Influence of genetic factors on long-term treatment related neurocognitive complications, and on anxiety and depression in survivors of childhood acute lymphoblastic leukemia: The Petale study

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Abstract

Background

A substantial number of survivors of childhood acute lymphoblastic leukemia suffer from treatment-related late adverse effects including neurocognitive impairment. While multiple studies have described neurocognitive outcomes in childhood acute lymphoblastic leukemia (ALL) survivors, relatively few have investigated their association with individual genetic constitution.

Methods

To further address this issue, genetic variants located in 99 genes relevant to the effects of anticancer drugs and in 360 genes implicated in nervous system function and predicted to affect protein function, were pooled from whole exome sequencing data of childhood ALL survivors (PETALE cohort) and analyzed for an association with neurocognitive complications, as well as with anxiety and depression. Variants that sustained correction for multiple testing were genotyped in entire cohort (n = 236) and analyzed with same outcomes.

Results

Common variants in *MTR*, *PPARA*, *ABCC3*, *CALML5*, *CACNB2* and *PCDHB10* genes were associated with deficits in neurocognitive tests performance, whereas a variant in *SLCO1B1*

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polymorphisms is fully available to the public at
Figshare (DOI: 10.6084/m9.figshare.8051573) as
well as the summary statistics for all
polymorphisms analyzed from the sequencing data
beyond those presented in the manuscript and
supplemental materials (DOI: 10.6084/m9.figshare.
8051825).

Abbreviations: ALL, Acute lymphoblastic leukemia;
PETALE, Prévenir les Effets tardifs des Traitements
de la leucémie Aiguë Lymphoblastique chez
l'Enfant; SJUHC, Sainte-Justine University Health
Center; LAEs, Late adverse effects; WES, Whole
exome sequencing; DFCI, Dana-Faber Cancer
Institute; CCSS, Childhood Cancer Survivors Study;
SJLIFE, St-Jude Lifetime cohort; CRT, Cranial
radiation therapy; 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;
MAF, Minor allele frequency; MTX, methotrexate;
CS, corticosteroids; SKAT-O test, Optimal
Sequence Kernel Association Test; FDR, False
discovery rate; SNP, Single nucleotide
polymorphism; SR, standard risk; HR, high risk;
OR, odds ratio; CI, confidence interval; CNS, central
nervous system; OATP, organic anion transporting
polypeptide.

and *EPHA5* genes was associated with anxiety and depression. Majority of associations
were modulated by intensity of treatment. Associated variants were further analyzed in an
independent SJLIFE cohort of 545 ALL survivors. Two variants, *rs1805087* in methionine
synthase, *MTR* and *rs58225473* in voltage-dependent calcium channel protein encoding
gene, *CACNB2* are of particular interest, since associations of borderline significance were
found in replication cohort and remain significant in combined discovery and replication
groups (OR = 1.5, 95% CI, 1–2.3; p = 0.04 and; OR = 3.7, 95% CI, 1.25–11; p = 0.01,
respectively). Variant *rs4149056* in *SLCO1B1* gene also deserves further attention since
previously shown to affect methotrexate clearance and short-term toxicity in ALL patients.

Conclusions

Current findings can help understanding of the influence of genetic component on long-term
neurocognitive impairment. Further studies are needed to confirm whether identified vari-
ants may be useful in identifying survivors at increased risk of these complications.

Introduction

Acute lymphoblastic leukemia (ALL) is the most frequent childhood cancer [1] accounting for
approximately 25% of all cases [2]. The five-year survival rate of childhood ALL is currently
greater than 85% due to the optimization of multi-agent risk-adapted treatment strategies [2,
3]. However, the exposure to specific chemotherapeutic agents and/or cranial radiation ther-
apy during a susceptible period of child development results in late-adverse effects (LAEs) [4–
6] including neurocognitive impairments [2]. Clinically significant deficits among ALL survi-
vors are most commonly found in attention [7–12], working memory [13], processing speed
[9, 14, 15] and executive functions, such as verbal fluency and cognitive flexibility [16]. Neuro-
cognitive impairment in childhood ALL survivors persist for many years after treatment [17,
18]. Large survey studies like the Childhood Cancer Survivors Study (CCSS) as well as other
studies conducted in childhood ALL survivors [7, 9, 19] have demonstrated higher risk of
depression, anxiety, behavioural difficulties, distress, as well as post-traumatic symptoms com-
pared to siblings [20–25]. Longitudinal follow-up in long-term survivors have indicated that
frequency of distress evolves over time, with more than 10% of survivors experiencing signifi-
cant increase in depression as well as in anxiety [26].

The childhood ALL survivor population is increasing in size and lifespan, and this specific
population needs an effective evaluation and targeted interventions [27]. Thus, better under-
standing of LAEs and factors contributing to their development is important to guide survi-
vorship health surveillance and strategies to prevent or remediate treatment-related toxicities
[2, 3]. Here we assessed the role of genetic factors in neurocognitive impairments as well as in
anxiety and depression by interrogating the relationship between above mentioned complica-
tions and genotypic profiling of 459 candidate genes obtained through whole exome sequenc-
ing (WES) of childhood ALL survivors.

Study population and methods

Discovery cohort

The discovery cohort included 236 patients diagnosed and treated for childhood ALL accord-
ing 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, a multidisciplinary research project with the goal to identify and comprehensively characterize associated predictive bio-markers of long-term treatment related complications in childhood ALL survivors [6]. Eligible participants were younger than 19 years old at diagnosis, at least 5 years after diagnosis of ALL and older than 12 years at evaluation, without history of relapse or refractory ALL or neurological condition or Down syndrome and had not received a hematopoietic stem cell transplant. The time from end of treatment to evaluation ranged from 5–26 year with a median of 13 years. The patients were classified to standard (SR) and high risk (HR) groups based on prognostic factors, including age, white blood cell count, immunophenotype, and central nervous system (CNS) status at diagnosis [28, 29]. The frequency of patients assigned to SR and HR groups during the treatment was 46.6% and 53.4%, respectively. They were almost exclusively of reported French Canadian descent (>95%). The HapMap genotype reference data [30, 31] was used for Principal component analysis (PCA) [30] to test and confirm predominant European ancestry (S1 Fig).

Replication cohort

The replication cohort consisted of 545 ALL survivors (274 males and 271 females) of European ancestry (based on genotype data) enrolled in the St. Jude Lifetime Cohort (SJLIFE) study and were evaluated using the same testing procedures as in PETALE cohort. Participants were younger than 19 years old at diagnosis and younger than 40 years old at SJLIFE evaluation, with no diagnosis of neurologic condition or Down syndrome, no history of relapse and had not received a hematopoietic stem cell transplant. The time from primary cancer diagnosis to the most recent date of neurocognitive evaluation ranged from 10.95–45.60 years with a median of 26.84 years. The risk group assignment during the treatment (SR and HR groups) was not available for this cohort.

Neuropsychological evaluation

A neurocognitive evaluation was performed using standardized testing procedures. It included three indices from two cognitive measures that reflect common impairments among childhood ALL survivors and are also good predictors of general neuropsychological outcomes [32]: Trail Making Test–Condition 4—Letter-Number Sequencing score and Verbal Fluency–Condition 1 –Letter fluency score from the Delis-Kaplan Executive Function System (D-KEFS) [33]; and Digit Span from the Wechsler Adult Intelligence Scale-Fourth Edition (WAIS-IV) [34] total score. Trail Making Test (D-KEFS) score is a measure that reflects processing speed, psychomotor speed, and cognitive flexibility [35]. Verbal Fluency (D-KEFS) score is a measure of phonological fluency in verbal modality [36]. Digit span (WAIS-IV) total score is a measure of verbal working memory [37]. All raw scores were converted to age-adjusted scaled scores based on population means [38]. The 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 [39], all other scores were considered non-impaired.

Emotional distress: Anxiety and depression

Participants were classified having emotional distress if they demonstrated elevated symptoms according to two measures referenced to age-specific norms. This was done in line with published recommendations [40, 41] and previous use of the instruments [21, 22, 26]. For younger participants (<19 years), we used anxiety and depression modules of the Beck Youth

Inventory (BYI), a self-report instrument to document psychological status in children from 7 to 18 years old [41]. For older participants (≥19 years) we used the Brief Symptom Inventory-18 (BSI-18 anxiety and depression score), an 18-item self-report questionnaire, assessing psychological distress in adults [40], previously also used in cohorts of young and older adult survivors of childhood cancer [42, 43]. Internal consistency coefficients measured by Cronbach's alphas were all satisfactory, >.80 [44]. Age-adjusted scores one standard deviation above the population mean were considered as impaired.

Sequencing and quality control

Whole-exome sequencing (WES) was performed on germline DNA, extracted from peripheral blood samples from a subset of 191 participants of PETALE cohort, using standard protocols as described previously [6]. 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 centre in pediatrics. Reads were aligned to the hg19 reference genome using SOLiD LifeScope software [45] for the SOLiD samples and BWA-MEM [46] for the samples sequenced on the Illumina system. PICARD [47, 48] was used to mark PCR duplicates and collect sequencing quality control metrics. Variant calling was performed using the Haplotype Caller and quality score recalibration was performed using Variant Recalibrator, both implemented in the Genome Analysis Tool Kit (GATK) [48]. Variants were selected based on the variant quality score (VQSR = PASS) and minimum depth of coverage (DP > = 10). The final germline variants were annotated by ANNOVAR [49] and the predicted functional impact of missense, nonsense and splicing common and rare variants was assessed in silico using Sift (<0.1) and PolyPhen2 (≥0.85) filters [50, 51]. Variants were defined as rare (minor allele frequency, MAF<5%) and common (MAF≥5%) according to the reported frequency for European populations in the 1000 Genomes [52] and ESP6500 datasets [53]. These variants were considered as potentially damaging and were used for analyses. Variants exceeding missing rate of 20%, with minor allele count<2 and not in Hardy-Weinberg Equilibrium (P<0.001) were excluded.

Association analyses

Two sets of candidate genes were selected—the first consisted of 99 genes implicated in the metabolism of methotrexate (MTX) and corticosteroids (CS) which are known to impact neurocognitive outcomes [54, 55] and the second consisted of 360 genes implicated in nervous system function, selected using the KEGG PATHWAY Database [56]. A total of 76 common variants (27 in MTX/CS pathway and 49 in nervous system function) and 1337 rare variants that satisfied all above filtering criteria were identified as functionally predicted and were used in association analyses. The analyses between common genetic variants and neurocognitive outcomes as well as with anxiety/depression were performed by the allelic chi-square or Fisher's exact test implemented in PLINK v.1.07 [57, 58]. Analyses were performed in 191 sequenced patients and stratified by sex, risk groups with different treatment intensity, and treatment with chemotherapy alone or chemotherapy and cranial radiation because these factors have an established role in modulating neurocognitive outcomes [4, 59]. The Benjamini-Hochberg procedure for false discovery rate (FDR) [60, 61] was used to adjust for multiple testing with a cut-off value of < 5% considered statistically significant. Selective genotyping of top-ranking common SNPs (based additionally on Bonferroni p-value corrected for the number of variants tested, p<0.001 and p<0.0019 for the neural and MTX/CS pathways, respectively) was carried out on the Sequenom platform at the McGill University and Génome

Québec Innovation Centre, Montreal, (Quebec), Canada, to confirm the results and extend the analysis to entire cohort (n = 236) including one hundred ninety-one patients analysed above (**S1 Table**). Associations of genotyped variants with the outcomes were assessed using chi-square or Fisher exact test in SPSS v.24.0.0.0 and appropriate genetic models, which were presented relative to the minor allele. Genotype-outcome association was represented as an odds ratio (OR) with a 95% confidence interval (CI). For the variants of MTX pathway for which the association showed similar trend in validation cohort, the modulation of the effect by cumulative drug dose was also analyzed. For that, cumulative drug dose was dichotomized to above and below the median and the association was analyzed in each subgroup. Additionally, logistic regression model was used in which main effect (genotype and drug dose) and interaction term were added. The detailed list of the studied polymorphisms **(DOI: 10.6084/m9. figshare.8051573)**, as well as the summary statistics for all polymorphisms analyzed from the sequencing data beyond those already presented in the regular and supplemental tables **(DOI: 10.6084/m9.figshare.8051825)** are provided.

For rare variants associations, we used the SKAT-O test (Optimal Sequence Kernel Association Test) [62, 63] implemented in SKAT package v.1.3.2.1 [64] with FDR < 5% considered statistically significant. Collapsing approach that combines several rare variants into a single variable [65, 66], with iterative exclusion of each single variant, was additionally performed to allow weighting variant contributions to association signals. These analyses were performed as exploratory and associated variants were not further analyzed by genotyping.

Replication analysis

Genotype data for selected variants were obtained from a larger effort to sequence whole-genomes of over three thousand long-term survivors participating in the SJLIFE cohort. For this replication analysis, we restricted inclusion to 545 ALL survivors of European ancestry. Associations of selected variants with respective neurocognitive outcomes were examined using chi-square or Fisher's exact tests, as appropriate, implemented in PLINK 1.9 [57, 58].

Results

Neurocognitive and emotional disturbances

The median age of ALL survivors at the time of evaluation was 21 years, with almost equal sex distribution, their demographics and clinical characteristics are presented in **Table 1**. The most prevalent deficit in neurocognitive test performance was noted for digit span (19.5%) followed by verbal fluency (18.6%) and trail making test (9.3%). Moderate-severe anxiety was noted in 10.1% survivors, whereas 11.5% of survivors were affected by moderate-severe depression, which was comparable to published normative groups on anxiety and depression [40–42].

Common variants

Among common variants implicated in nervous system function obtained from WES data, significant associations were detected for four of them (*CALML5*, *CACNB2*, *PCDHB10* and *EPHA5)* either in all survivors or following stratification according to sex, risk groups or CRT (**S2 Table**). These variants were further analyzed by genotyping in the entire PETALE cohort and the association was confirmed for all of them (**Table 2**). The analyses were performed for the same subgroups for which association was noted for WES data, and additionally in all participants. The neurocognitive deficit related to digit span task was associated in an additive manner with the minor allele of *rs58225473* in *CACNB2* gene either in all patients (p = 0.02),

Table 1. Patient demographics and clinical characteristics (N = 236).

		N	%
Sex			
	Male	115	48.7
	Female	121	51.3
Neuropsychological outcomes (affected patients)			
	Trial making test*	22	9.3
	Verbal fluency*	44	18.6
	Digit span*	46	19.5
	Moderate-severe anxiety	21	10.1
	Moderate-severe depression	24	11.5
DFCI protocol			
	87–01	18	7.6
	91–01	48	20.3
	95–01	71	30.1
	00–01	75	31.8
	05–01	24	10.2
Prognostic risk group			
	Standard risk (SR)	110	46.6
	High risk (HR)	126	53.4
Cranial radiation therapy			
	Yes**	131	55.5
	No	105	44.5
	Cumulative doses, mg/m2—median (range)		
	Parenteral/PO MTX (853.6–12750.5)	6576.5	
	IT MTX (0–279)	150.4	
	IV/PO corticosteroids*** (4425.7–24930.1)	8826.6	
	IT corticosteroids*** (2.05–87.19)	31.3	
Age at diagnosis—median (range)			
	Male (1–18)	5.0	
	Female (0–18)	4.0	
Age at follow—up median (range)			
	Male (12–36)	21.0	
	Female (12–38)	21.0	

DFCI, Dana-Farber Cancer Institute; IV, intravenous; PO, per os; IT, intrathecal; MTX, methotrexate

*Score at least 1.5 standard deviation below the norm was considered as impaired in all neuropsychological tests

** Median (range), 12 Gy (12-18Gy)

*** Cumulative corticosteroid doses are calculated as prednisone equivalents

https://doi.org/10.1371/journal.pone.0217314.t001

or those who received chemotherapy only (p = 0.004). Homozygotes for the minor C allele of *CALML5 rs10904516* were at higher risk of having deficit in verbal fluency score, whereas the neurocognitive deficit related to trail making test was associated with the minor allele of *rs2907323* in *PCDHB10* gene, both potentiated in HR participants (p = 0.03 and p = 0.01 respectively). The carriers of the minor C allele of *EPHA5 rs33932471* were at higher risk of both moderate-severe anxiety and depression, with the strongest effects seen in females (p = 0.02 and p = 0.003, respectively).

Among common variants implicated in MTX/CS pathway obtained from WES data, the significant associations were detected for 6 of them (*MTR*, *PPARA*, *ABCC3*, *SHMT1* and

Table 2. Frequency of associated genotypes in patients with and without neurocognitive or emotional distress, genes of relevance for nervous system function, PETALE cohort (N = 236).

Outcome	Genotype	Case[*], N (%)	Control[*], N (%)	Model	Case[*], N (%)	Control[*], N (%)	P[**]	OR (95%-CI)
Digit span				*CACNB2 rs58225473*				
				All patients				
	TT	25 (58.1)	128 (71.5)	**TT**	25 (58.1)	128 (71.5)	0.02[a]	2.0 (1.1–3.9)
	TG	16 (37.2)	51 (28.5)	**TG**	16 (37.2)	51 (28.5)		
	GG	2 (4.7)	0 (0.0)	**GG**	2 (4.7)	0 (0.0)		
				Chemotherapy only[***]				
	TT	4 (40.0)	63 (70.8)	**TT**	4 (40.0)	63 (70.8)	0.004[a]	5.0 (1.5–16.4)
	TG	4 (40.0)	26 (29.2)	**TG**	4 (40.0)	26 (29.2)		
	GG	2 (20.0)	0 (0.0)	**GG**	2 (20.0)	0 (0.0)		
Trial making test				*PCDHB10 rs2907323*				
				All patients				
	GG	11 (55.0)	155 (73.1)	**GG**	11 (55.0)	155 (73.1)		
	GC	8 (40.0)	57 (26.9)	**GC**	8 (40.0)	57 (26.9)	0.02[a]	2.5 (1.1–6.2)
	CC	1 (5.0)	0 (0.0)	**CC**	1 (5.0)	0 (0.0)		
				High risk				
	GG	6 (46.2)	85 (76.6)	**GG**	6 (46.2)	85 (76.6)		
	GC	6 (46.2)	26 (23.4)	**GC**	6 (46.2)	26 (23.4)	0.01[a]	4.3 (1.4–12.7)
	CC	1 (7.7)	0 (0.0)	**CC**	1 (7.7)	0 (0.0)		
Verbal fluency				*CALML5 rs10904516*				
				All patients				
	TT	18 (40.9)	93 (48.7)	**TT+TC**	37 (84.1)	178 (93.2)	0.05[r]	2.6 (1.0–6.9)
	TC	19 (43.2)	85 (44.5)					
	CC	7 (15.9)	13 (6.8)	**CC**	7 (15.9)	13 (6.8)		
				High risk				
	TT	8 (34.8)	52 (50.5)	**TT+TC**	18 (78.3)	96 (93.2)	0.03[r]	3.8 (1.1–13.3)
	TC	10 (43.5)	44 (42.7)					
	CC	5 (21.7)	7 (6.8)	**CC**	5 (21.7)	7 (6.8)		
Moderate-severe anxiety				*EPHA5 rs33932471*				
				Females				
	AA	5 (55.6)	82 (89.1)	**AA**	5 (55.6)	82 (89.1)	0.02[d]	6.6 (1.5–28.5)
	AC	4 (44.4)	8 (8.7)	**AC+CC**	4 (44.4)	10 (10.9)		
	CC	0 (0.0)	2 (2.2)					
Moderate-severe depression				*EPHA5 rs33932471*				
				All patients				
	AA	15 (78.9)	151 (88.8)	**AA**	15 (71.4)	151 (88.8)	0.03[d]	3.2 (1.1–9.2)
	AC	6 (28.6)	17 (10.0)	**AC+CC**	6 (28.6)	19 (11.2)		
	CC	0 (0.0)	2 (1.2)					
				Females				
	AA	7 (58.3)	80 (89.9)	**AA**	7 (58.3)	80 (89.9)	0.003[d]	6.3 (1.7–24.2)
	AC	5 (41.7)	7 (7.9)	**AC+CC**	5 (41.7)	9 (10.1)		
	CC	0 (0.0)	2 (2.2)					

CACNB2: Calcium Voltage-Gated Channel Auxiliary Subunit Beta2; *CALML5*: Calmodulin Like 5, *EPHA5*: EPH Receptor A5, Brain-Specific Kinase; OR, odds ratio; CI, confidence interval.

[*]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).

[***]Chemotherapy without cranial radiation therapy.

Table 3. Frequency of associated genotypes in patients with and without neurocognitive or emotional distress, genes implicated in methotrexate and corticosteroids pathways, PETALE cohort (N = 236).

Outcome	Genotype	Case*, N (%)	Control*, N (%)	Model	Case*, N (%)	Control*, N (%)	P**	OR (95%-CI)
Verbal fluency				*MTR rs1805087*				
				All patients				
	AA	25 (61.0)	120 (66.7)	**AA+AG**	37 (90.2)	178 (98.9)	0.01[r]	9.6 (1.7–54.5)
	AG	12 (29.2)	58 (32.2)					
	GG	4 (9.8)	2 (1.1)	**GG**	4 (9.8)	2 (1.1)		
				Males				
	AA	12 (52.2)	57 (66.3)	**AA+AG**	19 (82.6)	86 (100.0)	0.002[r]	
	AG	7 (30.4)	29 (33.7)					
	GG	4 (17.4)	0 (0.0)	**GG**	4 (17.4)	0 (0.0)		
				PPARA rs1800206				
				Standard risk				
	CC	13 (72.7)	76 (92.7)	**CC**	13 (72.7)	76 (92.7)	0.008[a]	4.6 (1.5–14.5)
	CG	3 (16.7)	6 (7.3)	**CG**	3 (16.7)	6 (7.3)		
	GG	2 (11.1)	0 (0.0)	**GG**	2 (11.1)	0 (0.0)		
				Chemotherapy only				
	CC	12 (75.0)	77 (92.8)	**CC**	12 (75.0)	77 (92.8)	0.02[a]	4.3 (1.3–13.5)
	CG	2 (12.5)	6 (7.2)	**CG**	2 (12.5)	6 (7.2)		
	GG	2 (12.5)	0 (0.0)	**GG**	2 (12.5)	0 (0.0)		
				ABCC3 rs12604031				
				High risk				
	AA	3 (13.0)	33 (33.3)	**AA+AG**	14 (60.9)	89 (89.9)	0.001[r]	5.7 (2.0–16.5)
	AG	11 (47.8)	56 (56.6)					
	GG	9 (39.1)	10 (10.1)	**GG**	9 (39.1)	10 (10.1)		
Moderate-severe depression				*SLCO1B1 rs4149056*				
				Chemotherapy only				
	AA	1 (14.3)	59 (75.6	**AA**	1 (14.3)	59 (76.5)	0.002[d]	18.6 (2.1–164.7)
	AG	5 (71.4)	19 (24.4)	**AG+GG**	6 (85.7)	19 (24.4)		
	GG	1 (14.3)	0 (0.0)					

MTR: 5-Methyltetrahydrofolate-Homocysteine Methyltransferase, *PPARA*: Peroxisome Proliferator Activated Receptor Alpha, *ABCC3*: ATP Binding Cassette Subfamily C Member 3, *SLCO1B1*: Solute Carrier Organic Anion Transporter Family Member 1B1.

*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).

SLCO1B1, (**S3 Table**). The variants in *MTR*, *PPARA*, *ABCC3* and *SLCO1B1* genes were further analyzed by genotyping in entire PETALE cohort and the association was confirmed for all of them (**Table 3**). The association between deficit in verbal fluency score and GG genotype of *MTR rs1805087* was seen for all survivors (p = 0.01) and male participants (p = 0.002). Deficits in verbal fluency performance were also associated with GG genotype of *ABCC3 rs12604031* among HR patients (p = 0.001) as well as with *rs1800206* in *PPARA* gene in low risk groups (p = 0.008). The risk of moderate-severe depression was highest among carriers of the minor G allele of *SLCO1B1 rs4149056* who received chemotherapy only (p = 0.002).

All variants found significantly associated with tested outcome (except those initially confined to risk subgroup such as those in *ABCC3* and *PCDHB10* genes) were further analyzed for an association with respective outcomes in an independent cohort of ALL survivors (SJLIFE

Table 4. Frequency of genotypes in patients with and without neurocognitive or emotional distress, genes of relevance for nervous system function, replication cohort of SJLIFE (N = 545).

Outcome	Genotype	Case* N (%)	Control* N (%)	Model	Case* N (%)	Control* N (%)	P**	OR (95%-CI)
Digit span				*CACNB2 rs58225473*				
				All patients				
	TT	23 (71.8)	279 (69.1)	**TT+TG**	29 (90.6)	393 (97.3)	0.08[r]	3.7 (1.0–13.9)
	TG	6 (18.8)	114 (28.2)					
	GG	3 (9.4)	11 (2.7)	**GG**	3 (9.4)	11 (2.7)		
				Chemotherapy only				
	TT	16 (66.7)	112 (67.1)	**TT+TG**	21 (87.5)	161 (96.4)	0.09[r]	3.8 (0.9–16.5)
	TG	5 (20.8)	49 (29.3)					
	GG	3 (12.5)	6 (3.6)	**GG**	3 (12.5)	6 (3.6)		
Verbal fluency				*CALML5 rs10904516*				
				All patients				
	TT	32 (45.7)	180 (40.0)	**TT+TC**	62 (88.6)	387 (86.0)	0.6[r]	0.8 (0.4–1.7)
	TC	30 (42.9)	207 (46.0)					
	CC	8 (11.4)	63 (14.0)	**CC**	8 (11.4)	63 (14.0)		
Moderate-severe anxiety				*EPHA5 rs33932471*				
				Females				
	AA	27 (84.4)	205 (87.2)	**AA**	27 (84.4)	205 (87.2)	0.7[d]	1.3 (0.5–3.5)
	AC	5 (15.6)	27 (11.5)	**AC+CC**	5 (15.6)	30 (12.8)		
	CC	0 (0.0)	3 (1.3)					
Moderate-severe depression				*EPHA5 rs33932471*				
				All patients				
	AA	58 (79.5)	399 (86.0)	**AA**	58 (79.5)	399 (86.0)	0.1[d]	1.6 (0.8–3.0)
	AC	15 (20.5)	60 (12.9)	**AC+CC**	15 (20.5)	65 (14.0)		
	CC	0 (0.0)	5 (1.1)					
				Females				
	AA	30 (90.9)	202 (86.3)	**AA**	30 (9.9)	202 (86.3)	0.5[d]	0.6 (0.2–2.2)
	AC	3 (9.1)	29 (12.4)	**AC+CC**	3 (9.1)	32 (13.7)		
	CC	0 (0.0)	3 (1.3)					

*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 (d: Dominant, r: Recessive).

cohort) (**Tables 4 and 5**). Two associations were noticeable. The association of borderline significance between deficit in verbal fluency score and the minor allele of *MTR rs1805087* was seen in all survivors (OR = 1.7; 95% CI, 1.0–2.8; p = 0.05). The association between deficit in digit span score and GG genotype of *CACNB2 rs58225473* showed similar trend as in PETALE cohort for all participants (OR = 3.7, 95% CI, 1.0–13.9), as well as for patients who received chemotherapy only (OR = 3.8, 95% CI, 0.9–16.5), however they did not reach significance (p = 0.08 and 0.09, respectively). The associations for *rs58225473* and *rs1805087* variants in *CACNB2* and *MTR* genes were significant for combined PETALE and SJLIFE cohorts (**S4 Table**). The association between deficit in digit span score and GG genotype of *CACNB2 rs58225473* was significant for all participants (OR = 3.7; 95% CI, 1.25–11; p = 0.01) and for patients who received chemotherapy only (OR = 7.2, 95% CI, 2.1–25; p = 0.0004). The association between deficit in verbal fluency score and the minor allele of MTR rs1805087 was seen in all survivors (OR = 1.5; 95% CI, 1–2.3; p = 0.04) and in male participants (OR = 1.8; 95% CI, 1–3.1; p = 0.04).

Table 5. Frequency of genotypes in patients with and without neurocognitive or emotional distress, genes implicated in methotrexate and corticosteroids pathways, replication cohort of SJLIFE (N = 545).

Outcome	Genotype	Case* N (%)	Control* N (%)	Model	Case* N (%)	Control* N (%)	P**	OR (95%-CI)
Verbal fluency				*MTR rs1805087*				
				All patients				
	AA	42 (59.2)	323 (70.7)	**AG+GG**	29 (40.8)	134 (29.3)	0.05[d]	1.7(1.0–2.8)
	AG	25(35.2)	121 (26.5)					
	GG	4 (5.6)	13 (2.8)	**AA**	42 (59.2)	323 (70.7)		
				Males				
	AA	23 (60.5)	164 (72.2)	**AG+GG**	15 (39.5)	63 (27.8)	0.1[d]	1.7 (0.8–3.5)
	AG	13 (34.2)	56 (24.7)					
	GG	2 (5.3)	7 (3.1)	**AA**	23 (60.5)	164 (72.2)		
				PPARA rs1800206				
				Chemotherapy only				
	CC	39 (81.2)	155 (85.6)	**CC**	39 (73.8)	155 (85.6)	0.5[d]	1.4 (0.6–3.2)
	CG	9 (18.8)	26 (14.4)	**CG+GG**	9 (23.8)	26 (14.4)		
	GG	0 (0)	0 (0)					
Moderate-severe depression				*SLCO1B1 rs4149056*				
				Chemotherapy only				
	AA	31 (73.8)	142 (72.4)	**AA**	31 (73.8)	142 (72.4)	0.9[d]	0.9 (0.4–2.0)
	AG	10 (23.8)	48 (24.5)	**AG+GG**	11 (26.2)	54 (27.6)		
	GG	1 (2.4)	6 (3.1)					

*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 (d: Dominant).

https://doi.org/10.1371/journal.pone.0217314.t005

Given that the *MTR* belongs to the MTX pathway, we further explored whether the effect of *rs1805087* was modulated by cumulative MTX doses, for which such data were available in the discovery group (**Table 1**). The relationship with the deficit in verbal fluency score was particularly obvious in patients who received higher overall cumulative doses (**Fig 1**, p = 0.01 for patients with cumulative doses above median vs. p = 0.3, for patients with cumulative doses below median).
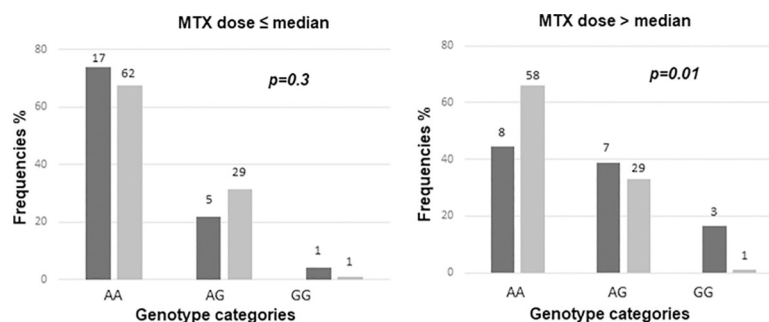

Fig 1. Interaction between MTR rs1805087 and cumulative methotrexate dose. The frequency of each genotype in affected and non-affected group defined by verbal fluency score is presented by black and gray bars, respectively, in the groups that received cumulative MTX below median (left panel) or above median (right panel). The number of individuals represented by each bar and p values are indicted on the plot. OR for interaction is 3.3, 95% CI 0.9–11.5, p = 0.07, as derived from logistic regression model including MTR genotype, cumulative MTX dose and interaction term.

https://doi.org/10.1371/journal.pone.0217314.g001

Table 6. SKAT-O analysis of the rare functional variants, PETALE cohort, WES data, demonstrated for the deficit in trail making test scores (N = 191).

Outcome	Gene	SNPs tested		MAF	P value	FDR	OR[1] [95% CI]
		position	rs number				
Trail making test	*SLCO2B1*	chr11:74875089–74875089		0.009	0.0002	0.004	8.7 [1.3–57.0]
		chr11:74880370–74880370	*rs35199625*	0.008			
		chr11:74899276–74899276	*rs377133671*	0.003			
	HSPA4	*****chr5:132387979–132387979**	***rs61745470***	0.017	0.004	0.027	*7.8 [1.7–36.4]
		chr5:132408967–132408967	*rs61755724*	0.042			
		*chr5:132412511–132412511		0.003			
		*chr5:132437499–132437499	*rs61749631*	0.005			
	GSTT1	chr22:24379402–24379402	*rs11550605*	0.003	0.003	0.027	19.7 [1.7–230.6]
		chr22:24381742–24381742		0.006			

SNP: single nucleotide polymorphism; OR: odds ratio; CI: confidence interval; *SLCO2B1*: Solute Carrier Organic Anion Transporter Family Member 2B1; *HSPA4*: Heat Shock Protein Family A (Hsp70) Member 4; *GSTT1*: Glutathione S-Transferase Theta 1. Individual contribution of variant *rs61745470* (noted in bold) was identified with OR = 19.5, 95%CI [2.97–128], p = 0.005.

[1] OR of significant combination, combination include all variants or variants noted with asterisk*.

Rare variants

The analysis of functionally predicted rare variants in PETALE cohort led to the detection of an association between the deficit in trail making test score and rare variants enrichment in *SLCO2B1*, *HSPA4* and *GSTT1* genes (p = 0.0002, p = 0.004 and p = 0.003, respectively, **Table 6**). Using the collapsing approach, we explored variant combinations that contributed to the observed association signal, identifying two variants in *GSTT1*, three in *HSPA4* and four in *SLCO2B1* gene. Replication analyses were not performed for these findings because information regarding these variants was not available in the SJLIFE cohort.

Discussion

Functionally predicted germline common variants in *MTR*, *PPARA*, *SLCO1B1*, *ABCC3*, *CALML5*, *CACNB2* and *PCDHB10* genes were found to be significantly associated with deficits in neurocognitive tests performance, whereas a variant in *EPHA5* gene was significantly associated with both anxiety and depression.

Neurocognitive performance

Among common variants associated with an impairment in neurocognitive function, *rs10904516* in the *MTR* gene, which was associated with a deficit in verbal fluency, seems particularly interesting given similar observation in the SJLIFE cohort. The *MTR* gene encodes a B12 dependent methionine synthase involved in remethylation of homocysteine (Hcy), which is the crucial step in methionine production in all types of cells [67]. Mutations in the *MTR* gene as well as severe deficiency of vitamin B12 could result in elevated concentration of Hcy in plasma and cerebrospinal fluid. Studies have shown that Hcy exerts a neurotoxic action and may participate in the mechanisms of neurodegeneration, such as excitotoxicity, oxidative stress, calcium accumulation, and apoptosis[68–70]. *MTR* gene is involved in the metabolic pathway of MTX. Administration of MTX was associated with acute and subacute neurotoxic effects; these detrimental effects may accumulate over time [69]. The detected common functional polymorphism (*rs1805087*) leading to Asp919Gly amino acid replacement in the *MTR* gene could affect enzymatic activity, thus increasing the level of Hcy [68, 69, 71]. Indeed, we

have shown interaction between *MTR rs1805087* and cumulative MTX dose in survivors with the deficit in verbal fluency score. This confirms previous finding: this variant together with polymorphisms of other genes that are implicated in the Hcy pathway were already studied in the context of MTX long-term neurotoxicity and has been found to affect neurocognitive function in childhood ALL survivors [72, 73].

CACNB2 rs58225473 variant was associated with the neurocognitive deficit as defined by digit span test, which measures working memory. Similar risk values, although not significant, were noted in the SJLIFE cohort in all participants and in the group of survivors who received chemotherapy only. The *CACNB2* gene encodes an auxiliary voltage-dependent subunit of L-type calcium-channel that is mainly expressed in brain and heart tissue. Voltage-dependent calcium channels are crucial for neuronal differentiation and maturation; they induce large number of intracellular events such as neurotransmitter release, neuronal excitability, synaptic plasticity and gene regulation [74]. Calcium influx mediated by those channels has both spatial and temporal components and encodes important signaling information [75]. Moreover, in a recent GWAS study *CACNB2* was identified among four significant risk loci underlying genetic effects shared between five major psychiatric disorders that included schizophrenia, autism spectrum disorder, attention deficit-hyperactivity disorder, bipolar and major depressive disorders [76]. Additionally, the rare variants in this gene were found in affected members of families with autism spectrum disease [77]. Given important role that *CACNB2* can play, it is not surprising that it was studied as a possible pharmacological target in treatment of mental disorders [78]. The *rs58225473* is c.1803T>G substitution (NM_201590.2) leading to Asp601Glu replacement, which is predicted to affect protein function and possibly calcium channel function. Common variants in several other genes influenced neurocognitive decline in PETALE cohort. *PPARA* gene belongs to PPARs receptor family of ligand-activated transcription factors involved in the regulation of inflammation [79]. Effects of glucocorticoids can be reinforced by PPAR ligands [80]. The enhanced heterodimer formations of PPARA could be associated with increased expression of brain and glial cell-derived neurotrophic factors [81]. The *rs1800206* variant in *PPARA* gene was associated with lower verbal fluency score in females and survivors assigned to SR group or chemotherapy only. Similar association was noted for *ABCC3 rs12604031*. *ABCC3* is a member of the superfamily of ATP-binding cassette (ABC) transporters, and the bioavailability of MTX may be affected by this transporter [82].

CALML5 gene is related to the calmodulin family of calcium binding proteins highly implicated in CNS function [83]. Its protective role and implication in inhibition of neuronal death was described in Alzheimer disease [84, 85]. We observed the significant association of variant *rs10904516* and deficit in verbal fluency score in all survivors with the stronger effect seen in the HR group. Similar association mostly confined to HR group was noted between lower score on trial making test and *rs2907323* in the *PCDHB10* gene. *PCDH* (protocadherin) genes, are expressed in the central and peripheral nervous systems and are required for their normal development. They mediate a variety of processes, including neuronal survival, morphogenesis and connectivity, synaptic maintenance, and spatial patterning of axons and dendrites [86]. The variants in *PCDH* genes have been reported to be associated with dyslexia and bipolar disorder [87, 88].

The neurotoxic effects of treatments in childhood ALL have been the subject of multiple investigations. These effects consist of central neurotoxicity clearly noticeable by encephalopathy and/or neurodevelopmental cognitive deficits [89–91], particularly in survivors exposed to a highly intensified treatment protocols with CNS-directed chemotherapy, even in the absence of CRT [4, 19, 92, 93]. Cognitive impairment and information processing have been associated with intensity and duration of CS treatment [94, 95]. Female survivors were reported to have more severe short-term memory impairment [59] and lower scores on attentional indices,

cognitive flexibility [96, 97] and visuomotor control [4]. Female childhood ALL survivors are more likely to present cerebral white matter damage [98] that may affect cognitive functioning. Congruent with these previous observations, several associations detected in the present study were modulated by sex and treatment intensity (reflected by presence or not of CRT or risk groups).

Anxiety and depression

We studied dimensions of internalized symptoms which are frequent in normative populations, namely anxiety and depression [99], highlighting impairment in mental quality of life of childhood ALL survivors [21, 22, 100]. Although anxiety and depression measures are not equivalent to clinical diagnosis derived from the gold standard systematic interview [101], moderate-severe levels are generally interpreted as a risk for having clinically relevant anxiety or depression.

The *rs11556218* in the *EPHA5* gene was associated with higher risk of both anxiety and depression that was further potentiated in female patients. The *EPHA5* gene codes for a brain-specific kinase that is selectively expressed in a subset of serotonin neurons during embryonic and postnatal development [102]. Receptors in the EPH subfamily modify the strength of existing synapses in the adult brain [103]. Divergent vulnerabilities between females and males could be explained by gender differences in brain maturation [104, 105], which might make females more vulnerable to the neurotoxic effects of chemotherapy. Other assumptions, such as endocrine factors, have also been hypothesized to explain sex differences in the susceptibility [59].

Moreover, we identified the association between depression and the presence of variant *rs4149056* in *SLCO1B1* gene. This association was detected in the group of survivors that received chemotherapy without CRT. *SLCO1B1* gene encodes a liver-specific member of the organic anion transporter family involved in hepatic uptake of MTX. This association deserved further attention given that the same variant was detected through genome wide association studies to contribute to inter-individual variability in the clearance of high-dose MTX [106]. It was subsequently replicated in independent cohorts and shown also as a predictor of short-term toxicity following MTX treatment [106–111]. MTX treatment has been associated with adverse emotional or behavioral outcomes [20], thus these results could justify further studies of *SLCO1B1* gene in related contexts.

Rare variants' analysis

The association between deficits in the trail making test score was identified in relation to rare variants enrichment in *HSPA4*, *SLCO2B1* and *GSTT1* genes, with a very strong individual contribution of *rs61745470* in *HSPA4* gene. This variant was recently associated with familial genetic risk for suicide (as well as with risk for psychiatric or substance abuse conditions) [112]. The *SLCO2B1* and *GSTT1* genes are highly implicated in physiological and pharmacological distribution of drugs and endogenous molecules. The *SLCO2B1*, a member of the organic anion transporting polypeptide (OATP) family, is involved in steroid hormone uptake and transport of steroid conjugates [113, 114]. *GSTT1* was recently associated with higher risk for early onset of severe mental and bipolar disorders [115]. We also evaluated the association of deletion polymorphisms of *GSTT1*[116] (found with the frequency of 23.7% in discovery cohort) with the deficits in the trail making test score. There was no association of *GSTT1* null genotype with the deficits in the trail making test score.

The impact of here identified rare functional variants requires further investigation.

Concluding remarks

Our study has certain limitations. Its limited sample size may affect the accuracy of the results, particularly in the context of the stratified analysis. Other unmeasured factors in this study, for example, inflammation and oxidative stress, could modulate or potentiate association with genetic factors. The candidate gene approach may have missed genetic markers potentially involved in neurocognitive decline and mood disturbances that could have been detected through unbiased approaches. Among associations detected in the PETALE cohort only two showed a similar trend in SJLIFE cohort. Despite matching both outcomes and patients' characteristics between the two cohorts, it is possible that small sample size, differences in treatment protocols or time of ALL diagnosis [117–120] contributed to the observed discrepancies. Likewise, stratification by risk group designation was not available for the SJLIFE cohort, precluding replication of the risk-based stratified analyses. Although the analyses in PETALE cohort were corrected for multiple testing, and confounding was reduced due to homogeneous population and uniform treatment, we cannot exclude that some of the associations have been obtained by chance.

In conclusion, using a comprehensive candidate gene approach and whole exome sequencing data we identified a panel of functionally predicted genetic variants significantly associated with neurocognitive deficits, anxiety and depression in childhood ALL survivors. Additional exome wide analysis might lead to the discovery of novel genes and genetic variants associated with neurocognitive LAEs as well as with the mood disorders.

While we acknowledge that the identified germline variants still need to be evaluated and validated through replication and functional studies, the current findings can help further understanding of the influence of genetic component on long-term complications related to cancer therapy.

Supporting information

S1 Fig. Principal component analysis (PCA). PCA analysis comparing sequencing data of 400 leukemia patients (including PETALE cohort) from Sainte-Justine University Health Center (SJUHC) to the HapMap genotype reference data (release 23) for Europeans (EUR), East Asians (EAS) and Africans (AFR).
PC1, Principal Component 1; PC2, Principal Component 2.
(TIF)

S1 Table. Genotyping: Identity of polymorphisms, details of PCR and ASO hybridization. R, reverse, F, forward. The base substitution that distinguishes the two variants of each polymorphism is given in bold for ASO probes. dbSNP number is provided. Ancestral allele is given in bold and minor allele is underlined. The polymorphisms are presented as a change from ancestral to derived allele, unless ancestral allele is not known, when the change is given from major to minor allele. SNPs in coding region leading or not to amino-acid substitutions are indicated.
(DOCX)

S2 Table. Significant results of association study of common variants from the candidate genes of relevance for nervous system function, PETALE cohort, WES data (n = 191). [a]Association test based on comparing allele frequencies between cases and controls. All associations have FDR-BH (Benjamini-Hochberg false discovery rate) lower than 5%. All also have p value lower than 0.001, which is Bonferroni cut-off value for the number of variants tested in nervous system function pathway.
[b]Stratified analyses according to sex and treatment intensity (standard vs high risk);

chemotherapy only vs chemotherapy and cranial radiation therapy (CRT).
Ref: reference allele; Var: variant allele; MAF: minor allele frequency, *PCDHB10*, protocadherin beta 10, *CALML5*: Calmodulin Like 5, *CACNB2*: Calcium Voltage-Gated Channel Auxiliary Subunit Beta2, *EPHA5*: EPH Receptor A5, Brain-Specific Kinase.
(DOCX)

S3 Table. Results of association study of common variants from methotrexate and corticosteroids pathways, PETALE cohort, WES data (n = 191). [a]Association test based on comparing allele frequencies between cases and controls. All associations have FDR-BH (Benjamini-Hochberg false discovery rate) lower than 5%.
[b]Stratified analyses according to sex and treatment intensity (standard vs high risk); chemotherapy only vs chemotherapy and cranial radiation therapy (CRT).
[c] SNPs or associations that did not qualify for genotyping with p value higher than 0.0019 (Bonferroni cut-off value for the number of genes tested in MTX/CS pathway).
Ref: reference allele; Var: variant allele; MAF: minor allele frequency; *MTR*: 5-Methyltetrahydrofolate-Homocysteine Methyltransferase, *PPARA*: Peroxisome Proliferator Activated Receptor Alpha, *ABCC3*: ATP Binding Cassette Subfamily C Member 3, *SHMT1*: Serine Hydroxymethyltransferase 1, *ADORA3*: Adenosine Receptor A3, *SLCO1B1*: Solute Carrier Organic Anion Transporter.
(DOCX)

S4 Table. The combined cohort represents the pooled samples from the discovery PETALE cohort and replication SJLIFE cohort (N = 781). Combined cohort analysis was performed for the variants in *CACNB2* and *MTR* genes. *Participants with and without indicated complications are defined as cases and controls, respectively.
**P values are calculated by Chi-square. The most representative genetic model used is indicated (d: Dominant, r: Recessive).
***Chemotherapy without cranial radiation therapy.
(DOCX)

Acknowledgments

The authors would like to thank all childhood ALL survivors and their parents for the participation in the PETALE study, as well as all study collaborators for their valuable contribution.

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References

1. Bhojwani D, Yang JJ, Pui CH. Biology of childhood acute lymphoblastic leukemia. Pediatr Clin North Am. 2015; 62(1):47–60. https://doi.org/10.1016/j.pcl.2014.09.004 PMID: 25435111; PubMed Central PMCID: PMC4250840.

2. Ward E, DeSantis C, Robbins A, Kohler B, Jemal A. Childhood and adolescent cancer statistics, 2014. CA Cancer J Clin. 2014; 64(2):83–103. https://doi.org/10.3322/caac.21219 PMID: 24488779.

3. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin. 2016; 66(1):7–30. Epub 2016/01/09. https://doi.org/10.3322/caac.21332 PMID: 26742998.

4. Buizer AI, de Sonneville LM, van den Heuvel-Eibrink MM, Veerman AJ. Chemotherapy and attentional dysfunction in survivors of childhood acute lymphoblastic leukemia: effect of treatment intensity. Pediatr Blood Cancer. 2005; 45(3):281–90. https://doi.org/10.1002/pbc.20397 PMID: 15806539.

5. Hudson MM, Ness KK, Gurney JG, Mulrooney DA, Chemaitilly W, Krull KR, et al. Clinical ascertainment of health outcomes among adults treated for childhood cancer. JAMA. 2013; 309(22):2371–81. https://doi.org/10.1001/jama.2013.6296 PMID: 23757085; PubMed Central PMCID: PMC3771083.

6. Marcoux S, Drouin S, Laverdiere C, Alos N, Andelfinger GU, Bertout L, et al. The PETALE study: Late adverse effects and biomarkers in childhood acute lymphoblastic leukemia survivors. Pediatr Blood Cancer. 2017; 64(6). Epub 2016/12/06. https://doi.org/10.1002/pbc.26361 PMID: 27917589.

7. Campbell LK, Scaduto M, Sharp W, Dufton L, Van Slyke D, Whitlock JA, et al. A meta-analysis of the neurocognitive sequelae of treatment for childhood acute lymphocytic leukemia. Pediatr Blood Cancer. 2007; 49(1):65–73. https://doi.org/10.1002/pbc.20860 PMID: 16628558.

8. Ashford J, Schoffstall C, Reddick WE, Leone C, Laningham FH, Glass JO, et al. Attention and working memory abilities in children treated for acute lymphoblastic leukemia. Cancer. 2010; 116(19):4638–45. https://doi.org/10.1002/cncr.25343 PMID: 20572038; PubMed Central PMCID: PMC2946517.

9. Iyer NS, Balsamo LM, Bracken MB, Kadan-Lottick NS. Chemotherapy-only treatment effects on long-term neurocognitive functioning in childhood ALL survivors: a review and meta-analysis. Blood. 2015; 126(3):346–53. https://doi.org/10.1182/blood-2015-02-627414 PMID: 26048910.

10. Kingma A, van Dommelen RI, Mooyaart EL, Wilmink JT, Deelman BG, Kamps WA. Slight cognitive impairment and magnetic resonance imaging abnormalities but normal school levels in children treated for acute lymphoblastic leukemia with chemotherapy only. J Pediatr. 2001; 139(3):413–20. https://doi.org/10.1067/mpd.2001.117066 PMID: 11562622.

11. Lofstad GE, Reinfjell T, Hestad K, Diseth TH. Cognitive outcome in children and adolescents treated for acute lymphoblastic leukaemia with chemotherapy only. Acta Paediatr. 2009; 98(1):180–6. https://doi.org/10.1111/j.1651-2227.2008.01055.x PMID: 18826490; PubMed Central PMCID: PMC2659382.

12. Rodgers J, Marckus R, Kearns P, Windebank K. Attentional ability among survivors of leukaemia treated without cranial irradiation. Arch Dis Child. 2003; 88(2):147–50. https://doi.org/10.1136/adc.88.2.147 PMID: 12538320; PubMed Central PMCID: PMC1719443.

13. Daams M, Schuitema I, van Dijk BW, van Dulmen-den Broeder E, Veerman AJ, van den Bos C, et al. Long-term effects of cranial irradiation and intrathecal chemotherapy in treatment of childhood leukemia: a MEG study of power spectrum and correlated cognitive dysfunction. BMC Neurol. 2012; 12:84. https://doi.org/10.1186/1471-2377-12-84 PubMed Central PMCID: PMC3517522. PMID: 22928913

14. Edelmann MN, Krull KR, Liu W, Glass JO, Ji Q, Ogg RJ, et al. Diffusion tensor imaging and neurocognition in survivors of childhood acute lymphoblastic leukaemia. Brain. 2014; 137(Pt 11):2973–83. https://doi.org/10.1093/brain/awu230 PMID: 25125614; PubMed Central PMCID: PMC4208463.

15. Mennes M, Stiers P, Vandenbussche E, Vercruysse G, Uyttebroeck A, De Meyer G, et al. Attention and information processing in survivors of childhood acute lymphoblastic leukemia treated with chemotherapy only. Pediatr Blood Cancer. 2005; 44(5):478–86. PMID: 15918215.

16. Carey ME, Haut MW, Reminger SL, Hutter JJ, Theilmann R, Kaemingk KL. Reduced frontal white matter volume in long-term childhood leukemia survivors: a voxel-based morphometry study. AJNR Am J Neuroradiol. 2008; 29(4):792–7. https://doi.org/10.3174/ajnr.A0904 PMID: 18184841.

17. Cole PD, Kamen BA. Delayed neurotoxicity associated with therapy for children with acute lymphoblastic leukemia. Ment Retard Dev Disabil Res Rev. 2006; 12(3):174–83. https://doi.org/10.1002/mrdd.20113 PMID: 17061283.

18. Krull KR, Brinkman TM, Li C, Armstrong GT, Ness KK, Srivastava DK, et al. Neurocognitive outcomes decades after treatment for childhood acute lymphoblastic leukemia: a report from the St Jude lifetime cohort study. J Clin Oncol. 2013; 31(35):4407–15. Epub 2013/11/06. https://doi.org/10.1200/JCO.2012.48.2315 PMID: 24190124; PubMed Central PMCID: PMCPMC3842908.

19. Cheung YT, Krull KR. Neurocognitive outcomes in long-term survivors of childhood acute lymphoblastic leukemia treated on contemporary treatment protocols: A systematic review. Neurosci Biobehav Rev. 2015; 53:108–20. Epub 2015/04/11. https://doi.org/10.1016/j.neubiorev.2015.03.016 PMID: 25857254; PubMed Central PMCID: PMCPMC4425605.

20. Schultz KA, Ness KK, Whitton J, Recklitis C, Zebrack B, Robison LL, et al. Behavioral and social outcomes in adolescent survivors of childhood cancer: a report from the childhood cancer survivor study. J Clin Oncol. 2007; 25(24):3649–56. https://doi.org/10.1200/JCO.2006.09.2486 PMID: 17704415.

21. Zeltzer LK, Recklitis C, Buchbinder D, Zebrack B, Casillas J, Tsao JC, et al. Psychological status in childhood cancer survivors: a report from the Childhood Cancer Survivor Study. J Clin Oncol. 2009; 27 (14):2396–404. https://doi.org/10.1200/JCO.2008.21.1433 PMID: 19255309; PubMed Central PMCID: PMC2677925.

22. Michel G, Rebholz CE, von der Weid NX, Bergstraesser E, Kuehni CE. Psychological distress in adult survivors of childhood cancer: the Swiss Childhood Cancer Survivor study. J Clin Oncol. 2010; 28 (10):1740–8. https://doi.org/10.1200/JCO.2009.23.4534 PMID: 20194864.

23. Stuber ML, Meeske KA, Krull KR, Leisenring W, Stratton K, Kazak AE, et al. Prevalence and predictors of posttraumatic stress disorder in adult survivors of childhood cancer. Pediatrics. 2010; 125(5): e1124–34. https://doi.org/10.1542/peds.2009-2308 PMID: 20435702; PubMed Central PMCID: PMC3098501.

24. Baytan B, Asut C, Cirpan Kantarcioglu A, Sezgin Evim M, Gunes AM. Health-Related Quality of Life, Depression, Anxiety, and Self-Image in Acute Lymphocytic Leukemia Survivors. Turk J Haematol. 2016; 33(4):326–30. Epub 2016/04/21. https://doi.org/10.4274/tjh.2015.0356 PMID: 27094799; PubMed Central PMCID: PMCPMC5204188 financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

25. Pepin AJ, Lippe S, Krajinovic M, Laverdiere C, Michon B, Sinnett D, et al. How to interpret high levels of distress when using the Distress Thermometer in the long-term follow-up clinic? A study with Acute Lymphoblastic Leukemia survivors. Pediatr Hematol Oncol. 2017; 34(3):133–7. Epub 2017/09/19. https://doi.org/10.1080/08880018.2017.1338805 PMID: 28922050.

26. Brinkman TM, Zhu L, Zeltzer LK, Recklitis CJ, Kimberg C, Zhang N, et al. Longitudinal patterns of psychological distress in adult survivors of childhood cancer. Br J Cancer. 2013; 109(5):1373–81. https://doi.org/10.1038/bjc.2013.428 PMID: 23880828; PubMed Central PMCID: PMC3778287.

27. Bitsko MJ, Cohen D, Dillon R, Harvey J, Krull K, Klosky JL. Psychosocial Late Effects in Pediatric Cancer Survivors: A Report From the Children's Oncology Group. Pediatr Blood Cancer. 2016; 63(2):337–43. https://doi.org/10.1002/pbc.25773 PMID: 26488337; PubMed Central PMCID: PMC4715481.

28. Moghrabi A, Levy DE, Asselin B, Barr R, Clavell L, Hurwitz C, et al. Results of the Dana-Farber Cancer Institute ALL Consortium Protocol 95–01 for children with acute lymphoblastic leukemia. Blood. 2007; 109(3):896–904. Epub 2006/09/28. https://doi.org/10.1182/blood-2006-06-027714 PMID: 17003366; PubMed Central PMCID: PMCPMC1785142.

29. Silverman LB, Stevenson KE, O'Brien JE, Asselin BL, Barr RD, Clavell L, et al. Long-term results of Dana-Farber Cancer Institute ALL Consortium protocols for children with newly diagnosed acute lymphoblastic leukemia (1985–2000). Leukemia. 2010; 24(2):320–34. Epub 2009/12/18. https://doi.org/10.1038/leu.2009.253 PMID: 20016537; PubMed Central PMCID: PMCPMC2820141.

30. Zheng X, Levine D, Shen J, Gogarten SM, Laurie C, Weir BS. A high-performance computing toolset for relatedness and principal component analysis of SNP data. Bioinformatics. 2012; 28(24):3326–8. Epub 2012/10/13. https://doi.org/10.1093/bioinformatics/bts606 PMID: 23060615; PubMed Central PMCID: PMCPMC3519454.

31. PLINK: Whole genome data analysis toolset 2018. Available from: http://zzz.bwh.harvard.edu/plink/index.shtml.

32. Krull KR, Okcu MF, Potter B, Jain N, Dreyer Z, Kamdar K, et al. Screening for neurocognitive impairment in pediatric cancer long-term survivors. J Clin Oncol. 2008; 26(25):4138–43. https://doi.org/10.1200/JCO.2008.16.8864 PMID: 18757327.

33. Delis DC KE, Kramer JH. Delis-Kaplan executive function system (D-KEFS). Psychological Corporation. 2001.

34. D W. Wechsler adult intelligence scale—Fourth Edition (WAIS-IV). San Antonio, TX: 2008.

35. Yochim B, Baldo J, Nelson A, Delis DC. D-KEFS Trail Making Test performance in patients with lateral prefrontal cortex lesions. J Int Neuropsychol Soc. 2007; 13(4):704–9. Epub 2007/05/25. https://doi.org/10.1017/S1355617707070907 PMID: 17521488.

36. Delis DC, Kramer JH, Kaplan E, Holdnack J. Reliability and validity of the Delis-Kaplan Executive Function System: an update. J Int Neuropsychol Soc. 2004; 10(2):301–3. Epub 2004/03/12. https://doi.org/10.1017/S1355617704102191 PMID: 15012851.

37. Erdodi LA, Abeare CA, Lichtenstein JD, Tyson BT, Kucharski B, Zuccato BG, et al. Wechsler Adult Intelligence Scale-Fourth Edition (WAIS-IV) processing speed scores as measures of noncredible responding: The third generation of embedded performance validity indicators. Psychol Assess. 2017; 29(2):148–57. Epub 2016/04/29. https://doi.org/10.1037/pas0000319 PMID: 27124099.

38. Harrison AG, Armstrong IT, Harrison LE, Lange RT, Iverson GL. Comparing Canadian and American normative scores on the Wechsler Adult Intelligence Scale-Fourth Edition. Arch Clin Neuropsychol. 2014; 29(8):737–46. Epub 2014/10/15. https://doi.org/10.1093/arclin/acu048 PMID: 25313225.

39. Boulet-Craig A, Robaey P, Laniel J, Bertout L, Drouin S, Krajinovic M, et al. DIVERGT screening procedure predicts general cognitive functioning in adult long-term survivors of pediatric acute lymphoblastic leukemia: A PETALE study. Pediatr Blood Cancer. 2018; 65(9):e27259. Epub 2018/05/26. https://doi.org/10.1002/pbc.27259 PMID: 29797640.

40. Derogatis LR. Brief Symptom Inventory (BSI) 18: Administration, scoring, and procedures manual. Minneapolis, MN: NCS Pearson; 2000.

41. Beck JS BA, Jolly JB, Steer RA. Beck Youth Inventories-Second Edition for children and adolescent's manual. San Antonio, TX: PsychCorp; 2005.

42. Recklitis CJ, Parsons SK, Shih MC, Mertens A, Robison LL, Zeltzer L. Factor structure of the brief symptom inventory—18 in adult survivors of childhood cancer: results from the childhood cancer survivor study. Psychol Assess. 2006; 18(1):22–32. https://doi.org/10.1037/1040-3590.18.1.22 PMID: 16594809.

43. Gianinazzi ME, Rueegg CS, Wengenroth L, Bergstraesser E, Rischewski J, Ammann RA, et al. Adolescent survivors of childhood cancer: are they vulnerable for psychological distress? Psychooncology. 2013; 22(9):2051–8. https://doi.org/10.1002/pon.3249 PMID: 23401292.

44. Streiner DL. Starting at the beginning: an introduction to coefficient alpha and internal consistency. J Pers Assess. 2003; 80(1):99–103. Epub 2003/02/14. https://doi.org/10.1207/S15327752JPA8001_18 PMID: 12584072.

45. Spinella JF, Healy J, Saillour V, Richer C, Cassart P, Ouimet M, et al. Whole-exome sequencing of a rare case of familial childhood acute lymphoblastic leukemia reveals putative predisposing mutations in Fanconi anemia genes. BMC Cancer. 2015; 15:539. Epub 2015/07/24. https://doi.org/10.1186/s12885-015-1549-6 PMID: 26201965; PubMed Central PMCID: PMCPMC4512039.

46. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009; 25(14):1754–60. Epub 2009/05/20. https://doi.org/10.1093/bioinformatics/btp324 PMID: 19451168; PubMed Central PMCID: PMCPMC2705234.

47. Picard Tools—By Broad Institute: Code ZIP File; 2018. Available from: https://broadinstitute.github.io/picard/.

48. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 2010; 20(9):1297–303. Epub 2010/07/21. https://doi.org/10.1101/gr.107524.110 PMID: 20644199; PubMed Central PMCID: PMCPMC2928508.

49. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res. 2010; 38(16):e164. https://doi.org/10.1093/nar/gkq603 PMID: 20601685; PubMed Central PMCID: PMC2938201.

50. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc. 2009; 4(7):1073–81. Epub 2009/06/30. https://doi.org/10.1038/nprot.2009.86 PMID: 19561590.

51. Ng PC, Henikoff S. Predicting the effects of amino acid substitutions on protein function. Annu Rev Genomics Hum Genet. 2006; 7:61–80. Epub 2006/07/11. https://doi.org/10.1146/annurev.genom.7.080505.115630 PMID: 16824020.

52. The 1000 Genomes Browsers | 1000 Genomes 2018. Available from: http://www.internationalgenome.org/1000-genomes-browsers/.

53. Exome Variant Server 2018. Available from: http://evs.gs.washington.edu/EVS/.

54. Mikkelsen TS, Thorn CF, Yang JJ, Ulrich CM, French D, Zaza G, et al. PharmGKB summary: methotrexate pathway. Pharmacogenetics and genomics. 2011; 21(10):679–86. https://doi.org/10.1097/FPC.0b013e328343dd93 PMID: 21317831; PubMed Central PMCID: PMC3139712.

55. Schlossmacher G, Stevens A, White A. Glucocorticoid receptor-mediated apoptosis: mechanisms of resistance in cancer cells. J Endocrinol. 2011; 211(1):17–25. https://doi.org/10.1530/JOE-11-0135 PMID: 21602312.

56. KEGG: Kyoto Encyclopedia of Genes and Genomes 2018. Available from: https://www.genome.jp/kegg/.

57. Whole genome association analysis toolset. Available from: http://zzz.bwh.harvard.edu/plink/index.shtml.

58. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007; 81 (3):559–75. Epub 2007/08/19. https://doi.org/10.1086/519795 PMID: 17701901; PubMed Central PMCID: PMCPMC1950838.

59. Waber DP, Tarbell NJ, Kahn CM, Gelber RD, Sallan SE. The relationship of sex and treatment modality to neuropsychologic outcome in childhood acute lymphoblastic leukemia. J Clin Oncol. 1992; 10 (5):810–7. https://doi.org/10.1200/JCO.1992.10.5.810 PMID: 1569453.

60. Benjamini Y, Drai D, Elmer G, Kafkafi N, Golani I. Controlling the false discovery rate in behavior genetics research. Behav Brain Res. 2001; 125(1–2):279–84. Epub 2001/10/30. PMID: 11682119.

61. Benjamini Y, Yekutieli D. Quantitative trait Loci analysis using the false discovery rate. Genetics. 2005; 171(2):783–90. Epub 2005/06/16. https://doi.org/10.1534/genetics.104.036699 PMID: 15956674; PubMed Central PMCID: PMCPMC1456787.

62. Lee S, Emond MJ, Bamshad MJ, Barnes KC, Rieder MJ, Nickerson DA, et al. Optimal unified approach for rare-variant association testing with application to small-sample case-control whole-exome sequencing studies. American journal of human genetics. 2012; 91(2):224–37. https://doi.org/10.1016/j.ajhg.2012.06.007 PMID: 22863193.

63. Wu MC, Kraft P, Epstein MP, Taylor DM, Chanock SJ, Hunter DJ, et al. Powerful SNP-set analysis for case-control genome-wide association studies. American journal of human genetics. 2010; 86 (6):929–42. https://doi.org/10.1016/j.ajhg.2010.05.002 PMID: 20560208.

64. CRAN—Package SKAT 2019. Available from: https://cran.r-project.org/web/packages/SKAT/index.html.

65. Dering C, Hemmelmann C, Pugh E, Ziegler A. Statistical analysis of rare sequence variants: an overview of collapsing methods. Genet Epidemiol. 2011; 35 Suppl 1:S12–7. Epub 2011/12/14. https://doi.org/10.1002/gepi.20643 PMID: 22128052; PubMed Central PMCID: PMCPMC3277891.

66. Sung YJ, Korthauer KD, Swartz MD, Engelman CD. Methods for collapsing multiple rare variants in whole-genome sequence data. Genet Epidemiol. 2014; 38 Suppl 1:S13–20. Epub 2014/08/13. https://doi.org/10.1002/gepi.21820 PMID: 25112183; PubMed Central PMCID: PMCPMC4558905.

67. Bellampalli R, Vohra M, Sharma K, Bhaskaranand N, Bhat KG, Prasad K, et al. Acute lymphoblastic leukemia and genetic variations in BHMT gene: Case-control study and computational characterization. Cancer Biomark. 2017. https://doi.org/10.3233/CBM-160186 PMID: 28582843.

68. Rozycka A, Jagodzinski PP, Kozubski W, Lianeri M, Dorszewska J. Homocysteine Level and Mechanisms of Injury in Parkinson's Disease as Related to MTHFR, MTR, and MTHFD1 Genes Polymorphisms and L-Dopa Treatment. Curr Genomics. 2013; 14(8):534–42. https://doi.org/10.2174/1389202914666131210210559 PMID: 24532985; PubMed Central PMCID: PMC3924248.

69. Robaey P, Krajinovic M, Marcoux S, Moghrabi A. Pharmacogenetics of the neurodevelopmental impact of anticancer chemotherapy. Dev Disabil Res Rev. 2008; 14(3):211–20. Epub 2008/10/17. https://doi.org/10.1002/ddrr.29 PMID: 18924160.

70. Cole PD, Finkelstein Y, Stevenson KE, Blonquist TM, Vijayanathan V, Silverman LB, et al. Polymorphisms in Genes Related to Oxidative Stress Are Associated With Inferior Cognitive Function After Therapy for Childhood Acute Lymphoblastic Leukemia. J Clin Oncol. 2015; 33(19):2205–11. Epub 2015/05/20. https://doi.org/10.1200/JCO.2014.59.0273 PMID: 25987702; PubMed Central PMCID: PMCPMC4477790.

71. Linnebank M, Malessa S, Moskau S, Semmler A, Pels H, Klockgether T, et al. Acute methotrexate-induced encephalopathy—causal relation to homozygous allelic state for MTR c.2756A>G (D919G)? J Chemother. 2007; 19(4):455–7. Epub 2007/09/15. https://doi.org/10.1179/joc.2007.19.4.455 PMID: 17855192.

72. Krull KR, Bhojwani D, Conklin HM, Pei D, Cheng C, Reddick WE, et al. Genetic mediators of neurocognitive outcomes in survivors of childhood acute lymphoblastic leukemia. J Clin Oncol. 2013; 31 (17):2182–8. Epub 2013/05/08. https://doi.org/10.1200/JCO.2012.46.7944 PMID: 23650422; PubMed Central PMCID: PMCPMC3731978.

73. Kamdar KY, Krull KR, El-Zein RA, Brouwers P, Potter BS, Harris LL, et al. Folate pathway polymorphisms predict deficits in attention and processing speed after childhood leukemia therapy. Pediatr Blood Cancer. 2011; 57(3):454–60. Epub 2011/05/28. https://doi.org/10.1002/pbc.23162 PMID: 21618410; PubMed Central PMCID: PMCPMC3134130.

74. Nakao A, Miki T, Shoji H, Nishi M, Takeshima H, Miyakawa T, et al. Comprehensive behavioral analysis of voltage-gated calcium channel beta-anchoring and -regulatory protein knockout mice. Front Behav Neurosci. 2015; 9:141. Epub 2015/07/03. https://doi.org/10.3389/fnbeh.2015.00141 PMID: 26136667; PubMed Central PMCID: PMCPMC4468383.

75. McEnery MW, Vance CL, Begg CM, Lee WL, Choi Y, Dubel SJ. Differential expression and association of calcium channel subunits in development and disease. J Bioenerg Biomembr. 1998; 30(4):409–18. Epub 1998/10/03. PMID: 9758336.

76. Cross-Disorder Group of the Psychiatric Genomics C. Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis. Lancet. 2013; 381(9875):1371–9. Epub 2013/03/05. https://doi.org/10.1016/S0140-6736(12)62129-1 PMID: 23453885; PubMed Central PMCID: PMCPMC3714010.

77. Breitenkamp AF, Matthes J, Nass RD, Sinzig J, Lehmkuhl G, Nurnberg P, et al. Rare mutations of CACNB2 found in autism spectrum disease-affected families alter calcium channel function. PLoS One. 2014; 9(4):e95579. https://doi.org/10.1371/journal.pone.0095579 PMID: 24752249; PubMed Central PMCID: PMC3994086.

78. Soldatov NM. CACNB2: An Emerging Pharmacological Target for Hypertension, Heart Failure, Arrhythmia and Mental Disorders. Curr Mol Pharmacol. 2015; 8(1):32–42. PMID: 25966706.

79. Klotz L, Schmidt S, Heun R, Klockgether T, Kolsch H. Association of the PPARgamma gene polymorphism Pro12Ala with delayed onset of multiple sclerosis. Neurosci Lett. 2009; 449(1):81–3. https://doi.org/10.1016/j.neulet.2008.10.066 PMID: 18977277.

80. Heberden C, Meffray E, Goustard-Langelier B, Maximin E, Lavialle M. Dexamethasone inhibits the maturation of newly formed neurons and glia supplemented with polyunsaturated fatty acids. J Steroid Biochem Mol Biol. 2013; 138:395–402. Epub 2013/08/03. https://doi.org/10.1016/j.jsbmb.2013.07.010 PMID: 23907015.

81. Fu Y, Zhen J, Lu Z. Synergetic Neuroprotective Effect of Docosahexaenoic Acid and Aspirin in SH-Y5Y by Inhibiting miR-21 and Activating RXRalpha and PPARalpha. DNA Cell Biol. 2017; 36(6):482–9. https://doi.org/10.1089/dna.2017.3643 PMID: 28346830.

82. Steinbach D, Legrand O. ABC transporters and drug resistance in leukemia: was P-gp nothing but the first head of the Hydra? Leukemia. 2007; 21:1172. https://doi.org/10.1038/sj.leu.2404692 PMID: 17429427

83. Mehul B, Bernard D, Simonetti L, Bernard MA, Schmidt R. Identification and cloning of a new calmodulin-like protein from human epidermis. J Biol Chem. 2000; 275(17):12841–7. https://doi.org/10.1074/jbc.275.17.12841 PMID: 10777582.

84. Matsuoka M. Protective effects of Humanin and calmodulin-like skin protein in Alzheimer's disease and broad range of abnormalities. Mol Neurobiol. 2015; 51(3):1232–9. https://doi.org/10.1007/s12035-014-8799-1 PMID: 24969584.

85. Hashimoto Y, Nawa M, Kurita M, Tokizawa M, Iwamatsu A, Matsuoka M. Secreted calmodulin-like skin protein inhibits neuronal death in cell-based Alzheimer's disease models via the heterotrimeric Humanin receptor. Cell Death Dis. 2013; 4:e555. https://doi.org/10.1038/cddis.2013.80 PMID: 23519124; PubMed Central PMCID: PMC3615737.

86. Hasegawa S, Kumagai M, Hagihara M, Nishimaru H, Hirano K, Kaneko R, et al. Distinct and Cooperative Functions for the Protocadherin-alpha, -beta and -gamma Clusters in Neuronal Survival and Axon Targeting. Front Mol Neurosci. 2016; 9:155. Epub 2017/01/10. https://doi.org/10.3389/fnmol.2016.00155 PMID: 28066179; PubMed Central PMCID: PMCPMC5179546.

87. Pedrosa E, Stefanescu R, Margolis B, Petruolo O, Lo Y, Nolan K, et al. Analysis of protocadherin alpha gene enhancer polymorphism in bipolar disorder and schizophrenia. Schizophr Res. 2008; 102 (1–3):210–9. Epub 2008/05/30. https://doi.org/10.1016/j.schres.2008.04.013 PMID: 18508241; PubMed Central PMCID: PMCPMC2862380.

88. Naskar T, Faruq M, Banerjee P, Khan M, Midha R, Kumari R, et al. Ancestral Variations of the PCDHG Gene Cluster Predispose to Dyslexia in a Multiplex Family. EBioMedicine. 2018; 28:168–79. Epub 2018/02/08. https://doi.org/10.1016/j.ebiom.2017.12.031 PMID: 29409727; PubMed Central PMCID: PMCPMC5835549.

89. Kadan-Lottick NS, Zeltzer LK, Liu Q, Yasui Y, Ellenberg L, Gioia G, et al. Neurocognitive functioning in adult survivors of childhood non-central nervous system cancers. Journal of the National Cancer Institute. 2010; 102(12):881–93. Epub 2010/05/12. https://doi.org/10.1093/jnci/djq156 PMID: 20458059; PubMed Central PMCID: PMC2886093.

90. Buizer AI, de Sonneville LM, Veerman AJ. Effects of chemotherapy on neurocognitive function in children with acute lymphoblastic leukemia: a critical review of the literature. Pediatr Blood Cancer. 2009; 52(4):447–54. https://doi.org/10.1002/pbc.21869 PMID: 19061221.

91. Janzen LA, Spiegler BJ. Neurodevelopmental sequelae of pediatric acute lymphoblastic leukemia and its treatment. Dev Disabil Res Rev. 2008; 14(3):185–95. https://doi.org/10.1002/ddrr.24 PMID: 18924154.

92. Edelstein K, D'Agostino N, Bernstein LJ, Nathan PC, Greenberg ML, Hodgson DC, et al. Long-term neurocognitive outcomes in young adult survivors of childhood acute lymphoblastic leukemia. J Pediatr Hematol Oncol. 2011; 33(6):450–8. https://doi.org/10.1097/MPH.0b013e31820d86f2 PMID: 21646917.

93. Waber DP, Queally JT, Catania L, Robaey P, Romero I, Adams H, et al. Neuropsychological outcomes of standard risk and high risk patients treated for acute lymphoblastic leukemia on Dana-Farber ALL consortium protocol 95–01 at 5 years post-diagnosis. Pediatr Blood Cancer. 2012; 58(5):758–65. https://doi.org/10.1002/pbc.23234 PMID: 21721112; PubMed Central PMCID: PMC3189432.

94. How J, Blattner M, Fowler S, Wang-Gillam A, Schindler SE. Chemotherapy-associated Posterior Reversible Encephalopathy Syndrome: A Case Report and Review of the Literature. Neurologist. 2016; 21(6):112–7. Epub 2016/11/02. https://doi.org/10.1097/NRL.0000000000000105 PMID: 27801773.

95. Stiefel FC, Breitbart WS, Holland JC. Corticosteroids in cancer: neuropsychiatric complications. Cancer Invest. 1989; 7(5):479–91. Epub 1989/01/01. PMID: 2695230.

96. Brown RT, Madan-Swain A, Walco GA, Cherrick I, Ievers CE, Conte PM, et al. Cognitive and academic late effects among children previously treated for acute lymphocytic leukemia receiving chemotherapy as CNS prophylaxis. J Pediatr Psychol. 1998; 23(5):333–40. https://doi.org/10.1093/jpepsy/23.5.333 PMID: 9782681.

97. Jain N, Brouwers P, Okcu MF, Cirino PT, Krull KR. Sex-specific attention problems in long-term survivors of pediatric acute lymphoblastic leukemia. Cancer. 2009; 115(18):4238–45. https://doi.org/10.1002/cncr.24464 PMID: 19536898.

98. Reddick WE, Taghipour DJ, Glass JO, Ashford J, Xiong X, Wu S, et al. Prognostic factors that increase the risk for reduced white matter volumes and deficits in attention and learning for survivors of childhood cancers. Pediatr Blood Cancer. 2014; 61(6):1074–9. https://doi.org/10.1002/pbc.24947 PMID: 24464947; PubMed Central PMCID: PMC4053257.

99. WHO | Depression and Other Common Mental Disorders. World Health Organization, 2017 2017-02-23 13:46:21. Report No.

100. Furlong W, Rae C, Feeny D, Gelber RD, Laverdiere C, Michon B, et al. Health-related quality of life among children with acute lymphoblastic leukemia. Pediatr Blood Cancer. 2012; 59(4):717–24. https://doi.org/10.1002/pbc.24096 PMID: 22294502; PubMed Central PMCID: PMC4123756.

101. Recklitis CJ, Blackmon JE, Chang G. Validity of the Brief Symptom Inventory-18 (BSI-18) for identifying depression and anxiety in young adult cancer survivors: Comparison with a Structured Clinical Diagnostic Interview. Psychol Assess. 2017; 29(10):1189–200. Epub 2017/01/13. https://doi.org/10.1037/pas0000427 PMID: 28080106; PubMed Central PMCID: PMCPMC5507754.

102. Teng T, Gaillard A, Muzerelle A, Gaspar P. EphrinA5 Signaling Is Required for the Distinctive Targeting of Raphe Serotonin Neurons in the Forebrain. eNeuro. 2017; 4(1). https://doi.org/10.1523/ENEURO.0327-16.2017 PMID: 28197551; PubMed Central PMCID: PMC5292598.

103. Murai KK, Pasquale EB. Can Eph receptors stimulate the mind? Neuron. 2002; 33(2):159–62. PMID: 11804564.

104. De Bellis MD, Keshavan MS, Beers SR, Hall J, Frustaci K, Masalehdan A, et al. Sex differences in brain maturation during childhood and adolescence. Cereb Cortex. 2001; 11(6):552–7. https://doi.org/10.1093/cercor/11.6.552 PMID: 11375916.

105. Schmithorst VJ, Holland SK, Dardzinski BJ. Developmental differences in white matter architecture between boys and girls. Hum Brain Mapp. 2008; 29(6):696–710. Epub 2007/06/29. https://doi.org/10.1002/hbm.20431 PMID: 17598163; PubMed Central PMCID: PMCPMC2396458.

106. Ramsey LB, Panetta JC, Smith C, Yang W, Fan Y, Winick NJ, et al. Genome-wide study of methotrexate clearance replicates SLCO1B1. Blood. 2013; 121(6):898–904. https://doi.org/10.1182/blood-2012-08-452839 PMID: 23233662.

107. Trevino LR, Shimasaki N, Yang W, Panetta JC, Cheng C, Pei D, et al. Germline genetic variation in an organic anion transporter polypeptide associated with methotrexate pharmacokinetics and clinical effects. J Clin Oncol. 2009; 27(35):5972–8. https://doi.org/10.1200/JCO.2008.20.4156 PMID: 19901119; PubMed Central PMCID: PMC2793040.

108. Abe T, Kakyo M, Tokui T, Nakagomi R, Nishio T, Nakai D, et al. Identification of a novel gene family encoding human liver-specific organic anion transporter LST-1. J Biol Chem. 1999; 274(24):17159–63. https://doi.org/10.1074/jbc.274.24.17159 PMID: 10358072.

109. Liu SG, Gao C, Zhang RD, Zhao XX, Cui L, Li WJ, et al. Polymorphisms in methotrexate transporters and their relationship to plasma methotrexate levels, toxicity of high-dose methotrexate, and outcome of pediatric acute lymphoblastic leukemia. Oncotarget. 2017; 8(23):37761–72. Epub 2017/05/20. https://doi.org/10.18632/oncotarget.17781 PMID: 28525903; PubMed Central PMCID: PMCPMC5514947.

110. Yang L, Wu H, Gelder TV, Matic M, Ruan JS, Han Y, et al. SLCO1B1 rs4149056 genetic polymorphism predicting methotrexate toxicity in Chinese patients with non-Hodgkin lymphoma. Pharmacogenomics. 2017; 18(17):1557–62. Epub 2017/11/03. https://doi.org/10.2217/pgs-2017-0110 PMID: 29095107.

111. Radtke S, Zolk O, Renner B, Paulides M, Zimmermann M, Moricke A, et al. Germline genetic variations in methotrexate candidate genes are associated with pharmacokinetics, toxicity, and outcome in childhood acute lymphoblastic leukemia. Blood. 2013; 121(26):5145–53. Epub 2013/05/09. https://doi.org/10.1182/blood-2013-01-480335 PMID: 23652803.

112. Coon H, Darlington T, Pimentel R, Smith KR, Huff CD, Hu H, et al. Genetic risk factors in two Utah pedigrees at high risk for suicide. Transl Psychiatry. 2013; 3:e325. Epub 2013/11/21. https://doi.org/10.1038/tp.2013.100 PMID: 24252905; PubMed Central PMCID: PMCPMC3849959.

113. Kock K, Koenen A, Giese B, Fraunholz M, May K, Siegmund W, et al. Rapid modulation of the organic anion transporting polypeptide 2B1 (OATP2B1, SLCO2B1) function by protein kinase C-mediated internalization. J Biol Chem. 2010; 285(15):11336–47. Epub 2010/02/18. https://doi.org/10.1074/jbc.M109.056457 PMID: 20159975; PubMed Central PMCID: PMCPMC2857012.

114. Yang M, Xie W, Mostaghel E, Nakabayashi M, Werner L, Sun T, et al. SLCO2B1 and SLCO1B3 may determine time to progression for patients receiving androgen deprivation therapy for prostate cancer. J Clin Oncol. 2011; 29(18):2565–73. Epub 2011/05/25. https://doi.org/10.1200/JCO.2010.31.2405 PMID: 21606417; PubMed Central PMCID: PMCPMC3138634.

115. Pejovic-Milovancevic MM, Mandic-Maravic VD, Coric VM, Mitkovic-Voncina MM, Kostic MV, Savic-Radojevic AR, et al. Glutathione S-Transferase Deletion Polymorphisms in Early-Onset Psychotic and Bipolar Disorders: A Case-Control Study. Lab Med. 2016; 47(3):195–204. https://doi.org/10.1093/labmed/lmw017 PMID: 27114251; PubMed Central PMCID: PMC4985766.

116. Krajinovic M, Labuda D, Sinnett D. Glutathione S-transferase P1 genetic polymorphisms and susceptibility to childhood acute lymphoblastic leukaemia. Pharmacogenetics. 2002; 12(8):655–8. Epub 2002/11/20. PMID: 12439226.

117. Krull KR, Zhang N, Santucci A, Srivastava DK, Krasin MJ, Kun LE, et al. Long-term decline in intelligence among adult survivors of childhood acute lymphoblastic leukemia treated with cranial radiation. Blood. 2013; 122(4):550–3. Epub 2013/06/08. https://doi.org/10.1182/blood-2013-03-487744 PMID: 23744583; PubMed Central PMCID: PMCPMC3724191.

118. Edelmann MN, Ogg RJ, Scoggins MA, Brinkman TM, Sabin ND, Pui CH, et al. Dexamethasone exposure and memory function in adult survivors of childhood acute lymphoblastic leukemia: A report from the SJLIFE cohort. Pediatr Blood Cancer. 2013; 60(11):1778–84. Epub 2013/06/19. https://doi.org/10.1002/pbc.24644 PMID: 23775832; PubMed Central PMCID: PMCPMC3928631.

119. Armstrong GT, Reddick WE, Petersen RC, Santucci A, Zhang N, Srivastava D, et al. Evaluation of memory impairment in aging adult survivors of childhood acute lymphoblastic leukemia treated with cranial radiotherapy. J Natl Cancer Inst. 2013; 105(12):899–907. Epub 2013/04/16. https://doi.org/10.1093/jnci/djt089 PMID: 23584394; PubMed Central PMCID: PMCPMC3687368.

120. Hudson MM, Ness KK, Nolan VG, Armstrong GT, Green DM, Morris EB, et al. Prospective medical assessment of adults surviving childhood cancer: study design, cohort characteristics, and feasibility of the St. Jude Lifetime Cohort study. Pediatr Blood Cancer. 2011; 56(5):825–36. Epub 2011/03/04. https://doi.org/10.1002/pbc.22875 PMID: 21370418; PubMed Central PMCID: PMCPMC3088729.