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Genetic Susceptibility to Hepatic Sinusoidal Obstruction Syndrome in Pediatric Patients Undergoing Hematopoietic Stem Cell Transplantation

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A B S T R A C T

Sinusoidal obstruction syndrome (SOS) is a well-recognized and potentially life-threatening complication of hematopoietic stem cell transplantation (HSCT). SOS arises from endothelial cell damage and hepatocellular injury mostly due to the transplantation conditioning regimens but also to other patient, disease, and treatment-related factors. Understanding risk factors associated with the development of SOS is critical for early initiation of treatment or prophylaxis. The knowledge about genetic contribution is limited; few studies investigated so far selected a set of genes. To get more comprehensive insight in the genetic component, we performed an exome-wide association study using genetic variants derived from whole-exome sequencing. The analyses were performed in a discovery cohort composed of 87 pediatric patients undergoing HSCT following a busulfan-containing conditioning regimen. Eight lead single-nucleotide polymorphisms (SNPs) were identified after correction for multiple testing and subsequently analyzed in a validation cohort (n = 182). Three SNPs were successfully replicated, including rs17146905 (P = .001), rs16931326 (P = .04), and rs2289971 (P = .03), located respectively in the *UGT2B10, BHLHE22*, and *KIAA1715* genes. *UGT2B10* and *KIAA1715* were retained in a multivariable model while controlling for nongenetic covariates and previously identified risk variants in the *GSTA1* promoter. The modulation of associations by conditioning regimens was noted; *KIAA1715* was dependent on the intensity of the conditioning regimen, whereas the effect of *UGT2B10* was equally applicable to all of them. Combined effect of associated loci was also observed (P = .00006) with a genotype-related SOS risk of 9.8. To our knowledge, this is the first study addressing the genetic component of SOS at an exome-wide level and identifying novel genetic variations conferring a higher risk of SOS, which might be useful for personalized prevention and treatment strategies.

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BACKGROUND

Hepatic sinusoidal obstruction syndrome (SOS), also known as veno-occlusive disease, is a life-threatening complication that occurs after hematopoietic stem cell transplantation

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(HSCT) [1]. It is mostly related to the intensity of the conditioning regimen and employed drugs, such as busulfan (Bu), cyclophosphamide (Cy), or melphalan (Mel), that cause sinusoidal endothelial cell damage and hepatocellular injury [2,3]. Other patient-, disease-, and treatment-related factors can also modify the risk of SOS, such as patient age, liver dysfunction, concomitant medication, alloreactivity, cytokine release due to inflammation, and engraftment [4-10]. Experimental models showed that endothelial damage triggers formation of gaps between sinusoidal endothelial cells and passage of erythrocytes to the perisinusoidal space [11]. Additionally, locally released cytokines induce activation of cell adhesion molecules, coagulation, and fibrinolytic pathways [12]. Fibrin deposition, clot formation, and erythrocyte extravasation have all been reported to contribute to the narrowing of the sinusoids and reduction of hepatic venous outflow, leading to central venular occlusion, hepatocellular necrosis, hepatic enlargement, and ultimately SOS [2]. Patients with severe forms can have significant complications, including multiorgan failure, and a high mortality rate. SOS is reported to occur in up to 18% of pediatric patients after HSCT [7]. Well-established risk factors mentioned above can influence the risk of SOS. Nevertheless, patients with similar treatments, disease, and demographics are not equally vulnerable to SOS development, suggesting a genetic contribution. Indeed, candidate gene studies have led to the identification of genetic factors contributing to SOS risk, including glutathione S-transferase (GST) polymorphisms, which might affect Bu metabolism, such as *GSTM1-null* genotype and *GSTA1 *B* haplotype, as well as polymorphisms that might affect glutathione levels and oxidative liver injury [13-18]. However, these studies focused on a selected set of candidate genes. To address the role of genetic susceptibility in a more comprehensive manner, we have used a hypothesis-free approach and assessed the relationship of SOS development in children undergoing HSCT with genetic variants obtained from whole-exome sequencing (WES). Top-ranking association signals were verified in a replication cohort, identifying novel genetic loci contributing to SOS.

PATIENTS AND METHODS

Patient Groups

Participants were recruited from the institutional HSCT biobank at Saint-Justine University Health Center (SJUHC), Montreal, Quebec, Canada, and in the context of a multicentric study by the European Society for Blood and Marrow Transplantation (ClinicalTrials.gov Identifier: NCT01257854) [19]. The discovery cohort included 87 patients who underwent allogeneic HSCT between 2000 and 2013 at SJUHC whose DNA was of sufficient quality and quantity to perform WES. The replication cohort was an independent cohort composed of 182 unselected patients, including 61 patients from SJUHC who underwent allogeneic or autologous HSCT and who were either not included in sequencing due to insufficient DNA quantity or were recruited after the sequencing had been performed (2013 to 2015). The replication cohort also included 121 pediatric patients who underwent allogeneic HSCT from 2001 to 2015 in 4 different centers in Europe and Canada (Geneva University Hospital; University Medical Center Utrecht; Leiden University Medical Center; Robert Debré Hospital, Paris; and Alberta Children's Hospital, Calgary) and were in part included in our previous study on *GSTA1* polymorphisms [19]. Written informed consent was obtained from every patient or parent/legal guardian. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the relevant institutional review boards or ethics committees. The characteristics of the discovery and replication cohorts are given in Tables 1 and 2, respectively, and are also provided in the supplemental material (Item S1). The information on SOS was collected from patients' medical charts. SOS was diagnosed according to the Modified Seattle Criteria [18], which include the occurrence of 2 of the following events: unexplained weight gain of more than 2% from baseline because of fluid accumulation, hyperbilirubinemia ≥2 mg/dL, hepatomegaly, or upper right quadrant pain of liver origin.

WES

WES in the discovery cohort was performed on germline DNA, extracted from peripheral blood or saliva samples before transplant as described previously [20]. Briefly, exomes were captured in solution with Agilent's (Agilent Technologies Canada Inc. Mississauga, ON, Canada) SureSelect Human All Exon V5 + UTRs kit and sequenced on the Illumina (Illumina Canada, Vancouver, BC, Canada. Used Illumina HiSeq2500 platform is located at the Integrated Centre for Pediatric Clinical Genomics at the SJUHC, Montreal, Canada) HiSeq2500 platform (mean coverage of 40×) at the SJUHC integrated clinical genomic center in pediatrics. Reads were aligned to the hg19 reference genome using Burrows-Wheeler Alignment tool-Maximum Exact Matches [21]. PICARD [22] was used to mark PCR duplicates and collect sequencing quality control metrics. Variant calling was performed using the Haplotype Caller and quality score recalibration was performed using the Variant Recalibrator, both implemented in the Genome Analysis Tool Kit [23]. Variants were selected based on the variant quality score (VQSR = PASS) and minimum overall depth of coverage (≥10). The annotation of the identified germline variants was performed using ANNOVAR [24]. Common single-

Table 1
Demographic and Treatment Characteristics of the Discovery Cohort (n = 87)

Demographic Characteristics	Patients	
	n	%
Sex		
Male	40	46
Female	47	54
Ethnicity		
Caucasian	62	71.3
Other	25	28.7
Diagnosis*		
Malignancies	45	51.7
Nonmalignancies	42	48.3
HLA compatibility		
Unrelated donor	50	57.5
Related donor	1	1.1
HLA identical sibling	36	41.4
Stem cell source*		
BM	43	49.4
PBSC	2	2.3
Cord blood	42	48.3
Conditioning		
Bu/Cy	62	71.3
Bu/Cy/VP16	5	5.7
Bu/Flu	19	21.8
Bu/Flu/Thiotepa	1	1.1
Busulfan protocol		
One dose per day	67	77
Four doses per day	20	23
Chemotherapy regimen*		
Myeloablative	68	78.2
Myeloablative with reduced toxicity	16	18.4
Nonmyeloablative	3	3.4
SOS		
Yes	12	13.8
No	75	86.2
Prophylaxis of SOS		
Ursodeoxycholic acid	87	100
Age in years, median (range)	7.4	(0.1-23.5)
cumAUC (mg × h/L), median (range)	59.6	(25.5-79.0)
GSTA1†	13 (14.9%)/74 (85.1%)	

BM indicates bone marrow; PBSC, peripheral blood stem cell; Bu, Busulfan; Cy, Cyclophosphamide; VP16, etoposide; Flu, Fludarabine; SOS, Sinusoidal Obstruction Syndrome; cumAUC, cumulative area under the curve; *GSTA1*, glutathione S-transferase A1.

* Further details of the discovery cohort, particularly regarding diagnosis, stem cell source, and chemotherapy regimens, are provided in supplemental material (Item S1).

† Number and frequency of diplotypes, as derived from genotype data, with and without reduced metabolic capacity.

Table 2
Demographic and Treatment Characteristics of the Replication Cohort (n = 182)

Characteristics	Patients	
	n	%
Centers		
St-Justine UHC, Montreal (Canada)	61	33.5
Geneva University Hospital, Geneva (Switzerland)	4	2.2
Robert Debré, University Hospital, Paris (France)	13	7.1
University Medical Center, Utrecht (Netherlands)	66	36.3
Alberta Children's Hospital, Calgary (Canada)	38	20.9
Sex		
Male	109	59.9
Female	73	40.1
Ethnicity		
Caucasian	150	82.4
Other	27	14.8
Not available data	5	2.8
Diagnosis*		
Hematologic malignancies	100	54.9
Neuroblastoma	10	5.5
Nonmalignancies	72	39.6
HLA compatibility		
Unrelated donor	121	66.5
Related donor	3	1.6
Autologous	14	7.7
HLA identical sibling	44	24.2
Stem cell source*		
BM	76	41.8
PBSC	42	23.1
Cord blood	63	34.6
BM + PBSC	1	0.5
Conditioning		
Bu/Cy	71	39.0
Bu/Cy/Mel	31	17.0
Bu/Cy/VP16	7	3.8
Bu/Mel	12	6.6
Bu/Mel/Ara-C	1	0.5
Bu/Mel/Gem	3	1.7
Bu/Flu	54	29.7
Bu/Flu/Thiotepa	3	1.7
Busulfan protocol		
One dose per day	122	67
Four doses per day	60	33
Chemotherapy regimen*		
Myeloablative	125	68.7
Myeloablative with reduced toxicity	50	27.5
Nonmyeloablative	7	3.8
Total body irradiation*		
Yes	28	15.4
No	154	84.6
SOS		
Yes	27	14.8
No	155	85.2
Prophylaxis of SOS		
Ursodeoxycholic acid	60	33.0
Defibrotide	5	2.7
Heparin	8	4.4
Defibrotide and ursodeoxycholic acid	4	2.2
Not available data	105	57.7
Age in years, median (range)	4.71	(0.0-21)
cumAUC (mg × h/L), median (range)	61.9	(31.6-118.7)
GSTA1†	24 (15.3%)/133 (84.7%)	

Mel indicates melphalan; Ara-C, cytarabine; Gem, gemcitabine.

* Further details of the replication cohort, particularly regarding diagnosis, stem cell source, chemotherapy regimen, and total body irradiation, are provided in supplemental material (Item S1).

† Number and frequency of diplotypes with and without reduced metabolic capacity.

nucleotide polymorphisms (SNPs) located in exons and UTRs with minor allele frequency higher than 5% were selected for the analyses. They were filtered to exclude variants exceeding a missingness rate of 20%, not in Hardy-Weinberg equilibrium ($P < .001$) [25], or with pairwise linkage disequilibrium ($r^2 > 0.8$). To further reduce the complexity of the analysis and focus on the coding variants with potential causal effects, only nonsynonymous variants with predicted functional effect, nonsense variants, and variants in splicing sites were conserved. The predicted effect of nonsynonymous variants on the protein function was assessed in silico using SIFT and PolyPhen-2 [26,27]. All above filtering resulted in 4946 common exonic variants and 28,540 common SNPs located in UTRs that were retained for the analyses.

Association Study

The analysis between genetic variants obtained from WES data and SOS was performed by allelic ratio implemented in PLINK v.1.07 [28] using chi-square or Fisher exact test, as applicable. Analyses were corrected for multiple testing using the Benjamini-Hochberg procedure [29] for the false discovery rate with a cutoff value of <5%. Variants significantly associated with SOS were subsequently analyzed by SPSS (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.) using cumulative incidence of SOS and 1 minus survival curves in the Kaplan-Meier framework according to the best genetic model presented relative to the minor allele. The difference between genotypes was assessed by log-rank test. Univariate Cox regression analysis was used to estimate a hazard ratio (HR) with 95% confidence interval (CI). Variants that were significantly associated with SOS were genotyped in the replication cohort using the Sequenom platform at McGill University, Montreal, Quebec, Canada, and Genome Quebec Innovation Centre or by PCR-coupled allele-specific oligonucleotide hybridization assay [30]. The pretransplant DNA was available for 175 patients. The amplification was not equally efficient for all loci, and the maximum number of individuals with successful genotyping data ranged from 167 to 175, depending on the locus analyzed. The analyses of cumulative incidence of SOS in relation to the genotypes were performed in the Kaplan-Meier framework, as explained above. Multivariable Cox regression (backward stepwise selection method) was used to estimate the impact of associated genotypes in the presence of other covariates in the replication cohort. Other covariates included age as a continuous variable, sex, diagnosis (nonmalignant disease, hematologic malignancies, and neuroblastoma), frequency of Bu administration (once or 4 times daily), type of conditioning regimen categorized according to the number of alkylating agents, and whether patients received total body irradiation (TBI) [31]. *GSTA1* haplotype groups defined previously to be a risk factor for SOS in the same cohorts [16,19] were also included in the multivariate analyses as well as dose-adjusted cumulative area under the curve (cumAUC, mg × h/L) estimated from the first-dose AUC and each individual dose received. *GSTA1* haplotype groups are defined by promoter polymorphisms (17), which are not available in WES data set; therefore, previously obtained genotypes were either used or obtained by PCR/allele-specific oligonucleotide and resulting haplotypes/diplotypes were recoded based on their metabolic capacity (slow metabolizers versus remaining groups). Stratified analyses according to conditioning regimen were also performed.

RESULTS

The association analysis using WES data in the discovery cohort revealed 8 loci significantly associated with SOS after multiple testing adjustment (Table 3; *P* ranged from 1.5×10^{-5} to 8×10^{-7}). All loci (*HADH,* rs17511319; *FAT3* rs11823754; *UGT2B10* rs17146905; *ZNF608* rs75323508; *AMPH* rs2810;

Table 3
Top-Ranking Loci of SOS Identified in the Discovery Cohort through Exome-Wide Association Study

Locus	SNP	Gene	MAF	Allelic *P* Value	Allelic* Ratio SOS+	Allelic Ratio SOS−	Allelic OR (95% CI)
chr2_176788639 (3'UTR)	rs2289971 (T>C)	*KIAA1715*	0.09	3.4×10^{-6}	8/16	7/143	10.2 (3.3-31.9)
chr4_108956331 (3'UTR)	rs17511319 (A>G)	*HADH*	0.059	1.2×10^{-5}	7/17	2/148	30.5 (5.9-158.6)
chr4_69696638 (3'UTR)	rs17146905 (A>G)	*UGT2B10*	0.11	6.9×10^{-6}	9/15	2/140	8.4 (3.0-23.9)
chr5_123973164 (3'UTR)	rs75323508 (C>T)	*ZNF608*	0.07	1.3×10^{-5}	7/17	6/144	9.9 (3.0-32.8)
chr7_38424328 (3'UTR)	rs2810 (T>C)	*AMPH*	0.09	1.1×10^{-5}	8/16	8/142	8.9 (2.9-26.9)
chr8_65495333 (3'UTR)	rs16931326 (G>A)	*BHLHE22*	0.09	1.1×10^{-5}	8/16	8/142	8.9 (2.9-26.9)
chr11_92625944 (3'UTR)	rs11823754 (G>T)	*FAT3*	0.10	8.3×10^{-7}	9/15	8/142	10.7 (3.6-31.7)
chr21_45403546 (3'UTR)	rs11537798 (A>G)	*AGPAT3*	0.07	1.3×10^{-5}	7/17	6/144	9.9 (3.0-32.8)

The results are obtained by allelic ratio implemented in PLINK.
MAF indicates minor allele frequency in entire cohort; OR, odds ratio; *KIAA1715*, lunapark (LNPK); *HADH*, hydroxyacyl-CoA dehydrogenase; *UGT2B10*, UDP, glucuronosyltransferase family 2 member B10; *ZNF608*, zinc finger protein 608; *AMPH*, amphiphysin; *BHLHE22*, basic helix-loop-helix family member e22; *FAT3*, FAT atypical cadherin 3; *AGPAT3*, 1-acylglycerol-3-phosphate O-acyltransferase 3.
* Allelic ratio is presented as ratio of minor versus major allele in patients with (+) and without (−) SOS.

BHLHE22 rs16931326; *AGPAT3* rs11537798; and *KIAA1715* rs2289971) were in 3′UTR of respective genes. The associated genes are implicated in different cellular functions such as transcriptional regulation, lipid homeostasis, or glucuronidation (Supplementary Table S1). Cumulative SOS incidence in relation to the genotypes is shown in Figure 1. In most cases, the dominant model was the most appropriate given the low number of homozygotes for the minor alleles, except for SNPs in the *UGT2B10* and *FAT3* genes, where the risk increased in an additive manner with each copy of the minor allele. The cumulative risk of SOS ranged from 4.8 to 12.8; HR for the carriers of the minor allele at *UGT2B10* rs17146905 was 4.8 (95% CI, 2.3 to 10.4; $P = 4 \times 10^{-6}$) and was 12.8 (95% CI, 4.1 to 40; $P = 1.5 \times 10^{-8}$) for rs17511319 in the *HADH* gene. There was only 1 case of SOS in Bu/fludarabine (Flu)-based conditioning regimens; thus, results mostly reflected association in patients who received Bu-Cy combinations. All associations were equally present in patients with malignant (n = 45) and nonmalignant disease (n = 42) and among patients of European ancestry (n = 62) (Supplementary Table S2).

The variants in these genes were further analyzed by genotyping in the replication cohort; the results are summarized in Table 4. The association was confirmed for *UGT2B10, BHLHE22*, and *KIAA1715* gene variants ($P < .05$). The frequency of risk genotype ranged from 23.1% to 34.6% in patients with SOS as compared to 10.3% to 11.4% seen in patients without this complication (Table 4). Although borderline significant association was also noted for *HADH* ($P = .05$), the direction of the effect was opposite as compared to the effect seen in the discovery cohort. This locus was not considered replicated. The cumulative incidence of SOS in relation to *KIAA1715, UGT2B10*, and *BHLHE22* ($P = .03$, .001, and .04, respectively) is also depicted in Supplementary Figure S1.

Multivariable analyses (Table 5) were subsequently performed and included *KIAA1715, UGT2B10*, and *BHLHE22* genotypes and nongenetic factors (age, sex, diagnosis, conditioning regimen, cumAUC, and TBI). Additionally, *GSTA1* haplotype groups (classified as the slow versus fast and normal metabolizers) were included in the model based on our previous studies showing in the discovery and replication cohort a higher

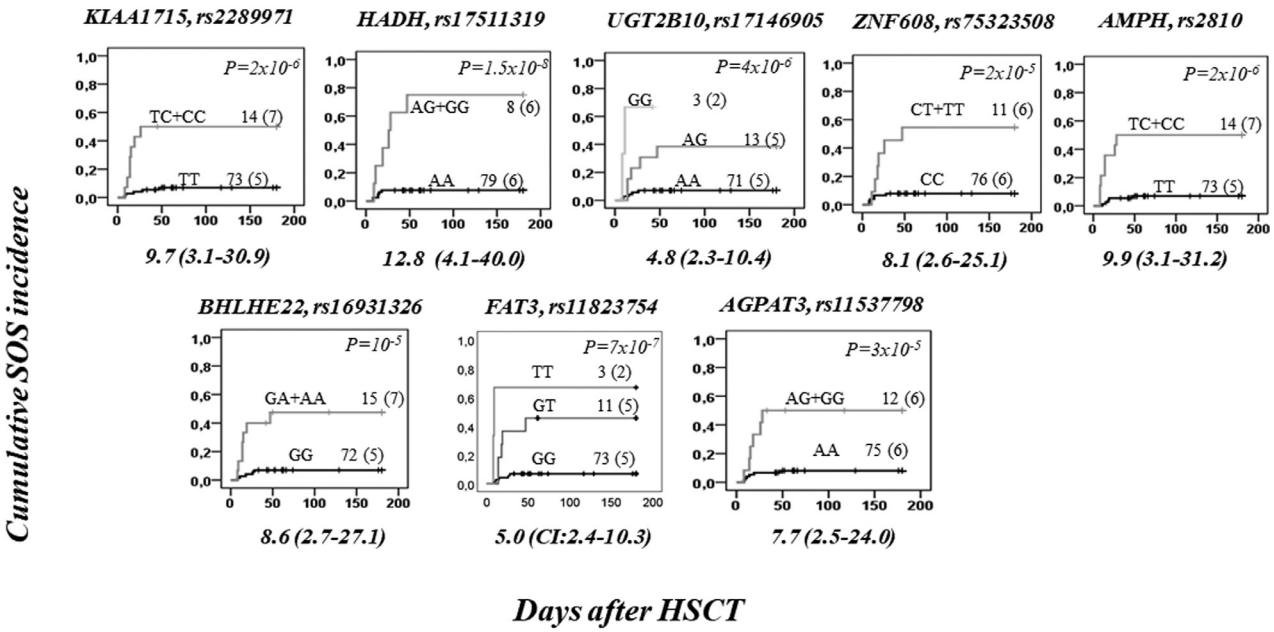

Figure 1. Cumulative incidence of SOS in relation to top-ranking loci identified through exome-wide association study of the discovery cohort. Cumulative incidence of SOS, plotted for indicated genotype groups according to dominant or additive model. The gene name and SNP rs number are given at the top of the panels. Total number of patients in each group with number of patients with SOS in brackets, and *P*value derived by log-rank, is depicted on all plots. Hazard ratio with 95% confidence interval in brackets is indicated below panels.

Table 4
Summary of the Analysis of Top-Ranking Association Signals in Replication Cohort.

Gene/SNP	No. of patients (P*)	SOS cases in genotype groups, n (%) **		Risk genotypes in SOS groups, n (%) ***	
KIAA1715 rs2289971	**N=175 (.03)**	7/24 (29.2)	19/151 (12.6)	7/26 (26.9)	17/149 (11.4)
HADH rs17511319	N=172 (.05)	0/21 (0)	26/151 (17.2)		
UGT2B10 rs17146905	**N=172 (.001)**	9/24 (37.5)	17/148 (11.5)	9/26 (34.6)	15/146 (10.3)
ZNF608 rs75323508	N=174 (.4)	1/14 (7.1)	25/161 (15.5)		
AMPH rs2810	N=173 (.9)	6/39 (15.4)	20/134 (14.9)		
BHLHE22 rs16931326	**N=169 (.04)**	6/21 (28.6)	20/148 (13.5)	6/26 (23.1)	15/143 (10.5)
FAT3 rs11823754	N=171 (1.0)	4/26 (15.4)	22/145 (15.2)		
AGPAT3 rs11537798	N=167 (.1)	2/31 (6.5)	25/136 (18.4)		

* P is obtained by log-rank test
** Analyses are done according to dominant model. Number of SOS cases in minor allele carriers (heterozygous and homozygous individuals combined) and non-carriers (homozygotes for major allele), with the frequency given in brackets.
*** Number of risk genotypes (presence of at least one copy of the minor allele, with the frequency in parentheses), in patients with and without SOS for significantly associated loci.

Table 5
Variables Retained in Stepwise Selection in Multivariate Cox Regression Model in the Replication Cohort

Variable	*P* Value	HR (95% CI)
UGT2B10 rs17146905	.0004	4.7 (2.0-11.5)
KIAA1715 rs2289971	.05	2.7 (1.0-7.5)
GSTA1	.02	3.1 (1.2-8.0)
Disease (hematologic malignancies)	.5	1.4 (0.5-3.7)
Disease (neuroblastoma)	.003	6.1 (1.9-20.1)
Conditioning regimen	.05	3.2 (1.0-9.9)

UGT2B10 and *KIAA1715* are analyzed according to the dominant model in which carriers of minor alleles are compared with major allele homozygotes. *GSTA1* diplotypes associated with reduced metabolic capacity are compared with remaining groups. Conditioning regimens are categorized into more than 1 versus 1 alkylating agent (latter corresponding to Bu only or Flu-containing regimen). Disease indication is categorized into nonmalignant disease, hematologic malignancies, and neuroblastoma. Presented HRs reflect risk of hematologic malignancies and neuroblastoma (P = .009) if the risk is compared across disease categories. Other covariables that were not retained in the final model included age at HSCT and dose-adjusted cumAUC as continues variables, sex, frequency of Bu administration (1 versus 4 times a day), use or not of total body irradiation, and *BHLHE22* rs16931326 genotype.

risk of SOS for *GSTA1* diplotypes defining slow metabolizing capacity [16,19]. Please note that *GSTA1* promoter polymorphisms defining metabolic capacity were not available in WES data set and could not be revealed through the WES data analyses in the discovery cohort. In the multivariable analysis, *UGT2B10* and *KIAA1715* remained associated with a higher risk of SOS (HR = 4.7; 95% CI, 2.0 to 11.2; P = .0004 and HR = 2.7; 95% CI, 1.0 to 7.5; P = .05; Table 5), while controlling for other explanatory covariates. Other factors that remained in the final model included *GSTA1* haplotype groups, disease diagnosis (categorized as nonmalignant disease, hematologic malignancies, or neuroblastoma), and conditioning regimen in which 2 or more alkylating agents were classified against Bu only, with the higher risk noted for neuroblastoma or a more intensive conditioning regimen. The maximal number of cases with available genotypes for nonmalignant diseases, hematologic malignancies, and neuroblastoma was 73, 92 and 10, and the respective number of SOS cases was 8, 13 and 5. Fifty-seven patients received conditioning regimens with 1 alkylating agent (Bu in Bu-Flu-based regimens), 4 cases had SOS; 118 patients received more than 1 alkylating agent mostly represented by Bu/Cy, Bu/Mel, and Bu/Cy/Mel combinations, of which 22 had SOS.

We previously reported that the association of the *GSTA1* genotypes with SOS was present in a double or triple alkylator setting [16,19], and Table 6 presents stratified analyses in the replication cohort by conditioning regimen relative to the genotypes retained in the multivariable model. Beside *GSTA1*, the effect of *KIAA1715* was dependent on the number of alkylating agents (P = .01), whereas the effect of *UGT2B10* was seen irrespectively of the type of conditioning regimen and was also present in Bu-Flu-based protocols (P = .001). A model combining risk alleles of *KIAA1715*, *UGT2B10*, and *GSTA1* was then built and tested in a more intensive conditioning regimen (Figure 2). The risk of SOS increased in an additive manner from 3.9 (95% CI, 1.4 to 10.4; P = .007) in patients with 1 risk genotype at any locus (group 1) to 9.8 (95% CI, 2.8 to 33.8; P = .0003) in patients with 2 risk genotypes (group 2) when compared to those with no risk genotypes (group 0). The significance for the overall difference across genotypes was .00006. There were no patients with 3 risk genotypes.

DISCUSSION

This study reports identification of the genetic contribution to SOS in pediatric patients undergoing HSCT. To our knowledge, no other study has performed genome- or exome-wide data analyses in either pediatric or adult patients undergoing HSCT. SOS has been reported as one of the most serious life-threatening complications in the post-transplantation period

Table 6
Analysis of SOS Associated Genotypes in the Replication Cohort in Relation to Conditioning Regimen.

Gene/SNP	One alkylating agent (Bu)			Two and more alkylating agents		
	No. of Patients (P*)	SOS Cases in Genotype Groups, n (%) **		No. of Patients (P*)	SOS Cases in Genotype Groups, n (%) **	
KIAA1715 rs2289971	N= 57 (.6)	1/9 (11.1)	3/48 (6.3)	**N= 118 (.01)**	6/15 (40.0)	16/103(15.5)
UGT2B10 rs17146905	**N= 56 (.001)**	3/9 (33.3)	1/47(2.1)	**N= 116 (.04)**	6/15 (40.0)	16/101(15.8)
*GSTA1****	N= 50 (.3)	0/10 (0)	4/40 (10.0)	**N= 107 (.003)**	6/14 (42.9)	14/93 (15.1)

* P is obtained by log-rank test
** Number of SOS cases in patients with and without the minor allele with the frequencies given in parentheses.
*** *GSTA1*, diplotypes with and without reduced metabolic capacity.

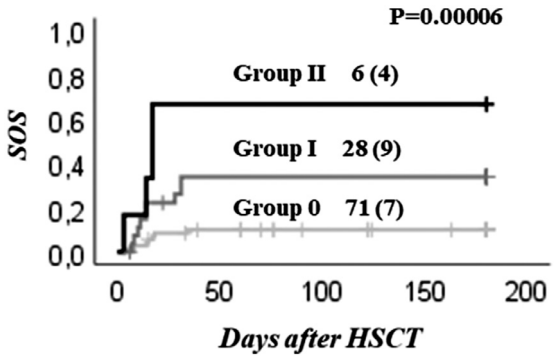

Figure 2. Model combining risk alleles of KIAA1715, UGT2B10, and GSTA1 in the intensive conditioning regimen. Cumulative incidence of SOS in patients who received 2 or more alkylating agents and had 0, 1, or 2 risk genotypes at any locus. Risk loci are minor alleles for *UGT2B10* rs17146905 and *KIAA1715* rs2289971, and diplotypes associated with reduced *GSTA1* metabolic capacity. *P* value derived by log-rank test for the difference across genotypes groups is indicated on the plot. HR (95% CI) for group 1 versus group 0 is 3.9 (1.4 to 10.4, *P* = .007) and for group 2 versus group 0 is 9.8 (2.8 to 33.8, *P* = .0003). Group 1 are patients with 1 risk genotype at any locus, group 2 are patients with any 2 risk genotypes, and group 0 are patients with no risk genotypes; there were no patients with 3 risk genotypes.

[1,7]. Identification of patient- and transplantation-specific risk factors for the development of SOS can help guide prophylaxis and treatment of this complication [12]. Our analyses identified 3 replicated loci, notably *BHLHE22, KIAA1715*, and *UGT2B10*, of which the last 2 were retained in the multivariable model, while controlling for other risk factors such as intensity of conditioning regimen, disease indication, Bu exposure, TBI, and *GSTA1* haplotypes. None of these genes was previously related to SOS, and the exact mechanism of their potential effect is not yet clear. They may contribute to SOS development through mechanisms underlying SOS pathophysiology, conditioning regimen drug pathways, or even pathology of the disease for which HSCT is performed.

KIAA1715 is coding for the endoplasmic reticulum (ER) junction formation factor also known as a lunapark (LNPK), ubiquitously expressed in a number of tissues [32]. The rs2289971 in *KIAA1715* is an expression quantitative trait locus with a higher expression noted for the minor allele (Supplementary Figure S2), as shown by the analyses of data available through the Genotype-Tissue Expression Project [33,34]. Loss-of-function mutations in LNPK lead to aberrant ER structures and increased luminal mass density [32]. When cells are subjected to changes in their extracellular environment, unfolded proteins accumulate in the ER, causing ER stress [35]. This initiates the unfolded protein response, a signal transduction cascade aimed at restoring cellular homeostasis, which is involved in the pathogenesis of many diseases, including chronic liver diseases [32,35,36]. Among other pathways, the unfolded protein response plays a significant role in vascular endothelial growth factor A (VEGFA) regulation, which is upregulated in the presence of endothelial damage [37]. Association of VEGFA levels after the conditioning regimen was noted with nonrelapse mortality and importantly with SOS, thus confirming VEGFA significance as an endothelial damage marker in the setting of HSCT [38]. Whether LNPK change in expression can be linked to VEGFA-level modulation or to other mechanisms resulting from ER stress remains to be determined. *KIAA1715* rs2289971 variant was associated in our study with SOS in patients who received a more intensive conditioning regimen.

The UDP-glucuronosyltransferase (UGT) 2B enzymes are important in the detoxification of a variety of endogenous and exogenous compounds, including many hormones, drugs, and carcinogens. Recent observations revealed that human *UGT2B10*, mostly expressed in liver, catalyzes *N*-glucuronidation of amine-containing compounds [39]. This eventually can affect the levels of biologically active metabolites of Cy and Mel [40]. There is also evidence that UGT expression in the context of chronic lymphocytic leukemia treatment can be affected by fludarabine-containing regimens [41]. It is worth noting that *UGT2B10* rs17146905 was the only variant significantly associated in our study with SOS in the Flu-based conditioning regimen. *UGT2B10* is extensively regulated through alternative splicing [42] but also by microRNA (miR) [43]. Interestingly, rs17146905 SNP is predicted to lead to target gain for hsa-miR-454-5p [44].

The *BHLHE22* gene encodes a protein that belongs to the basic helix-loop-helix (bHLH) family of transcription factors that regulate cell fate determination, proliferation, and differentiation [45]. This gene is thought to play a role mostly in neural circuit assembly [46]. However, its downregulation by microRNA was recently reported in the formation of insulin-producing cells [47]. It was also 1 of 3 genes whose methylation was most predictive for endometrial cancer [48], suggesting a wider regulatory role of this gene in different tissues. How this protein can be connected to SOS is not clear. One of the possibilities is a crosstalk with hypoxia-induced factors, as shown for the other bHLH members [49]. The hypoxia-induced factor pathway, in turn, is well known for its regulation of vascular endothelial growth factor [35]. The other possibility might lie in the proximity of *BHLHE22* to *CYP7B1*, whose hepatic activity is implicated in the inactivation of oxysterols [50] and could suggest a linkage disequilibrium between their variants. Loss of *CYP7B1* activity is associated with liver failure in children [51-53]. Nevertheless, the *BHLHE22* rs16931326 variant was not retained in a multivariable model, which might suggest its relatively minor role.

Among nongenetic factors included in the multivariable model, the highest risk of SOS was noted for neuroblastoma and the more intensive conditioning regimen. Both factors were previously reported to be associated with a higher incidence of SOS [4,5].

Our study suffers from certain drawbacks such as limited sample size, heterogeneity in terms of diagnosis and conditioning regimen within and between cohorts, and a study design that did not include the promoter variants. Despite uniform SOS diagnostic criteria across study centers, it is worth noting the retrospective nature of the study with a wide period for patients' enrollment, which ended in 2015; therefore, the study did include last up-to-date diagnostic criteria [6], what might have biased the estimate of SOS incidence. Likewise, the information on SOS prophylaxis was missing in most patients of the replication cohort, which is why this covariate was not included in the multivariable model.

CONCLUSIONS

We used WES data to perform whole-exome/adjacent UTR analysis of the genetic component of SOS in pediatric patients undergoing HSCT. Despite certain study drawbacks, 3 loci conferring a higher risk of SOS were successfully replicated in an independent patient group. Most of the associations were found in more intensive conditioning regimens (double or triple alkylator setting) and could increase the risk of SOS through a combined gene effect, including previously identified *GSTA1* diplotypes underlying low metabolic capacity.

These loci have not been previously identified as potential SOS predictors. Although we acknowledge that they still need to be investigated through functional assays, additional replication studies (eg, with larger sample size, different diseases, and adult population), and prospective evaluation with up-to-date SOS diagnostic criteria, the current findings could help further understand the role of the genetic component on the pathophysiology of SOS. Combining these genetic markers with known risk factors may lead to prediction models to identify children who might be highly susceptible to SOS in an HSCT setting and could possibly benefit from early prophylactic intervention.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.bbmt.2019.11.026.

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