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ORIGINAL REPORT

Polymorphisms in Genes Related to Oxidative Stress Are Associated With Inferior Cognitive Function After Therapy for Childhood Acute Lymphoblastic Leukemia

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ABSTRACT

Purpose
Survivors of childhood acute lymphoblastic leukemia (ALL) exhibit increased rates of neurocognitive deficits. This study was conducted to test whether interpatient variability in neurocognitive outcomes can be explained by polymorphisms in candidate genes conferring susceptibility to neurocognitive decline.

Methods
Neurocognitive testing was conducted in 350 pediatric leukemia survivors, treated on Dana-Farber Cancer Institute ALL Consortium Protocols 95-01 or 00-01. Genomic DNA was isolated from bone marrow collected at remission. Candidate polymorphisms were selected on the basis of prior literature, targeting genes related to drug metabolism, oxidative damage, altered neurotransmission, neuroinflammation, and folate physiology. Single nucleotide polymorphisms were detected using either a customized multiplexed Sequenom MassARRAY assay or polymerase chain reaction–based allelic discrimination assays. Multivariable logistic regression models were used to estimate the effects of genotype on neurocognitive outcomes, adjusted for the effects of demographic and treatment variables. False-discovery rate correction was made for multiple hypothesis testing, indicated as a Q value.

Results
Inferior cognitive or behavioral outcomes were associated with polymorphisms in three genes related to oxidative stress and/or neuroinflammation: *NOS3* (IQ, $Q = 0.008$; Vocabulary $Q = 0.011$; Matrix Reasoning $Q = 0.008$), *SLCO2A1* (IQ $Q = 0.043$; Digit Span $Q = 0.006$; Block Design $Q = 0.076$), and *COMT* (Behavioral Assessment System for Children-2 Attention $Q = 0.080$; and Hyperactivity $Q = 0.084$). Survivors homozygous for *NOS3* 894T, with at least one *SLCO2A1* variant G allele or with at least one GSTP1 variant allele, had lower mean estimated IQ scores than those without these genotypes.

Conclusion
These data are consistent with the hypothesis that oxidative damage contributes to chemotherapy-associated neurocognitive decline among children with leukemia.

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INTRODUCTION

Although the majority of children with acute lymphoblastic leukemia (ALL) can be cured,[1] treatment-related toxicity remains a persistent challenge. Outcome studies document increased rates of deficits in attention, working memory, and processing complex novel information among childhood leukemia survivors,[2-11] with the potential for lifelong impact on academic performance, occupational outcomes, and quality of life. There exists considerable interpatient variability in neurocognitive outcomes, some of which has been related to demographic factors (eg, female sex and young age[4,12]) or treatment variables (eg, cranial irradiation therapy,[4,13,14] high-dose intravenous methotrexate[2,15]). These factors do not, however, reliably identify all childhood ALL survivors with neurocognitive deficits.

Over the past two decades, investigators have begun to explore genetic influences that may account for individual variation in neurocognitive outcomes.[16] Krajinovic et al[17] reported that the *NOS3* 894TT genotype was associated with significant change in IQ scores after completion of childhood ALL treatment, particularly among those

patients who had been treated with cranial radiation. More recently, Krull et al[18] identified associations among laboratory and questionnaire measures of attention and genetic polymorphisms in methionine synthase, monoamine oxidase A, apolipoprotein E, and glutathione *S*-transferases GSTT1 and GSTP1.

The aim of the this study was to expand our understanding of associations between prevalent genetic polymorphisms that could plausibly influence treatment-related toxicity and neurocognitive outcomes measured in a large cohort of childhood ALL survivors. Our selection of genetic variants was guided by an understanding of pathophysiologic mechanisms,[19-22] focused on those likely to influence neurologic function. We thus selected prevalent variants within genes related to the two agents most frequently implicated in neurotoxicity of therapy for childhood ALL: methotrexate[2,23] and corticosteroids.[12] Methotrexate, by inhibiting methylation reactions dependent on folic acid, leads to an increase in the toxic amino acid homocysteine in blood and cerebrospinal fluid.[24,25] Homocysteine may contribute to neurotoxicity through induction of oxidative damage to neuronal tissue and vascular endothelium,[26,27] and through further metabolism to excitotoxic glutamate analogs.[28,29] Steroids directly influence mood and memory through glucocorticoid receptors in the frontal cortex and hippocampus.[12,30] Consequently, we probed polymorphisms in genes relevant to folate physiology, glucocorticoid pharmacodynamics, defense against oxidative stress,[27,31] neuroinflammation,[32] and neurotransmission.[21] This panel of targeted variants also included genes that have previously been linked to IQ[17] or attention[18] among patients with childhood leukemia, as well as others that have not been previously investigated in this context.

METHODS

Patients

Children and adolescents between the ages of 1 and 18 years with newly diagnosed ALL were eligible for enrollment onto Dana-Farber Cancer Institute (DFCI) ALL Consortium Protocols 95-01[33] and 00-01.[34] The protocols were approved by the institutional review boards of each participating institution, and informed consent was obtained from parents or guardians before study enrollment and the initiation of therapy. Patient characteristics, details of therapy, and disease outcomes have been described for both protocols.[33,34] A total of 983 evaluable patients were enrolled. After completion of therapy, 381 patients (170 treated on 00-01[35] and 211 on 95-01[36]) had neurocognitive testing, and 350 of these also had genomic DNA available for analysis. Patient characteristics are shown in Table 1, and a comparison with the remaining patients treated on 00-01 or 95-01 is shown in Appendix Table A1 (online only).

Table 1. Patient Characteristics

Characteristic	No. (%)
Total	350
DFCI ALL consortium treatment protocol	
95-01	209 (60)
00-01	141 (40)
Age at initial diagnosis, years; median (range)	4.2 (1-17.99)
< 5	210 (60)
5-9.99	93 (27)
10-17.99	47 (13)
Sex	
Female	159 (45)
Male	191 (55)
Risk group	
Standard risk	225 (64)
High risk	125 (36)
Race/ethnicity	
White	308 (88)
Black or African American	8 (2)
Asian	6(2)
Other/unknown	28 (8)
Hispanic	25 (7)
CNS prophylaxis	
Intrathecal chemotherapy	176 (50)
Cranial radiation (12 or 18 Gy)	156 (45)
Not recorded/not given	18 (5)
Corticosteroids	
Prednisone	277 (79)
Dexamethasone	73 (21)
None	0 (0)
Age at time of neurocognitive testing, years; median (range)	10 (6-25)
Years from diagnosis to testing, median (range)	5 (3-9)
Mother's education	
High school graduate/GED or less	89 (25)
Some college or more	251 (72)
Unknown	10 (3)

NOTE. Demographic and treatment variables are shown for patients included in the genetic analyses.
Abbreviations: ALL, acute lymphoblastic leukemia; DFCI, Dana-Farber Cancer Institute; GED, general equivalency diploma.

Neurocognitive Assessments

The neuropsychological testing protocols and outcomes have been described previously.[35,36] Briefly, testing was initiated approximately 5 years after diagnosis. Patients with pre-existing neurodevelopmental problems that could impair cognitive function were excluded, as were patients who did not remain in remission. Informed consent for the neurocognitive assessment was obtained from each participant or each participant's guardian, and assent was obtained from minors.

We used a relatively brief neuropsychological battery to enhance reliability and comparability of data across institutions and to encourage compliance. Testing was conducted in Spanish or French for native speakers of those languages, using published instruments and language-specific norms. IQ was estimated on the basis of two subtests from the Wechsler scales. For the 95-01 protocol, IQ was estimated from the Vocabulary and Block Design subtests of the Wechsler Intelligence Scale for Children–III or Wechsler Adult Intelligence Scale.[37] For the 00-01 protocol, IQ was estimated from the Vocabulary and Matrix Reasoning subtests from the Wechsler Abbreviated Scale of Intelligence. We also included the age-appropriate Wechsler Digit Span,[38] a measure of working memory, and the Rey-Osterrieth Complex Figure,[39] a measure of the ability to integrate complex material. To assess psychosocial adjustment in everyday life, we administered the Behavioral Assessment System for Children–2 (BASC-2),[40] a structured parent questionnaire that yields scales characterizing mood and behavior symptoms as well as positive adaptation. The BASC-2 scales that assess attention symptoms, Attention and Hyperactivity, were included in the present analysis because of prior reports of genetic associations with attention problems. Parents were asked whether the child was currently receiving special education services.

Genomic DNA

Genomic DNA was isolated from bone marrow collected at the end of induction for the purpose of detecting minimal residual disease. All samples were deidentified and coded before shipment for this study. Consequently, the investigators conducting the genetic analyses (P.D.C., V.V.) remained blinded to demographic information and neurocognitive outcomes.

Candidate Gene Selection and Target Genotypes

Candidate genetic variants were selected through a nonexhaustive literature review, with the following criteria: (1) variants present in genes related to

Table 2. Targeted Genetic Variants

Gene	refSNP	Method	Total Calls, No. (%)	Target Versus Nontarget Genotype	Wild Type		Heterozygous		Homozygous	
						No. (%)		No. (%)		No. (%)
Genes Related to Folate Metabolism										
MTHFR	1801131	iPLEX	295 (84%)	AC/CC *v* AA	AA	152 (51%)	AC	117 (40%)	CC	26 (9%)
MTHFR	1801133	TaqMan	274 (78%)	TT *v* CC/CT	CC	100 (36%)	CT	142 (52%)	TT	32 (12%)
SLC19A1	1051266	iPLEX	283 (81%)	AA *v* AG/GG	GG	81 (29%)	AG	131 (46%)	AA	71 (25%)
TCN2	1801198	iPLEX	307 (88%)	GG *v* CC/CG	CC	113 (37%)	CG	136 (44%)	GG	58 (19%)
TS	28bp repeat	PCR	207 (69%)*	3R/3R *v* 2R/2Ror2R/3R	2R/2R	36 (17%)	2R/3R	133 (64%)	3R/3R	38 (18%)
Genes Related to Glucocorticoids										
ABCB1	1045642	iPLEX	301 (86%)	TT *v* CC/CT	CC	91 (30%)	CT	140 (47%)	TT	70 (23%)
NR3C1	41423247	iPLEX	296 (85%)	CG/GG *v* CC	CC	123 (42%)	CG	140 (47%)	GG	33 (11%)
Genes Related to Neurotransmission										
COMT	4680	TaqMan	280 (80%)	AA *v* AG/GG	GG	76 (27%)	AG	161 (58%)	AA	43 (15%)
GRIN2B	10193895	iPLEX	302 (86%)	AG/GG *v* AA	AA	279 (92%)	AG	22 (7%)	GG	1 (1%)
MAOA	1137070	TaqMan	267 (76%)	CT/TT *v* CC	CC	159 (60%)	CT	55 (21%)	TT	53 (20%)
SLC6A4	1042173	TaqMan	243 (69%)	AA *v* CA/CC	CC	43 (18%)	CA	135 (56%)	AA	65 (27%)
Genes Related to Inflammation or Oxidative Damage										
APOE	7412	TaqMan	281 (80%)	CT/TT *v* CC	CC	242 (86%)	CT	39 (14%)	TT	0 (0%)
APOE	429358	TaqMan	325 (93%)	CC/CT *v* TT	TT	249 (77%)	TC	73 (22%)	CC	3 (1%)
GSTP1	1138272	TaqMan	280 (80%)	CT/TT *v* CC	CC	220 (79%)	CT	48 (17%)	TT	12 (4%)
GSTP1	1695	TaqMan	251 (72%)	AG/GG *v* AA	AA	121 (48%)	AG	111 (44%)	GG	19 (8%)
HFE	1799945	iPLEX	304 (87%)	CG/GG *v* CC	CC	235 (77%)	CG	63 (21%)	GG	6 (2%)
HFE	1800562	iPLEX	302 (86%)	AA/AG *v* GG	GG	269 (89%)	AG	33 (11%)	AA	0 (0%)
IL1B	1143627	TaqMan	269 (77%)	GG *v* AA/AG	AA	104 (39%)	AG	73 (27%)	GG	92 (34%)
IL1B	1143634	TaqMan	327 (93%)	AA *v* AG/GG	GG	175 (54%)	AG	100 (31%)	AA	52 (16%)
IL1RN	380092	TaqMan	291 (83%)	TT *v* AA/AT	AA	131 (45%)	AT	123 (42%)	TT	37 (13%)
NOS3	1799983	iPLEX	300 (86%)	TT *v* GG/GT	GG	137 (46%)	GT	133 (44%)	TT	30 (10%)
NQO1	1800566	TaqMan	312 (89%)	CT/TT *v* CC	CC	189 (61%)	CT	115 (37%)	TT	8 (3%)
SLCO2A1	7625035	iPLEX	300 (86%)	AG/GG *v* AA	AA	182 (61%)	AG	93 (31%)	GG	25 (8%)

NOTE. Targeted genetic variants studied in 350 patients with neurocognitive data. Twenty-three genetic polymorphisms were included in these analyses. The number of patients in whom a genotype could be called is indicated, along with the observed distribution of genotypes. The target genotypes, hypothesized to be associated with increased risk of inferior neurocognitive outcomes, are indicated.

Abbreviations: PCR, polymerase chain reaction; SNP, single nucleotide polymorphism.

*For analysis of *TS*, sufficient DNA remained for testing in 301 cases.

pathways presumed to be relevant to treatment-induced cognitive deficits, including antifolate or corticosteroid pharmacodynamics, oxidative stress, neurotransmission, or neuroinflammation; (2) variants known to be associated with altered function of the gene product; and (3) variants with a population prevalence of at least 10%. Twenty-eight genetic variants were selected for analysis (Table 2). For each polymorphism, an a priori hypothesis was established regarding a target genotype (ie, whether risk of neurocognitive deficits would be associated with being homozygous for the variant allele or with having ≥ one copy of the variant allele).

Genotyping

Thirteen single-nucleotide polymorphisms (SNPs) were detected using Sequenom MassARRAY iPLEX Platform (Sequenom Bioscience, San Diego, CA), a customized multiplexed assay. Briefly, initial multiplexed polymerase chain reaction (PCR) was performed in 5-μL reactions on 384-well plates containing 5 ng of genomic DNA, 0.5 U HotStart Taq polymerase (Qiagen, Valencia, CA), 100 nmol/L primers, 1.25× HotStart Taq buffer, 1.625 mmol/L MgCl2, and 500 μmol/L deoxyribonucleotide triphosphates. After enzyme activation at 94°C for 15 minutes, DNA was amplified with 45 cycles of 94°C × 20 seconds, 56°C × 30 seconds, 72°C × 1 minute, followed by a 3-minute extension at 72°C. Single-base extension (SBE) was carried out in 9-μL reactions, by addition of SBE primers, iPLEX enzyme and buffers (Sequenom). SBE products were then assayed using the MassARRAY Compact system, and mass spectra were analyzed using TYPER software (Sequenom), to generate genotype calls and allele frequencies. An additional 14 SNPs were detected using PCR-based allelic discrimination assays (Life Technologies, Grand Island, NY), following the manufacturer's instructions. Genotype calls and allele frequencies were analyzed with Applied Biosystems TaqMan Genotyper Software v1.3 (Life Technologies). SNP genotypes with questionable or low-probability calls (ie, where a specific genotype could not be assigned) were repeated with additional DNA, or excluded from further analysis.

The number of 28-base pair (bp) nucleotide repeats within the 5′ untranslated region of the gene for thymidylate synthase was assessed by PCR-product length analysis. PCR primer sequences were 5′-GTGGCTCCT-GCGTTTCCCCC-3′ 5′-GCTCCGAGCCGGCCACAGGCATGGCGCGG-3′. The 20-μL PCR reaction mixture contained 12.5 μL of master mix, 1.5 μL of the primer mix, and 2 ng of genomic DNA. Each of the 35 PCR cycles consisted of 1 minute at 96°C, 30 seconds at 60°C and 1 minute at 72°C. After the reaction, the mixture was incubated at 72°C for 5 minutes. The amplified DNA fragments were analyzed by electrophoresis on a 4% agarose gel. The sizes of the amplified fragments (250 or 220 bp for the 3R and 2R products, respectively) were determined by reference to molecular weight markers.

Statistical Analyses

The neurocognitive outcome variables were dichotomized for purposes of analysis. Impaired performance on the Rey-Osterrieth Complex Figure test was defined by scores less than or equal to those for the 25th percentile for age. For all other tests, impaired function was defined by scores more than one

Table 3. Summary Statistics for Neurocognitive and Behavioral Outcomes

Measure	No.	Median (range)	Percentile (25th, 75th)	Mean (SD)	Impairment, No. (%; 95% CI)	*P*
IQ	344	103.0 (59-152)	94.0, 113.0	103.3 (15.1)	39 (11; 8 to 15)	.99
Wechsler Digit Span	349	9.0 (1-18)	7.0, 11.0	9.2 (3.0)	103 (30; 25 to 35)	< .0001
Wechsler Vocabulary	347	11.0 (2-19)	9.0, 13.0	10.7 (3.1)	51 (15; 11 to 19)	.79
Wechsler Block Design	209	10.0 (2-19)	8.0, 12.0	10.3 (3.2)	36 (17; 13 to 23)	.34
Wechsler Matrix Reasoning	141	11.0 (3-67)	9.0, 13.0	11.2 (7.0)	25 (18; 12 to 25)	.32
Rey-Osterrieth Complex Figure Organization	325	25.0 (10-100)	10.0, 50.0	41.7 (31.6)	184 (57; 51 to 62)	< .0001
BASC-2 Attention	316	47.0 (29-92)	41.0, 60.0	49.2 (11.9)	85 (27; 22 to 32)	< .0001
BASC-2 Hyperactivity	316	50.0 (31-86)	41.0, 55.0	51.2 (11.8)	54 (17; 13 to 22)	.32

NOTE. The Block Design test was used for patients enrolled onto DFCI protocol 95-01 and the Matrix Reasoning test was used for patients in 00-01. The number and percentage of tested patients who exhibited abnormal neurocognitive or behavioral scores, as defined in the Methods, is shown. The probability that the proportion exhibiting abnormal scores exceeded the expected proportion in the normative population was computed with a one-sided binomial test.
Abbreviations: BASC-2; Behavioral Assessment System for Children–2; DFCI, Dana-Farber Cancer Institute; SD, standard deviation.

standard deviation from the reference population mean. Operationally, this was defined as follows: IQ ≤ 85; Digit Span, Vocabulary, Block Design, or Matrix Reasoning ≤ 7; and BASC-2 Attention and Hyperactivity T scores ≥ 60. The probability that the proportion exhibiting abnormal scores exceeded the expected proportion in the normative population was computed with a one-sided binomial test.

Univariable and multivariable logistic regression models were constructed for impaired function on each neurocognitive outcome measure. Multivariable logistic regression models were adjusted for age at diagnosis (< 5, 5-9.99, ≥ 10 years.), sex (male *v* female), risk group (standard risk *v* high risk), race (white *v* nonwhite), CNS treatment (intrathecal methotrexate *v* 120-180 Gy *v* none), type of corticosteroid (prednisone *v* dexamethasone), socioeconomic status (mother's education, high school or less *v* some college or more), and genotypes. Although similar, Block Design and Matrix Reasoning assess distinct domains of neurocognitive function. Therefore, they were considered separately in logistic regression modeling. For each polymorphism, the estimated IQ, Vocabulary, and Digit Span scores were also evaluated as continuous variables using linear regression modeling that included the patient characteristics and genotype as described above and were compared univariately using a Wilcoxon rank sum test. All *P* values were two-sided and considered significant at the .05 level.

The targeted genetic variants were selected a priori on the basis of prior literature and/or hypothesized associations with treatment outcomes. A post hoc Benjamini-Hochberg false discovery rate (FDR) correction for multiple hypothesis testing was made considering the eight neurocognitive outcomes, using the nominal *P* values obtained from the multivariable logistic regression models. Adjusted *P* values are denoted as *Q* values.

RESULTS

Neurocognitive Outcomes

Table 3 shows the summary statistics for each neurocognitive measure. Estimated IQ in this cohort did not differ from normative expectations. However, relative to population norms, more patients exhibited impaired Digit Span scores, Rey-Osterrieth Complex Figure organization, and Attention ratings. In addition, 90 (29%) of the 312 patients who were under 18 years of age at the time of testing were enrolled in special education, a prevalence that is substantially higher than the national rate (8.4%; $P < .001$, one-sided binomial test).[41]

Genotyping Call Rates

For each target polymorphism, a specific genotype could be assigned in 44% to 94% of patients (median, 83%). Three polymorphisms (in *MTR, GSTT1,* and *IL1A*) were excluded from further analysis because of genotyping call rates less than 60%. Two polymorphisms (*GSTM1* and *MAOA* rs1799835) were excluded from further analysis because no patients carried the minor allele. Allele frequencies for the remaining 23 targets are shown in Table 2. For all targets, the distribution of genotypes was consistent with Hardy-Weinberg equilibrium, and the minor allele frequencies were similar to published values.

Associations Between Genetic Variants and Neuropsychological Outcomes

Polymorphisms in two genes (*NOS3* and *SLCO2A1*) were significantly associated with risk for inferior outcomes on five intelligence measures (IQ, Digit Span, Vocabulary, Block Design, and Matrix Reasoning) in multivariable logistic regression modeling (Table 4). The *SLCO2A1* polymorphism was also significantly associated with risk for parental reporting of behavioral symptoms of inattention. The *COMT* polymorphism was marginally associated with risk for parental reporting of both inattention and hyperactivity. Two polymorphisms in *HFE* were associated with marginally increased risk of hyperactivity behaviors; however, these associations were not significant after FDR adjustment Data Supplement).

The odds of impairment for the measured neurocognitive outcomes were approximately 2 to 5 times higher for patients who carried these target polymorphisms than for those without the target polymorphism in univariable modeling. The odds of IQ impairment were five times higher (adjusted odds ratio = 5.07; 95% CI, 1.80 to 14.28; $Q = 0.008$) for survivors homozygous for the variant *NOS3* 894T allele in an adjusted model.

Figure 1 illustrates the associations among genetic variants and neurocognitive outcomes measured as continuous variables. Patients with the target *NOS3* TT genotype had lower mean scores for estimated IQ (95.8; 95% CI, 89.9 to 101.7; n = 30 *v* 103.9; 95% CI, 102.1 to 105.8; n = 264; multivariable $P = .011$) and Vocabulary (9.5; 95% CI, 8.4 to 10.6; n = 30 *v* 10.9; 95% CI, 10.5 to 11.3; n = 268; $P = .049$) than patients without this genotype. Patients who carry the variant *SLCO2A1* G allele also had lower mean estimated IQ than those who were homozygous for the wild-type A allele (100.4; 95% CI, 97.5 to 103.2; n = 117 *v* 104.6; 95% CI, 102.4 to 107; n = 177; $P = .014$), and lower mean Digit Span scores (8.8; 95% CI, 8.2 to 9.4; n = 118 *v* 9.5; 95% CI, 9.1 to 10; n = 182; $P = .027$). Patients with at least one *GSTP1* T allele also had lower mean Digit Span scores (8.3; 95% CI, 7.5 to 9.1; n = 60) than those who were homozygous for the wild-type C allele (9.5; 95% CI, 9.1 to 9.9; n = 219; $P = .006$) and marginally lower IQ

Table 4. Logistic Regression Modeling of Neurocognitive Impairment Outcomes for Targeted SNPs

Neurocognitive Outcome	Gene Symbol (refSNP)	Univariable OR (95% CI)	*P*	Multivariable OR (95% CI)	*P*	*Q*
Wechsler IQ	*NOS3* (rs1799983)	3.33 (1.35 to 8.32)	.009	5.07 (1.80 to 14.28)	.002	0.008
	SLCO2A1 (rs7625035)	2.23 (1.09 to 4.55)	.028	2.67 (1.20 to 5.92)	.016	0.043
Wechsler Digit Span	*SLCO2A1* (rs7625035)	2.35 (1.41 to 3.91)	<.001	2.55 (1.48 to 4.40)	<.001	0.006
Wechsler Vocabulary	*NOS3* (rs1799983)	3.21 (1.39 to 7.41)	.006	3.94 (1.54 to 10.09)	.004	0.011
Wechsler Block Design	*SLCO2A1* (rs7625035)	2.00 (0.92 to 4.34)	.080	2.40 (1.05 to 5.48)	.038	0.076
Wechsler Matrix Reasoning	*NOS3* (rs1799983)	4.33 (1.30 to 14.42)	.017	15.65 (2.80 to 87.66)	.002	0.008
Attention (BASC-2)	*COMT* (rs4680)	2.24 (1.12 to 4.48)	.023	2.61 (1.26 to 5.45)	.010	0.080
	SLCO2A1 (rs7625035)	1.99 (1.16 to 3.40)	.013	2.04 (1.17 to 3.57)	.013	0.043
Hyperactivity (BASC-2)	*COMT* (rs4680)	2.07 (0.94 to 4.55)	.070	2.71 (1.17 to 6.31)	.021	0.084

NOTE. Univariable and multivariable ORs for abnormal scores, as defined in the methods, were calculated, adjusting for the effects of age at diagnosis, sex, risk group, race, CNS prophylaxis (radiation *v* intrathecal chemotherapy), corticosteroid (prednisone *v* dexamethasone), and socioeconomic status. For each neurocognitive or behavioral outcome, genetic variants with an FDR *Q* value < 0.10 multivariable OR are shown, and a complete table is included as a Data Supplement.

Abbreviations: BASC-2, Behavioral Assessment System for Children–2; FDR, false discovery rate; OR, odds ratio; SNP, single nucleotide polymorphism.

scores (99.9; 95% CI, 95.7 to 104.2; n = 59 *v* 103.9; 95% CI, 101.9 to 106.0; n = 217; univariable $P = .08$), although the association with impairment was not significant after FDR adjustment ($P = .029$; $Q = 0.23$; Data Supplement).

DISCUSSION

This study demonstrates associations between prevalent genetic polymorphisms and inferior neurocognitive outcomes after treatment for childhood ALL, potentially explaining some of the observed interpatient variability. Our study benefited from a larger sample size than has been included in similar recent studies,[17,18,42] with 350 pediatric patients treated on two consecutive cooperative group trials. Of note, all of the polymorphisms identified as predictors of inferior neurocognitive outcome have been associated with increased susceptibility to oxidative stress and/or neuroinflammation: endothelial nitric oxide synthase (*NOS3*), catechol-*O*-methyltransferase (*COMT*), hemochromatosis (*HFE*), glutathione *S*-transferase pi (*GSTP1*), and the prostaglandin transporter (*SLCO2A1*).

The *NOS3* polymorphism emerged as the variant most strongly related to low IQ among these leukemia survivors. Endothelial nitric oxide production regulates vascular tone and contributes to protection from oxidative damage. This is relevant to patients with leukemia, because we and others have shown in both animal models[43] and in patients[25] that treatment with methotrexate leads to an increase in homocysteine within the CNS. Homocysteine may contribute to neurotoxicity by inducing the production of oxygen radicals, leading to oxidative damage of vascular endothelium and neuronal tissue.[26,27] Homozygosity for the T allele in *NOS3* results in decreased enzyme activity, and consequently a diminished capacity for protection against oxidative stress. This polymorphism has been previously linked to decline in IQ after treatment for leukemia in a cohort that included a subset of the patients included in this study (N = 66).[17] Here we expand on this finding, in a larger cohort of survivors treated on the same or subsequent treatment protocol, confirming a significant association between the *NOS3* variant and decreased IQ measured cross-sectionally 5 years after completion of treatment.

Mutations in both *HFE*[44] and *GSTP1*[45] have also been associated with increased susceptibility to oxidative stress within the CNS. Here we describe a novel association between *GSTP1* polymorphisms and lower estimated IQ and Digit Span scores. The *HFE* variants were marginally associated with increased reports of hyperactive behavior, although the associations were no longer significant after FDR adjustment.

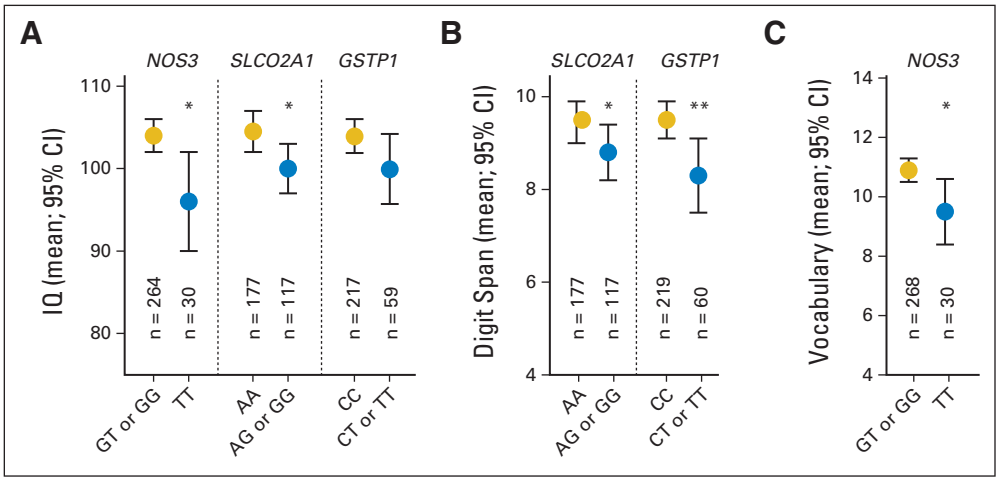

Fig 1. Genetic variants associated with (A) decreased estimated IQ, (B) Wechsler Vocabulary score, and (C) Wechsler Digit Span score. Mean (± SEM) estimated IQ, Digit Span, and Vocabulary scores are shown for patients with (blue circles) or without (gold circles) the target genotype. Mean estimated IQ was lower among patients with the target *NOS3* or *SLCO2A1* genotype. Mean Digit Span score was lower among patients with the target *SLCO2A1* or *GSTP1* genotype. Mean Vocabulary score was lower among patients with the target *NOS3* genotype. (*) $P < .05$; (**) $P < .01$.

The function of the *COMT* gene has primarily been associated with the inactivation of catecholamine neurotransmitters, including dopamine. However, like *NOS3, HFE,* and *GSTP1,* it may also relate to susceptibility to oxidative stress. The methyl donor for reactions catalyzed by the gene product, catechol-*O*-methyltransferase, is *S*-adenosylmethionine, a folate-dependent cofactor that decreases after methotrexate exposure.[46] The val158Met polymorphism in *COMT* further decreases enzyme activity,[47] and the subsequent reduction in *O*-methylated catechols diminishes protection against oxygen radicals.[48,49] We observed a marginal association between the val158Met *COMT* polymorphism and both attention and hyperactivity symptoms among childhood leukemia survivors. These results contrast with a recent report[18] that did not note such an association. However, this study used different instruments to detect attention problems (Conners Continuous Performance Test and Conners Parent Ratings Scale) than those used in our study (BASC-2 parent report), and had less statistical power to detect this association as a result of a smaller sample size.

In contrast with the genes discussed above, *SLCO2A1* has not been as directly associated with oxidative stress. The prostaglandin transporter encoded by this gene facilitates the movement of prostaglandins across the blood brain barrier. Prostaglandins modulate many brain activities by regulating cerebral blood flow, synaptic transmission, neurotrophin production, angiogenesis, and gene expression. Prostaglandins also influence inflammatory processes within the CNS. Because oxygen radicals are produced in the setting of chronic inflammation, it is probable that a functional polymorphism affecting prostaglandin entry into the CNS alters the balance between reactive oxygen species and protective mechanisms.

There were potential limitations to this study. By design, we examined associations with polymorphisms in candidate genes hypothesized to relate to treatment-induced cognitive decline. Unlike an agnostic approach, our study design could not uncover novel polymorphisms linked to cognitive decline, nor point to pathways not previously expected to contribute to toxicity. Second, neurocognitive testing was conducted at a single time point approximately 5 years after treatment, when neurocognitive sequelae are presumably no longer developing. However, because a baseline assessment of cognitive function was not done, associations between genetic variants and inferior cognitive function do not necessarily prove a causal relationship with treatment-induced decline in cognitive function. The consistency with the Krajinovic study, however, which included a subsample of our cohort and did measure change over time, supports our findings. Third, there was no nontreated control group to address the possibility that these polymorphisms influence neurocognitive function independently of ALL or chemotherapy. Fifth, pathway and combinatorial analyses were not explored in this study, because statistical power to eliminate false positive associations was limited by the sample size. Finally, there may have been unintended selection bias, as only about half of the patients who were eligible agreed to the neuropsychological testing. It is possible that those patients suspected of having abnormal cognitive function were more likely to consent for testing, falsely increasing the prevalence of cognitive dysfunction in our cohort. However, it seems unlikely that any such selection bias would have systematically altered relationships between cognitive function and genetic variants.

Despite these limitations, the observed associations among genetic polymorphisms and impaired neurocognitive function among leukemia survivors provide additional support for the hypothesis that oxidative stress contributes to treatment-induced cognitive decline among children with ALL. Patients who carry these polymorphisms may be more vulnerable to oxidative stress and thus might benefit from concurrent antioxidant therapy.

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Disclosures provided by the authors are available with this article at www.jco.org.

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Polymorphisms in Genes Related to Oxidative Stress Are Associated With Inferior Cognitive Function After Therapy for Childhood Acute Lymphoblastic Leukemia

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Appendix

Table A1. Comparison of Patients Included in Genetic Analyses and Remaining Patients Enrolled Onto 95-01 or 00-01

Characteristic	Neurocognitive and Genetic Testing Done, No. (%)	Remaining Patients, No. (%)	*P*
No.	350	633	
DFCI ALL consortium treatment protocol			
95-01	209 (60)	282 (45)	< .001
00-01	141 (40)	352 (55)	
Age at initial diagnosis, years; median (range)	4.2 (1-17.99)	5.1 (1-17.9)	
< 5	210 (60)	313 (49)	< .001
5-9.99	93 (27)	168 (27)	
10-17.99	47 (13)	152 (24)	
Sex			
Female	159 (45)	287 (45)	.99
Male	191 (55)	346 (55)	
DFCI risk group			
Standard risk	225 (64)	329 (52)	< .001
High risk	125 (36)	304 (48)	
Race/ethnicity			
White	308 (88)	537 (85)	.053
Black or African American	8 (2)	37 (6)	
Asian	6 (2)	7 (1)	
Other/unknown	28 (8)	52 (8)	
Hispanic	25 (7)	73 (12)	.034
CNS prophylaxis			
Intrathecal chemotherapy	176 (50)	245 (39)	
Cranial radiation (12 or 18 Gy)	156 (45)	276 (44)	
Did not receive	18 (5)	112 (18)	
Corticosteroids			
Prednisone	277 (79)	489 (77)	.69
Dexamethasone	73 (21)	139 (22)	
Did not receive	0 (0)	5 (1)	
Age at time of neurocognitive testing, years; median (range)	10 (6, 25)	—	
Mother's education			
High school graduate/GED or less	89 (25)	—	
Some college or more	251 (72)	—	
Unknown	10 (3)	—	
Time from diagnosis to neurocognitive testing, years; median (range)	5 (3-9)		

NOTE. The observed differences between these cohorts are a function of the study design. Neurocognitive testing was conducted among survivors, 5 years after diagnosis. Patients with induction failure, death, or early relapse were therefore excluded from analysis. Consequently, one would expect high-risk features (eg, older age) to be more prevalent among the group who did not undergo neurocognitive testing. Nevertheless, it seems unlikely that any selection bias would have systematically altered relationships among genetic variants and neurocognitive outcomes. Dashes indicate data not collected.

Abbreviations: ALL, acute lymphoblastic leukemia; DFCI, Dana-Farber Cancer Institute; GED, general educational development.