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ORIGINAL REPORT

Genetic Mediators of Neurocognitive Outcomes in Survivors of Childhood Acute Lymphoblastic Leukemia

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ABSTRACT

Purpose
Survivors of childhood acute lymphoblastic leukemia (ALL) are at increased risk for neurocognitive problems, with significant interindividual variability in outcome. This study examined genetic polymorphisms associated with variability in neurocognitive outcome.

Patients and Methods
Neurocognitive outcomes were evaluated at the end of therapy in 243 survivors treated on an institutional protocol featuring risk-adapted chemotherapy without prophylactic cranial irradiation. Polymorphisms in genes related to pharmacokinetics or pharmacodynamics of antileukemic agents, drug metabolism, oxidative stress, and attention problems in noncancer populations were examined as predictors of outcome, using multiple general linear models and controlling for age at diagnosis, sex, race, and treatment intensity.

Results
Compared with national norms, the cohort demonstrated significantly higher rates of problems on direct assessment of sustained attention ($P = .01$) and on parent ratings of attention problems ($P = .02$). Children with the A2756G polymorphism in methionine synthase (*MS*) were more likely to demonstrate deficits in attentiveness ($P = .03$) and response speed ($P = .02$), whereas those with various polymorphisms in glutathione S-transferase demonstrated increased performance variability ($P = .01$) and reduced attentiveness ($P = .003$). Polymorphisms in monoamine oxidase (T1460CA) were associated with increased attention variability ($P = .03$). Parent-reported attention problems were more common in children with the Cys112Arg polymorphism in apoliopoprotein E4 ($P = .01$).

Conclusion
These results are consistent with our previous report of association between attention problems and MS in an independent cohort of long-term survivors of childhood ALL treated with chemotherapy only. The results also raise the possibility of an impact from genetic predispositions related to oxidative stress and CNS integrity.

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INTRODUCTION

Survivors of childhood acute lymphoblastic leukemia (ALL) are at risk for neurocognitive problems, generally characterized by reduced attention and processing speed.[1] Although neurocognitive problems are clearly linked to cranial radiation therapy, they can also be associated with chemotherapy.[2] We recently demonstrated increased frequency of attention problems in survivors of childhood ALL who were treated with chemotherapy only.[3] Neurocognitive problems in survivors of childhood ALL have been associated with increased treatment intensity, younger age at treatment exposure, and female sex,[4,5] factors which account for only a proportion of the variability in outcome, suggesting additional risk factors.

One potential source of outcome variability is genetic polymorphisms that affect key enzyme pathways associated with pharmacokinetics or pharmacodynamics of antileukemic agents, particularly antifolates (eg, lower folate availability, higher homocysteine). We recently reported a link between polymorphisms associated with the folate pathway and parent-reported attention problems and neurocognitive measures in long-term survivors of childhood ALL.[6,7] Among these survivors treated with chemotherapy only, those who had germline polymorphisms of 10-methylenetetrahydrofolate reductase (*MTHFR*) demonstrated elevated ratings on parent-reported attention problems, whereas children with polymorphisms in either *MTHFR* or the methionine synthase (*MS*) gene demonstrated reduced performance on direct measures of attention and processing speed.

Other potential genetic polymorphisms affecting neurocognitive outcome in ALL survivors include those associated with glucocorticoids (eg, glucocorticoid receptor gene, nuclear receptor subfamily 3 [*NR3C1*]),[8] metabolism of additional chemotherapeutic agents (eg, cytochrome P450 family 3 [*CYP3A*]),[9] and/or regulators of oxidative stress generated by chemotherapy (eg, glutathione S-transferases [GSTs]).[10] Although polymorphisms in many of these genes have been examined for their contribution to genetic risk for leukemia and survival,[11,12] their association with neurocognitive functional outcomes has not been reported.

Specific germline polymorphisms have been linked to problems with attention in noncancer populations. Polymorphisms in dopamine receptor and monoamine oxidase A (*MAOA*) genes have been associated with developmental attention deficits,[13] whereas polymorphisms in the catechol-O-methyltransferase gene (*COMT*) have been linked to the development of impaired attention regulation.[14,15] Variants in apoliopoprotein E (*APOE*) have been implicated in mediating early-onset dementia,[16] neurocognitive outcome after traumatic brain injury[17] (presumably through oxidative stress and CNS response to injury[18]), and neurocognitive outcome in survivors of breast cancer and adult-onset lymphoma.[19] The impact of these polymorphisms in mediating neurocognitive impairment in survivors of childhood cancer exposed to potentially neurotoxic chemotherapy has not been well explored.

The aim of our study was to examine, in a large cohort of leukemia survivors, the association between neurocognitive outcome and genetic polymorphisms related to antifolate and glucocorticoid chemotherapy and oxidative stress, as well as those commonly associated with attention problems in noncancer populations. Consistent with our previous reports,[6,7] we hypothesized that polymorphisms in the folate pathway would be associated with attention problems.

PATIENTS AND METHODS

Participants

All participants were treated on the Total XV therapeutic protocol for childhood ALL, which has been previously reported.[20] Briefly, on the basis of presenting features and response to initial remission induction therapy, patients were assigned to either low-risk or standard-/high-risk treatment arms.[20] Children in the low-risk arm received 13 to 18 intrathecal (IT) treatments with methotrexate (MTX), hydrocortisone, and cytarabine; high-dose (HD) intravenous (IV) MTX at 2.5 gm/m^2 per dose for four doses; and dexamethasone pulses at 8 mg/m^2 per day for 5 days, in addition to other chemotherapeutic agents. Children in the standard-/high-risk arm received 16 to 25 IT injections, HD IV MTX at 5.0 gm/m^2 per dose for four doses, and dexamethasone pulses at 12 mg/m^2 per day for 5 days. Prophylactic cranial irradiation was not administered to any patient, regardless of presenting features, including the presence of CNS leukemia at diagnosis. None of the patients had received therapeutic cranial radiation therapy for CNS relapse before the collection of outcome measures. Patients were excluded from analyses if they had been diagnosed with a neurodevelopmental disorder (eg, Down syndrome), if their primary language was not English, or if they had developed CNS relapse before the neurocognitive testing. This study was approved by the institutional review board at St Jude Children's Research Hospital. Informed consent was obtained from the parent or guardian, and assent was obtained from the patient when appropriate.

The Total XV protocol enrolled 408 patients at St Jude Children's Research Hospital. Of these, 345 (84.6%) participated in a neurocognitive assessment at least once during the course of therapy, and 243 of these had a single assessment (70.4%) 2 years after completion of consolidation therapy (ie, approximately 2.3 years from diagnosis). No differences were apparent in demographic, disease, or treatment characteristics among survivors who completed the assessments versus those who did not (Appendix Table A1, online only). This time point was used in the current analyses because it represented the latest point of assessment for the majority of the group and thus the point most likely to be associated with chronic effects of treatment. DNA was collected from peripheral blood cells during remission and was available for 346 of the patients who completed the therapeutic protocol (84.8%), and 210 of the patients who were evaluated at the end of therapy (86.4%).

Genotyping

DNA was provided by the laboratory of Mary Relling, PharmD. Forty-two single nucleotide polymorphisms (SNPs) were a priori selected based on their know contribution to: one, the folate pathway; two, steroid receptors, general drug metabolism, or oxidative stress; or three, attention deficits in the general population. Whole-genome amplification was conducted with GenomePlex WGA kits (Sigma-Aldrich, St Louis, MO). Purified genomic DNA was plated into 96-well polymerase chain reaction (PCR) plates. Silicon-based multiplexing was performed to divide SNPs into groups. PCR was carried out in multiplex on an MJ Research DNA engine (St Bruno, Quebec, Canada). Reactions were carried out in multiplex using the SNaPshot Multiplex kit (Applied Biosystems, Foster City, CA). The reaction included the extension of an oligonucleotide probe designed to lie adjacent to the SNP of interest by one of four fluorescently labeled dideoxynucleotides complementary to the base found at the SNP site. Oligonucleotide probes were designed to be of different lengths by the addition of neutral sequence. After the SNaPshot reaction, the mixture was treated with 1.0 unit of shrimp alkaline phosphatase to remove the 5′-phosphoryl groups. After digestion, 1.0 μL of PCR product was added to a mixture of 8.5 μL of formamide (Invitrogen, Carlsbad, CA) and 0.5 μL of fluorescently labeled standard-size LIZ120 (Applied Biosystems). The fluorescently extended oligonucleotides were separated on an ABI 3730xl capillary electrophoresis system (Applied Biosystems). PCR fragment-size analysis using flurorescently labeled primer pairs was conducted for variations in *DHFR* and *TYMS* (RS70991108 and RS34489327, respectively). PCR was performed with 50 ng of genomic DNA and the Takara Bio Ex Taq Hot Start PCR system (Otsu, Japan) in a 25-μL reaction on an MJ Research DNA engine. Thermocycling conditions were adjusted specifically for the gene to be amplified. After amplification, 1.0 μL of PCR product was added to a mixture of 8.5 μL of formamide (Invitrogen) and 0.5 μL of fluorescently labeled standard-size ROX 400HD (Applied Biosystems). The samples were denatured for 5 minutes at 95°C and then loaded onto an ABI 3730xl DNA analyzer (Applied Biosystems). Allele determination and fragment-size analysis were performed using GeneMapper 4.0 software (Applied Biosystems). SNP genotypes with questionable call rates ($<$ 90%) were repeated using additional DNA. Forward and reserve primers for all genes are listed in Appendix Table A2 (online only).

Neurocognitive Evaluations

All participants completed a standard neurocognitive battery 2 years after completion of consolidation therapy. Specific neurocognitive domains evaluated (and corresponding age-appropriate tests) included general intelligence (Wechsler Preschool and Primary Scale of Intelligence–Revised,[21] Wechsler Intelligence Scale for Children–Third Edition [WISC-III],[22] or Wechsler Adult Intelligence Scale–Third Edition [WAIS-III][23]); processing speed (processing speed index from WISC-III[22] or WAIS-III[23]); working memory (freedom from distractibility index from WISC-III[22] or WAIS-III[23]); sustained attention (beta [response speed], D-prime [attentiveness], and SE of reaction time [variability] indices from Conners' Continuous Performance Test [CPT][24]); and parent-reported attention problems (Conners' Parent Rating Scale[25]). These indices of attention represent related although separate constructs[26] and are associated with a vast network of neural activation.[27] Age-adjusted standard scores were calculated for each measure according to standard procedures outlined in the test manuals.

Covariates

Patient demographic and clinical treatment variables were considered as covariates. Patient variables included age at diagnosis (dichotomized into $<$ 5 *v* $\geq$ 5 years, based on previous research demonstrating increased sensitivity at $<$ 5 years[3]) and sex (male *v* female). The impact of race and ethnicity was considered, given their potential association to differences in allele frequency.

Table 1. Survivor Demographic and Clinical Characteristics

Characteristic	Survivors	
	No.	%
Sex		
Male	131	53.9
Female	112	46.1
Race		
White	194	79.8
Black	39	16.1
Other	10	4.1
Risk arm		
Low	126	51.9
Standard/high*	117	48.1

Characteristic	Survivors			
	No.	Mean	SD	Range
Age at diagnosis, years	243	6.6	4.39	1.0-18.7
HD IV MTX dose, gm/m^2†				
Low risk	126	11.7	2.14	3.6-18.0
Standard/high risk	117	18.6	3.64	7.4-29.3
IT MHA dose, ml‡				
Low risk	126	150.1	69.75	94.0-856.0
Standard/high risk	117	204.6	50.12	80.0-375.0
Dexamethasone, mg/m^2†				
Low risk	126	1,008.2	177.96	175.5-1,302.6
Standard/high risk	117	1,212.6	374.11	60.3-1,690.1

Abbreviations: HD IV MTX, high-dose intravenous methotrexate; IT MHA, intrathecal injection of MTX plus hydrocortisone plus cytarabine; SD, standard deviation.
*Children identified as standard and high risk were treated in same therapeutic arm.
†Cumulative doses listed.
‡Cumulative doses listed; 1 mL contains MTX 1 mg, hydrocortisone 2 mg, and cytarabine 3 mg.

Hispanic origin was identified in only nine patients, an insufficient number for analysis of this ethnicity. Race was included as a covariate, with 39 black patients identified. IT and HD IV MTX have been previously identified as potential treatment factors associated with neurocognitive problems. However, the intensity and frequency of these treatments are determined by risk stratum. Furthermore, risk stratum was identified as a more consistent predictor of neurocognitive outcome in our previous report.[28] For these reasons, we used risk stratum in lieu of specific treatment doses to capture the combined effect of treatment intensity.

Statistical Analyses

Descriptive statistics were generated for patient and treatment characteristics as well as for neurocognitive outcome measures. Impairment on neurocognitive outcome was defined as attaining a standard score falling in the lowest 15% of the normal distribution. Rates of impairment within the sample were compared with the expected 15% in the general population. The Hochberg and Benjamini[29] adaptive step-down Bonferroni method was used to adjust for multiple comparisons. Only those neurocognitive measures with impairment rates significantly higher than expected were included in linkage analyses. Common and rare allele frequencies were determined for each of the SNPs of interest. SNPs with limited frequency of rare alleles were dropped from additional analyses. Univariate associations were examined among each of the remaining SNPs and relevant neurocognitive outcomes. Unless otherwise specified, SNPs significantly associated with a neurocognitive outcome at the $P < .10$ level in the univariate analyses were included in a multiple general linear model, along with risk, sex, age at diagnosis, and race. Separate multivariate models were generated for each neurocognitive outcome with an elevated impairment rate. All analyses were performed using SAS 9.2 software (SAS Institute, Cary, NC).

RESULTS

Demographic and treatment characteristics of participants are listed in Table 1. Performance levels and rates of impairment on neurocognitive outcome measures are summarized in Table 2. Survivors demonstrated significantly elevated rates of impairment on measures of sustained attention, including inattentiveness, slow response speed, and high variability (all $P < .01$) on the Conners' CPT. Parents reported elevated rates of attention problems ($P = .02$). On the basis of these results, a phenotype of impaired attention, defined by CPT performance and parent-reported attention problems, was used to explore associations with genetic polymorphisms.

Frequency results for the 42 polymorphisms identified as potential mediators of neurocognitive outcome are listed in Table 3. Limited or no variation was observed in dihydrofolate reductase, *CYP3A*, and *MAOA*. The remaining 39 genomic variations were used in univariate linkage analysis with the four attention outcomes. Allele frequency differed by race for the following genes: *CYP3A5*, *DBH*, *DRD2*, *FPGS*, *GSTP1*, *GSTT1*, *MDR1*, *MTHFD1*, and *TYMS*. Given these multiple differences by race and the relatively small sample of nonwhite participants (ie, 20.2% of 243 patients), race was used as an independent variable in the multiple regression analyses. Because three of the attention outcomes (ie, attentiveness, response speed, and variability) were derived from a common test (ie, Conners' CPT) and are correlated with one another, 500 multiple permutations were conducted to examine the reliability of associations with these three measures.

Table 2. Performance and Rates of Impairment on Outcome Measures

Neurocognitive Outcome	Population Mean	Population SD	Subset Mean	Subset SD	95% CI	Impaired (%)*	*P*	Adjusted *P*†
Wechsler scales‡								
Intelligence	100	15	96.0	15.78	93.9 to 98.0	22.9	.058687696	.176063
Working memory	100	15	96.0	14.46	93.5 to 98.4	24.3	.093919976	.18784
Processing speed	100	15	99.8	17.00	96.9 to 102.7	17.2	1.00	1.00
Conners' CPT§								
Attentiveness	50	10	60.0	10.43	58.3 to 61.6	44.9	< .001	< .001
Response speed	50	10	71.7	19.17	68.7 to 74.8	63.5	< .001	< .001
Variability	50	10	58.7	13.61	56.6 to 60.9	46.2	< .001	< .001
Conners' parent report‖								
Attention problems	50	10	53.2	14.19	51.4 to 55.1	24.8	.024548342	.073645
Impulsivity/hyperactivity	50	10	50.0	10.79	48.6 to 51.5	16.2	1.00	1.00
Hyperactivity index	50	10	50.2	11.65	48.7 to 51.8	15.8	1.00	1.00

Abbreviations: CPT, Continuous Performance Test; SD, standard deviation.
‡Age-appropriate version of Wechsler intelligence scales (ie, Wechsler Preschool and Primary Scale of Intelligence–Revised,[21] Wechsler Intelligence Scale for Children–Third Edition,[22] or Wechsler Adult Intelligence Scale–Third Edition[23]); lower scores reflect worse performance.
§Conners' CPT[24]: variability, SE of reaction time; attentiveness, D-prime; slow response speed, beta. Higher scores reflect worse performance. Low scores on beta can also reflect problem behavior, specifically impulsive response style; however, such abnormally fast responses were infrequent and not considered in impairment classification.
‖Conners' Parent Rating Scale[24]; higher scores reflect more problems.
*Impairment defined as score falling > one SD below population mean.
†*P* values adjusted using Holm-Bonferroni step-down method to account for multiple comparisons and reduce risk of type I error.[29]

Polymorphisms associated with an average $P < .05$ across the 500 permutations were included in multivariate analyses. These polymorphisms included MS, MAOA, and GST. Polymorphisms associated with parent-reported attention problems in univariate analyses included *GST, APOE, CYP3A*, and immunophilin protein.

Table 4 summarizes the results of multivariate general linear models for each of the four attention outcomes. Controlling for risk, sex, age at diagnosis, and race, problems with attentiveness were demonstrated in children with polymorphisms in MS ($P = .03$) and GST ($P = .003$ and $P = .002$ for *GSTT1*0* and *GSTP1*, respectively). Slower response speed was demonstrated in children with polymorphism in *MS* ($P = .02$), whereas increased variability was demonstrated in children with polymorphisms in GST ($P = .01$) and *MAOA* ($P = .03$). With regard to parent-reported symptoms, increased attention problems were identified in children with polymorphism in *APOE* ($P = .01$). Table 4 also lists estimates of the effect size associated with each polymorphism, expressed in standard score units on an age-adjusted scale (mean, 50; standard deviation, 10). Positive values ≥ 10 points (ie, one standard deviation) would generally be considered clinically significant.

Children with the variant G allele in MS demonstrated a progressive increase in problems with attentiveness and response speed (Fig 1). Mean performance on the attention measures and parent reports are listed in Appendix Table A3 (online only) according to allele pattern.

DISCUSSION

This study demonstrates a common phenotype of attention problems in survivors of childhood ALL, manifested through direct assessment of sustained attention (ie, inattentiveness, slow response speed, and increased variability) and parent-reported behavior. Although the onset of these problems may occur earlier in cancer therapy, they are present in a significant percentage of survivors 2 years after completion of consolidation therapy. The attention problems seem to be mediated by multiple factors, including treatment intensity as well as polymorphisms in genes related to antifolate chemotherapy, oxidative stress, and CNS integrity. Treatment intensity was related to parent-reported attention problems only and not to direct assessment, likely because the latter is more sensitive and negatively affected, even at lower-intensity therapies.

Polymorphisms in *MS* were associated with decreased attentiveness and slowed response speed. We recently demonstrated an association between attention and processing-speed problems in long-term survivors of childhood ALL and polymorphisms in the *MS* gene.[7] Although the current attention test differed from that used in the previous study, and the current cohort was tested earlier in the survival process, the identified association between *MS* and attention outcome is consistent with the initial discovery in the previous report. *MS* is involved in the conversion of homocysteine to methionine, and polymorphisms in *MS* are associated with hyperhomocysteinemia.[30] Excess homocysteine increases risk for vascular abnormalities, including CNS stroke.[31,32] Survivors with *MS* polymorphisms who were treated with MTX, which could increase homocysteine levels, may be at increased risk for these abnormalities.

Specific attention problems were also associated with polymorphisms in additional genes. GSTs are enzymes involved in sequestering reactive oxygen species,[33] among other things, and polymorphisms in the *GST* gene may interfere with the ability to respond to oxidative stress. *MAOA* is involved in serotonin and norepinephrine catabolism,[34,35] and low activity of this gene has been associated with increased norepinephrine and overactivation of the sympathetic nervous system.[36] This process may result in increased anxiety and/or physiologic stress, which have also been associated with attention problems.[37,38] The association with polymorphisms in

Table 3. Frequency of Targeted Pathway Polymorphisms Examined As Mediators of Neurocognitive Outcomes

Gene Description	Gene Symbol	Genomic Variation	RSID	Detection Method	Wild Type*		Hetero-zygous*		Homozygous*	
					No.	%	No.	%	No.	%
Folate pathway polymorphisms										
Dihydrofolate reductase	*DHFR*	Intron-1 19bp del	70991108	PCR fragment-size analysis	334	100			1	< 1
Folylpolyglutamate synthase	*FPGS*	G144A	10760502	SNaPshot	187	55	117	35	33	10
		A1994G	10106	SNaPshot	87	30	153	52	54	18
Gamma-glutamyl hydrolase	*GGH*	C452T	11545078	SNaPshot	281	84	47	14	5	2
Methionine synthase	*MS*	A2756G	1805087	SNaPshot	202	63	105	33	12	4
Methionine synthase reductase	*MTRR*	A66G	1801394	SNaPshot	136	44	86	28	85	28
Methylenetetrahydrofolate dehydrogenase	*MTHFD1*	G1958A	2236225	SNaPshot	209	67	88	28	15	5
		T401C	1950902	SNaPshot	116	38	142	46	50	16
Methylenetetrahydrofolate reductase	*MTHFR*	C677T	1801133	SNaPshot	137	43	140	44	39	12
		A1298C	1801131	SNaPshot	135	47	122	42	31	11
Reduced folate carrier	*SLC19A1*	G80A	1051266	SNaPshot	72	24	184	62	41	14
Serine hydroxymethyltransferase	*SHMT*	C1420T	1979277	SNaPshot	149	45	149	45	30	9
Thymidylate synthase	*TYMS*	1494del6	34489327	PCR fragment-size analysis	258	91	25	9	1	< 1
Polymorphisms related to steroid receptors, drug metabolism, or oxidative stress										
Cytochrome P450 family 3	*CYP3A4*	CYP3A4*1B	3091339	SNaPshot	325	100				
	CYP3A5	CYP3A5*3	776746	SNaPshot	133	42	136	43	47	15
Glucocorticoid receptor	*NR3C1*	G1088A	56149945	SNaPshot	294	95	14	5		
	NR3C1	Asn363Ser	6195	SNaPshot	320	95	14	4	3	1
Glutathione S-transferase	*GSTP1*	G313A	1695	SNaPshot	109	39	125	45	45	16
		C341T	1138272	SNaPshot	239	81	45	15	10	3
	GSTM1	GSTM1*0	2071487	SNaPshot	185	56	142	43		
	GSTT1	GSTT1*0	2266637	SNaPshot	312	97	6	2	3	1
Multidrug resistance protein 1	*MDR1*	T3435C	1045642	SNaPshot	88	29	151	50	62	21
		A2677G	2032582	SNaPshot	128	40	135	43	54	17
Immunophilin protein	*FKBP5*	AC	3800373	SNaPshot	156	48	136	41	35	11
		GA	9296158	SNaPshot	263	86	33	11	9	3
		CT	1360780	SNaPshot	148	45	147	45	31	10
		CT	9470080	SNaPshot	122	39	151	48	41	13
Vitamin D receptor	*VDR*	Intron 8 G→A	1544410	SNaPshot	114	37	152	49	44	14
		VDR foklstartsite T→C	2228570	SNaPshot	115	36	181	57	23	7
Polymorphisms related to attention deficit phenotype										
Apoliopoprotein E	*APOE4*	Cys112Arg	429358	SNaPshot	235	73	80	25	7	2
Brain-derived neurotrophic factor	*BDNF*	Val66Met	6265	SNaPshot	215	68	87	28	12	4
Catechol-O-methyltransferase	*COMT*	Val158Met	4680	SNaPshot	99	32	168	53	47	15
Dopamine beta hydroxylase	*DBH*	C-1021T	1611115	SNaPshot	197	74	66	25	2	1
		Taq1 (intron 5)	2519152	SNaPshot	152	45	134	40	50	15
Dopamine receptor	*DRD2*	A241G	6277	SNaPshot	107	35	147	48	50	17
		Taq1 A	1800496	SNaPshot	313	94	19	6	1	< 1
	DRD4	Val194Gly	1800443	SNaPshot	189	97	5	3		
Monoamine oxidase A	*MAOA*	G941T	1799835	SNaPshot	281	100				
		T1460C	1137070	SNaPshot	156	57	72	26	45	17
Serotonin receptor	*HTR1B*	G861C	6296	SNaPshot	224	73	71	23	12	4
Synaptosomal-associated protein 25	*SNAP25*	T1065G	3746544	SNaPshot	129	39	107	32	94	29
		T1069C	10513112	SNaPshot	199	65	75	24	33	11

Abbreviations: PCR, polymerase chain reaction; RSID, rapid stain identification series for *Homo sapiens*.
*Allele frequency differed by race for following genes: *CYP3A5*, *DBH*, *DRD2*, *FPGS*, *GSTP1*, *GSTT1*, *MDR1*, *MTHFD1*, and *TYMS*. Given these multiple differences by race and relatively small sample of nonwhite participants (20.2% of 243 patients), race was used as independent variable in multiple regression analyses.

MAOA may suggest a predisposition for attention problems in a subset of survivors, one that may not be related to specific chemotherapy agents. However, given the discovery nature of these associations, validation is required.

APOE-4 was associated with parent report of attention problems. *APOE* is involved in lipoprotein metabolism,[39,40] and the E4 polymorphism is a risk factor for dementia.[41] *APOE-4* has been associated with age-related myelin breakdown[42] and risk of neurocognitive

Table 4. Multiple Regression Models* Predicting Attention Deficits in Survivors of Childhood ALL

Parameter	Attentiveness†		Response Speed†		Variability†		Attention Problems‡	
	Estimate§	*P*	Estimate§	*P*	Estimate§	*P*	Estimate§	*P*
Risk (low *v* standard/high)	−0.28	.90	−3.30	.42	−0.69	.82	6.34	**.005**
Sex (female *v* male)	−0.34	.88	−0.09	.98	0.01	.99	−0.59	.78
Age at diagnosis (< 5 *v* ≥ 5 years)	0.38	.88	−5.94	.21	2.43	.49	3.25	.14
Race (black *v* white)	−1.75	.60	13.31	**.02**	0.19	.96	−3.25	.29
Methionine synthase (A2756G)	3.93	**.03**	7.00	**.02**	1.42	.54		
Monoamine oxidase A (T1460C)	1.31	.35	1.54	.53	3.95	**.03**		
Glutathione S-transferase (GSTT1*0)	21.75	**.003**	23.10	.07	24.44	**.01**		
Glutathione S-transferase (GSTP1)	8.09	**.002**	8.73	.06	1.22	.72		
Glutathione S-transferase (GSTM1*0)							2.99	.16
Apoliopoprotein E (Cys112Arg)							4.92	**.01**
Cytochrome P450 family 3 (CYP3A5*3)							1.45	.36
Immunophilin protein (FKBP5 GA)							1.68	.50

NOTE. Bold font indicates significance.
Abbreviation: ALL, acute lymphoblastic leukemia.
*Separate multiple regression models were conducted on each outcome (ie, attentiveness, response speed, variability, attention problems) using multiple independent variables identified under each column.
†Conners' Continuous Performance Test[24]: variability, SE of reaction time; attentiveness, D-prime; response speed, beta.
‡Conners' Parent Rating Scale.[25]
§Estimate represents unit change in age-adjusted standard score (mean, 50; standard deviation, 10) associated with each parameter.

impairment after traumatic brain injury,[17] HIV infection,[43] and obstructive sleep apnea in children.[44] The association between *APOE-4* and attention problems in survivors of childhood ALL would also suggest a predisposition that may be independent of specific chemotherapy agents, although this too would require validation.

The finding that direct assessment of attention and parent report of attention problems were related to different polymorphisms suggests that these measures assess different aspects of a complex phenotype. This phenomenon is not unexpected; previous studies have reported limited correspondence between direct assessment and behavioral ratings.[45] However, both sources of assessment are relevant, as evidenced by their independent correspondence to magnetic resonance imaging of brain integrity.[46] This specificity reinforces the need for comprehensive assessment, including direct measures and patient-reported outcomes, to fully capture the complex phenotype of attention problems.

This study is not without limitation. Although the neurocognitive assessment occurred at the same point in treatment for all survivors, an advantage over most other reports in the literature, the survivorship phase was slightly earlier than those of the other reports and may not reflect patterns seen in long-term survivors (ie, > 5 years after diagnosis). Still, attention problems are a common long-term outcome, and these deficits at the end of therapy are likely to continue. A second limitation is the inability to confirm associations for all of the significant polymorphisms. Our previous study focused only on polymorphisms in the folate pathway, and our current study confirmed the association between *MS* and attention problems. Recruitment of an independent cohort will be necessary to validate associations with *GST*, *MAOA*, and *APOE-4*.

Despite these limitations, the current results support the conclusion that a phenotype of attention problems is a common outcome in survivors of childhood ALL and that the etiology of this phenotype is multifactorial. Risk for this outcome is associated with treatment intensity and polymorphisms in the folate pathway and may also be increased by predispositions that influence response to physiologic stress and CNS integrity. Given the potential influence of pre-existing factors, early cognitive intervention focused on enhancing attention networks to prevent post-therapy decline seems warranted. We recently described a pilot study of such a preventative approach[47] and recommend further research along these lines. Knowledge of specific polymorphisms contributing to neurocognitive outcome may also assist in development of personalized interventions. For example, contributions from polymorphisms in *GST* may warrant preventive antioxidant trials targeting those affected.

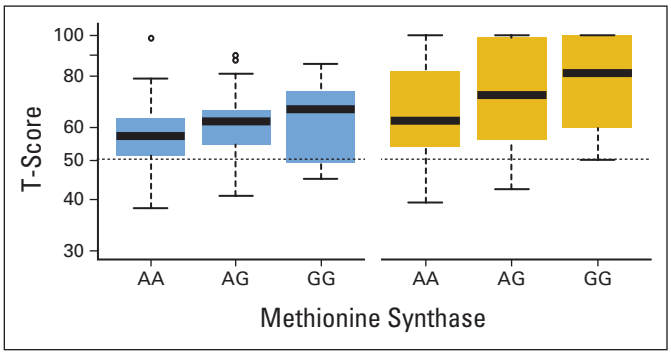

Fig 1. Box plots of age-adjusted standard scores on measures of attentiveness (left) and response speed (right) by genomic variation in methionine synthase. Dashed line represents mean T score on Conners' Continuous Performance Test[24] for normative sample.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

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REFERENCES

1. Krull KR, Okcu MF, Potter B, et al: Screening for neurocognitive impairment in pediatric cancer long-term survivors. J Clin Oncol 26:4138-4143, 2008
2. Kadan-Lottick NS, Brouwers P, Breiger D, et al: Comparison of neurocognitive functioning in children previously randomly assigned to intrathecal methotrexate compared with triple intrathecal therapy for the treatment of childhood acute lymphoblastic leukemia. J Clin Oncol 27:5986-5992, 2009
3. Conklin HM, Krull KR, Reddick WE, et al: Cognitive outcomes following contemporary treatment without cranial irradiation for childhood acute lymphoblastic leukemia. J Natl Cancer Inst 104:1386-1395, 2012
4. Jain N, Brouwers P, Okcu MF, et al: Sex-specific attention problems in long-term survivors of pediatric acute lymphoblastic leukemia. Cancer 115: 4238-4245, 2009
5. Kadan-Lottick NS, Zeltzer LK, Liu Q, et al: Neurocognitive functioning in adult survivors of childhood non-central nervous system cancers. J Natl Cancer Inst 102:881-893, 2010
6. Krull KR, Brouwers P, Jain N, et al: Folate pathway genetic polymorphisms are related to attention disorders in childhood leukemia survivors. J Pediatr 152:101-105, 2008
7. Kamdar KY, Krull KR, El-Zein RA, et al: Folate pathway polymorphisms predict deficits in attention and processing speed after childhood leukemia therapy. Pediatr Blood Cancer 57:454-460, 2011
8. van Rossum EF, Roks PH, de Jong FH, et al: Characterization of a promoter polymorphism in the glucocorticoid receptor gene and its relationship to three other polymorphisms. Clin Endocrinol (Oxf) 61:573-581, 2004
9. Eichelbaum M, Burk O: CYP3A genetics in drug metabolism. Nat Med 7:285-287, 2001
10. Hayes JD, Pulford DJ: The glutathione S-transferase supergene family: Regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. Crit Rev Biochem Mol Biol 30:445-600, 1995
11. Borst L, Wallerek S, Dalhoff K, et al: The impact of CYP3A5*3 on risk and prognosis in childhood acute lymphoblastic leukemia. Eur J Haematol 86:477-483, 2011
12. Davies SM, Bhatia S, Ross JA, et al: Glutathione S-transferase genotypes, genetic susceptibility, and outcome of therapy in childhood acute lymphoblastic leukemia. Blood 100:67-71, 2002
13. Thapar A, O'Donovan M, Owen MJ: The genetics of attention deficit hyperactivity disorder. Hum Mol Genet 14:R275–R282, 2005
14. Krämer UM, Cunillera T, Càmara E, et al: The impact of catechol-O-methyltransferase and dopamine D4 receptor genotypes on neurophysiological markers of performance monitoring. J Neurosci 27:14190-14198, 2007
15. Lipsky RH, Sparling MB, Ryan LM, et al: Association of COMT Val158Met genotype with executive functioning following traumatic brain injury. J Neuropsychiatry Clin Neurosci 17:465-471, 2005
16. Balasa M, Gelpi E, Antonell A, et al: Clinical features and APOE genotype of pathologically proven early-onset Alzheimer disease. Neurology 76:1720-1725, 2011
17. Shadli RM, Pieter MS, Yaacob MJ, et al: APOE genotype and neuropsychological outcome in mild-to-moderate traumatic brain injury: A pilot study. Brain Inj 25:596-603, 2011
18. Jordan BD: Genetic influences on outcome following traumatic brain injury. Neurochem Res 32:905-915, 2007
19. Ahles TA, Saykin AJ, Noll WW, et al: The relationship of APOE genotype to neuropsychological performance in long-term cancer survivors treated with standard dose chemotherapy. Psychooncology 12:612-619, 2003
20. Pui CH, Campana D, Pei D, et al: Treating childhood acute lymphoblastic leukemia without cranial irradiation. N Engl J Med 360:2730-2741, 2009
21. Wechsler D: Wechsler Preschool and Primary Scales of Intelligence: Revised. New York, NY, Harcourt Brace Jovanovich, 1989
22. Wechsler D: Wechsler Intelligence Scale for Children (ed 3). San Antonio, TX, Psychological Corporation, 1991
23. Wechsler D: Wechsler Adult Intelligence Scale (ed 3). San Antonio, TX, Psychological Corporation, 1997
24. Conners CK: Conners' Continuous Performance Test. Toronto, Ontario, Canada, Multi-Health Systems, 1995
25. Conners CK: Conners' Rating Scales: Revised User's Manual. New York, NY, Multi-Health Systems, 1997
26. Strauss E, Sherman EMS, Spreen O: A Compendium of Neuropsychological Tests: Administration, Norms, and Commentary (ed 3). Oxford, United Kingdom, Oxford University Press, 2006, pp 562-575
27. Ogg RJ, Zou P, Allen DN, et al: Neural correlates of a clinical continuous performance test. Magn Reson Imaging 26:504-512, 2008
28. Conklin HM, Krull KR, Reddick WE, et al: Cognitive outcomes following contemporary treatment without cranial irradiaiton for childhood acute lymphoblastic leukemia. J Natl Cancer Inst 104:1386-1395, 2012
29. Hochberg Y, Benjamini Y: More powerful procedures for multiple significance testing. Stat Med 9:811-818, 1990
30. Watkins D, Ru M, Hwang HY, et al: Hyperhomocysteinemia due to methionine synthase deficiency, cblG: Structure of the MTR gene, genotype diversity, and recognition of a common mutation, P1173L. Am J Hum Genet 71:143-153, 2002
31. Almawi WY, Khan A, Al-Othman SS, et al: Case-control study of methylenetetrahydrofolate reductase mutations and hyperhomocysteinemia and risk of stroke. J Stroke Cerebrovasc Dis 18:407-408, 2009
32. Parnetti L, Caso V, Santucci A, et al: Mild hyperhomocysteinemia is a risk-factor in all etiological subtypes of stroke. Neurol Sci 25:13-17, 2004
33. Strange RC, Spiteri MA, Ramachandran S, et al: Glutathione-S-transferase family of enzymes. Mutat Res 482:21-26, 2001
34. Cases O, Lebrand C, Giros B, et al: Plasma membrane transporters of serotonin, dopamine, and norepinephrine mediate serotonin accumulation in atypical locations in the developing brain of monoamine oxidase A knock-outs. J Neurosci 18:6914-6927, 1998
35. Kumagae Y, Matsui Y, Iwata N: Deamination of norepinephrine, dopamine, and serotonin by type A monoamine oxidase in discrete regions of the rat brain and inhibition by RS-8359. Jpn J Pharmacol 55:121-128, 1991
36. Gershon MD, Sherman DL, Pintar JE: Type-specific localization of monoamine oxidase in the enteric nervous system: Relationship to 5-hydroxytryptamine, neuropeptides, and sympathetic nerves. J Comp Neurol 301:191-213, 1990
37. Ferreri F, Lapp LK, Peretti CS: Current research on cognitive aspects of anxiety disorders. Curr Opin Psychiatry 24:49-54, 2011
38. Lane SJ, Reynolds S, Thacker L: Sensory over-responsivity and ADHD: Differentiating using electrodermal responses, cortisol, and anxiety. Front Integr Neurosci 4:8, 2010
39. Alvim RO, Freitas SR, Ferreira NE, et al: APOE polymorphism is associated with lipid profile, but not with arterial stiffness in the general population. Lipids Health Dis 9:128, 2010
40. Park TS, Panek RL, Rekhter MD, et al: Modulation of lipoprotein metabolism by inhibition of sphingomyelin synthesis in ApoE knockout mice. Atherosclerosis 189:264-272, 2006
41. Jin YP, Østbye T, Feightner JW, et al: Joint effect of stroke and APOE 4 on dementia risk: The Canadian Study of Health and Aging. Neurology 70:9-16, 2008
42. Ryan L, Walther K, Bendlin BB, et al: Age-related differences in white matter integrity and cognitive function are related to APOE status. Neuroimage 54:1565-1577, 2011
43. Spector SA, Singh KK, Gupta S, et al: APOE epsilon4 and MBL-2 O/O genotypes are associated with neurocognitive impairment in HIV-infected plasma donors. AIDS 24:1471-1479, 2010
44. Gozal D, Capdevila OS, Kheirandish-Gozal L, et al: APOE epsilon 4 allele, cognitive dysfunction, and obstructive sleep apnea in children. Neurology 69:243-249, 2007
45. Edwards MC, Gardner ES, Chelonis JJ, et al: Estimates of the validity and utility of the Conners' Continuous Performance Test in the assessment of inattentive and/or hyperactive-impulsive behaviors in children. J Abnorm Child Psychol 35:393-404, 2007
46. Mahone EM, Martin R, Kates WR, et al: Neuroimaging correlates of parent ratings of working memory in typically developing children. J Int Neuropsychol Soc 15:31-41, 2009
47. Moore IM, Hockenberry MJ, Anhalt C, et al: Mathematics intervention for prevention of neurocognitive deficits in childhood leukemia. Pediatr Blood Cancer 59:278-284, 2011

Appendix

Table A1. Clinical Features Between Tested Versus Nontested Patient Cases

Characteristic	Tested (%)	Nontested (%)	*P**
Sex			1.00
Male	55.36	55.56	
Female	44.64	44.44	
Race			.32
White	77.68	84.13	
Black	18.55	11.11	
Other	3.77	4.76	
Risk			.27
Low	49.28	41.27	
Standard/high	50.72	58.73	
Age group, years			.28
< 5	47.25	39.68	
≥ 5	52.75	60.32	

*Exact χ^2 test with two-sided comparison.

Table A2. Forward and Reverse Primers Used for Sequencing Genomic Variation

Gene Description	Gene Symbol	Genomic Variation	RSID	Forward Primer	Reverse Primer
Apoliopoprotein E	*APOE4*	Cys112Arg	429358	TGTCCAAGGAGCTGCAGGCG	TCATCGGCATCGCGGAGGAG
Brain-derived neurotrophic factor	*BDNF*	Val66Met	6265	AGGCTTGACATCATTGGCTGACAC	AGGCTCCAAAGGCACTTGACTACT
Catechol-O-methyltransferase	*COMT*	Val158Met	4680	ATCACCCAGCGGATGGTGGATTT	GGGCCTGGTGATAGTGGGTTT
Cytochrome P450 family 3	*CYP3A4*	CYP3A4*1B	3091339	TGAAGACTTGAGTGGCTCCTGTGT	TGCTTACCCTCCGGTTTGTGAAGA
	CYP3A5	CYP3A5*3	776746	CCAACTGCCCTTGCAGCATTTAGT	AGGGTAATGTGGTCCAAACAGGGA
Dopamine beta hydroxylase	*DBH*	C-1021T	1611115	AGCTGGAGGGATCAAGCAGAATGT	TGAATTTGAAGCCTCTCAGGGCAG
		Taq1 (intron 5)	2519152	GCATCTGGCAGCTTCCCTTATGAA	GCTGTCCATCTTCCATGGCTGT
Dihydrofolate reductase	*DHFR*	Intron-1 19bp del	70991108	AATCCGGGCAGAAATCAGCAACTG	AGAACATGGGCATCGGCAAGAA
Dopamine receptor	*DRD2*	Taq1 A	1800496	TGTGGTGTTTGCAGGAGTCTTCAG	GTGGTCTTTGGCATGCCCATTCTT
		A241G	6277	AGGAGCTGGAGATGGAGATGCT	ATGCCCATTCTTCTCTGGTTTGGC
	DRD4	Val194Gly	1800443	TACTGTGCGGCCTCAACGA	TAGGAAGAAGGAGCACACGGACGA
Immunophilin protein	*FKBP5*	CT	1360780	GCCCTTATTCTATAGCTGCAAGTCCC	TCTCTTGTGCCAGCAGTAGCAAGT
		GA	9296158	CGTTCTGTTATACTCATTCCATGCCC	CCCTAGTGTCTACACCATTTCTGT
		AC	3800373	ACACAGTACTTCCTCCCAGCATTG	AGAACAGAGAAGCTTGACAGGGCA
		CT	9470080	GAACAGTACCTTATTCTACAGATACGGAG	TTGGCCTCCCAGAGTGTTAGGATT
Folylpolyglutamate synthase	*FPGS*	A1994G	10,106	AATCTACCACCCAGCACATGGCAA	CCCATGAACTTACATACTAGGTGCC
		G144A	10760502	ACGCTGCGCTGATTGGCT	GCCTGATACCTGGTACTCCATGCT
Gamma-glutamyl hydrolase	*GGH*	C452T	11545078	GGGCACATGCCTTGGATTTGAAGA	TGCTACTTACTAATCCTGCCCAGC
Glutathione S-transferase	*GSTM1*	GSTM1*0	2071487	TGGAGGTTCCAGCCCACATATTCT	TGGGCTCAAATATACGGTGGAGGT
	GSTP1	G313A	1695	AGTGACTGTGTGTTGATCAGGCG	AACCCTGGTGCAGATGCTCACATA
		C341T	1138272	GATGATACATGGTGGTGTCTGGCA	TCTCCCACAATGAAGGTCTTGCCT
	GSTT1	GSTT1*0	2266637	AGAGTTGGATGTGACCCTGCAGTT	AAGCAGGACTTCAGCAACTAGCCA
Serotonin receptor	*HTR1B*	G861C	6296	AGCGAATCCGGATCTCCTGTGTAT	AGCCAACACACAATAAAGGCTCCC
Monoamine oxidase A	*MAOA*	T1460C	1137070	GCTAGCAGGGCCTTGAATCTGTAGAA	TGCCCAGAGTCACCAAACTTACCT
		G941T	1799835	TCCGACCTTGACTGCCAAGATTCA	TAGCAGCCTACCCTTCTTCTTCCA
Multidrug resistance	*MDR1*	T3435C	1045642	ACATTGCCTATGGAGACAACAGCC	CGATGAAGGCATGTATGTTGGCCT
		A2677G	2032582	CCCATCATTGCAATAGCAGGAGTTG	AGAGCATAGTAAGCAGTAGGGAGT
Methionine synthase	*MS*	A2756G	1805087	GGGAGAAGAAATGAAGTTAAGGAAGCC	CTACCACTTACCTTGAGAGACTCAT
Methylenetetrahydrofolate dehydrogenase	*MTHFD1*	T401C	1950902	TCCAAGCATCCCTTAGGCGTACAA	AGTGTGGCTTGGCTTCCTATGTCT
		G1958A	2236225	TCCAAATCCTGCTTCCGTCACTGT	AACATCGCACATGGCAATTCCTCC
Methylenetetrahydrofolate reductase	*MTHFR*	A1298C	1801131	GGGCAGAATTTACAGGAATGGCCT	CCAGCATCACTCACTTTGTGACCA
		C677T	1801133	TCTCTTCATCCCTCGCCTTGAACA	ATGTCGGTGCATGCCTTCACAAAG
Methionine synthase reductase	*MTRR*	A66G	1801394	ACATGCCTTGAAGTGATGAGGAGG	CGGCTCTAACCTTATCGGATTCAC
Glucocorticoid receptor	*NR3C1*	G1088A	56149945	AGCATCCCTTTCTCAACAGCAGGA	TGTTCGACCAGGGAAGTTCAGAGT
		Asn363Ser	6195	AGCATCCCTTTCTCAACAGCAGGA	TGTTCGACCAGGGAAGTTCAGAGT
Serine hydroxymethyltransferase	*SHMT*	C1420T	1979277	TTGGAGCAGCTCATCCATCTCTCA	TGTCAGAGCCACCCTGAAAGAGTT
Reduced folate carrier	*SLC19A1*	G80A	1051266	AGACCATCTTCCAAGGTGCCCTGA	AGCCGTAGAAGCAAAGGTAGCACA
Synaptosomal-associated protein 25	*SNAP25*	T1065G	3746544	AAAGGACCGTGGCAGTAACTCTGT	TGGTCATTTGGTGGCTCTAACTCC
		T1069C	10513112	TGGTCATTTGGTGGCTCTAACTCC	AAAGGACCGTGGCAGTAACTCTGT
Thymidylate synthase	*TYMS*	1494del6	34489327	GGAGCTGAGTAACACCATCGATCA	AGGAACTGAGCAGATAAGTGGCAG
Vitamin D receptor	*VDR*	VDR fokIstartsite T→C	2228570	AGCCAGCTATGTAGGGCGAATCAT	TGAAGAAGCCTTTGCAGCCTTCAC
		Intron 8 G→A	1544410	AGAGGTCAAGGGTCACTGCACATT	AACTAGATAAGCAGGGTTCCTGGG

Abbreviation: RSID, rapid stain identification series for *Homo sapiens*.

Table A3. Age-Adjusted Standard Scores* on Measures of Attention by Genotype

SNP	Type	No.	Attentiveness†		Response Speed†		Variability†		Attention Problems‡	
			Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
Apoliopoprotein E (Cys112Arg)	CC	97	60	58 to 62	71	67 to 75	59	56 to 62	51	49 to 53
	CT	30	58	55 to 62	69	63 to 75	56	51 to 61	**58**	**53 to 62**
	TT	5	58	53 to 63	69	52 to 85	61	45 to 76	**61**	**43 to 78**
Cytochrome P450 family 3 (CYP3A5*3)	GG	50	59	56 to 62	70	64 to 75	56	52 to 59	54	51 to 57
	AG	53	60	57 to 63	71	66 to 76	59	55 to 63	53	49 to 56
	AA	25	59	56 to 63	74	66 to 82	**62**	**57 to 67**	**48**	**44 to 51**
Immunophilin protein (FKBP5 GA)	GG	102	59	57 to 61	70	66 to 74	58	55 to 61	52	50 to 55
	AG	18	61	54 to 67	71	63 to 79	61	56 to 67	53	49 to 58
	AA	2	55	−89 to 199	74	−50 to 197	58	−116 to 233	**68**	**46 to 90**
Glutathione S-transferase (GSTM1*0)	CC	72	60	57 to 63	71	66 to 75	59	56 to 63	54	51 to 57
	CT	55	58	56 to 61	69	64 to 75	57	54 to 61	50	48 to 53
Glutathione S-transferase (GSTP1)	CC	94	58	56 to 60	69	65 to 72	58	55 to 61	52	49 to 54
	CT	19	**65**	**58 to 71**	78	68 to 87	60	54 to 65	**55**	**48 to 61**
	TT	5	**61**	**55 to 67**	85	59 to 111	**62**	**47 to 78**	**58**	**46 to 70**
Glutathione S-transferase (GSTT1*0)	CC	121	59	58 to 61	71	67 to 74	58	56 to 60	53	51 to 55
	CT	3	**70**	**29 to 110**	84	13 to 154	**77**	**52 to 103**	52	31 to 73
Monoamine oxidase A (T1460C)	CC	56	58	56 to 60	67	62 to 71	55	52 to 58	54	50 to 58
	CT	29	58	53 to 63	71	63 to 78	**62**	**56 to 68**	52	48 to 55
	TT	20	**64**	**59 to 69**	**78**	**69 to 87**	**62**	**56 to 68**	50	45 to 55
Methionine synthase (A2756G)	AA	69	58	55 to 60	68	63 to 72	57	54 to 60	51	49 to 54
	AG	53	**62**	**59 to 64**	**74**	**69 to 80**	**61**	**57 to 65**	54	50 to 58
	GG	5	**64**	**43 to 85**	**78**	**50 to 107**	55	34 to 76	**57**	**29 to 85**

NOTE. Bold font identifies scores in survivors with minor alleles that fall outside 95% CI for survivors with wild-type allele.

*Standard score scale results in expected mean of 50 and standard deviation of 10 in general population.

†Conners' Continuous Performance Test[24]: variability, SE of reaction time; attentiveness, D-prime; slow response speed, beta; lower scores reflect better performance.

‡Conners' Parent Rating Scale[25]; lower scores reflect fewer problems.