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

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Supplemental Methods, Tables and Figures

Contents

1 Supplemental Methods

Details on patients treated according to the Nordic Society of Pediatric Hematology and Oncology ALL2008 protocol

The patients were diagnosed and treated at 22 pediatric oncology centers in Sweden, Norway, Denmark, Finland, Iceland, Estonia, and Lithuania. Patients with bi-lineage ALL, Philadelphia-positive ALL or Down syndrome were excluded from the study. Patients with central nervous system (CNS) toxicities were identified in the Nordic Society of Pediatric Hematology and Oncology (NOPHO) ALL2008 registry. CNS toxicities were verified through complementary data collection via a comprehensive questionnaire sent to all participating pediatric oncology centers. Phenotypes were partly re-evaluated by SA, SR, AHS and ME.

Classification of CNS toxicites

Patients with posterior reversible encephalopathy syndrome (PRES), cerebrovascular events, seizures, hypertensive encephalopathy, methotrexate-related stroke-like syndrome (SLS), encephalopathy (defined as personality/behavioral changes oriented or disoriented to person, time and place *or* drowsiness/excessive sleepiness *or* obtundation/coma), aseptic meningitis and steroid psychosis were labeled as having “defined CNS toxicites”. Seizures, PRES, SLS and sinus venous thrombosis were determined according to NOPHO ALL2008 protocol and Schmiegelow *et al.* Delphi consensus definitions [1-4]. Other CNS toxicities were determined according to the current established neurological classifications [5-11].

Statistical methods

Statistical analyses were performed using R (versions 3.6.0 and 4.0.2) and SPSS, Version 26.0 for Windows (SPSS Inc., Chicago, IL) [12]. Cox proportional hazards models were used to evaluate the association between the incidence of CNS toxicity and age at diagnosis (≥ 10 years vs. < 10 years), sex, central nervous system (CNS) status at diagnosis (CNS2+3 vs. CNS1), induction therapy (dexamethasone +/- prednisolone pre-phase vs. prednisolone), and immunophenotype (T–cell vs. B-cell precursor). The cumulative incidence of CNS toxicity was calculated and visualized using the method of Gray (1988), with death, relapse, stem cell transplantation, and secondary malignant neoplasm as competing events, censoring at last follow-up[13].

The differences in the hazards for overall survival and event free survival for patients with CNS toxicities and controls were estimated using an illness-death model and Cox proportional hazards. A stratified model was used as the hazard for the transition to toxicity was not proportional to the other transitions. The transition probabilities were estimated using the Aalen-Johansen estimator[14].

Genetic data collection

As a part of the NOPHO ALL2008 study, all patients were invited to participate in genome wide association studies (GWAS) on host genome variants in association with any pharmacology, efficacy or toxicity [15,16]. Genotyping was performed on germline DNA obtained after clinical remission on exome-enriched Illumina Infinium Omni2.5exome-8-BeadChip arrays. Genotype data strand alignment and quality control were performed using PLINK2/1.90beta3 (see details in **Quality control of genotype data**) [17,18]. In total, 2,146,021 single nucleotide polymorphisms (SNPs) qualified for association analyses.

Quality control of genotype data.

The genotype data was aligned to the PLUS strand using William Rayner strand files as reference and human genome build 37[19]. SNPs that were not present in the reference and the SNPs mapped to multiple locations were excluded. The quality control excluded individuals with *(i)* missing data at more than 2% of SNPs, *(ii)* mismatch in sex information and genotypic sex as estimated by the inbreeding coefficient (*F*) where female sex is assigned when $F < 0.2$ and male is assigned when $F > 0.8$, *(iii)* excess heterozygosity or homozygosity which removes possibly contaminated samples and population substructure excludes individuals with *F* more than 4 standard deviations away from the mean of *F*, and finally *(iv)* individuals with high degree of relatedness using the three identity-by-descent (IBD) coefficients, which represented the probability of having 0, 1 or 2 inherited alleles in common, to estimate the degree of relatedness. The proportion of IBD allowed for unrelated patients was less than or equal to 0.1875, as estimated by *p=PIBD=2+12*P(IBD=1)*. For highly related pairs, the individual with lower amount of missing data is kept. SNPs were excluded if they were missing in more than 2% of the individuals. The number of patients and SNPs included after each step of quality control is described in **Supplemental Table S1 and Supplemental Table S2**.

Genome-wide association analysis

Genome-wide association analysis was performed in PLINK2/1.90beta6.18 [18] using logistic regression adjusted for age, sex, CNS leukemia (CNS2 or 3), and genetic ancestry by the first four principal components [18]. Age and the four genetic principal components were normalized by z-score standardization.

Genotype was treated as an additive effect (0, 1, or 2 per minor allele). A suggestive threshold of $P < 5 \times 10^{-6}$ and a Bonferroni-corrected P-value $< 2 \times 10^{-8}$, which were regarded as significant, were used to explore the top findings from the GWAS. Manhattan and qq plots were generated using the R package 'qqman' in R (v4.0.0) [20]. When no SNPs reached genome-wide significance, the top 50 SNPs were assessed for biological function of the affected genes for all the different CNS toxicities and seizures.

GWAS analyses were performed on three phenotype groups: all different CNS toxicites, PRES, and seizures. However, the group of patients with PRES showed signs of genomic inflation and were therefore not considered for further analysis. The most frequently identified SNPs were annotated using the variant effect predictor (GRCh37.p13) [21]. This SNP gene annotation was checked using the Ensembl GRCh37 database for possible matches to genes, disorders, and populations; GeneCards database was used for gene information [22,23].

Candidate single nucleotide polymorphism for seizures analysis

We selected 22 SNPs previously being associated with epilepsy and methotrexate-related central CNS toxicity, based on previously published data (**Supplemental Table 3**), to test for association with seizures [24,25]. Three SNPs (rs4665630, rs11890028, and rs4794333) were available on the exome-enriched Illumina Infinium Omni2.5exome-8-BeadChip array post quality control. The remaining SNPs were imputed using IMPUTE2[26]. Pre-phasing of haplotypes was estimated using SHAPEIT2 (v2.r790) with an effective population size of 15000 (default setting) and the 1000 Genomes haplotypes - Phase 3 integrated data set (build 37) release was used as the reference genome [27,28]. For the imputation, each chromosome was imputed in sets of 5MB using an effective population size of 20000 (default setting), and a buffer region of 250 kB to increase imputation quality. Two SNPs (rs887696 and rs1044352)

were not imputed. Post-imputation filtering of SNPs left 19 SNPs for candidate SNP analysis. Each SNP was tested for association with seizures using logistic regression, assuming an additive genetic model and a missing data likelihood score test (method: score) using SNPTEST, as previously described [29]. Corrections for multiple testing were performed by the Benjamini-Hochberg procedure.

Polygenic risk scores of candidate SNPs

In this study, we have estimated weighted and unweighted polygenic risk scores (PRS). An un-weighted PRS was calculated for all 19 candidate SNPs (**Supplemental Table 3**) with an additive assumption of the number of effect alleles, meaning that for each SNP in the score homozygotes major allele received a value of 0, heterozygotes received the value 1 and homozygotes for the minor/effect allele received the value 2. These values were then summarized for each patient across the 19 candidate SNPs. The weighted PRS uses the SNPs, effect alleles and log-transformed effect sizes (OR) given by Mateos et al and an additive assumption of the effect allele (SNPs are named in **Supplemental Table 3**, "Methotrexate-related central CNS toxicity")[25]. The weighted PRS is per patient given by: $PRS = \sum_{i=1}^{N} \log(OR_i) * n_{effect\ alleles}$[25]. Hazard ratios for the PRSs were estimated with cox regression models.

Validation study

Validation was sought within an external Australian dataset, which has been previously described in relation to methotrexate-related central CNS toxicity[25]. In brief, the cohort was assembled as part of the ERASE (**E**valuation of **R**isk of **A**cute Lymphoblastic Leukaemia-related **S**ide-**E**ffects) Study. Ethical approval was granted by the Hunter New England Human Research Ethics Committee (HNEHREC Reference Number: 12/11/21/4.01).

For the GWAS, patients aged 1-18 years treated on consecutive Berlin-Frankfurt-Munster (BFM)-based ALL treatment regimens in Australia from 1998 to 2013 were evaluated (n=1021); germline data were available for 932 patients. Following sample quality control and filtering for European ancestry, 707 individuals were available for analysis[25]. Separate GWAS were run for methotrexate-related central CNS toxicity and central CNS toxicity (GWAS results unpublished) in patients with European ancestry (Spanish IBS, Italian TSI, British GBR, White European from Utah CEU, Finnish FIN as defined by1000 Genomes)[25,30]. For the purposes of candidate SNP validation using the results of patients from NOPHO ALL2008, all 19 SNPs were available for assessment in the ERASE cohort. Of these, 16 SNPs passed filtering (information score >0.4), and were thus assessed further. The ERASE GWAS was conducted using age, sex and principal components as covariates.

For the methotrexate-related central CNS toxicity cohort, there were 48 cases and 537 controls. For the central CNS toxicity cohort, there were 103 cases and 537 controls. These CNS toxicities (n=103) were classified as per Schmiegelow *et al.* Delphi consensus definitions and current established neurological classifications[1,25]. Patients who experienced peripheral CNS toxicity, without central CNS toxicity, were excluded from the GWAS analysis for both phenotypes.

2 Supplemental Tables and Figures

Supplemental Table S1 Quality control of genotype data: overview of preprocessing and quality control (QC) on individually genotyped batches. The batches were genotyped using different versions of the same chips, therefore these were initially quality controlled separately.

Batch	***1, 2, 3***		***5***		***2019***		***2020***	
Genotyping chip	*HumanOmni2-5Exome-8-v1-1-A*		*InfiniumOmni2-5Exome-8v1-3_A1*		*InfiniumOmni2-5Exome-8v1-3_A1*		*InfiniumOmni2-5Exome-8v1-4_A1*	
	Patients	**SNPs**	**Patients**	**SNPs**	**Patients**	**SNPs**	**Patients**	**SNPs**
NOPHO genotypes	1 519	2 546 527	334	2 612 357	362	2 612 357	135	2 617 655
NOPHO genotypes with consent	1 384	2 546 528	325	2 612 357	362	2 612 357	135	2 617 656
Strand alignment	1 384	2 498 653	325	2 560 920	362	2 560 920	135	2 566 350
Remove duplicate samples and SNPs	1 321	2 453 541	311	2 514 171	362	2 515 787	135	2 523 930
QC step 1: SNP and sample missingness (2 %)	1 232	2 383 820	234	2 314 622	345	2 434 130	134	2 462 363
QC step 2: Sex discrepancy	1 220	2 383 820	225	2 314 622	340	2 434 130	134	2 462 363
QC step 3: Remove individuals with excess heterozygosity and homozygosity	1 202	2 383 820	219	2 314 622	334	2 434 130	133	2 462 363
QC step 4: Relatedness (keep identity-by-descent ≤ 0.1875)	1 194	2 383 820	219	2 314 622	331	2 434 130	133	2 462 363

QC: quality control, SNP: single nucleotide polymorphism, NOPHO: Nordic Society of Pediatric Hematology and Oncology

Supplemental Table S2 Quality control of genotype data: overview of quality control on merged genotype batches.

	Patients	**SNPs**
Remove duplicate samples and SNPs	1 842	2 534 414
QC step: SNP and sample missingness	1 842	2 146 021
QC step: Relatedness (keep identity-by-descent ≤ 0.1875)	1 837	2 146 021
QC step: Remove individuals with excess homozygosity	1 828	2 146 021

QC: quality control, SNP: single nucleotide polymorphism

Supplemental table S3 Candidate SNPs associated with epilepsy and methotrexate related central CNS toxicity.

SNP	*Chromo-some*	*Gene*	*Minor allele*	*MAF*	*P**	*Phenotype*
rs4671319	2	*FANCL BCL11A*	G	0.44	8.1E–09	All epilepsy
rs6432877	2	*SCN3A SCN2A TTC21B SCN1A*	G	0.26	1.7E–13	All epilepsy
rs4638568	16	*HEATR3, BRD7*	A	0.06	4.0E–08	All epilepsy
rs2212656	2	*SCN3A SCN2A TTC21B SCN1A*	A	0.26	7.3E–09	Focal epilepsy
*rs4665630***	2	None**	C	0.13	4.3E–08	Generalized epilepsy
rs1402398	2	*FANCL BCL11A*	G	0.36	1.2E–11	Generalized epilepsy
rs11890028	2	*SCN3A SCN2A TTC21B SCN1A*	G	0.27	4.7E–08	Generalized epilepsy
rs887696	2	*STAT4*	C	0.34	3.0E–08	Generalized epilepsy
rs1044352	4	*PCDH7*	T	0.42	2.2E-09	Generalized epilepsy
rs11943905	4	*GABRA2*	T	0.27	3.9E–08	Generalized epilepsy
rs4596374	5	*KCNN2*	C	0.45	7.2E–10	Generalized epilepsy
rs68082256	6	*ATXN1*	A	0.20	1.7E–09	Generalized epilepsy
*rs13200150****	6	None***	G	0.30	5.9E–09	Generalized epilepsy
rs4794333	17	*PNPO*	C	0.38	6.8E–09	Generalized epilepsy
rs2833098	21	*GRIK1*	G	0.38	1.7E–08	Generalized epilepsy
rs4712462	6	*MBOAT1*	A	0.35	2.54E-07	Methotrexate-related central CNS toxicity
rs2241357	19	*GIPC1*	A	0.2	3.60E-07	Methotrexate-related central CNS toxicity
rs1106479	3	*ZDHHC19*	T	0.16	4.08E-07	Methotrexate-related central CNS toxicity

rs35307996	17	*NXN*	CC	0.2	5.70E-07	Methotrexate-related central CNS toxicity
rs74956940	19	*PKN1*	G	0.23	6.19E-07	Methotrexate-related central CNS toxicity
rs62576054	9	*HMGB1P37*	G	0.18	7.50E-07	Methotrexate-related central CNS toxicity
rs9590003	13	None	A	0.11	9.73E-07	Methotrexate-related central CNS toxicity

CNS=central nervous system, SNP=single nucleotide polymorphism, MAF=minor allele frequency. *P value according to previously published studies, ** rs4665630: updated data show that the SNP is located in KLHL29 gene, *** rs13200150: updated data show that the SNP is located in PTPRK gene.

Supplemental table S4 All CNS toxicities studied with genome wide associations analyses.

CNS toxicity	**Number of patients**
PRES	46
SVT	22
Isolated seizures	12
Methotrexate SLS	6
Hypertensive encephalopathy	4
CNS infection	3
Encephalopathy	3
ICH	2
Aseptic meningitis	2
Electrolyte disturbances	2
Hypoxic brain injury	1
Seizures secondary to multiorgan failure	1
Elevated ICP	1
Hypoglycemia	1
Myelinolysis of pons	1
Steroid psychosis	1
Symptoms from cognition	1
Total	**109**

CNS=central nervous system, PRES=posterior reversible encephalopathy syndrome, SVT=sinus venous thrombosis, SLS=stroke like syndrome, ICH=intracranial hemorrhage, ICP=intracranial pressure

Supplemental table S5 50 most important SNPs for cases with all CNS toxicity cases and cases with seizures.

	SNP	CHR	Gene	Consequence (most important first)	BP	Effect allele (minor)	Reference allele (major)	MAF	OR	P	Phenotype of neurological, neuropsychological or developmental disorder
All CNS toxicities											
1.	rs72798143	2	*AC068490.2*	Intron variant; non coding transcript variant	22448244	A	C	0.08	2.88	1.11E-06	-
2.	rs79459815	4	-	Intergenic variant	180706970	A	G	0.01	11.51	2.29E-06	-
3.	rs13407218	2	*CTNNA2*	Intron variant	80600505	T	C	0.02	5.61	3.50E-06	Cortical dysplasia. complex. with other brain malformations; cerebral palsy. ataxic. autosomal recessive
4.	rs35916740	7	-	Regulatory region variant	93028950	G	T	0.09	2.63	3.71E-06	-
5.	rs62325077	4	-	Intergenic variant	162120255	C	A	0.11	2.43	4.89E-06	-
6.	rs114884102	6	-	Intergenic variant	8685785	T	C	0.01	7.08	5.85E-06	-
7.	rs79566233	6	-	Intergenic variant	8623113	G	A	0.01	6.96	6.89E-06	-
8.	rs192842795	1	*SYDE2*	Non coding transcript exon variant	85656421	T	C	0.01	8.83	7.62E-06	Multiple sclerosis; progressive supranuclear palsy
9.	rs114310506	1	*HHLA3*	Downstream gene variant	70855979	G	T	0.01	5.77	8.96E-06	Progressive supranuclear palsy
10.	rs55895260	5	-	Intergenic variant	62775330	A	T	0.10	2.40	9.55E-06	-

11.	rs78817171	11	*MRE11A*	Intron variant	94166352	A	G	0.05	2.96	9.81E-06	Ataxia-Telangiectasia-Like Disorder; ataxia. early-onset. with oculomotor apraxia and hypoalbuminemia; Nijmegen Breakage syndrome-like disorder
12.	rs78725566	10	*CACNB2*	Intron variant	18626839	T	C	0.01	7.28	1.03E-05	-
13.	rs112620400	5	*CTC-347C20.2*	Intron variant; non coding transcript variant	71893394	G	A	0.01	8.34	1.11E-05	-
14.	rs4598957	17	*MYO1D*	Intron variant	31062850	C	T	0.03	3.84	1.17E-05	Canavan disease
15.	rs17681215	15	*RP11-624L4.1*	Intron variant; non coding transcript variant	39369688	A	G	0.07	2.66	1.18E-05	-
16.	rs255024	5	-	Intergenic variant	178779612	T	G	0.20	2.10	1.22E-05	-
17.	rs826230	3	*THRB*	Intron variant	24306302	G	A	0.13	2.21	1.33E-05	-
18.	kgp6084995	3	*THRB*	Intron variant	24306831	T	G	0.13	2.20	1.39E-05	-
19.	rs130539	22	*SYN3*	Intron variant	33176450	C	T	0.11	2.41	1.45E-05	Seizure disorder; Alzheimer's disease progression score.
20.	rs4549468	4	-	Intergenic variant	115225571	A	G	0.01	6.19	1.63E-05	-
21.	rs2692472	7	*AC011288.2*	Intron variant; non coding transcript variant	13567908	T	G	0.17	2.04	1.76E-05	-
22.	rs76120612	6	-	Intergenic variant	49036257	C	T	0.02	4.19	1.81E-05	-
23.	rs9494981	6	*KIAA1244*	Intron variant	138558718	G	A	0.08	2.45	1.87E-05	-
24.	rs255020	5	-	Regulatory region variant	178793549	A	G	0.16	2.16	2.01E-05	-

25.	rs79190189	13	-	Intergenic variant	90895884	C	T	0.13	2.20	2.11E-05	
26.	kgp9569483	3	*THRB*	Intron variant	24316463	T	C	0.13	2.16	2.16E-05	-
27.	rs28809811	8	*SDCBP*	Intron variant	59475078	T	G	0.31	1.86	2.31E-05	Neurofibromatosis II
28.	rs11911591	21	*CHODL*	Intron variant	19594000	T	C	0.06	2.60	2.46E-05	Noonan Syndrome 5; Amyotrophic lateral sclerosis (sporadic)
29.	rs4888254	16	*GLG1*	Intron variant	74595649	G	A	0.17	2.03	2.48E-05	Amyotrophic Lateral Sclerosis 1
30.	rs12506016	4	-	Intergenic variant	166757964	T	C	0.16	2.03	2.58E-05	-
31.	rs340417	5	*ADAMTS2*	Intron variant	178762064	A	C	0.15	2.12	2.60E-05	-
32.	rs12357198	10	*ABI1*	Intron variant	27142382	C	T	0.05	2.82	2.63E-05	-
33.	rs13391262	2	AC018685.1	Downstream gene variant	2642893	T	C	0.20	2.00	2.75E-05	Unipolar depression. mood disorder
34.	rs55974243	8	-	Intergenic variant	59265949	A	C	0.09	2.31	2.75E-05	-
35.	rs76998416	10	*CACNB2*	Intron variant	18725730	T	C	0.01	7.98	2.90E-05	Lambert-Eaton myasthenic syndrome
36.	rs2073134	6	*KIF6*	Intron variant	39464712	T	C	0.30	1.85	2.91E-05	Attention deficit hyperactivity disorder symptoms
37.	rs79349206	10	*ABI1*	Intron variant	27074911	G	A	0.06	2.80	2.94E-05	Spinocerebellar ataxia. autosomal recessive 3
38.	rs11015279	10	*ABI1*	Intron variant	27064648	C	T	0.06	2.80	2.94E-05	Spinocerebellar ataxia. autosomal recessive 3
39.	rs2874792	4	-	Intergenic variant	166752233	A	C	0.17	2.03	2.97E-05	-
40.	rs2161277	5	-	Intergenic variant	62866262	T	C	0.11	2.25	3.00E-05	-
41.	rs12435954	14	*CEP128*	Intron variant	81222739	C	T	0.02	4.20	3.00E-05	Epilepsy; seizures; neurodevelopmental disorder
42.	rs74450405	21	-	Intergenic variant	18262773	G	A	0.03	3.61	3.02E-05	-
43.	kgp650977	1	*ZNF697*	Intron variant	120187040	A	C	0.17	1.99	3.10E-05	-

44.	rs12231568	12	*GLT1D1*	Intron variant	129454718	T	G	0.13	2.15	3.29E-05	Schizophrenia
45.	rs9367014	6	*KIF6*	Intron variant	39475060	T	C	0.30	1.84	3.33E-05	-
46.	rs17044392	2	-	Intergenic variant	53510962	A	G	0.16	2.07	3.49E-05	-
47.	rs80260186	4	*NR3C2*	Intron variant. NMD transcript variant	149314482	T	C	0.02	3.85	3.65E-05	Autism spectrum disorder
48.	rs12565092	1	*CNIH3*	Intron variant	224818616	A	G	0.26	1.93	3.68E-05	Schizophrenia
49.	rs10511725	9	-	Intergenic variant	23426193	C	A	0.50	1.87	3.80E-05	-
50.	rs17149881	9	*AK8*	Intron variant	135745274	A	G	0.04	3.13	3.81E-05	-
											-
Seizures											
1.	rs75487096	3	*KIAA0226*	Intron variant	197436685	C	T	0.02	7.01	2.11E-06	Spinocerebellar ataxia. autosomal. recessive; pathologic nystagmus; alacrima. achalasia and mental retardation syndrom; disease of mental health
2.	rs16936423	9	-	Intergenic variant	2000098	G	A	0.03	4.68	2.27E-06	-
3.	rs116011797	5	-	Intergenic variant	121924081	T	C	0.02	7.36	2.46E-06	-
4.	rs114884102	6	-	Intergenic variant	8685785	T	C	0.01	9.24	2.78E-06	-
5.	rs79566233	6	-	Intergenic variant	8623113	G	A	0.01	9.23	2.81E-06	-
6.	rs78682412	8	-	Regulatory region variant	142606705	A	G	0.05	3.62	2.97E-06	-

7.	rs17641985	13	*AL355390.1*	Intron variant	74990916	C	T	0.01	8.03	3.48E-06	-
8.	rs16936230	9	*RP11-443B9.1 pseudogene*	Upstream gene variant	1981979	G	A	0.03	4.48	4.09E-06	-
9.	rs1528779	2	-	Intergenic variant	22969224	C	T	0.48	0.39	4.14E-06	-
10.	rs353999	19	*SUMO1P4 peudogene*	Downstream gene variant	49782621	A	G	0.29	2.32	4.24E-06	-
11.	rs10478527	5	*RP11-510I6.2 pseudogene*	Downstream gene variant	120954047	G	A	0.32	2.35	4.87E-06	-
12.	rs12340816	9	-	Intergenic variant	2005105	G	T	0.03	4.41	4.91E-06	-
13.	rs9686533	5	*RP11-510I6.2*	Downstream gene variant	120955493	C	A	0.33	2.35	5.22E-06	-
14.	rs353988	19	*SLC6A21P pseudogene*	Upstream gene variant	49761199	T	C	0.28	2.29	5.29E-06	-
15.	rs6890291	5	*RP11-510I6.2*	Upstream gene variant	120951788	C	T	0.23	2.39	6.30E-06	-
16.	rs28662973	9	-	Intergenic variant	2005904	T	G	0.03	4.21	6.34E-06	-
17.	kgp10034446	2	-	Intergenic variant	22968522	G	A	0.44	0.38	7.12E-06	-
18.	rs28673928	5	*RP11-510I6.2*	Downstream gene variant	120956631	T	C	0.23	2.34	9.29E-06	-

19.	rs74450405	21	-	Intergenic variant	18262773	G	A	0.03	4.76	9.76E-06	-
20.	rs77196177	6	*SMOC2*	Intron variant	169057623	T	C	0.05	3.44	9.87E-06	-
21.	rs117691319	11	*DEAF1*	Intron variant	686888	A	G	0.01	7.31	1.09E-05	Neurodevelopmental disorder with hypotonia and impaired expressive language with or without seizures; autism. intellectual disability. basal ganglia dysfunction and epilepsy; intellectual disability-epilepsy-extrapyramidal syndrome; Vulto-Van Silfhout-De Vries Syndrome; autosomal dominant non-syndromic intellectual disability; Smith-Magenis syndrome; autism spectrum disorder
22.	rs10044992	5	*RP11-510I6.2*	Non coding transcript exon variant	120952441	T	G	0.23	2.32	1.11E-05	-
23.	rs117843355	17	-	Intergenic variant	51950533	G	C	0.02	5.66	1.41E-05	-
24.	rs73244559	6	*SMOC2*	Intron variant	169061552	C	T	0.05	3.36	1.41E-05	-
25.	rs12357198	10	*ABI1*	Intron variant	27142382	C	T	0.05	3.46	1.44E-05	Spinocerebellar ataxia. autosomal recessive 3
26.	kgp11122788	2	-	Intergenic variant	22975606	T	C	0.46	0.41	1.45E-05	-
27.	rs13407218	2	*CTNNA2*	Intron variant	80600505	T	C	0.02	6.44	1.47E-05	Cortical dysplasia. complex. with other brain malformations; cerebral palsy. ataxic. autosomal recessive
28.	rs35916740	7	-	Regulatory region variant	93028950	G	T	0.09	2.96	1.48E-05	-
29.	rs114310506	1	*HHLA3*	Downstream gene variant	70855979	G	T	0.01	6.93	1.50E-05	-
30.	rs77817759	11	*RPS27P20 pseudogene*	Upstream gene variant	129406692	A	C	0.03	4.73	1.53E-05	Unipolar depression

31.	rs79349206	10	*ABI1*	Intron variant	27074911	G	A	0.05	3.43	1.63E-05	Spinocerebellar ataxia. autosomal recessive 3
32.	rs11015279	10	*ABI1*	Intron variant	27064648	C	T	0.05	3.43	1.63E-05	Spinocerebellar ataxia. autosomal recessive 3
33.	rs34514944	3	*PPM1M*	Upstream gene variant	52278944	C	T	0.07	3.10	1.66E-05	Autism spectrum disorder or schizophrenia; bipolar disorder; schizophrenia
34.	rs76120612	6	-	Intergenic variant	49036257	C	T	0.02	4.86	1.66E-05	-
35.	rs186932437	14	-	Intergenic variant	95356482	A	G	0.01	15.81	1.69E-05	-
36.	rs12435954	14	*CEP128*	Intron variant	81222739	C	T	0.02	5.21	1.71E-05	Epilepsy; seizures; neurodevelopmental disorder
37.	rs78817171	11	*MRE11A*	Intron variant	94166352	A	G	0.05	3.45	1.73E-05	Ataxia-telangiectasia-like disorder; ataxia. early-onset. with oculomotor apraxia and hypoalbuminemia; Nijmegen Breakage syndrome-like disorder
38.	rs3924202	2	-	Intergenic variant	23015895	T	C	0.45	0.41	1.77E-05	-
39.	rs45488095	14	*CEP128*	Missense variant	81259336	C	T	0.01	10.37	1.91E-05	Epilepsy; seizures; neurodevelopmental disorder
40.	rs34096397	20	-	Regulatory region variant	22780848	A	G	0.05	3.32	1.94E-05	-
41.	rs77426433	2	-	Intergenic variant	139393692	T	C	0.02	5.01	2.02E-05	-
42.	rs35506008	20	-	Intergenic variant	22780795	G	A	0.05	3.30	2.12E-05	-
43.	rs3026603	20	*EDN3*	Intron variant	57878937	A	G	0.02	5.76	2.21E-05	Ondine syndrome
44.	rs6075967	20	-	Regulatory region variant	22782178	A	C	0.05	3.28	2.28E-05	-

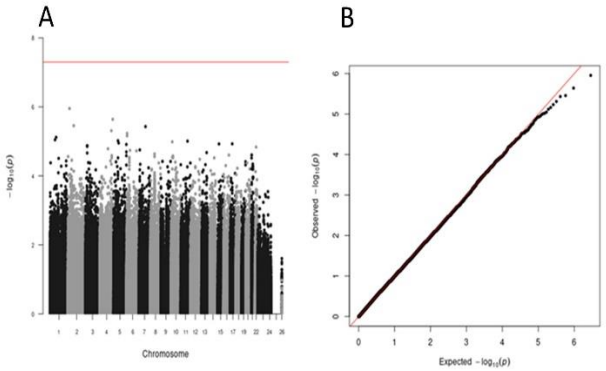
45.	rs79980218	13	*LINC00348*	Intron variant; non coding transcript variant	71601466	T	C	0.01	9.51	2.31E-05	-
46.	rs12641748	4	*KCNIP4*	Intron variant	20806697	T	G	0.06	3.30	2.34E-05	Amyotrophic lateral sclerosis; attention deficit disorder with hyperactivity; Parkinson disease; stroke; suicidal ideation
47.	rs76005629	4	-	Intergenic variant	117666718	T	C	0.07	3.13	2.34E-05	-
48.	rs6544204	2	-	Intergenic variant	23004918	G	A	0.47	0.43	2.37E-05	-
49.	rs118118987	13	*LINC00348*	Intron variant; non coding transcript variant	71600941	A	C	0.01	9.47	2.38E-05	-
50.	rs76569948	2	*AC012501.2*	Intron variant; non coding transcript variant	154408401	A	C	0.01	12.81	2.53E-05	-

SNP=single nucleotide polymorphism, CNS=central nervous system, CHR=chromosome, BP=base pair, NMISS=number of observations, OR=odds ratio, NMD=Nonsense-mediated mRNA decay.

Supplemental Figure 1

Supplementary Figure 1. Genome wide association studies

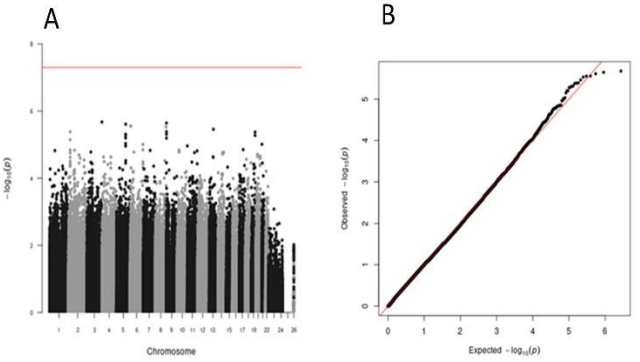
I. Genome wide association study on diverse acute CNS toxicities (109 are cases and 1057 are controls).



A. Manhattan plot for GWAS on acute CNS toxicitites (109 are cases and 1055 are controls) adjusted for age, sex, CNS leukemia and genetic ancestry by the first four principal components. Red line = genome-wide significance (p=5e-8). Genomic inflation est. lambda (based on median chisq) =1.04185.

***B.** QQ-plot for GWAS on acute CNS toxicitites (109 are cases and 1055 are controls) adjusted for age, sex, CNS leukemia and genetic ancestry by the first four principal components.*

II. Genome wide association study on seizures (67 are cases and 1057 are controls).



***A.** Manhattan plot for GWAS on seizures (67 are cases and 1055 are controls) adjusted for age, sex, CNS leukemia and genetic ancestry by the first four principal components. Red line = genome-wide significance (p=5e-8). Genomic inflation est. lambda (based on median chisq) = 1.02651.*

***B.** QQ-plot for GWAS on seizures (67 are cases and 1055 are controls) adjusted for age, sex, CNS leukemia and genetic ancestry by the first four principal components.*

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