Acute central nervous system toxicity during treatment of pediatric acute lymphoblastic leukemia: phenotypes, risk factors and genotypes

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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: 1.11x10^-9), 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.5,6-11 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.12,13 Recent research has used genome-wide association studies (GWAS) to identify single nucleotide polymorphisms (SNP) related to methotrexate-induced leukoencephalopathy in pediatric ALL.14 Mateos et al. identified SNP in genes regulating neuronal growth, differentiation, and cytoskeletal organization at a significance level of P<1.0×10^-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.23 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<5×10^-6 and a Bonferroni-corrected P<2×10^-4, 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.18-22 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).20,22 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 toxicities.
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).

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

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**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.
toxicities were alive. Eight patients with CNS toxicities (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 conditions with CNS toxicities** |               |
| Elevated Intracranial 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 fulfill 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<2×10^-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 <5×10^-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-

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).
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 \((HR=0.94\text{ per weighted risk allele}, 95\%\ CI: 0.85-1.05; P=0.29)\). We have subsequently calculated an unweighted additive 19-SNP genetic score based on candidate SNP that were associated with seizures or methotrexate-related CNS toxicity.

**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.

| CNS status**, N (%) | 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-
ity including stroke-like syndrome is a well-known entity but it is possible that mild cases may have been under-detected 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 $P<5 \times 10^{-6}$.

| 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              |                             | -                                                                            | 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              |                             | -                                                                            | 142606705| A                     | G                        | 0.05 | 3.62 | 2.97e-06 |
| rs17641985     | 13         | Intron variant, Upstream gene variant  | AL355390.1 LINC00381 (2394)  | 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                | RP11-5106.2 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.
evaluation with neuroimaging. Patients with CNS leukemia were excluded from some studies on the occurrence of CNS toxicities.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{29,34,35} Immunophenotype (T-cell) and induction therapy (dexamethasone) were significant in univariate analyses, but did not reach signifi-

| SNP ID     | Gene                  | Chromosome | Phenotype       | Position          | Effect allele | Reference allele | Info score | MAF (minor allele) | OR (P-value) | FDR  |
|------------|-----------------------|------------|-----------------|-------------------|---------------|------------------|------------|--------------------|--------------|------|
| rs6432877  | SCN3A, SCN2A, TTC21B, | 2          | All epilepsy    | 166998767         | G             | C                | 1.00       | 0.23 (G)           | 1.07         | 0.92 |
| rs4671319  | FANCL, BCL11A         | 2          | All epilepsy    | 57950346          | A             | G                | 1.00       | 0.47 (G)           | 0.89         | 0.45 |
| rs4638568  | HEATR3, BRD7          | 16         | All epilepsy    | 50045839          | A             | G                | 0.99       | 0.05 (A)           | 0.55         | 0.23 |
| rs2212656  | SCN3A, SCN2A, TTC21B, | 2          | Focal epilepsy  | 167000843         | A             | C                | 1.00       | 0.23 (A)           | 1.07         | 0.93 |
| rs4665630  | KLHL29                | 2          | Generalized epilepsy | 23898317       | T             | C                | 1.00       | 0.10 (C)           | 1.02         | 0.98 |
| rs11943905 | GABRA2                | 4          | Generalized epilepsy | 46397617       | T             | C                | 1.00       | 0.28 (T)           | 0.98         | 0.87 |
| rs13200150 | PTPRK                 | 6          | Generalized epilepsy | 128309768      | G             | A                | 1.00       | 0.33 (G)           | 0.99         | 0.86 |
| rs1402398  | FANCL, BCL11A         | 2          | Generalized epilepsy | 58042241       | A             | G                | 1.00       | 0.37 (G)           | 0.95         | 0.71 |
| rs4596374  | KCNN2                 | 5          | Generalized epilepsy | 114221505      | T             | C                | 0.98       | 0.48 (T)           | 0.91         | 0.59 |
| rs4794333  | PNPO                  | 17         | Generalized epilepsy | 46045495       | C             | T                | 1.00       | 0.41 (C)           | 1.19         | 0.24 |
| rs11890028 | SCN3A, SCN2A, TTC21B, | 2          | Generalized epilepsy | 166943277      | G             | T                | 1.00       | 0.26 (G)           | 0.76         | 0.22 |
| rs2833098  | GRIK1                 | 21         | Generalized epilepsy | 32183996       | A             | G                | 1.00       | 0.37 (G)           | 0.71         | 0.04 |
| rs68082256 | ATXN1                 | 6          | Generalized epilepsy | 16971575       | A             | G                | 0.99       | 0.19 (A)           | 0.47         | 0.01 |
| rs47712462 | 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.
cance in the multivariate model, probably due to co-vari-
ation. Epilepsy as a late effect after PRES or other CNS toxicity in childhood ALL has been described in previous studies.\textsuperscript{2,5,6,10,12-23} We do not have data on epilepsy among controls, but the incidence of epilepsy among ALL pa-
tients with CNS toxicity in our study was higher than that in the general population in developed countries.\textsuperscript{26} This was true even if we assumed that none of the controls in this ALL cohort had a diagnosis of epilepsy and the 12 re-
ported patients with epilepsy would represent the overall prevalence of epilepsy in this population.\textsuperscript{26} 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 se-
quel is more common in ALL patients who displayed CNS toxici-
ties.\textsuperscript{26} ATXN1, encoding ataxin-1 protein, is the gene underly-
ing spinocerebellar ataxia type 1 and is implicated in sei-
zures.\textsuperscript{12,27} 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 re-

clects 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 pre-


disposition and give insight into the pathogenesis. Cognitive impairment was reported in 12 patients, but for-
normal 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.\textsuperscript{38,39} The mortality of patients from CNS toxicity in our study was lower than that previously described, but still considerable.\textsuperscript{5,28} This study did not reveal any novel genome-wide signifi-
cant 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 sys-
tem, to neurite growth, stability and the morphological plasticity of synapses; both genes are related to neuro logical disorders.\textsuperscript{38,39,40} 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, MRETT1A rs78817171 and HHLA3 rs114310506) were present in both groups, reflecting the overlap of pa-
tients (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 neurologi-
cal, neuropsychological and developmental disorders but since they did not reach genomic significance, the sug-
gestive $P$ threshold or any functional enrichment by gene set overlap analysis this finding is non-specific.\textsuperscript{36,37} None of the previously described genes associated with CNS toxicity in pediatric ALL was replicated with GWAS in our study.\textsuperscript{70} 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 sei-
zures in childhood ALL possibly includes genetic aspects. Genome-wide association analyses and validation studies in larger cohorts of pediatric patients with ALL are war-
nanted to further study genetic predisposition to CNS toxicities among ALL patients.\textsuperscript{70} 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 interpre-
tation of the results. MKM provided clinical data and ge-
notype-phenotype correlations for the Australian cohort of patients and contributed to the interpretation of the re-


sults, MAE supervised the interpretation of neurological findings. KS provided access to genetic data and con-
tributed 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, super-
vised the writing of the manuscript and interpretation of results. All authors reviewed and approved the final version of the manuscript.
Acknowledgments
The authors acknowledge the Sydney Children’s Tumour Bank Network, which provided samples for the Australian GWAS that was used for validation purposes.

Funding
This work was supported by the Swedish Childhood Cancer Fund (grants KP2017-0010, TJ2020-0082, TJ2019-0031), Stockholm county, the Danish Childhood Cancer Foundation (TRAVERSE, 2018-3755) and the Interregional Childhood Oncology Precision Medicine Exploration (iCOPE), a cross-Oresund collaboration between University Hospital Copenhagen, Rigshospitalet, Lund University, Region Skåne and Technical University Denmark (DTU), supported by the European Regional Development Fund. This work was part of Childhood Oncology Network Targeting Research, Organisation & Life expectancy (CONTROL) and supported by the Danish Cancer Society (R-257-A14720) and the Danish Childhood Cancer Foundation (2019-5934). This work was also supported by a Cancer Institute NSW Fellowship (grant ECF181430).

Data-sharing statement
Peer investigators wishing to see the study data may contact the corresponding author.

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