with PCNSL. In the first study, the Tc2 c.776C>G polymorphism was over-represented among patients with WMC, though significance did not remain after correction for multiple testing[37]. However, as previously mentioned, a combined risk haplotype defined as presence of at least one of the genotypes Tc2 c.776C>G, MTHFR c.677C>T and MTR c.2756A>G, conferred a relative risk for CNS WMC of 4.7. In the second study, the GG genotype of Tc2 c.776C>G independently predicted the occurrence of WMC[34].

Thymidylate synthase in the form of enhancer region repeats (TSER)

Thymidylate synthase catalyzes the conversion of deoxyuridine monophosphate to deoxythymidine monophosphate[79]. The promotor enhancer region of the TS gene may contain two (2R) or three (3R) 28-bp tandem repeat sequences that function as transcriptional enhancer elements. The TSER 3R/3R yields higher gene expression levels in vitro and higher enzyme activity in vivo than the 2R/2R genotype[86]. The 3R/3R genotype is associated with reduced folate and higher homocysteine levels, particularly in individuals with low dietary folate intake[87].

We identified three studies in which TSER polymorphisms were examined[25,31,34].

Only Kamdar et al. found a significant association, with pediatric ALL survivors with TSER 2R/3R and 3R/3R genotypes exhibiting worse performance on shifting attention and processing speed tasks, compared to those with the TSER 2R/2R genotypes.

Adenosine A2A receptor (ADORA2A) gene

MTX and its metabolites lead to increased levels of adenosine which binds to ADORA1 and ADORA2A in neural, glial and endothelial cells in the CNS[88]. ADORA1 confers a neuroprotective effect while activation of ADORA2A contributes to neurotoxicity, by enhancing excitatory neurotransmitter release such as glutamate and by downregulation of the neuroprotective effect of ADORA1[89]. Moreover, aminophylline, a competitive agonist against adenosine, has been used for treatment and prevention of MTX-related neurotoxicity[90].

Only Tsujimoto et al. (2016) assessed the effect of ADORA2A polymorphisms[33]. The authors found that in patients with lower cumulative doses of MTX (total cumulative dose less than 20g/m²), the ADORA2A rs2298383 CC genotype was associated with an increased risk of leukoencephalopathy compared with the CT and TT genotypes combined. In the high cumulative dose group (total cumulative dose 20g/m²), ADORA2A rs2298283 CC showed a

tendency for an association with leukoencephalopathy but was not statistically significant, suggesting that high cumulative doses of MTX might attenuate the effect of the SNP.

GWAS

The study by Bhojwani et al. (2014) is the only GWAS that has been carried out to date in this research area[28]. Serial brain MRI screening for leukoencephalopathy was performed in a large cohort of children with ALL treated with high-dose MTX and triple intrathecal therapy. The authors revealed polymorphisms in genes related to neurodevelopment (e.g., TRIO, SSPN, PRKG1, DKK2, ANK1, COL4A2, NTN1) with plausible mechanistic roles in neurotoxicity which were not previously investigated in candidate gene studies. However, findings remained largely speculative since none of the SNPs reached genome-wide significance, possibly because of limited sample size.

DISCUSSION

The aim of this review was to summarize literature findings on the genetic modulation of cancer treatment-related neurocognitive impairment, with a special focus on those variants that could be used in the pediatric cancer setting. Results of our study indicate that several polymorphisms across proposed pathophysiological pathways, i.e. (1) neural plasticity and repair, (2) neuroinflammation and defenses against oxidative stress, (3) neurotransmission, and (4) folate metabolism pathway, may be involved in the complex, apparently polygenic, susceptibility to cancer treatment-induced neurotoxicity. In particular, SNPs in ApoE, the gluthation-S-transferase gene family (GSTM1, GSTT1, GSTP1), COMT, MTHFR and MTR emerge as promising candidates, given the positive associations with neurocognitive impairment or neuroimaging abnormalities in a number of studies. However, considering that the number of studies in this research area is still poor and studies often report discordant findings, it is not yet possible to draw definite conclusions. The included studies were very heterogeneous with respect to study design, sample size, ethnicity, cancer types, treatment regimens, assessment techniques (i.e., neuroimaging vs. neurocognitive evaluation; performance vs. self-report measures) and domains assessed (i.e., global vs. specific processes), and post-treatment intervals. This precludes pooling of data for larger analyses. Moreover, several important limitations should be noted which warrant further discussion.

A first shortcoming concerns the lack of longitudinal information. The ideal research design to understand the effect of cancer treatment on neurocognitive outcome through survivorship would be a longitudinal design with baseline cognitive assessments and/or baseline neuroimaging. However, many studies did not include pre-treatment assessment data, probably due to practical limitations inherent to closely following patients over time. Lack of pretreatment assessment limits the conclusions than can be drawn from these studies for two reasons. First, pre-treatment evaluation is essential to prove that cognitive changes or neuroimaging abnormalities are truly related to cancer treatment and not to pre-existing differences in neurocognitive function or neuroanatomy. Secondly, since cognitive dysfunction caused by cancer treatment can be subtle, post-treatment assessment scores could still fall in the normal range although the cognitive decline may represent a clinically significant difference. Such subtle cognitive changes would not be detected without pre-treatment evaluation, therefore leading to false-negative findings. In addition to a longitudinal design with baseline assessments, patients are ideally compared to appropriate control groups which undergo the same assessments in the same timeframe as the group of interest.

Secondly, study sizes were often rather small. Many studies were not prospectively designed but were conducted post hoc using a convenience sampling, which is supported by the fact that none of the studies mentioned a sample size calculation. Ideally, to ensure sufficient statistical power in genetic association studies, sample size should be determined a priori by the minor allele frequency of the investigated variant and its effect size. According to our study results, the occurrence of treatment-related neurocognitive impairment probably reflects a polygenic trait, with cumulative risk determined by multiple common risk alleles with low-to-medium effect sizes. However, reliable detection of such associations with low-to-medium effect sizes require (far) more participants than most of the reviewed studies had[91,92]. Therefore, replication and validation of current findings in sufficiently large homogeneous pediatric cancer cohorts, which could be achieved by establishing large (inter)national consortia, will be necessary before clinical implementation of this knowledge is possible.

Furthermore, timing of assessment varied widely from one study to the next. We selected six months post-(consolidation) treatment as the cut-off to ensure that ample time had elapsed for determination of neurocognitive function. However, studies in which the survivorship phase was relatively early (i.e., six months post-treatment) may still not reflect patterns seen in long-term survivors (i.e., > five years post-treatment). Moreover, the included studies were also very heterogeneous with respect to assessment approach (i.e., neuropsychological tests versus neuroimaging, performance versus self-report measures, global versus specific processes). A standardized, uniform approach to these two key features, i.e. timing and tools of assessment, will be critical in constructing (inter-)national consortia to tackle this issue. There has already been some preliminary consensus on a core set of neuropsychological tests with adequate psychometric properties that cover key domains with established relevance to cancer and its treatment[10,93]. Considering that neuroimaging studies have provided important neurobiological evidence for the impact of cancer treatment on neurocognition, a multifaceted approach that integrates both neuropsychological assessments and neuroimaging is essential to achieve a thorough understanding of treatment-related neurocognitive impairment. In the reviewed studies that investigated leukoencephalopathy as the outcome of interest, MRI T2 and FLAIR sequences were typically used. However, more advanced MRI techniques (i.e., functional MRI, diffusion-weighted imaging and anatomical volumetric studies) may provide greater sensitivity to treatment-related CNS changes. Incorporating these advanced neuroimaging techniques into research on the genetic modulation of treatment-induced cognitive impairment may therefore lead to further progress in this clinically relevant area.

Another important consideration is that most of the included studies used a targeted approach, focusing on specific candidate variants believed to play a role in cancer treatment-related neurotoxicity. As mentioned previously, the occurrence of treatment-related neurocognitive impairment probably reflects a polygenic trait. Certainly not all genetic variation can be explained by variants studied by targeted approaches so far. Future genetic research will need a broader approach by performing GWASs. In contrast to targeted approaches, GWASs provide an 'agnostic' approach in which no a priori biological hypothesis is needed and novel polymorphisms in previously unsuspected genes could be identified across the entire genome. Moreover, subsequent pathway analysis - grouping identified genes into biological pathways - may point to pathways not previously expected to contribute to neurotoxicity. As such, the underlying pathophysiology may be further clarified. Despite these substantial advantages, the number of GWASs is clearly lagging behind in this research field, considering the fact that only one GWAS has been performed to date. Given that the number of investigated SNPs in a GWAS ranges from 100,000 to approximately 5 million on newer SNP arrays, the likelihood of false positive discovery is very high. Genome-wide significance is therefore defined by very low p-

values ($p < 10^{-8}$), and very large cohorts are required to create enough power to identify relevant variants. This, once again, emphasizes the need for national and international collaboration.

CONCLUSION

Considering these recommendations for future studies, we believe that further genetic research holds great promise for minimizing adverse neurodevelopmental outcomes in pediatric cancer patients by enabling (1) prognostication of neurocognition, followed by risk-adapted, genotype-tailored treatment regimens and prophylactic interventions, and (2) identification of targets for rational interventions to protect the nervous system. Large scale, collaborative GWAS integrating both neurocognitive assessments and advanced neuroimaging techniques will become highly important. However, it should be noted that not the complete spectrum of neurocognitive outcome can be predicted by genetic variation. As the etiology of cognitive impairment in cancer survivors is likely multifactorial, another challenge will be to determine how to integrate genetic information with other risk factors related to interpatient variability in neurocognitive outcomes.

CONFLICT OF INTEREST

None.

ROLE OF FUNDING SOURCE

None.

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