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Genetic factors and long-term treatment-related neurocognitive deficits, anxiety, and depression in childhood leukemia survivors: an exome-wide association study

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Abstract

Background: An increased risk of neurocognitive deficits, anxiety, and depression has been reported in childhood cancer survivors.

Methods: We analyzed associations of neurocognitive deficits, as well as anxiety and depression, with common and rare genetic variants derived from whole-exome sequencing data of acute lymphoblastic leukemia (ALL) survivors from the PETALE cohort. In addition, significant associations were assessed using stratified and multivariable analyses. Next, top-ranking common associations were analyzed in an independent SJLIFE replication cohort of ALL survivors.

Results: Significant associations were identified in the entire discovery cohort (N=229) between the *AK8* gene and changes in neurocognitive function, whereas *PTPRZ1, MUC16, TNRC6C-AS1* were associated with anxiety. Following stratification according to sex, the *ZNF382* gene was linked to a neurocognitive deficit in males, whereas *APOL2* and *C6orf165* were associated with anxiety and *EXO5* with depression. Following stratification according to prognostic risk groups,

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the modulatory effect of rare variants on depression was additionally found in the *CYP2W1* and *PCMTD1* genes.

In the replication SJLIFE cohort (N=688), the male-specific association in the *ZNF382* gene was not significant, however, a p-value<0.05 was observed when the entire SJLIFE cohort was analyzed. *ZNF382* was significant in males in the combined cohorts as shown by meta-analyses as well as the depression-associated gene *EXO5*.

Conclusions: Further research is needed to confirm whether the current findings, along with other known risk factors, may be valuable in identifying patients at increased risk of these long-term complications.

Impact: Our results suggest that specific genes may be related to increased neuropsychological consequences.

INTRODUCTION

The survival rates in children diagnosed with acute lymphoblastic leukemia (ALL)(1, 2), the most frequent childhood cancer(3), have dramatically increased over the past decades due to the introduction of multi-agent risk-adapted treatment regimens and outstanding improvements in care delivery. However, exposure to cytotoxic therapy during a vulnerable period of child development can have long-term consequences, including impaired neurocognitive functions(4, 5), and mood disorders(6, 7). Furthermore, childhood and adolescence are periods characterized by intensive development of the central nervous system(8, 9), which is pertinent in the context of the impact of cancer treatment on the integrity of the white matter(10–14). Indeed, numerous studies conducted in childhood ALL survivors(4, 15, 16) have reported an increased risk of neurocognitive deficits(6) in attention(15–20), working memory(21), processing speed(16, 22, 23), and executive functions, such as verbal fluency and cognitive flexibility(24); as well as depression, anxiety, behavioral difficulties, distress, and post-traumatic symptoms compared with siblings(25–30).

Varying degrees of neurocognitive dysfunction and levels of emotional distress associated with cancer treatment have been observed that differ by patient characteristics, such as age and sex, and possibly reflecting different underlying mechanisms(5, 31, 32). Moreover, while some survivors may not experience any of these complications, others may have more than one. Factors contributing to this variability, include the type of treatment, the characteristics of the malignancy, the lifestyle, and the genetic makeup of the patient(33).

We examined whether common and rare genetic polymorphisms contribute to this variability by altering the risk of treatment-related neurocognitive deficits, as well as anxiety and depression in combination with non-genetic factors.

We previously analyzed these complications in a well-described cohort of ALL survivors (PETALE)(34) using a candidate gene approach(33); two associations between the *MTR* and *CACNB2* genes and neurocognitive deficit were validated in an independent St. Jude Lifetime Cohort (SJLIFE) replication cohort (St. Jude Children's Research Hospital, Memphis, USA)(33).

Here, the association analyses are extended to a hypothesis-free approach – an exome-wide association study, which could identify additional genes as potential risk modulators of these complications.

MATERIALS AND METHODS

Discovery cohort

The discovery cohort included 229 patients diagnosed and treated for childhood ALL according to Dana Farber Cancer Institute (DFCI) ALL 87-01 to 05-01 protocols at Sainte-Justine University Health Center (SJUHC), Montreal, (Quebec), Canada. The participants were recruited during 2013–2015 in the context of the PETALE study(34). Written informed consent was obtained from every patient or parent/legal guardian. The study was conducted in accordance with the Declaration of Helsinki and the protocol was approved by the Ethics Committee of SJUHC.

Eligible participants were of European descent, younger than 19 years old at diagnosis and older than 12 years at evaluation, at least 5 years after diagnosis of ALL, without a history of relapse or refractory ALL or Down syndrome and had not received a hematopoietic stem cell transplant. The median age of patients at the time of diagnosis was 4 years, the time from the end of treatment to evaluation ranged from 3–24 years with a median of 13 years (for 76.0% of participants, it was ≥10 years), both sexes were equally represented (51.1% of females). The patients were classified to standard (SR) and high relapse risk (HR) groups based on prognostic factors, including age, white blood cell count, immunophenotype, and central nervous system (CNS) status at diagnosis(35, 36). The frequency of patients assigned to SR and HR groups during the treatment was 45.9% and 54.1%, respectively.

Neuropsychological evaluation

A neurocognitive evaluation was performed using standardized testing procedures and scores from the Delis-Kaplan Executive Function System (D-KEFS)(37) and the Wechsler Adult Intelligence Scale-Fourth Edition (WAIS-IV)(38), and included the Trail Making Test (D-KEFS) score, the Verbal Fluency (D-KEFS) score, and the Digit span (WAIS-IV) total score, as described previously(39). The conversion of raw scores to age-adjusted scaled scores was based on population means(40); subsequently, neurocognitive outcomes were transformed into dichotomous variables and studied accordingly. For each of these variables, scores lower than one and a half standard deviations below the mean of the normative dataset were indicative of impairment(41), all other scores were considered non-impaired.

Anxiety and Depression

Categorization of participants into anxiety or depression groups was based on their symptoms exceeding age-specific norms as described previously(39). For participants under the age of 19, we employed the anxiety and depression modules of the Beck Youth Inventories - Second Edition (BYI) (42). For older participants (≥19 years) the Brief Symptom Inventory-18 (BSI-18 anxiety and depression score) was applied (43). The Cronbach’s alpha coefficients for internal consistency demonstrated satisfactory levels, all

exceeding 0.80(44). Scores adjusted for age that were one standard deviation above the population mean were regarded as indicative of impairment.

Sequencing and quality control

Whole-exome sequencing (WES) was performed on germline DNA, extracted from peripheral blood samples from participants in the PETALE cohort, using standard protocols as described previously(33). Whole exomes were captured in solution with Agilent's SureSelect Human All Exon 50Mb kits and sequenced on either Life Technologies SOLiD System 4.0 (mean coverage = 40X) or Illumina HiSeq 2500 platform (mean coverage = 113.1X) at SJUHC integrated clinical genomic center in pediatrics as described previously(39, 45). Only missense, nonsense, and splicing common and rare variants with predicted functional impact (Sift (<0.1) and/or PolyPhen2 (≥0.85)) were considered(46, 47). Variants were defined as rare (minor allele frequency, MAF<5%) and common (MAF≥5%) according to the reported frequency for European populations in public datasets(48). Variants exceeding a missing rate of 20%, not in Hardy-Weinberg Equilibrium (p=0.05/number of tests)(49), and common variants with pairwise linkage disequilibrium (LD, r2≥0.8) were excluded. Therefore, 5312 common genetic variants corresponding to 3793 genes, and 58924 rare genetic variants corresponding to 11441 genes that satisfied the above-mentioned filtering criteria entered the association analyses.

Association analyses

The P-value threshold of 5×10^{-8}, commonly used to identify an association between a common genetic variant and an outcome of interest in a typical GWAS(50–52) is not applicable to our study since variants for analysis were selected from WES data to focus on those predicted by various instruments to affect coding protein function(46, 47). As a result, 5312 common variants were analyzed, which is less than the typical number of GWAS variants; and the Benjamini-Hochberg procedure for false discovery rate (FDR) (53, 54) was used to adjust for the number of variants tested with an adjusted cut-off value of <5% considered to be statistically significant(52). The analyses between common genetic variants and neurocognitive outcomes, and anxiety/depression were performed by the allelic chi-square or Fisher's exact test implemented in PLINK v.1.07(55, 56). Analyses were performed in 229 sequenced patients and stratified by sex and risk groups with different treatment intensities because these factors have an established role in modulating neurocognitive outcomes(57, 58). For top-ranking associations, the best genotyping model was subsequently used in the univariate model as well as in multivariable logistic regression analysis to assess the effect of each genotype when controlling for non-genetic covariates. In cases when several genetic variants were associated with the same outcome, additional multivariable analyses were performed in which all genetic variants associated with that outcome were analyzed with non-genetic covariates in a single model. Non-genetic co-variables included: age at the time of diagnosis (continuous variable); time since the end of treatment (continuous variable); sex: males/females (categorical variable); DFCI Protocol: 87-01=1, 91-01=2, 95-01=3, 00-01=4, 05-01=5 (categorical variable); risk: SR/HR (categorical variable); treatment variable with patients who received only chemotherapy and patients who received chemotherapy and cranial radiation (categorical variable). The associations that remained statistically significant through multivariable regression models

were retained for further analyses. The effect of genotype was quantified by odds ratio (OR) with 95% CI according to the best-fitting genetic model, which was either dominant or recessive in all cases. In addition, the potential additive effect of combining risk loci by recoding genotypes as having none, one, or two and more risk alleles has also been explored.

The effects of rare variants were evaluated using the SKAT-O test (Optimal Sequence Kernel Association Test)(59–61) implemented in SKAT package v.2.0.1(62). Only genes with at least two variants that satisfied filtering criteria were retained for the SKAT-O test. To evaluate individual variant contributions to association signals, a collapsing approach(63, 64), with the iterative exclusion of every single variant, was additionally executed in SPSS v.25.0. Similar to the common variant analyses, genetic associations were assessed for each of the neurocognitive outcomes in the entire cohort, in different sex groups, as well as in SR and HR groups, and through multivariable models adjusted for the covariates described above. The multiple test adjustment (FDR) for the number of genes tested was included in all analyses.

Replication cohort

The replication cohort consisted of 688 childhood ALL survivors of European ancestry enrolled in the SJLIFE study with whole-genome sequencing data. The maximum number of participants in the replication cohort with available outcome data was 675; the total number varied depending on the outcomes and subgroup studied. Participants were selected to resemble the discovery cohort based on demographics and treatment characteristics. They were younger than 19 years at diagnosis, older than 12 years at evaluation, with no history of relapse within 5 years of the primary ALL diagnosis date, Down syndrome, or hematopoietic stem cell transplantation. The median age at diagnosis was 4.9 years, the median time since 5-year survival from ALL diagnosis was 25.8 years for neurocognitive evaluation, and 25.6 years for anxiety and depression evaluation; 50.4% of participants were males. All outcome measures were the same as in the discovery cohort with the exception of the Patient-Reported Outcomes Measurement Information System (PROMIS) Anxiety and Depression Scales(65, 66) that were used to assess anxiety and depression in participants under 18 years of age. Associations that remained significant in the discovery cohort using multivariable regression models were analyzed in the replication cohort by Fisher’s exact test for allelic contingency tables and logistic regression adjusting for continuous age at diagnosis, sex, the continuous time between the date of becoming a 5-year ALL survivor and date of test measurement, whether the survivor was treated with chemotherapy only versus chemotherapy plus radiation, and the top 20 principal components adjusting for genetic ancestry. Variant rs750295511 (*MUC16*) did not pass quality control and was excluded from the analysis. Stratification by the risk group designation was not available for the SJLIFE cohort. Rare variant replication analyses were not performed since rare variant associations in the discovery cohort were only detected in the risk group stratified analyses. The combined effect in the discovery and replication cohort was addressed through meta-analysis performed using the Mantel-Haenszel method implemented in *MedCalc* software and assuming a fixed-effects model.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon request and revision of the projects for which the data might be used.

RESULTS

Discovery cohort characteristics

Demographics and clinical characteristics of PETALE participants are presented in Table 1. The analyses were performed in either all patients or subgroups. These included patients assigned to standard (SR, 45.9%) and high risk (HR, 54.1%) groups, males (48.9%) and females (51.1%). The median age of ALL survivors at the time of evaluation was 21 years. The most prevalent deficit in neurocognitive test performance was noted for digit span (19.7%) followed by verbal fluency (19.2%) and trail making test (8.7%). Moderate-severe anxiety was noted in 9.2% survivors, whereas 10.5% of survivors were affected by moderate-severe depression.

Common variants

Among the common genetic variants, the top-ranking associations obtained using PLINK (Supplementary Tables S1–S3) were assessed through multivariate regression models that also included non-genetic co-variables. Only the associations that remained significant in these models were retained for further analyses (Supplementary Tables S4–S7).

Accordingly, significant associations were detected between Trail making test and rs17407084 variant in the *AK8* gene (OR=7.3, 95% CI, 2.7–19.7; p=4.52E-04), as well as between Moderate-severe anxiety and the following variants: rs740965 in *PTPRZ1* (OR=5.1, 95% CI, 1.98–12.9; p=1.00E-03), rs750295511 in *MUC16* (OR=8.3, 95% CI, 3.1–22.5; p=3.10E-05), and rs2748431in *TNRC6C-AS1* (OR=6.1, 95% CI, 2.0–18.1; p=1.00E-03). These association are presented in their best genetic models (Tables 2 and 3) as well as by Manhattan plots (Supplementary Figures S1 and S2). Additionally, the combined effect of the rs740965 (*PTPRZ1*), rs750295511 (*MUC16*), and rs2748431 (*TNRC6C-AS1)* variants was identified by recoding genotypes as having none, one or two and more alleles at risk (Supplementary Figure S3). Following stratification according to sex we identified variant rs61732180 in the *ZNF382* gene that was associated with the increased risk of deficit scores in the Trail making test in male participants (OR=20.2, 95% CI, 4.3–95.4; p=2.62E-04, Table 2). Male carriers of the variant alleles in the rs7285167 (*APOL2*) and rs61731441 (*C6orf165*) genes were more prone to Moderate-severe anxiety (OR=9.6, 95% CI, 2.3–40.5; p=3.00E-03 and OR=10.9, 95% CI, 2.5–47.0; p=2.00E-03, respectively, Table 3), whereas male carriers of the variant allele in the rs35672330 (*EXO5*) gene were more prone to Moderate-severe depression (OR=20.3, 95% CI, 4.1–99.8; p=4.90E-04, Table 4). Additionally, the combined effect of the rs7285167 (*APOL2*) and rs61731441 (*C6orf165*) variants was seen when they were tested by recoding genotypes as having none, one or two and more alleles at risk (Supplementary Figure S4).

We did not find any significant common variant association that would satisfy multiple testing adjustments when performing an exome-wide association with the remaining neurocognitive phenotypes either in entire group or following stratification.

Replication results.

Demographics and clinical characteristics of participants from the replication SJLIFE cohort are presented in Supplementary Table S8. Of the eight common variant associations that remained statistically significant in the discovery cohort using multivariable regression models, seven variants passed SJLIFE quality control (except for a variant rs750295511 in *MUC16*) and were further analyzed for an association using the respective phenotype measures in the independent cohort of ALL survivors in the SJLIFE cohort (Tables 5). An association between deficit in the trail making test performance and the minor allele of *ZNF382* rs61732180 was not observed in males, however, it was seen in all survivors (OR=1.4; 95% CI, 1.04–1.90; p=0.025) with statistical significance in both allelic Fisher's exact test and adjusted logistic regression analyses (Supplementary Table S9). Additionally, variant rs61732180 in the *ZNF382* gene remained statistically significant in male participants of the combined discovery and replication set when assessed through meta-analysis (Figure 1a).

An allelic association between moderate-severe anxiety and *APOL2* rs7285167 was seen with p-value<0.05 in the male-restricted adjusted logistic regression analysis and the full SJLIFE cohort allelic and adjusted logistic regression analyses (Table 5 and Supplementary Table S9); however, it had the opposite effect and therefore cannot be considered as replicated. In addition, although an association between moderate-severe depression and the minor allele of *EXO5* rs35672330 was not observed in males, the effect of this allele was detected in the pooled discovery and replication cohort through the meta-analysis (Figure 1b).

Rare variants

The analysis of functionally predicted rare variants in PETALE cohort led to the detection of the associations between the moderate-severe anxiety and rare variants in the *PCMTD1* and *CYP2W1* genes in the HR patients (p=9.4E-6 and p=1.3E-5, respectively, Table 6). Using the collapsing approach, we explored variant combinations that contributed to the observed association signal, consequently identifying two variants in *PCMTD1* (rs201786115 and rs200377849), and one in *CYP2W1* (rs3735684). No significant association was obtained for moderate-severe depression and neurocognitive outcomes through the rare variants' analysis.

The detailed description of common and rare significant variants, including rs identifiers and their functional implication, is provided in Supplementary Table S10.

DISCUSSION

Neurocognitive function

In our study, functionally predicted germline common variants in the *AK8* and *ZNF382* genes were found to be significantly associated with deficits in the performance of the trail making test in PETALE participants. The association with the *AK8* gene was detected when the entire cohort of survivors was analyzed and also in the standard-risk group, whereas that of *ZNF382* was context-dependent, and was detected upon sex stratification. The detailed description of the *AK8* gene function is provided in Supplementary Material (Supplementary Material S1).

Although the male specific ZNF382 association was not observed in males alone, it was detected within the entire replication cohort. Zinc finger protein 382, encoded by the *ZNF382* gene, is a member of the largest family of transcriptional regulators - Krüppel-associated box domain (KRAB) zinc finger proteins(67). It plays critical roles as a transcription inhibitor and has been suggested to be a tumor suppressor in various types of human cancer, including pediatric acute myeloid leukemia(68, 69). Interestingly, ZNF382 inhibits the activating protein 1 (AP-1) and nuclear factor kappa-B (NF-kB) signaling. NF-κB involvement was detected in the different categories of neurons including both excitatory (glutamatergic) and inhibitory (GABAergic), as well as in the neural sub-compartment of the synapse; thus suggesting that neuronal NF-κB signaling pathway functions under normal physiological conditions to promote synaptic growth and to improve synaptic activity and long-lasting forms of plasticity(70). Moreover, its activation by excitatory neurotransmission and participation in multiple forms of structural and synaptic plasticity is probably at the basis of the function of this transcription factor in cognitive behaviors(70). On the other hand, NF-κB, a key regulator of innate immunity, is over-activated in a number of neurodegenerative diseases, including Alzheimer's disease(71). Although the male-specific association between the deficit score in neurocognitive test performance and the minor rs61732180 *ZNF382* allele identified in our study was not observed in males from the SJLIFE replication cohort, a p-value<0.05 was obtained for the full SJLIFE population and in males of the pooled discovery and replication cohorts. Therefore, the involvement of the *ZNF382* gene in neurocognitive function may warrant further investigation since the genetic variant might potentially play a role in the outcome regardless of gender.

Childhood ALL patients who have received CNS-directed chemotherapy demonstrate persistent and significant neurocognitive impairment that manifests after treatment(5, 72). Specifically, treatment of ALL may result in smaller gray and white matter volumes as well as alterations in white matter microstructure, often associated with decreased neurocognitive performance(73). Our previous work identified, using a candidate gene approach, a panel of several genes that showed an effect on neurocognitive decline in the PETALE cohort. Two variants, rs1805087 in the *MTR* gene and rs58225473 in the *CACNB2* gene, deserve mention as these associations were confirmed in the independent SJLIFE replication cohort.

It is worth noting that standard-risk childhood ALL patients typically receive less intensive treatment than high-risk patients(4, 74, 75) and the modulation of the genetic association by the treatment might be more obvious in the high-risk group. On the other hand, some of the

drug doses did not differ between the two groups(74, 76). Moreover, epidemiological and neuroimaging results indicate that ALL survivors who received contemporary therapeutic protocols (which consist of intensified intravenous and intrathecal administration of chemotherapeutic drugs for standard risk patients(74, 77)) were still at risk of neurocognitive problems(4).

Interestingly, genes identified in the present study through a hypothesis-free approach regarding the impaired neurocognitive function are associated to varying degrees with oxidative stress (*AK8* gene) or with immune regulation (*ZNF382* gene); and all of them, in one way or another, are also related to the function of the CNS. There is a lot of evidence to suggest that the brain is very susceptible to oxidative damage due to its high metabolic demand(78, 79). The oxidative stress, which is related to elevated intracellular levels of reactive oxygen species (ROS) is a key mediator of neuroinflammation, metabolic changes, bioenergetic deficiency, and neuronal apoptosis (80). ROS generated during chemotherapy may be associated with various harmful events, including neurotoxicity(81). For example, methotrexate (MTX) promotes oxidative stress in several organs, including the brain(81). In addition, MTX inhibits the activation of NF-κB, the already mentioned protein complex, which, among other functions, plays a central role in DNA transcription and the regulation of inflammation(82, 83).

Anxiety and Depression

We found that functionally predicted germline common variants in the *PTPRZ1, MUC16, TNRC6C-AS1, APOL2,* and *C6orf165* genes were significantly associated with moderate-severe anxiety in ALL survivors; whereas the *EXO5* gene was associated with the increased risk of moderate-severe depression. Noteworthy, most of the associations were sex-specific and were detected in males, which may be partly explained by the fact that self-reported anxiety/depression includes more social variance in females than in males(84). Additionally, an association was found between the moderate-severe anxiety in HR patients and rare variants enrichment in the *PCMTD1* and *CYP2W1* genes. The *EXO5* association deserves special mention since its effect was also seen in the pooled discovery and replication cohort.

EXO5 (Exonuclease 5) is a single-stranded DNA-specific bidirectional exonuclease that functions in the repair of nuclear DNA(85). In a recent study, the *EXO5* gene was identified as a risk gene involved in prostate tumorigenesis(86). Although there is no data available on the potential involvement of *EXO5* in mood disorders or CNS function, it is interesting to note that the effect of the *EXO5* gene detected in our cohort was also gender-specific and has been identified in male individuals.

The detailed description of the gene functions of the remaining associations is provided in Supplementary Material (Supplementary Material S1).

Emotional challenges that arise from diagnosis and treatment of cancer in childhood can be seen as a highly traumatic event and can have long-term consequences. There is growing evidence that exposure to psychological distress at an early age can seriously affect brain maturation and development(87); in addition, childhood stress can increase vulnerability to later development of mental disorders, such as depression and anxiety(88).

Particularly, during peri-adolescence, brain areas critical for emotional regulation, such as the prefrontal cortex, hippocampus, and amygdala are still developing and are highly sensitive to stress(87). In a similar stressful environment, individuals will respond to stress differently as only part of them will demonstrate vulnerability, while others will remain resilient(30, 89).

The loci reported in the current study have not previously been identified as potential risk predictors of anxiety and/or depression susceptibility, however, given their important biological role, may warrant further study. In addition, the significant combined effect demonstrated in our study indicates that a single marker and/or single genotype may not be sufficient to explain the etiology of psychological distress phenotypes, given their complexity and environmental influences.

We acknowledge that our study has certain limitations. For example, limited sample size may affect the accuracy of the results, since the power of the study and magnitude of the effect might be affected in the context of a stratified analysis. Some phenotypes, such as trail making test, appear to occur with the similar frequency as in the general population, but the cause of their development, including genetic predisposition, may differ between patients exposed to the treatment and untreated individuals(90). At the same time, it is also important to note that survivors may tend to systematically report lower or normal distress rates as a result of a tendency to over normalize their situation. If this is the case in the present group, then it is likely that those with moderate-severe levels experience feelings to an even more significant degree. Thus, despite the small number of affected individuals in the study group it is nevertheless legitimate to explore why some individuals are more vulnerable. The association results obtained for rare variants in the discovery cohort (not evaluated in the SJLIFE cohort) should be taken with caution given their low number. Most of the common variant associations found in the PETALE cohort were not replicated in the SJLIFE cohort. This can be explained by several reasons. Mainly, despite the use of similar inclusion/exclusion criteria and the use of similar outcomes between the two cohorts, it is possible that the small sample sizes in both cohorts, differences in treatment protocols, lack of evaluation according to risk groups and/or timing between ALL diagnosis and evaluation contributed to the observed discrepancies. Finally, we can not disregard the possibility that some of the associations observed in the discovery cohort could have been obtained by chance.

In conclusion, using WES data and a hypothesis-free approach, we identified several genes as potential modulators of the risk of developing treatment-related neurocognitive complications, as well as anxiety and depression. The association between deficit in the trail making test and variant rs61732180 in the *ZNF382* and rs35672330 in *EXO5* genes are of particular interest since associations were also found in the replication cohort or meta-analysis of discovery and replication cohorts.

Multiple evidence has been collected nowadays for potential genetic and epigenetic risk markers of the long-term treatment-related neurocognitive and emotional complications in survivors of childhood cancer. In addition, accumulating data suggest that genetic factors contribute significantly to resilient responses to trauma and stress(91). Large genome-wide

association studies on the genetic architecture of mental disorders indicate its polygenic nature(92–94). Therefore, future studies will be required not only to verify current results, but also for multilevel integration of several approaches including polygenic score models along with other non-genetic factors, in order to identify markers of neurocognitive and emotional disorders and implement them into clinical practice.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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LIST OF ABBREVIATIONS

ALL	Acute lymphoblastic leukemia
PETALE	**P**révenir les **E**ffets tardifs des **T**raitements de la leucémie **A**iguë **L**ymphoblastique chez l'**E**nfant
SJUHC	Sainte-Justine University Health Center
SJLIFE	St. Jude Lifetime Cohort
WES	Whole exome sequencing
DFCI	Dana-Faber Cancer Institute
SJLIFE	St-Jude Lifetime cohort
D-KEFS	Delis-Kaplan Executive Function System
WAIS-IV	Wechsler Adult Intelligence Scale-Fourth Edition
BYI	Beck Youth Inventory
BSI-18	Brief Symptom Inventory-18

PROMIS	Patient-Reported Outcomes Measurement Information System
MAF	Minor allele frequency
EAF	Effect allele frequency
SKAT-O test	Optimal Sequence Kernel Association Test
FDR	False discovery rate
SR	standard risk
HR	high risk
OR	odds ratio
CI	confidence interval
CNS	central nervous system
NF-kB	nuclear factor kappa-B
ROS	reactive oxygen species
ZNF382	Zinc finger protein 382
EXO5	Exonuclease 5
AK8	Adenylate Kinase 8 ATP-AMP Transphosphorylase 8
KIR3DL1	Killer Cell Immunoglobulin Like Receptor
PPARG	Peroxisome Proliferator Activated Receptor Gamma
PLAUR	Urokinase Plasminogen Activator Surface Receptor
DUOXA1	Dual Oxidase Maturation Factor 1
DUOX2	Dual Oxidase 2
PTPRZ1	Protein Tyrosine Phosphatase Receptor Type Z1
MUC16	Mucin 16, Cell Surface Associated
TNRC6C-AS1	TNRC6C antisense RNA 1
MICB	MHC Class I Polypeptide-Related Sequence B
ARL16	ADP Ribosylation Factor Like GTPase 16
APOL2	Apoliprotein L2
C6orf165	Cilia And Flagella Associated Protein 206

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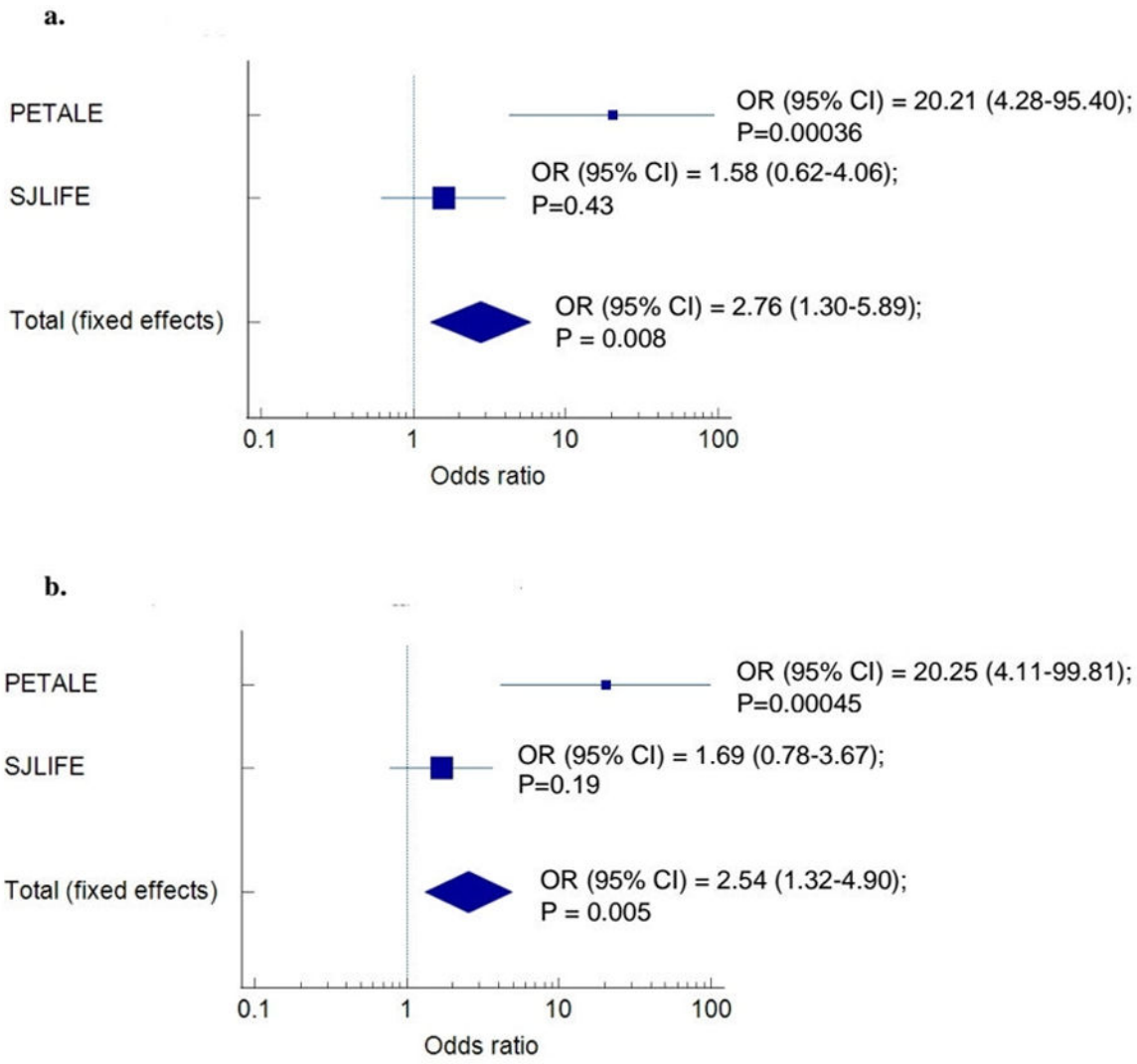

Figure 1.

Meta-analysis of the effect of the variant rs61732180 *ZNF382* on the neurocognitive deficit (a) and rs35672330 *EXO5* on moderate-severe depression (b) in male participants of combined discovery and replication set.

Plot represents the associations in the discovery cohort (PETALE), the replication cohort (SJLIFE) and the combined cohort (Total). Odd ratios (OR) comparing carriers to non-carriers, along with the 95% confidence intervals (95% CI) and the p-values of the associations are provided next to the combined patient set.

Table 1.

Patient demographics and clinical characteristics, Discovery PETALE cohort, N=229.

	N	%
Sex		
Males	112	48.9
Females	117	51.1
Prognostic risk group		
Standard risk	105	45.9
High risk	124	54.1
Criteria for High-risk stratification *		
Age	41	33.1
WBC	33	26.6
T-cell markers	5	4.1
Combination Age and/or WBC and/or T-cell markers	21	16.9
CNS involvement	16	12.9
MRD+	2	1.6
Other **	6	4.8
DFCI protocol		
(87–1)	18	7.9
(91–1)	47	20.5
(95–01)	68	29.7
(00–1)	72	31.4
(5–1)	24	10.5
Cranial radiation therapy (median 18 Gy)		
Yes	134	58.5
No	95	41.5
Age at diagnosis, median (range)		
		4 (0–18)
Time since the end of treatment		
More than 10 years	174	76
Less than 10 years	55	24
Median (range)		13 (3–24)
Tested outcomes		

	Affected	Unaffected	Missing
Neurocognitive outcomes			
Trail making test	20 (8.7%)	209 (91.3%)	0 (0.0%)
Verbal fluency	44 (19.2 %)	184 (80.4%)	1 (0.4%)
Digit span	45 (19.7%)	187 (80.3%)	0 (0.0%)
Emotional distress outcomes			
Moderate-severe anxiety	21 (9.2%)	180 (78.6%)	28 (12.2%)
Moderate-severe depression	24 (10.5%)	177 (77.3%)	28 (12.2%)

DFCI, Dana-Farber Cancer Institute. WBC: White Blood Cell; CNS: Central Nervous System; MRD: Minimal Residual Disease.

*Criteria for High-risk stratification was mainly attributed based on age, white blood cell count, immunophenotype (presence of T-cell markers) and combination of these factors; as well as central nervous system (CNS) status and Minimal residual disease at diagnosis.

**This category represents patients with chromosomal abnormalities and/or combination of factors.

Table 2.

Top-ranking associations of the common variants analysis regarding the performance of the Trail making test.

Genotype	Case*, N (%)	Control*, N (%)	Model	Case*, N (%)	Control*, N (%)	P value**	OR [95%-CI]	P value adj***
				***AK8* rs17407084**				
				All cohort, N=229				
TT	11 (55.0)	188 (90.0)	**TT**	11 (55.0)	188 (90.0)			
TC	7 (35.0)	21 (10.0)	**TC+CC**	9 (45.0)	21 (10.0)	2.16E-04[d]	7.3 [2.7–19.7]	4.52E-04
CC	2 (10.0)	0 (0.0)						
				***ZNF382* rs61732180**				
				Males, N=112				
CC	3 (27.3)	65 (64.4)	**CC+CT**	6 (54.5)	97 (96.0)			
CT	3 (27.3)	32 (31.6)	**TT**	5 (45.5)	4 (4.0)	3.58E-04[r]	20.2 [4.3–95.4]	2.62E-04
TT	5 (45.4)	4 (4.0)						
				***AK8* rs17407084**				
				Standard risk, N=105				
TT	2 (28.6)	90 (91.8)	**TT**	2 (28.6)	90 (91.8)			
TC	4 (57.1)	8 (8.2)	**TC+CC**	5 (71.4)	8 (8.2)	2.44E-04[d]	28.1 [4.7–168.8]	4.40E-04
CC	1 (14.3)	0 (0.0)						

AK8: Adenylate Kinase 8; ZNF: Zinc Finger Protein 382; FDR: false discovery rate; OR: odds ratio. TT indicates homozygosity for the T allele; TC represents heterozygosity, with one copy of the T allele and one copy of the C allele; CC indicates homozygosity for the C allele.

* Participants with and without indicated complications are defined as cases and controls, respectively.

** P values are calculated by chi-square or Fisher exact test, as appropriate. The most representative genetic model used is indicated (a: Additive; d: Dominant, r: Recessive).

*** P value adj: p value from logistic regression adjusted for age at diagnosis, sex, time since the end of treatment, protocol, and treatment variable (chemotherapy only or chemotherapy and radiotherapy).

Table 3.

Top-ranking associations between common variants and Moderate-severe anxiety.

Genotype	Case*, N (%)	Control*, N (%)	Model	Case*, N (%)	Control*, N (%)	P value**	OR [95%-CI]	P value adj***
PTPRZ1 rs740965								
All cohort, N=200								
TT	10 (47.6)	147 (82.1)	**TT**	10 (47.6)	147 (82.1)	1.00E-03	5.1 [1.98–12.9]	1.00E-03
TG	8 (38.1)	31 (17.3)	**TG+GG**	11 (52.4)	32 (17.9)			
GG	3 (14.3)	1 (0.6)						
MUC16 rs750295511								
All cohort, N=197								
AA	10 (50.0)	158 (89.3)	**AA**	10 (50.0)	158 (89.3)	7.20E-05	8.3 [3.1–22.5]	3.10E-05
TA	10 (50.0)	19 (10.7)	**AT+TT**	10 (50.0)	19 (10.7)			
TT	0 (0.0)	0 (0.0)						
TNRC6C-AS1 rs2748431								
All cohort, N=167								
GG	12 (63.2)	135 (91.2)	**GG**	12 (63.2)	135 (91.2)	2.00E-03	6.1 [2.0–18.1]	1.00E-03
GA	5 (26.3)	11 (7.4)	**GA+AA**	7 (36.8)	13 (8.8)			
AA	2 (10.5)	2 (1.4)						
APOL2 rs7285167								
Males only, N=95								
GG	5 (50.0)	77 (90.6)	**GG**	5 (50.0)	77 (90.6)	4.00E-03	9.6 [2.3–40.5]	3.00E-03
GA	4 (40.0)	8 (9.4)	**GA+AA**	5 (50.0)	8 (9.4)			
AA	1 (10.0)	0 (0.0)						
C6orf165 rs61731441								
Males only, N=95								
GG	3 (30.0)	70 (82.4)	**GG**	3 (30.0)	70 (82.4)	1.00E-03	10.9 [2.5–47.0]	2.00E-03
GA	6 (60.0)	15 (17.6)	**GA+AA**	7 (70.0)	15 (17.6)			
AA	1 (10.0)	0 (0.0)						

PTPRZ1: Protein Tyrosine Phosphatase Receptor Type Z1; MUC16: Mucin 16, Cell Surface Associated; TNRC6C-AS1: TNRC6C antisense RNA 1; APOL2: Apolipoprotein L2; C6orf165: Cilia And Flagella Associated Protein 206; FDR: false discovery rate; OR: odds ratio. TT indicates homozygosity for the T allele; TG represents heterozygosity, with one copy of the T allele and one copy of the G allele; GG indicates homozygosity for the G allele; AA indicates homozygosity for the A allele; TA represents heterozygosity, with one copy of the T allele and one copy of the A allele; GA represents heterozygosity, with one copy of the A allele and one copy of the G allele.

*Participants with and without indicated complications are defined as cases and controls, respectively.

**P values are calculated by chi-square or Fisher exact test, as appropriate. Given the low frequency of homozygotes for minor alleles, genetic model in all cases was dominant.

***P value adj: p value from logistic regression adjusted for age at diagnosis, sex, time since the end of treatment, protocol, and treatment variable (chemotherapy only or chemotherapy and radiotherapy).

Table 4.

Top-ranking associations between common variants and Moderate-severe depression.

Genotype	Case*, N (%)	Control*, N (%)	Model	Case*, N (%)	Control*, N (%)	P value**	OR [95%-CI]	P value adj***
EXO5 rs35672330								
Males only, N=94								
TT	4 (44.4)	81(94.2)	**TT**	4 (44.4)	81(94.2)	4.52E-04[d]	20.3 [4.1–99.8]	4.90E-04
TC	4 (44.4)	5 (5.8)	**TC+CC**	5 (55.6)	5 (5.8)			
CC	1 (11.2)	0 (0.0)						

EXO5: Exonuclease 5; FDR: false discovery rate; OR: odds ratio. TT indicates homozygosity for the T allele; TC represents heterozygosity, with one copy of the T allele and one copy of the C allele; CC indicates homozygosity for the C allele.

*Participants with and without indicated complications are defined as cases and controls, respectively.

**P values are calculated by chi-square or Fisher exact test, as appropriate. Given the low frequency of homozygotes for minor alleles, genetic model in all cases was dominant.

***P value adj: p value from logistic regression adjusted for age at diagnosis, sex, time since the end of treatment, protocol, and treatment variable (chemotherapy only or chemotherapy and radiotherapy).

Table 5.

Replication results, SJLIFE cohort, N=688*.

Gene	rs number	EAF	cases			controls			Allelic Fisher exact test		Logistic regression**	
			n.2	n.1	n.0	n.2	n.1	n.0	OR [95%CI]	P value	OR [95%CI]	LRT P value
Trail making test												
All cohort, N= 675*												
AK8	rs17407084	0.05	0	12	140	1	52	470	0.76 [0.36–1.45]	0.45	0.75 [0.36–1.44]	0.39
Males only, N= 337												
ZNF382	rs61732180	0.26	7	36	40	14	94	146	1.36 [0.90–2.04]	0.13	1.40 [0.92–2.15]	0.12
Moderate-severe anxiety												
All cohort, N= 675*												
PTPRZ1	rs740965	0.15	2	20	45	13	150	445	1.29 [0.77–2.09]	0.31	1.52 [0.90–2.50]	0.11
TNRC6C-AS1	rs2748431	0.07	0	9	58	5	77	526	0.93 [0.40–1.92]	1.00	0.86 [0.38–1.70]	0.67
Males only, N= 340												
APOL2	rs7285167	0.08	0	2	32	0	52	254	0.33 [0.04–1.29]	0.15	0.24 [0.04–0.94]	0.040
C6orf165	rs61731441	0.1	1	3	30	1	61	244	0.69 [0.21–1.80]	0.53	0.71 [0.23–1.83]	0.50
Moderate-severe depression												
Males only, N= 339												
EXO5	rs35672330	0.06	0	10	45	0	33	251	1.62 [0.69–3.50]	0.20	2.18 [0.91–4.97]	0.08

AK8: Adenylate Kinase 8; ZNF: Zinc Finger Protein 382; PTPRZ1: Protein Tyrosine Phosphatase Receptor Type Z1; TNRC6C-AS1: TNRC6C antisense RNA 1; APOL2: Apolipoprotein L2; C6orf165: Cilia And Flagella Associated Protein 206; EXO5: Exonuclease 5.

EAF: Effect allele frequency; n.2: 2 copies of the effect allele; n.1: 1 copy of the effect allele; n.0: 0 copies of the effect allele; OR: odds ratio for each additional copy of the effect allele; CI: confidence interval; LRT P value: Likelihood ratio test P value.

*While the total replication SJLIFE cohort consisted of 688 participants, it's noteworthy that when participants with non-missing data were selected for the Trail making test and Moderate-severe anxiety regressions, the number included happened to be identical, with 675 survivors in each analysis. However, these participants were not the exact same survivors in both analyses.

**Logistic regression analyses adjusted for continuous age at diagnosis, sex, continuous time between date of becoming a 5-year ALL survivor and date of test measurement, whether the survivor was treated with chemotherapy only versus chemotherapy plus radiation, and the top 20 principal components adjusting for genetic ancestry.

Table 6.

Top-ranking associations of the rare variants identified through the SKAT-O test regarding the Moderate-severe anxiety in HR patients, N=124.

Gene	SNVs tested		MAF	P value SKAT-O	FDR-BH	11/12+22		11	12+22	P value Fisher test	OR [95% CI]
	position (hg19)	rs number									
PCMTD1	**chr8:52732981** *	rs201786115	0.013	9.4E-06	0.03	Significant combination of collapsed variants **					**21.5 (4.4–105.9)** *
	chr8:52733110		0.008								
	chr8:52733164	rs149898988	0.021								
	chr8:52733209	rs202074278	0.022			91/9	Affected	5 (50.0%)	5 (50.0%)	4E-04	
	chr8:52733214 *	rs200377849	0.025				Unaffected	86 (95.6%)	4 (4.4%)		
	chr8:52733227 *		0.016								
CYP2W1	**chr7:1024855** *	rs3735684	0.038	1.3E-05	0.03	Individual contribution *					**21.9 (4.2–114.8)** *
	chr7:1024874		0.005			85/8	Affected	6 (54.5%)	5 (45.5%)	4E-04	
	chr7:1024921		0.005				Unaffected	79 (96.3%)	3 (3.7%)		

PCMTD1: Protein-L-Isoaspartate (D-Aspartate) O-Methyltransferase Domain Containing 1; CYP2W1: Cytochrome P450 Family 2 Subfamily W Member 1; SNV: single nucleotide variation; *MAF*: minor allele frequency; *FDR-BH: Benjamini–Hochberg false discovery rate; OR: odds ratio; CI: confidence interval.*

* SNVs that are identified as the most important contributors to the association signal are highlighted.

** Collapsed variants (carriers of at least one of rare variants were included into the model, variants with missing values were excluded.

Genotypes were recoded as follows:11-homozygote wild type; 12-heterozygote variant; 22-homozygote variant.