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Abstract

Central nervous system (CNS) toxicity is common at diagnosis and during treatment of pediatric acute lymphoblastic leukemia (ALL). We studied CNS toxicity in 1,464 children aged 1.0–17.9 years, diagnosed with ALL and treated according to the Nordic Society of Pediatric Hematology and Oncology ALL2008 protocol. Genome-wide association studies, and a candidate single-nucleotide polymorphism (SNP; n=19) study were performed in 1,166 patients. Findings were validated in an independent Australian cohort of children with ALL (n=797) in whom two phenotypes were evaluated: diverse CNS toxicities (n=103) and methotrexate-related CNS toxicity (n=48). In total, 135/1,464 (9.2%) patients experienced CNS toxicity for a cumulative incidence of 8.7% (95% confidence interval: 7.31–10.20) at 12 months from diagnosis. Patients aged ≥10 years had a higher risk of CNS toxicity than had younger patients (16.3% *vs*. 7.4%; *P*<0.001). The most common CNS toxicities were posterior reversible encephalopathy syndrome (n=52, 43 with seizures), sinus venous thrombosis (n=28, 9 with seizures), and isolated seizures (n=16). The most significant SNP identified by the genome-wide association studies did not reach genomic significance (lowest *P*-value: 1.11×10^{-6}), but several were annotated in genes regulating neuronal functions. In candidate SNP analysis, *ATXN1* rs68082256, related to epilepsy, was associated with seizures in patients <10 years (*P*=0.01). *ATXN1* rs68082256 was validated in the Australian cohort with diverse CNS toxicities (*P*=0.04). The role of *ATXN1* as well as the novel SNP in neurotoxicity in pediatric ALL should be further explored.

Introduction

Therapeutic advances in recent decades have greatly improved the outcome of pediatric acute lymphoblastic leukemia (ALL), with a current survival rate above 90%.[1] However, severe acute adverse events involving the central nervous system (CNS), from here on referred to as CNS toxicities, still have an incidence of up to 18.4% with significant morbidity and mortality and remain a significant challenge in ALL treatment.[2-5] Certain chemotherapy regimens, individual patients' vulnerability, underlying comorbidities, drug-drug interactions, and the distribution and tumor load of the ALL itself may predispose patients to CNS toxicities, while the role of CNS leukemia in CNS toxicities is still unclear.[2,3,6-11] Accumulating data on pharmacogenetic associations with CNS toxicities and the genetic background of neurological diseases, such as seizures and epilepsy, support possible existence of genetic susceptibility to CNS toxicities during ALL treatment.[7,10,12] Recent research has used genome-wide association studies (GWAS) to identify single nucleotide polymorphisms (SNP) related to methotrexate-induced leukoencephalopathy in pediatric ALL.[7,10] Mateos *et al.* identified SNP in genes regulating neuronal growth, differentiation, and cytoskeletal organization at a significance level of $P<1.0x10^{-6}$.[10]

In this study, we explored the phenotypes, outcome, clinical and genetic risk factors for all severe acute CNS toxicities in pediatric patients with ALL. We applied both GWAS and candidate SNP analyses to identify genotypes associated with CNS toxicities.[10,12] We hypothesized that genetic variants that predispose healthy children to epilepsy may also predispose them to seizures during ALL treatment and studied multi-SNP genetic risk scores for the risk of seizures in ALL patients.[13] We collaborated with an independent Australian research group to test whether our most significant findings would be validated in a cohort of pediatric ALL patients in whom two phenotype groups, diverse CNS toxicities and methotrexate-related CNS toxicity, were studied.

Methods

Patients

The study included all children aged between 1 and 17.9 years at diagnosis of B-cell precursor or T–cell ALL between 2008 and 2015. All patients were treated according to the Nordic Society of Pediatric Hematology and Oncology (NOPHO) ALL2008 protocol, which has a prospective online registration system encompassing detailed data on patients' characteristics, treatment, response to treatment, and toxicities (including any CNS toxicity, posterior reversible encephalopathy syndrome [PRES] and seizures).[14-16] Patients with CNS toxicities were identified in the NOPHO ALL2008 registry (*Online Supplementary Methods*).

Classification of central nervous system toxicities

CNS toxicities were classified as "defined" or "other", based on systemic or unclear conditions according to established nomenclature, as previously described (*Online Supplementary Methods*).[2]

Statistical methods

Statistical analyses were performed using R and SPSS. Time to CNS toxicity was counted as days from diagnosis until the first CNS toxicity, or censored at the time of relapse, stem cell transplantation, second malignant neoplasm, death, or last follow-up, whichever came first. Overall survival was defined as days from diagnosis until death or last follow-up. Event-free survival was defined as days from diagnosis to the last follow-up, relapse, stem cell transplantation, or second malignant neoplasm (*Online Supplementary Methods*).

Genome-wide association and candidate single nucleotide polymorphism analyses

Genotype associations were explored using GWAS, candidate SNP analysis and polygenic risk scoring (*Online Supplementary Methods*). Genome-wide association analysis on the SNP array data was performed in PLINK2/1.90beta6.18 using logistic regression adjusted for age, sex, CNS leukemia, and genetic ancestry by the first four principal components.[17] A suggestive threshold of $P<5\times10^{-6}$ and a Bonferroni-corrected $P<2\times10^{-8}$, which were regarded as significant, were used to explore the top findings from the GWAS.

Genome-wide associations were analyzed on three phenotype groups: all CNS toxicities, PRES, and seizures. The group of patients with PRES showed signs of genomic inflation and were excluded from further analyses. The most significant SNP were annotated using the variant effect predictor (GRCh37.p13) and genes were checked using the Ensembl GRCh37 and GeneCards genetic databases for function and related disorders.[18-20] Genes were further tested for functional enrichment by gene set overlap analysis (GSEA).[21]

Nineteen SNP previously found to be associated with epilepsy and methotrexate-related central CNS toxicity qualified for testing for association with seizures in imputed genotype data (*Online Supplementary Table S3*).[10,12] Of them, those SNP reaching statistical significance for association with seizures ($P<0.05$) before corrections were also tested separately for children with seizures <10 years and ≥10 years of age. Two polygenic risk scores were estimated for risk for seizures based on all candidate SNP and on six SNP associated with methotrexate-related CNS

toxicity. The candidate 19-SNP polygenic risk score were unweighted, and for the 6-SNP polygenic risk score, each SNP was weighted by the log-transformed odds ratio (OR) from Mateos *et al.*[10]

Validation study

Our GWAS findings passing the suggestive threshold and candidate SNP showing a trend for association with seizures were evaluated in the Australian cohort including patients who displayed either diverse CNS toxicities (n=103) or methotrexate-related CNS toxicity (n=48) (*Online Supplementary Methods*).[10]

Ethical approval

The ALL2008 study (EudraCT 2008-003235-20) was approved by the scientific ethical review boards of the involved countries. The genetic study was approved by local ethical review boards with separate verbal and written consent. The genetic data were compiled in Denmark (Danish Data Protection Agency j.nr.: 2012-58-0004; Regional Ethical Institutional Review Board in the capital region of Denmark, protocol number: H-2-2010-002). The Australian study was approved by Hunter New England Human Research Ethics Committee (reference number: 12/11/21/4.01).

Results

The study group consisted of 1,464 children, 1 274 with B-cell precursor and 190 with T–cell ALL. The median follow-up time for survivors was 5.04 years (range, 0.05–9.28, n=1,351).

In total, 135 children had acute CNS toxicities, of whom 120 had a defined CNS toxicity and 15 had other CNS toxicities (Table 1). Ten patients with CNS toxicity had a neurological or neurodevelopmental disorder prior to the diagnosis of ALL, including febrile seizures (n=3), intellectual disability (n=3), epilepsy (n=2), migraine (n=1), and attention-deficit hyperactivity disorder (n=1).

The most common defined CNS toxicity was PRES (n=52), followed by sinus venous thrombosis (n=28) and isolated seizures (n=16); the most common neurological symptoms were seizures (n=82) (Table 1, Figure 1). In the Australian cohort, the most common CNS toxicity was methotrexate-related stroke-like syndrome (Table 2).

Incidence and clinical risk factors

Overall, 9.2% (135/1,464) of the patients displayed at least one CNS toxicity during the course of ALL treatment. The majority of CNS toxicities occurred during the first 6 months of treatment (110/135), while eight cases of first CNS toxicity were reported after the first year (Figure 2). The cumulative incidence of CNS toxicities at 2 months was 4.8% (95% confidence interval [95% CI]: 3.77–5.97), at 6 months it was 7.5% (95% CI: 6.24–8.95), and at 1 year the cumulative incidence was 8.7% (95% CI: 7.31–10.20). Older age, T-cell immunophenotype, CNS leukemia, and therapy induction with dexamethasone were associated with a higher risk of CNS toxicity in univariate analyses. Older age remained a statistically significant risk in a multivariate analysis adjusting for age, sex, immunophenotype, CNS status, and therapy induction (Table 3, Figure 3). Stratification into block treatment at the end of induction was a significant risk factor for CNS toxicity in univariate analysis (hazard ratio [HR]=1.81; 95% CI: 1.21–2.70, *P*=0.004) but not in a multivariate analysis adjusting for age group, sex, immunophenotype, induction therapy and CNS status (HR=1.29; 95% CI: 0.81–2.06, *P*=0.28).

Table 1. Acute severe central nervous system toxicities reported in children with acute lymphoblastic leukemia treated with the NOPHO ALL2008 protocol.

CNS toxicity	N of patients
Defined leukemia and/or treatment-related CNS toxicities	
PRES	52
Sinus venous thrombosis	28
Isolated seizures	16
Hypertensive encephalopathy	8
Methotrexate-related SLS	6
Encephalopathy NOS	4
Intracranial hemorrhage	3
Aseptic meningitis	2
Steroid psychosis	1
Systemic or unclear conditions with CNS toxicities	
CNS infection	4
Seizures secondary to hyponatremia (sodium <125 mmol/L)	3
Seizures secondary to hypoglycemia (glucose <2.5 mmol/L)	2
Other or unclear symptoms (visual field defects, elevated ICP, cognitive difficulties)	3
Severe anoxic brain injury secondary to cardiac arrest	1
Seizures secondary to multi-organ failure	1
Pontine myelinolysis secondary to hypernatremia (sodium >160 mmol/L)	1
Total	**135**

NOPHO: Nordic Society of Pediatric Hematology and Oncology; ALL: acute lymphoblastic leukemia; CNS: central nervous system; PRES: posterior reversible encephalopathy syndrome; SLS: stroke-like syndrome; ICP: intracranial pressure; NOS: not otherwise specified.

Survival

At the last follow-up, 121/135 (89.6%) patients with CNS

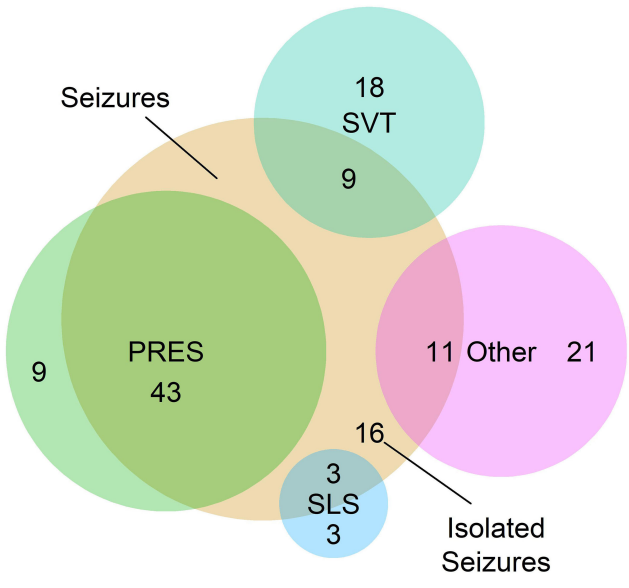

Figure 1. Venn diagram showing total cases with seizures and underlying central nervous system toxicities. Data on seizures were not available for one patient with sinus venous thrombosis and one patient with other central nervous system toxicity. PRES: posterior reversible encephalopathy syndrome; SVT: sinus venous thrombosis; SLS: stroke-like syndrome (methotrexate-related).

toxicities were alive. Eight patients with CNS toxi cities(5.9%) died within 15 days of the episode of toxicity (median 5 days; range, 0–15 days). CNS toxicity was reported to be the cause of death in 2/14 cases. There was no statistically significant difference in overall survival or event-free survival between patients with and without CNS toxicities (both considering defined CNS toxicities or any CNS toxicities; *data not shown*).

Table 2. Acute severe central nervous system toxicities reported in children with acute lymphoblastic leukemia in the Australian cohort.

CNS toxicity	N of patients
Defined leukemia and/or treatment-related CNS toxicities	
Methotrexate-related SLS	28
Possible methotrexate-related SLS*	17
Isolated seizures	14
Motor deficits central**	11
Leukoencephalopathy	10
Encephalopathy NOS	8
PRES	4
Intracranial hemorrhage	3
Aseptic meningitis	1
Systemic or unclear conditionswith CNS toxicities	
Elevated Intracranical pressure	4
Symptoms from cognition***	3
Total	**103**

CNS: central nervous system; SLS: stroke-like syndrome, NOS: not otherwise specified; PRES: posterior reversible encephalopathy syndrome. *Patients with methotrexate leukoencephalopathy who did not fit strict methotrexate-related SLS definition, **two patients with stroke/transient ischemic attack and patients with motor deficits of central origin, such as cerebellar ataxia, ***altered mental state that did not fulfil criteria for encephalopathy NOS (1=suspected infarct, 1=non-specific changes on magnetic resonance imaging 1=progressive neurocognitive deterioration).

Sequelae

After recovery from the first CNS toxicity, 12/103 (11.7%, data missing for 32 patients) patients were reported to have been diagnosed with epilepsy. One patient with intracranial hemorrhage had spastic tetraparesis and one patient with PRES had right-sided hemiplegia. Systematic neurocognitive evaluation was reported in only two cases showing impaired working memory, but clinical suspicion of impaired cognition was reported in a total of 12/110 (10.9%, data missing for 25 patients) patients at the time of data gathering.

Genome-wide association studies of central nervous system toxicities

One hundred and nine of the 135 patients with CNS toxicities participated in GWAS, of whom 67 had seizures (*Online Supplementary Table S4*). In total, 2,146,021 SNP qualified for GWAS of the two phenotype groups: all CNS toxicities (n=109 patients) and seizures (n=67 patients). The two groups were tested against 1,057 controls. No Bonferroni-corrected genome-wide significant hits ($P<2\times10^{-8}$) were obtained. GWAS on all CNS toxicities or seizures showed no signs of genomic inflation (*Online Supplementary Figure S1*). When no SNP reached genome-wide significance, the top 50 SNP were assessed for biological function of the affected genes for the two groups, all CNS toxicities and seizures (*Online Supplementary Table S5*).

In the group of patients with all CNS toxicities, five of the 50 most important SNP passed the suggestive *P*-value threshold of $<5\times10^{-6}$, of which one was mapped in a gene related to neurological functions (Table 4). In the seizure group, 12 of the 50 most important SNP passed the suggestive *P*-value; one was mapped in a gene related to autophagy and regulation of proinflammatory cytokine production (Table 4).

Overall, 18 of the 50 most important SNP in the all CNS toxicities group and 13 of the 50 most important SNP in the seizures group were mapped in genes related to neur-

ological, neuropsychological and developmental disorders (*Online Supplementary Table S5*). Functional enrichment testing of genes in which SNP related to all CNS toxicities or seizures were located showed no significant gene set overlaps.

Association of candidate single nucleotide polymorphisms with seizures

We tested the seizure group for 19 candidate SNP, of which 13 were associated with epilepsy and six with methotrexate-related CNS toxicity, against 1,057 controls (Table 5, *Online Supplementary Table S3*). Two SNP, rs2833098 located in *GRIK1* and rs68082256 located in *ATXN1*, both associated with generalized epilepsy, had significant ($P<0.05$) associations. One SNP associated with methotrexate-related CNS toxicity, rs4712462 located in *MBOAT1*, showed a trend for association but without reaching statistical significance ($P=0.07$). However, the statistical significance of the associations did not survive after adjusting for multiple testing by Benjamini-Hochberg for any of these three SNP (Table 5).

A weighted additive genetic score based on six SNP associated with methotrexate-related CNS toxicity was not significantly associated with seizures (HR=0.94 per weighted risk allele, 95% CI: 0.85-1.05; $P=0.29$). We have subsequently calculated an unweighted additive 19-SNP

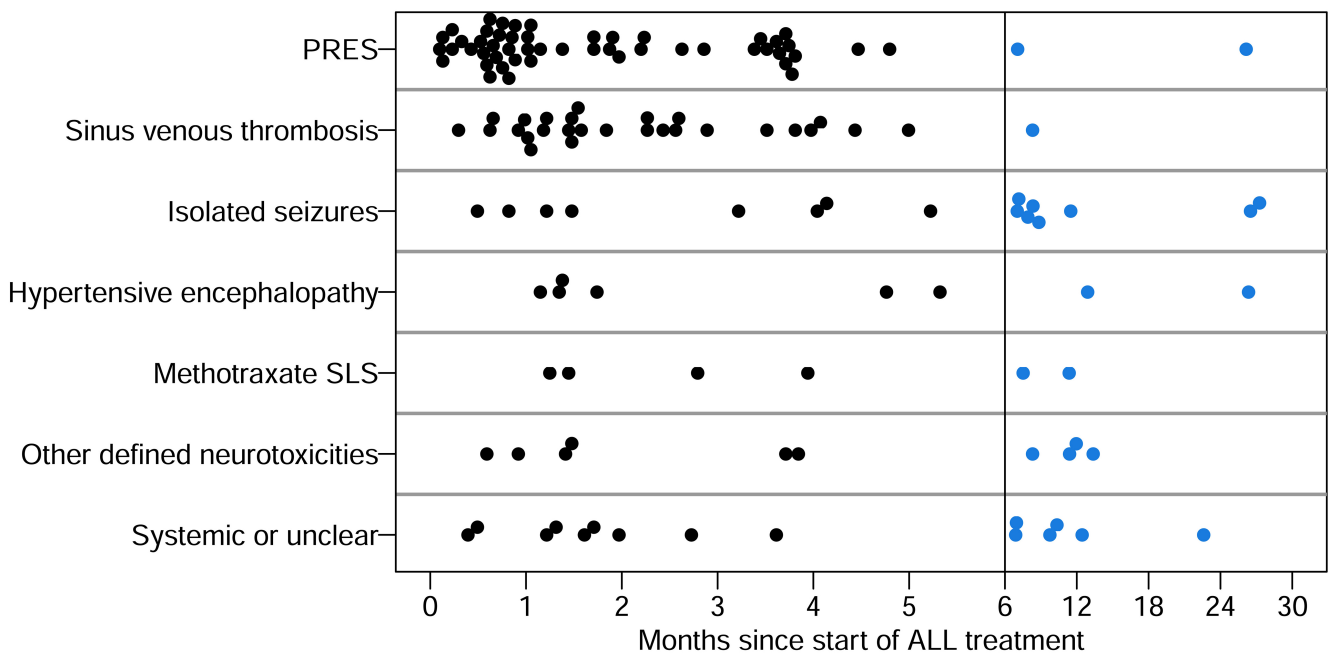

Figure 2. Distribution of central nervous system toxicities over time after the diagnosis of acute lymphoblastic leukemia. PRES: posterior reversible encephalopathy syndrome; SLS: stroke-like syndrome (methotrexate-related); ALL: acute lymphoblastic leukemia.

Table 3. Clinical characteristics of patients with and without central nervous system toxicities and risk factors for these toxicities.

	Controls (N=1,329)	CNS toxicities (N=135)	Univariable HR (95% CI; *P*)	Multivariable HR (95% CI; *P*)*
Age group, N (%)				
1-9 years	1,078 (81.1)	86 (63.7)	Ref	Ref
10-17 years	251 (18.9)	49 (36.3)	2.38 (1.68-3.38; <0.001)	2.22 (1.55-3.19; <0.001)
Sex, N (%)				
Male	726 (54.6)	66 (48.9)	Ref	Ref
Female	603 (45.4)	69 (51.1)	1.25 (0.89-1.75; 0.19)	1.37 (0.97-1.93; 0.07)
Immunophenotype, N (%)				
BCP ALL	1,168 (87.9)	106 (78.5)	Ref	Ref
T–cell	161 (12.1)	29 (21.5)	1.99 (1.32-3.00; 0.001)	1.33 (0.65-2.72 ; 0.43)
CNS status**, N (%)				
CNS 1	1,159 (87.2)	110 (81.5)	Ref	Ref
CNS 2 or 3	166 (12.5)	25 (18.5)	1.59 (1.03-2.46; 0.04)	1.42 (0.91-2.22; 0.12)
Induction therapy***, N (%)				
Prednisolone	1,080 (81.3)	96 (71.1)	Ref	Ref
Dexamethasone	237 (17.8)	39 (28.9)	1.84 (1.27-2.67; 0.001)	1.26 (0.66-2.40; 0.48)

CNS: central nervous system; HR: hazard ratio; 95% CI: 95% confidence interval; BCP-ALL: B-cell precursor acute lymphoblastic leukemia. *Including age group, sex, immunophenotype, induction therapy, CNS status. **Four missing values for the controls. ***Three controls received other induction, nine missing values for the controls.

genetic score based on all candidate SNP which was not significantly associated with seizures (HR=0.94 per risk allele, 95% CI: 0.86-1.03; *P*=0.17).

Stratification by age group showed a significant association with seizures for *ATXN1* rs68082256 (*P*=0.01) in patients <10 years (n=44, controls: n=867) and a trend for a significant association with seizures in this group of patients' for *GRIK1* rs2833098 (*P*=0.06). The difference in findings between the two age groups might depend on different group sizes (patients ≥10 years old: cases with seizures, n=23, controls: n=190) and therefore heterogeneity between effect sizes of the age groups was tested by adding an interaction term between age group and SNP to the logistic regression model. No interaction term was statistically significant, and thus heterogeneity between the effect sizes could not be supported by our data.

Validation study

The most significant SNP from GWAS that passed the suggestive threshold, 12 related to seizures and five related to all CNS toxicities, as well as the two candidate SNP which showed significant association with seizures before multiple correction were included in the validation study (Tables 4 and 5, *Online Supplementary Data*). *ATXN1* rs6802256 was replicated in the diverse CNS toxicities cohort (*P*=0.04).

Discussion

We previously studied the occurrence of PRES and seizures in children treated according to the NOPHO ALL2008 protocol, and found that PRES and seizures are relatively common during ALL treatment, and that a diagnosis of epilepsy is occasionally reported during long-term follow-up.[6,22] Here, we expanded the scope and explored the incidence, phenotypes, possible long-term effects, and risk factors for all severe acute CNS toxicities. In total, 9.2% of pediatric patients with ALL had at least one episode of CNS toxicity during the course of their disease and treatment; PRES was the most common CNS toxicity (38.5%). CNS toxicities occurred most often within the first 6 months of treatment, confirming previous findings that CNS toxicities are common during the first months of ALL treatment, especially during induction.[2,5,6,22] Children aged ≥10 years had a higher risk of CNS toxicities. None of the SNP identified by GWAS reached genome-wide significance, but *GRIK1* rs2833098 and *ATXN1* rs68082256 identified by the candidate SNP approach, may play a role in predisposition to seizures in children with ALL. The replication of *ATXN1* rs68082256 in the Australian cohort with diverse CNS toxicities further supports this hypothesis.

The incidence of CNS toxicities in children with ALL has previously been found to vary between 3.6% and 18.4%.[2,3,5,23-27] This wide spectrum of incidences mirrors different study designs, treatment protocols, and potential differences in documentation and classification of CNS toxicities; for example, in the Australian cohort with diverse CNS toxicities cases of sinus venous thrombosis were not included because they were examined in a separate study (Tables 1 and 2).[2,3,5,23-26,28,29] The chemotherapeutic agents most often associated with CNS toxicity in ALL are methotrexate, glucocorticosteroids, vincristine, asparaginase, and cytarabine.[30-32] The NOPHO ALL2008 protocol includes intensive treatment with vincristine, high-dose methotrexate and asparaginase, which might have contributed to a higher incidence of CNS toxicity compared to that in patients treated with other protocols.[14-16,22,33] In our study the incidence of PRES, which was reported separately, was high whereas methotrexate-related stroke-like syndrome was rare, contrasting with the findings in the Australian cohort in which the incidence of methotrexate-related stroke-like syndrome was clearly higher (Tables 1 and 2).[22] Methotrexate-related CNS toxic-

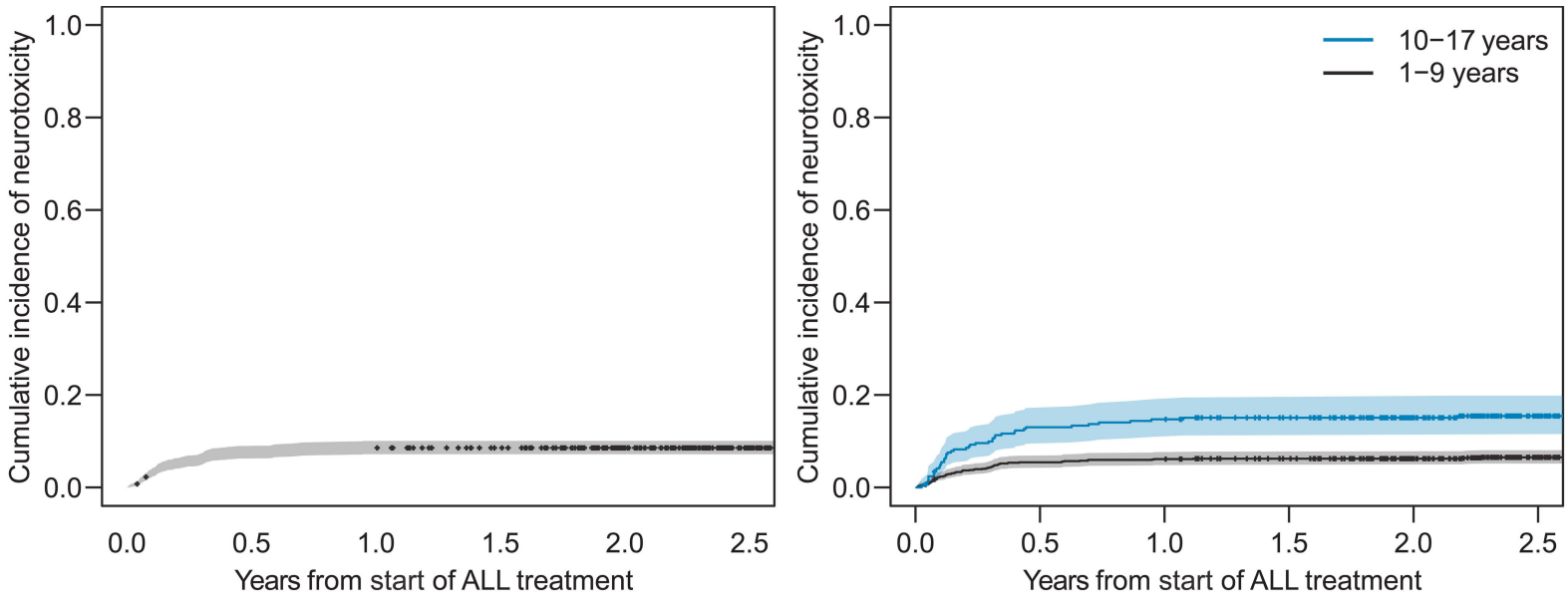

Figure 3. Cumulative incidence of central nervous system toxicities (neurotoxicity). ALL: acute lymphoblastic leukemia.

ity including stroke-like syndrome is a well-known entity but it is possible that mild cases may have been undetected and not registered as CNS toxicity or they might have been registered as isolated seizures without further

Table 4. Top single nucleotide polymorphisms identified by genome-wide association studies related to all central nervous system toxicities and seizures with significance level $P<5x10^{-6}$.

SNP	Chromosome	Consequence (most severe shown first)	Gene (bp distance from gene)	Gene function	Position	Effect allele (minor)	Reference allele (major)	MAF	OR	*P*
All CNS toxicities										
rs72798143	2	Intron variant, Non coding transcript variant	AC068490.2	Unknown	22448244	A	C	0.08	2.88	1.11e-06
rs79459815	4	Intergenic variant	-	-	180706970	A	G	0.01	11.5	2.29e-06
rs13407218	2	Intron Non coding transcript variant	CTNNA2	Regulation of stability and plasticity of synapses, differentiation in the nervous system, neuronal migration, neurite growth	80600505	T	C	0.02	5.61	3.50e-06
rs35916740	7	Regulatory region variant Intergenic variant	-	-	93028950	G	T	0.09	2.63	3.71e-06
rs62325077	4	Intergenic variant	-	-	162120255	C	A	0.11	2.43	4.89e-06
Seizures										
rs75487096	3	Intron variant, Non coding transcript variant Regulatory region variant	KIAA0226	Negative regulation of autophagy. Negative regulation of pro-inflammatory cytokine production following fungal or viral infection	197436685	C	T	0.02	7.01	2.11e-06
rs16936423	9	Intergenic variant	-	-	2000098	G	A	0.03	4.68	2.27e-06
rs116011797	5	Intergenic variant	-	-	121924081	T	C	0.02	7.36	2.46e-06
rs114884102	6	Intergenic variant	-	-	8685785	T	C	0.01	9.24	2.78e-06
rs79566233	6	Intergenic variant	-	-	8623113	G	A	0.01	9.23	2.81e-06
rs78682412	8	Regulatory region variant Intergenic variant	-	-	142606705	A	G	0.05	3.62	2.97e-06
rs17641985	13	Intron variant Upstream gene variant	AL355390.1 LINC00381 (2394)	Unknown Unknown	74990916	C	T	0.01	8.03	3.48e-06
rs16936230	9	Upstream gene variant	RP11-443B9.1 pseudogene (3432)	-	1981979	G	A	0.03	4.48	4.09e-06
rs1528779	2	Intergenic variant	-	-	22969224	C	T	0.48	0.39	4.14e-06
rs353999	19	Downstream gene variant	SUMO1P4 pseudogene (3944)	-	49782621	A	G	0.29	2.32	4.24e-06
rs10478527	5	Downstream gene variant	RP11-510I6.2 pseudogene (1545)	-	120954047	G	A	0.32	2.35	4.87e-06
rs12340816	9	Intergenic variant	-	-	2005105	G	T	0.03	4.41	4.91e-06

SNP: single nucleotide polymorphism; bp: base pairs; MAF: minor allele frequency, OR: odds ratio; CNS: central nervous system; OR=odds ratio.

Table 5. Results of candidate single nucleotide polymorphism approach in children with acute lymphoblastic leukemia and seizures.

SNP ID	Gene	Chromosome	Phenotype	Position	Effect allele	Reference allele	Info score	MAF (minor allele)	OR	*P*- value	FDR
rs6432877	SCN3A, SCN2A, TTC21B, SCN1A	2	All epilepsy	166998767	G	C	1.00	0.23 (G)	1.07	0.92	0.98
rs4671319	FANCL, BCL11A	2	All epilepsy	57950346	A	G	1.00	0.47 (G)	0.89	0.45	0.86
rs4638568	HEATR3, BRD7	16	All epilepsy	50045839	A	G	0.99	0.05 (A)	0.55	0.23	0.75
rs2212656	SCN3A, SCN2A, TTC21B, SCN1A	2	Focal epilepsy	167000843	A	C	1.00	0.23 (A)	1.07	0.93	0.98
rs4665630	KLHL29	2	Generalized epilepsy	23898317	T	C	1.00	0.10 (C)	1.02	0.98	0.98
rs11943905	GABRA2	4	Generalized epilepsy	46397617	T	C	1.00	0.28 (T)	0.98	0.87	0.98
rs13200150	PTPRK	6	Generalized epilepsy	128309768	G	A	1.00	0.33 (G)	0.99	0.86	0.98
rs1402398	FANCL, BCL11A	2	Generalized epilepsy	58042241	A	G	1.00	0.37 (G)	0.95	0.71	0.97
rs4596374	KCNN2	5	Generalized epilepsy	114221505	T	C	0.98	0.48 (T)	0.91	0.59	0.86
rs4794333	PNPO	17	Generalized epilepsy	46045495	C	T	1.00	0.41 (C)	1.19	0.24	0.75
rs11890028	SCN3A, SCN2A, TTC21B, SCN1A	2	Generalized epilepsy	166943277	G	T	1.00	0.26 (G)	0.76	0.22	0.75
rs2833098	GRIK1	21	Generalized epilepsy	32183996	A	G	1.00	0.37 (G)	0.71	0.04	0.36
rs68082256	ATXN1	6	Generalized epilepsy	16971575	A	G	0.99	0.19 (A)	0.47	0.01	0.13
rs4712462	MBOAT1	6	Methotrexate related central CNS toxicity	20196934	G	A	0.95	0.28 (A)	1.52	0.07	0.44
rs2241357	GIPC1	19	Methotrexate related central CNS toxicity	14590919	A	G	0.95	0.15 (A)	0.85	0.45	0.86
rs1106479	ZDHHC19	3	Methotrexate related central CNS toxicity	195925355	T	C	0.93	0.14 (T)	1.16	0.58	0.86
rs35307996	NXN	17	Methotrexate related central CNS toxicity	747700	G	GC	0.96	0.18 (G)	0.86	0.48	0.86
rs74956940	PKN1	19	Methotrexate related central CNS toxicity	14571966	G	C	0.93	0.20 (G)	0.80	0.34	0.86
rs9590003	none	13	Methotrexate related central CNS toxicity	95072136	A	G	1.00	0.09 (A)	0.88	0.55	0.86

SNP: single nucleotide polymorphism; ID: identify; CNS: central nervous system; MAF: minor allele frequency; OR: odds ratio; FDR: false discovery rate.

evaluation with neuroimaging. Patients with CNS leukemia were excluded from some studies on the occurrence of CNS toxicities.[5,23] Here, we included patients with CNS leukemia to explore whether CNS involvement at diagnosis was associated with a higher risk of CNS toxicity. Unlike in a recent study of Finnish children with ALL, leukemic involvement of the CNS was not an independent risk factor for CNS toxicity in our cohort or the Australian cohort studying methotrexate-related CNS toxicity.[2,10] Older age was associated with a higher risk of CNS toxicity, which is in line with previous findings that older age is a risk factor for PRES, seizures, and methotrexate-induced CNS toxicity.[6,10,22] Moreover, older age has previously been shown to be a risk factor for non-CNS treatment-related toxicities such as thrombosis, pancreatitis, and osteonecrosis in childhood ALL.[29,34,35] Immunophenotype (T–cell) and induction therapy (dexamethasone) were significant in univariate analyses, but did not reach signifi-

cance in the multivariate model, probably due to co-variation.

Epilepsy as a late effect after PRES or other CNS toxicity in childhood ALL has been described in previous studies.[2,5,6,10,22,33] We do not have data on epilepsy among controls, but the incidence of epilepsy among ALL patients with CNS toxicity in our study was higher than that in the general population in developed countries.[36] This was true even if we assumed that none of the controls in this ALL cohort had a diagnosis of epilepsy and the 12 reported patients with epilepsy would represent the overall prevalence of epilepsy in this population.[36] In the latest Australian study 3/95 (3.2%) children with methotrexate-related CNS toxicity had epilepsy at last follow-up, as compared to 4/427 (1.0%) controls with epilepsy at last follow-up, which further illustrates that epilepsy as a sequel is more common in ALL patients who displayed CNS toxicities.[10] *ATXN1*, encoding ataxin-1 protein, is the gene underlying spinocerebellar ataxia type 1 and is implicated in seizures.[12,37] The replication of *ATXN1* rs68082256 in the group of younger patients and in the Australian cohort suggests a genetic predisposition to seizures in younger ALL patients, even if we cannot conclude whether it reflects risk for CNS toxicity or comorbidity with epilepsy. Larger studies might clarify the role of *ATXN1* rs68082256 in seizures in ALL and of age as a mediator of genetic predisposition and give insight into the pathogenesis.

Cognitive impairment was reported in 12 patients, but formal neuropsychiatric assessment was performed in only two cases. Accumulating data indicate a risk of cognitive sequelae in patients with ALL, including working memory difficulties, highlighting the need for more standardized neurocognitive follow-up of pediatric patients with ALL.[38,39] The mortality of patients from CNS toxicity in our study was lower than that previously described, but still considerable.[5,28]

This study did not reveal any novel genome-wide significant genetic associations with CNS toxicities, probably due to the limited number of patients with CNS toxicities, diverse underlying conditions, and available phenotype data. When the suggestive *P*-value was applied, *KIAA0226* rs75487096 was associated with seizures and *CTNNA2* rs13407218 was associated with all CNS toxicities. Notably, the *KIAA0226* gene is a negative regulator in autophagy which is involved in neuron function and the *CTNNA2* gene may contribute to the differentiation of the nervous system, to neurite growth, stability and the morphological plasticity of synapses; both genes are related to neuro logical disorders.[18,19,40] *CTNNA2* rs13407218 was among the 50 most important SNP even in the seizures group. Among the 50 most important SNP, seven (*CTNNA2* rs13407218, *ABI1* rs12357198, *ABI1* rs11015279, *ABI1* rs79349206, *CEP128* rs12435954, *MRE11A* rs78817171 and *HHLA3* rs114310506) were present in both groups, reflecting the overlap of patients (*Online Supplementary Table S5*). Overall, 18 SNP in the all CNS toxicities group and 13 SNP in the seizures group were mapped in genes associated with neurological, neuropsychological and developmental disorders but since they did not reach genomic significance, the suggestive *P* threshold or any functional enrichment by gene set overlap analysis this finding is non-specific.[18,19] None of the previously described genes associated with CNS toxicity in pediatric ALL was replicated with GWAS in our study.[7,10] Similarly, none of SNP passing the suggestive threshold was replicated in the independent Australian cohort, which might reflect small sizes of the cohorts or variation between the phenotypes. The replication of *ATXN1* rs68082256 in both our cohort and the Australian cohort does, however, indicate that pathogenesis of seizures in childhood ALL possibly includes genetic aspects. Genome-wide association analyses and validation studies in larger cohorts of pediatric patients with ALL are warranted to further study genetic predisposition to CNS toxicities among ALL patients.[7,10]

In conclusion, CNS toxicities are common and potentially life-threatening complications of pediatric ALL treatment which occur most commonly during the first 6 months of treatment. Age is a modifier of CNS toxicity with an overall higher risk of CNS toxicity in children 10 years and older, and a possible genetic predisposition to seizures in children younger than 10 years. Our findings motivate further GWAS and validation studies in larger cohorts of pediatric patients with ALL.

Disclosures

No conflicts of interest to disclose.

Contributions

SA collected phenotype data, wrote the manuscript and contributed to the interpretation of the results. RLN, MH, BW and SA collected the genetic data, analyzed the GWAS and contributed to the interpretation of the results. IMM and AW contributed with statistical analyses. BA-N, JB, IMJ, OGJ, SM, RN, MT and GV provided phenotype data from all countries participating in the study. SM and CM conducted additional GWAS analyses and contributed to the interpretation of the results. MKM provided clinical data and genotype-phenotype correlations for the Australian cohort of patients and contributed to the interpretation of the results, MAE supervised the interpretation of neurological findings. KS provided access to genetic data and contributed to the study design and interpretation of results. MMH contributed to the study design and interpretation of results. SR and AH-S conceived the study concept, supervised the writing of the manuscript and interpretation of results. All authors reviewed and approved the final version of the manuscript.

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Data-sharing statement

Peer investigators wishing to see the study data may contact the corresponding author.

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