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ORIGINAL REPORT

# Neurocognitive Functioning of Children Treated for High-Risk B-Acute Lymphoblastic Leukemia Randomly Assigned to Different Methotrexate and Corticosteroid Treatment Strategies: A Report From the Children's Oncology Group

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## ABSTRACT

### Purpose
Survivors of childhood acute lymphoblastic leukemia (ALL) are at risk for neurocognitive deficits that are associated with treatment, individual, and environmental factors. This study examined the impact of different methotrexate (MTX) and corticosteroid treatment strategies on neurocognitive functioning in children with high-risk B-lineage ALL.

### Methods
Participants were randomly assigned to receive high-dose MTX with leucovorin rescue or escalating dose MTX with PEG asparaginase without leucovorin rescue. Patients were also randomly assigned to corticosteroid therapy that included either dexamethasone or prednisone. A neurocognitive evaluation of intellectual functioning (IQ), working memory, and processing speed (PS) was conducted 8 to 24 months after treatment completion (n = 192).

### Results
The method of MTX delivery and corticosteroid assignment were unrelated to differences in neurocognitive outcomes after controlling for ethnicity, race, age, gender, insurance status, and time off treatment; however, survivors who were age < 10 years at diagnosis (n = 89) had significantly lower estimated IQ ($P < .001$) and PS scores ($P = .02$) compared with participants age ≥ 10 years. In addition, participants who were covered by US public health insurance had estimated IQs that were significantly lower ($P < .001$) than those with US private or military insurance.

### Conclusion
Children with high-risk B-lineage ALL who were age < 10 years at diagnosis are at risk for deficits in IQ and PS in the absence of cranial radiation, regardless of MTX delivery or corticosteroid type. These data may serve as a basis for developing screening protocols to identify children who are at high risk for deficits so that early intervention can be initiated to mitigate the impact of therapy on neurocognitive outcomes.

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## INTRODUCTION

Children who are treated for acute lymphoblastic leukemia (ALL) without cranial radiation are at risk for neurocognitive late effects, including deficits in processing speed (PS), visual-motor abilities, attention, working memory (WM), and executive function.[1-6] Intellectual functioning (IQ) commonly remains within the average range,[7,8] although mean IQs may decline significantly within the average range.[9] Younger age at diagnosis,[1,4] female gender,[3,10] Hispanic/Latino ethnicity,[11] and lower socioeconomic status (SES)[12] have emerged as risk factors for poorer neurocognitive outcomes. Although many survivors exhibit stable functioning, 20% to 40% develop cognitive difficulties over time that impact overall adaptive functioning, learning, and adjustment.[13]

Intravenous (IV) methotrexate (MTX) is an important component of therapy, although doses and schedules of MTX infusions and leucovorin rescue vary across regimens. Some evidence suggests that higher doses of MTX increase the risk for neurocognitive impairment[14-16]; however,

ASSOCIATED CONTENT

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Appendix
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Data Supplement
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children who received extremely high-dose MTX (HDMTX; 33.6 g/m$^2$) have exhibited stable verbal IQs and improvements in performance IQ over time,[17] which suggests that dose alone does not predict outcome. When examining the impact of dexamethasone versus prednisone, few significant differences have been found in IQ, attention, PS, or WM, although a higher percentage of children who are treated with dexamethasone receive special education services.[18] Thus, the differential impact of variations in MTX and corticosteroid delivery on neurocognitive outcome is unclear. Much of the published data that examine these variables have been derived from single- or limited institution trials, or from retrospective samples in which key components of therapy vary or were not randomized. The Children's Oncology Group (COG) AALL0232[19] trial randomly assigned patients with high-risk B-lineage ALL (HR B-ALL) to receive therapy that included a 2-month block of either HDMTX with leucovorin rescue or a lower, escalating dose MTX without leucovorin rescue, plus asparaginase.

Results demonstrated a significant increase in the 5-year event-free survival for participants who received HDMTX (79.6%) versus the escalating dose regimen (75.2%).[19] Children age 1 to 9 years were also randomly assigned to receive 14 days of dexamethasone versus 28 days of prednisone during induction therapy, with superior survival observed for those randomly assigned to receive dexamethasone plus HDMTX compared with other regimens. This trial provided an opportunity to evaluate the relative impact of two different approaches to MTX and corticosteroid delivery while examining the moderating role of age on neurocognitive functioning.

The primary aim of this study was to evaluate the differences in estimated IQ, WM, and PS between children who were randomly assigned to receive HDMTX with leucovorin rescue versus those who received escalating dose MTX with asparaginase. The effect of the corticosteroid—dexamethasone versus prednisone—delivered during induction therapy was also evaluated. An exploratory aim was to identify germline host polymorphisms that may predict which individuals are at increased risk for neurocognitive toxicity. This study represents the first evaluation of patients randomly assigned to different MTX and corticosteroid regimens within a common therapeutic trial.

## METHODS

COG AALL06N1 was designed to evaluate the neurocognitive impact of treatment delivered in AALL0232. Slow accrual led to several amendments that were designed to facilitate enrollment, including changing from a longitudinal design to a cross-sectional design, expanding the assessment window to 8 to 24 months after completion of AALL0232 therapy, and revising the evaluation from a $\geq$ 4-hour comprehensive neuropsychological battery to a screening battery (approximately 1 hour) administered by a psychometrist, as in COG ALTE07C1.[20]

As amended, eligibility criteria for AALL06N1 included enrollment in COG AALL0232, age at diagnosis of 1 to 18 years, and a primary language of English or Spanish. Exclusion criteria included preexisting neurodevelopmental disability, significant sensory impairment, central nervous system (CNS) involvement, treatment with cranial radiation, or recurrent disease. AALL06N1 was approved by the National Cancer Institute and the institutional review boards of participating institutions. Informed consent was obtained in accordance with Department of Health and Human Services guidelines. All participants had also enrolled in AALL03B1. This trial included the procurement of remission peripheral blood samples for the determination of host polymorphisms. Single-nucleotide polymorphism (SNP) genotyping was performed on germline DNA by using the Illumina Human Exome BeadChip v1.1 (Illumina, San Diego, CA) and Affymetrix GeneChip Human Mapping Array 6.0 (Affymetrix, Santa Clara, CA), and imputation was performed according to the 1000 Genome Project as previously described.[21]

### Participants

The study enrolled 230 eligible participants (Fig 1), with a final sample of 192 participants (83.5%) who submitted valid neurocognitive data. Fifty-four percent were female (n = 104), 82% (n = 157) self-identified as White, 20% (n = 38) Hispanic, and 46% (n = 89) were age $<$ 10 years at diagnosis (mean age, 8.8 years; standard deviation, 5.1). Insurance status was used as a proxy for SES. Insurance designations were collapsed into the following categories for analysis: US public (n = 52), US private or military (n = 106), non-US (n = 27), and unknown or self-pay (n = 7; Table 1).

### Treatment

A detailed treatment schema for AALL0232 has been published.[19] In brief, eligible consenting patients were randomly assigned to 14 days of dexamethasone or 28 days of prednisone (induction), and to four courses of HDMTX with leucovorin rescue or five doses of escalating dose MTX with PEG asparaginase (interim maintenance). Both random assignments were restricted or halted before study closure on the basis of response data accrued during the trial.[19] Approximately 4 years after study activation, excessive osteonecrosis among patients who were age $>$ 10 years at diagnosis led to the non–random assignment of these patients to prednisone; younger patients continued to be randomly assigned. Girls received 23 doses and boys 27 doses of intrathecal MTX, with one dose of intrathecal cytarabine on day 1 of therapy for CNS prophylaxis. Patients with overt CNS leukemia and those with a slow early response to induction therapy received cranial radiation and were excluded from AALL06N1.

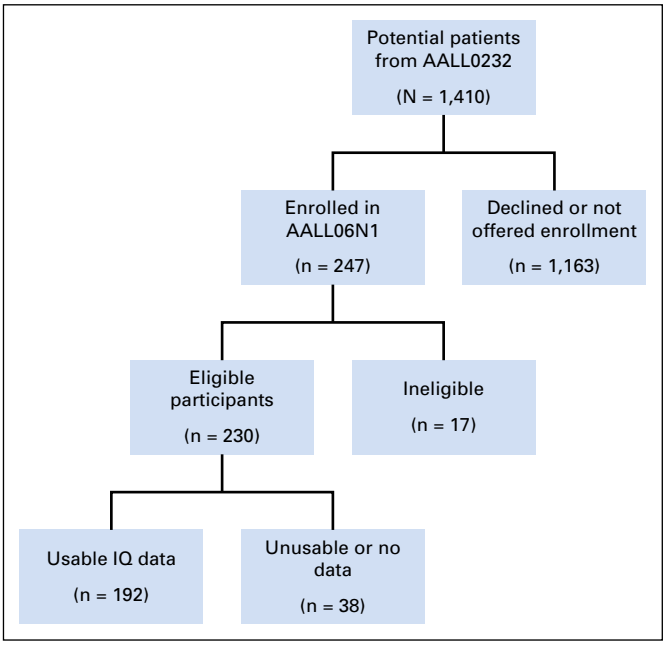


**Fig 1.** AALL06N1 flow diagram (excluding two enrollments from AALL0434). IQ, intelligence quotient.

**Table 1.** Demographic and Clinical Characteristics of the Sample

| Characteristic | No. (%) | Mean ± SD |
|---|---|---|
| Sex | | |
| Male | 88 (45.8) | |
| Female | 104 (54.2) | |
| Race | | |
| White | 157 (81.8) | |
| Asian | 6 (3.1) | |
| Black | 6 (3.1) | |
| Other/unknown | 23 (12.0) | |
| Ethnicity | | |
| Hispanic or Latino | 38 (19.8) | |
| Not Hispanic or Latino | 143 (74.5) | |
| Unknown or not reported | 11 (5.7) | |
| Insurance status | | |
| US Public | 52 (27.1) | |
| US private or military | 106 (55.2) | |
| Non-US | 27 (14.1) | |
| Unknown (includes self-pay) | 7 (3.6) | |
| Age at diagnosis, years | | 8.8 ± 5.1 |
| < 10 | 89 (46.4) | 3.9 ± 2.1 |
| ≥ 10 | 103 (53.6) | 13.1 ± 2.2 |
| Age at assessment, years | | 12.9 ± 5.1 |
| Time off therapy, months | | 14.4 ± 4.0 |

Abbreviation: SD, standard deviation.

### *Procedures*

Neurocognitive assessments were conducted 8 to 24 months after completion of therapy with widely used clinical measures with well-established validity and reliability, normalized on large representative samples. To assess the primary domains of estimated IQ, WM, and PS, age-appropriate versions of the Wechsler Intelligence Scales were used.[22-24] Estimated IQ was derived by using vocabulary and block design subtests. Within the Wechsler series, this short-form combination correlates highly with full-scale IQ, with validity coefficients of 0.85 to 0.88.[25] WM was assessed with digit span, and PS was assessed with symbol search and coding from the Wechsler scales.

### *Statistical Analysis*

Descriptive statistics were calculated for clinical and demographic characteristics, including gender, race, ethnicity, insurance status, age at diagnosis, age at assessment, MTX delivery method, corticosteroid type, time off therapy, estimated IQ, PS, and WM. Primary analysis was performed with multiple linear regression that included the covariates gender, age at diagnosis (age >10 years or age < 10 years), race, ethnicity, insurance status, and time between completion of treatment and assessment. Primary independent variables were MTX delivery method and type of corticosteroid, and the primary outcome was post-treatment estimated IQ score. PS and WM were analyzed as secondary outcome variables using the same model. Models with interactions terms for MTX dosing and the type of corticosteroid as well as for age at diagnosis and MTX dosing or corticosteroid were also considered on the basis of prior outcome results.[19] Statistical significance was defined as $P < .05$. A sample size of 192 gives 79% and 78% power, respectively, to detect a 0.4 standard deviation difference in post-treatment estimated IQ for MTX and corticosteroid comparisons on the basis of attained sample sizes per group. All analyses were performed by using SAS (SAS/STAT User's Guide, Version 9.4; SAS Institute, Cary, NC).

Genome-wide association study was performed with estimated IQ score or PS as dependent variables and using linear regression models that adjusted for covariates using PLINK (Version 1.9, Center for Human Genetic Research, Boston, MA). Covariates included age, corticosteroid use (dexamethasone *v* prednisone), MTX use (HDMTX *v* escalating dose), insurance status, and ancestry treated as a continuous variable (percent European, African, Asian, or Native American). Ancestry was determined by using STRUCTURE (Version 2.2.3).[21]

## RESULTS

We compared demographic characteristics between eligible participants in AALL06N1 and eligible individuals from AALL0232 who did not enroll in AALL06N1. Compared with patients in AALL0232 (n = 1,163), participants in AALL06N1 were younger—at diagnosis—by an average of 1.2 years ($P < .01$) and fewer identified as Hispanic/Latino (19% *v* 22%; $P = .02$).

After controlling for age, gender, race, ethnicity, time since diagnosis, and type of insurance, there were no significant differences in estimated IQ, PS, or WM scores 8 to 24 months postcompletion of therapy for children who received HDMTX versus escalating dose MTX, nor were there significant differences on the basis of induction corticosteroid type (all $P \geq .20$; Table 2). The interaction between MTX dosing and the type of corticosteroid reflected no significant differences for any of the cognitive outcomes (all $P \geq .45$), including when analysis was restricted to only patients whose corticosteroid treatment was randomly assigned (Appendix Table A1, online only). Interaction between age at diagnosis and MTX dosing or corticosteroid was also examined with nonsignificant results (all $P \geq .17$).

In multivariable models with the other covariates, both age and insurance status were found to be significant predictors of post-treatment estimated IQ (both $P < .001$); age was predictive of post-treatment PS ($P = .02$; Fig 2); and insurance type was predictive of post-treatment WM ($P < .001$; Fig 3). Specifically, children who were age < 10 years at diagnosis exhibited significantly lower post-treatment estimated IQ (adjusted difference, 11.4; standard error [SE], 2.2) and PS (adjusted difference, 5.3; SE, 2.2) scores compared with those age ≥ 10 years (Table 3). Using age at diagnosis as a continuous variable in a multivariable logistic model, a single year of increase in age at diagnosis was associated with a 14% decrease in the odds of having an IQ score ≥ 1 standard deviation below the normative mean. In addition, US public insurance was associated with lower post-treatment IQ scores (adjusted difference, −11.3; SE, 2.9) and WM (adjusted difference, −2.4; SE, 0.6) compared with US private and military insurance (Table 3).

As a group, these survivors showed estimated IQ, PS, and WM scores within the average range (Table 3). Regardless, 21.4% of participants demonstrated impairment in IQ and 28.6% had impaired PS as defined by scores of ≥ 1 standard deviation below the mean—versus 15.9% of individuals in the normative samples for each Wechsler measure (two-sided $P = 2.04$ and $< .01$, respectively). In contrast, the number of participants with WM impairment was not different than expectations on the basis of the standardization sample (15.9% vs. 15.9; $P > 0.99$). Figures 2-4, Appendix Figure A1 (online only) and the Data Supplement show the distributions of outcomes by treatment and key demographic features.

An exploratory aim sought to determine whether neurocognitive outcomes were influenced by identifiable germline SNPs. We tested the association between germline genotypes and

  

**Table 2.** Cognitive Outcomes by MTX Dosing and Corticosteroid Treatment

| Variable | Age at Diagnosis (years) | IQ | PS | WM |
|---|---|---|---|---|
| MTX dosing | | | | |
| β (SE) | | 2.5 (2.1) | 2.5 (2.2) | 0.3 (0.5) |
| *P* | | .24 | .25 | .53 |
| High dose | | 103.0 | 97.6 | 9.4 |
| n = 91 (47%) | < 10; n = 40 (44%) | 97.9 | 94.5 | 8.7 |
| | ≥ 10; n = 51 (56%) | 107.1 | 100.0 | 9.8 |
| Escalating dose | | 99.7 | 95.1 | 9.1 |
| n = 101 (53%) | < 10; n = 49 (49%) | 92.6 | 92.7 | 8.8 |
| | ≥ 10; n = 52 (51%) | 106.4 | 97.3 | 9.4 |
| Corticosteroid | | | | |
| Adjusted mean difference (SE) | | 1.1 (2.2) | 1.2 (2.3) | 0.6 (0.5) |
| *P* | | .61 | .61 | .20 |
| Dexamethasone | | 101.0 | 96.1 | 9.5 |
| n = 84 (44%) | < 10; n = 46 (55%) | 95.0 | 94.6 | 8.9 |
| | ≥ 10; n = 38 (45%) | 108.4 | 98.0 | 10.1 |
| Prednisone | | 101.5 | 96.4 | 9.1 |
| n = 108 (56%) | < 10; n = 43 (40%) | 95.0 | 92.5 | 8.6 |
| | ≥ 10; n = 65 (60%) | 105.8 | 99.0 | 9.3 |

NOTE. Separate models were run for each outcome and for MTX dosing and corticosteroid. Models containing interaction terms were considered, with all *P* values nonsignificant. Interaction terms were removed for main effects testing. All models included the following covariates: Age at diagnosis (dichotomous), gender, ethnicity, race, insurance status, and time off treatment. β reflects the model-based adjusted mean differences for high-dose – escalating dose and dexamethasone – prednisone. *P* values reflect main effects tests (nonzero β). No. (%) are for overall sample n = 192 with valid estimated IQ scores. Finally, note that in 2008, the corticosteroid random assignment stopped for patients age ≥ 10 years at diagnosis because of an increased risk of avascular necrosis.
Abbreviations: IQ, intellectual quotient; MTX, methotrexate; PS, processing speed; SD, standard deviation; WM, working memory.

estimated IQ or PS scores in 172 patients with genotypes available at more than 6 million SNPs. Our sample size was significantly underpowered for a genome-wide association study; thus, there were no germline SNPs associated with estimated IQ or PS that reached a genome-wide significance threshold of $P < 5 \times 10^{-8}$. The top 10 loci are listed in Appendix Tables A2 and A3 (online only). We also examined SNPs in candidate genes that were previously associated with decreased attention (COMT, MTHFR, GSTP, MS, MAOA),[15,26-28] slower PS (MAOA, MTHFR, MS),[15,27,28] and decreases in IQ (SLCO2A1).[26] None of these SNPs was significant at the $P < .05$ level in our study (Appendix Table A4, online only). We additionally examined all 530 SNPs—75 independent loci, on the basis of r2 for linkage disequilibrium of < 0.5 in our study population—that were located within 5,000 base pairs of these candidate genes (Appendix Table A5, online only). No germline genomic variants were associated with IQ or PS at a genome-wide level of significance, although COMT variants rs5993882 and rs174680 were associated with PS and IQ at $P = .005$ and .01, respectively.

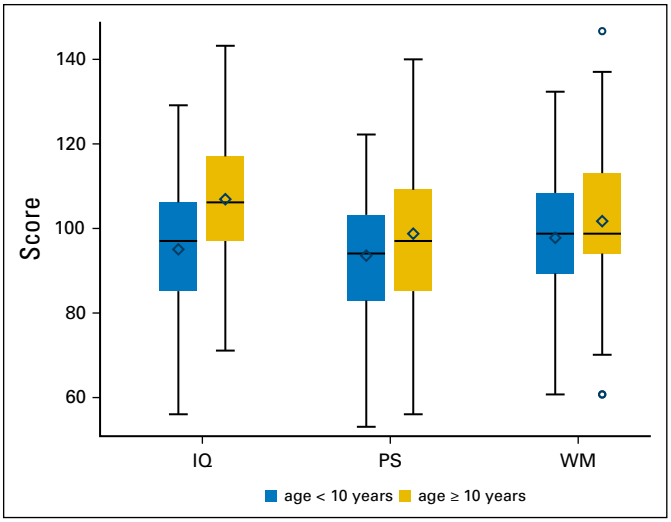


**Fig 2.** Cognitive outcomes by age at diagnosis. Colored boxes represent the middle 50% of the sample with the median represented as a horizontal line and mean as diamond. The ends of the whiskers are the minimum and maximum values discounting outliers that are beyond 1.5 interquartile ranges above or below the 75th and 25th percentiles, respectively, which are delineated separately as circles. For presentation purposes, working memory (WM) was scaled to have a sample mean of 100 and a sample standard deviation of 15. IQ, intellectual quotient; PS, processing speed.

## DISCUSSION

The majority of children with HR B-ALL will be cured, which makes it imperative that we understand how components of therapy impact long-term neurocognition. This trial is the first to our knowledge to assess neurocognitive function 8 to 24 months after the completion of therapy among patients with HR B-ALL who were treated without cranial irradiation and randomly assigned to two different methods of MTX and corticosteroid delivery. Neither MTX delivery method nor corticosteroid assignment was associated with neurocognitive outcome 3 to 5 years after diagnosis; however, regardless of treatment arm, patients who were age < 10 years at diagnosis were at a greater risk for neurocognitive toxicity, with significantly lower estimated IQ and PS scores compared with older participants after controlling for ethnicity, race, gender, insurance status, and time off treatment. In addition, participants covered by US public insurers had estimated IQs that were approximately three fourths of a standard deviation lower than participants with US private or military insurance.

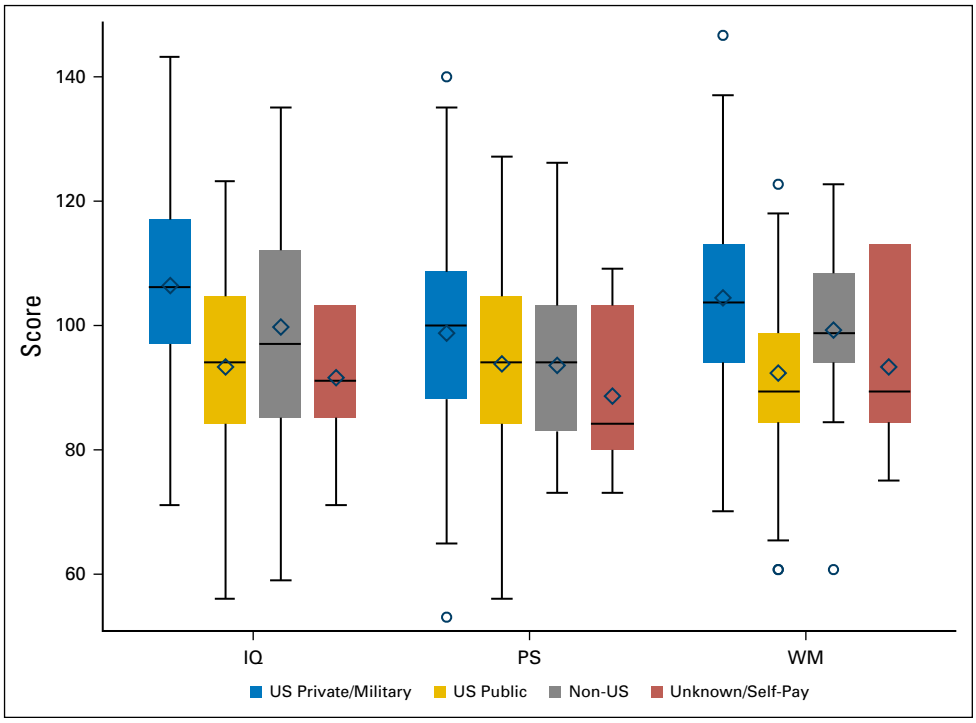


**Fig 3.** Cognitive outcomes by insurance status. Colored boxes represent the middle 50% of the sample with the median represented as a horizontal line and mean as diamond. The ends of the whiskers are the minimum and maximum values discounting outliers that are beyond 1.5 interquartile ranges above or below the 75th and 25th percentiles, respectively, which are delineated seprately as circles. For presentation purposes, working memory (WM) was scaled to have a sample mean of 100 and a sample standard deviation of 15. IQ, intellectual quotient; PS, processing speed.

Detailed meta-analysis, reviews, and large single-institution trials have demonstrated neurocognitive deficits in survivors of ALL who were treated without cranial radiation.[6,8,29] An integral component of all curative regimens for patients with ALL, MTX has been associated with acute, subacute, and chronic neurotoxicities.[6,8,29-33] Acute neurotoxicity and leukoencephalopathy[31,34] have been described after repetitive exposure to low doses of oral MTX and after IV doses of only 1 g/m$^2$ per dose, with the frequency of toxicity lessened by increasing leucovorin rescue.[31,34] Similarly, infants who were treated with IV infusions of MTX 33.6 g/m$^2$ had little acute neurotoxicity and stable verbal IQs, with improvements in performance IQ over time,[17] when therapy included early, high-dose leucovorin (200 mg/m$^2$ at

**Table 3.** Cognitive Outcomes as Predicted by Demographic Factors

| | IQ | | PS | | WM | |
|---|---|---|---|---|---|---|
| Variable | Mean | Adjusted Mean Difference (SE) | Mean | Adjusted Mean Difference (SE) | Mean | Adjusted Mean Difference (SE) |
| Total sample (SD) | 101.3 (16.6) | | 96.3 (15.2) | | 9.3 (3.1) | |
| Age at diagnosis, years | | $P < .001$ | | $P = .02$ | | $P = .14$ |
| < 10 | 95.0 | Ref | 93.5 | Ref | 8.8 | Ref |
| ≥10 | 106.7 | 11.4 (2.2) | 98.6 | 5.3 (2.2) | 9.6 | 0.7 (0.5) |
| Sex | | $P = .19$ | | $P = .64$ | | $P = .68$ |
| Male | 102.5 | Ref | 95.7 | Ref | 9.3 | Ref |
| Female | 100.2 | −2.9 (2.2) | 96.8 | 1.0 (2.2) | 9.2 | −0.2 (0.5) |
| Ethnicity | | $P = .61$ | | $P = .33$ | | $P = .33$ |
| Not Hispanic | 102.7 | Ref | 96.5 | Ref | 9.6 | Ref |
| Hispanic | 95.7 | −2.5 (3.3) | 96.9 | 3.6 (3.3) | 8.1 | −0.7 (0.7) |
| Unknown | 102.5 | −4.7 (5.5) | 91.6 | −3.7 (5.6) | 8.7 | −1.6 (1.1) |
| Race | | $P = .68$ | | $P = .14$ | | $P = .37$ |
| White | 100.9 | Ref | 95.8 | Ref | 9.2 | Ref |
| Other | 103.3 | −0.4 (4.5) | 105.9 | 8.9 (4.5) | 9.9 | 0.2 (0.9) |
| Unknown | 102.8 | 3.5 (4.0) | 94.3 | −0.4 (4.1) | 9.2 | 1.2 (0.9) |
| Insurance status | | $P < .001$ | | $P = .10$ | | $P < .001$ |
| US private or military | 106.2 | Ref | 98.7 | Ref | 10.2 | Ref |
| US Public | 93.4 | −11.3 (2.9) | 93.8 | −5.6 (2.9) | 7.6 | −2.4 (0.6) |
| Non-US | 99.6 | −5.9 (3.3) | 93.6 | −3.6 (3.4) | 9.1 | −1.3 (0.7) |
| Unknown/self | 91.6 | −15.7 (5.8) | 88.6 | −10.7 (5.9) | 7.9 | −2.2 (1.2) |

NOTE. *P* values are from multivariable models fitted for each of the three outcomes with covariates, including age at diagnosis (dichotomous), gender, ethnicity, race, insurance status, and time off treatment. Non-White race categories were combined because of small sample sizes. β reflects the model-based adjusted mean differences compared with the reference group. Overall *P* values are given for ethnicity (*df* = 2), race (*df* = 2), and insurance status (*df* = 3).
Abbreviations: IQ, intellectual quotient; PS, processing speed; Ref, reference; SD, standard deviation; WM, working memory.

  

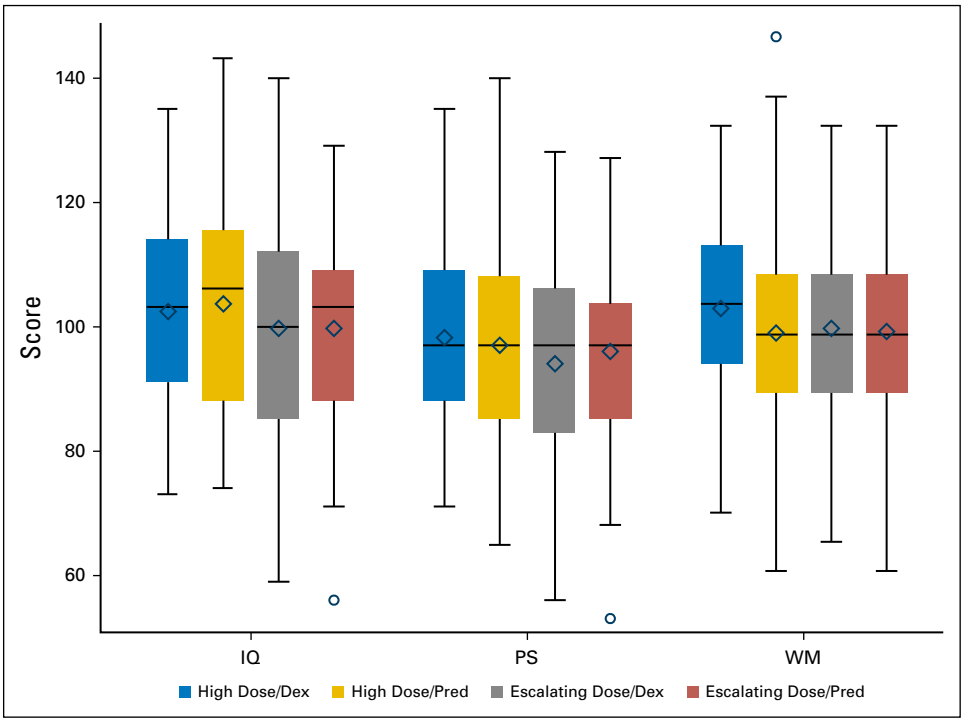


**Fig 4.** Cognitive outcomes by methotrexate dosing and corticosteroid treatment. Colored boxes represent the middle 50% of the sample with the median represented as a horizontal line and mean as diamond. The ends of the whiskers are the minimum and maximum values discounting outliers that are beyond 1.5 interquartile ranges above or below the 75th and 25th percentiles, respectively, which are delineated separately as circles. For presentation purposes, working memory (WM) was scaled to have a sample mean of 100 and a sample standard deviation of 15. Dex, dexamethasone; IQ, intellectual quotient; Pred, prednisone; PS, processing speed.

hour 36; 12 mg/m$^2$ every 3 hours $\times$ 6, then every 6 hours until $[MTX]_{plasma} < 0.08$ μM). Thus, the impact of MTX dose is influenced by the timing and quantity of leucovorin. Our finding of no significant difference in neurocognitive outcomes among patients who were randomly assigned to HDMTX with leucovorin versus escalating dose MTX with PEG asparaginase does not support an association between MTX dose and neurocognitive effects, although this is confounded by the use of leucovorin rescue in one arm and PEG asparaginase in the other arm.

There were also no significant corticosteroid-related differences in estimated IQ, PS, or WM. Similarly, no significant difference in overall neurocognitive function was observed among children who were randomly assigned to dexamethasone versus prednisone in two prior studies, although one found a difference in word reading[35] and the other, a difference in fluid reasoning.[18] Considering all available data, there seems to be no robust corticosteroid-related differences in neurocognitive outcomes, although there may be differences in specific cognitive domains that merit further study.

Although treatment-related variables did not predict neurocognitive outcomes in our sample, demographic factors significantly predicted differences in estimated IQ and PS. Insurance status—used as a proxy for SES—correlated with both estimated IQ and WM, with an increased risk of lower scores associated with US public insurance status. This relationship between socioeconomic disadvantage and cognitive outcome has been established for typically developing children.[36-40] Future work would benefit from the careful assessment of SES, including parental education, resource insecurity, caregiver occupation, and family structure[41] as potential moderators that interact with disease or treatment factors to mitigate or enhance neurocognitive deficits among children with ALL. Regardless of etiology, it may be that children with lower cognitive reserve, particularly younger children, may benefit from cognitive enrichment strategies during and after chemotherapy. Indeed, there is some evidence to indicate that enhancement of cognitive or academic skills during this period can mitigate declines over time in children with ALL.[42] It is also possible that interventions that have been known to remediate difficulties in the survivorship period could be effective in preventing or delaying the onset of cognitive difficulties.[43-45]

In this small sample, there were no germline SNPs associated with estimated IQ or PS that reached a genome-wide level of significance. *COMT* encodes for catechol-*O*-methyltransferase and its substrates include neurotransmitters. Nonsynonymous variants in *COMT* (eg, rs4680) have been associated with neuropsychiatric difficulties[46-48] and, specifically, with attention and hyperactivity in survivors of ALL.[26] Although rs4680 was not associated with estimated IQ or PS in our study (Appendix Table A3), there were other *COMT* variants (all intronic) that were marginally associated with PS and estimated IQ (Appendix Table A4). Likewise, a variant (rs7625035) in *SLCO2A1*—a prostaglandin transporter—was associated with lower scores of tests of IQ, digit span, and block design in a prior study of ALL,[26] but not in our study. Additional functional studies or replication with larger samples are required to better understand the relationship between specific SNPs and neurocognition.

The data presented here must be interpreted in light of the limitations of this trial. The population enrolled in AALL06N1 represents less than 20% of those who were enrolled in AALL0232 who were eligible for this trial. Comparing those who did and did not enroll, those evaluated in AALL06N1 were younger and less ethnically diverse; however, among the patients who were evaluable for AALL06N1, sociodemographic characteristics among those who were randomly assigned HDMTX versus escalating dose MTX were comparable, thus preserving the primary aim of the trial—to determine whether MTX or corticosteroid random assignment was

predictive of outcome. The cross-sectional design of the trial is also a limitation, as we could not assess a change in neurocognitive function over time. Thus, the finding that public insurance status—used as a proxy for SES—was associated with a lower mean estimated IQ and WM may reflect a premorbid discrepancy that stems from long-term socioeconomic disadvantage. Longitudinal data are needed to determine whether cognitive losses are greater for children who begin treatment with an SES disadvantage. Finally, we present findings that pertain to estimated intellectual functioning only. It will be important to analyze additional measures that evaluate memory, functional outcomes, and psychosocial functioning completed by this sample to have a fuller picture of possible late effects and contributing risk factors. Despite these limitations, the identification of deficits in younger patients, regardless of therapy delivered, led to the funding of an ongoing, longitudinal, computer-based assessment of neurocognitive functioning of patients enrolling in the current COG therapeutic trial for children who are diagnosed with HR B-ALL (AALL1131) with the aim of clarifying the timing and trajectory of neurocognitive deficits in this population.

In summary, our findings suggest that more than 70% of children with HR B-ALL who are treated with contemporary therapies that do not include cranial radiation have estimated IQ, PS, and WM scores within the average range. Neither MTX nor corticosteroid random assignment impacted outcomes, but when evaluated in the context of age, those who were age $<$ 10 years at diagnosis were found to be at risk for lower estimated IQ and PS scores. Future work must focus on identifying patients for whom therapy can be reduced further without eroding the excellent event-free survival that has been achieved with contemporary therapy as well as collecting longitudinal data that include sensitive evaluations of IQ, PS, WM, and SES, so that trajectories can be examined. Although complex, simultaneous assessments of SES, host genetics, and chemotherapy-induced metabolic insults would provide information that is critical to understanding the etiology of neurocognitive dysfunction and identifying children at risk.

## AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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

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## AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

**Neurocognitive Functioning of Children Treated for High-Risk B-Acute Lymphoblastic Leukemia Randomly Assigned to Different Methotrexate and Corticosteroid Treatment Strategies: A Report From the Children's Oncology Group**

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## *Appendix*

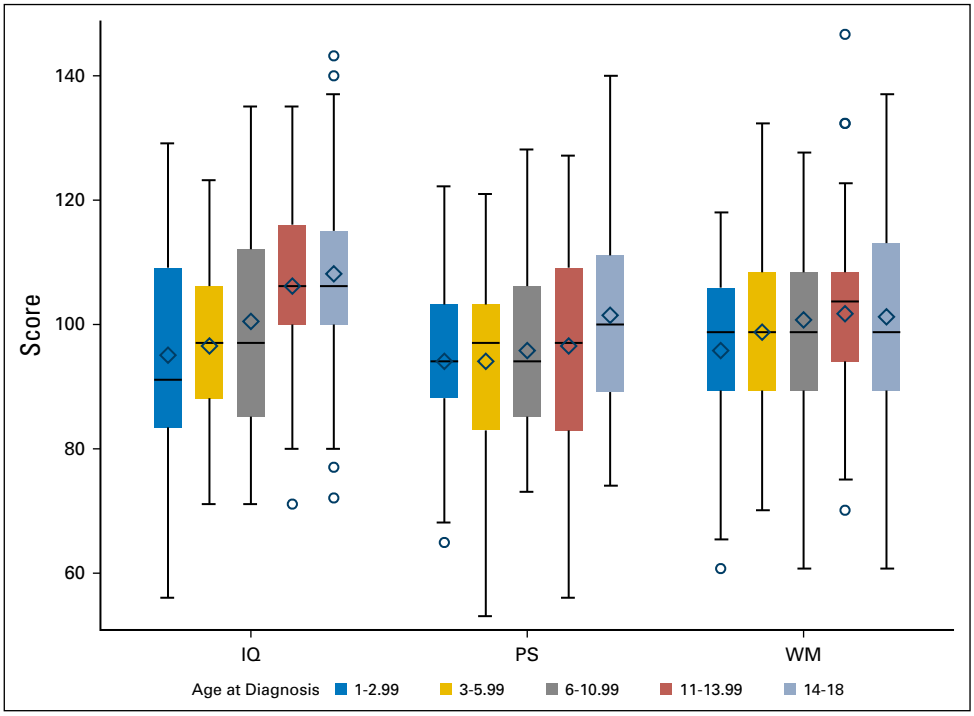


**Fig A1.** Cognitive outcomes by age at diagnosis. Colored boxes represent the middle 50% of the sample with the median represented as a horizontal line and the mean as a diamond. The ends of the whiskers are the minimum and maximum values discounting outliers that are beyond the 1.5 interquartile ranges above or below the 75th and 25th percentiles, respectively, which are delineated separately as circles. For presentation purposes, WM was scaled to have a sample mean of 100 and a sample SD = 15. IQ, intelligence quotient; PS, processing speed; SD, standard deviation; WM, working memory.

**Table A1.** Cognitive Outcomes by Corticosteroid Treatment Using Only Corticosteroid Randomly Assigned Patients

| Corticosteroid | Age at Diagnosis (years) | IQ | PS | WM |
|---|---|---|---|---|
| Adjusted mean difference (SE) | | 1.6 (2.3) | 2.0 (2.4) | 0.7 (0.5) |
| *P* | | .49 | .41 | .15 |
| Dexamethasone | | 101.0 | 96.1 | 9.5 |
| n = 84 (51%) | < 10; n = 46 (55%) | 95.0 | 94.6 | 8.9 |
| | ≥ 10; n = 38 (45%) | 108.4 | 98.0 | 10.1 |
| Prednisone | | 99.9 | 94.5 | 8.9 |
| n = 82 (49%) | < 10; n = 43 (52%) | 95.0 | 92.5 | 8.6 |
| | ≥ 10; n = 39 (48%) | 105.4 | 96.9 | 9.2 |

NOTE. Supplemental with omission of 26 patients who were deterministically given prednisone after corticosteroid random assignment stopped for patients age ≥ 10 years at diagnosis because of an increased risk of avascular necrosis. Models containing interaction terms for Age*Steroid were considered, with all *P* values nonsignificant. Interaction terms were removed for main effects testing. All models included the following covariates: age at diagnosis (dichotomous), gender, ethnicity, race, insurance status, and time off treatment. β reflects the model-based adjusted mean differences for dexamethasone – prednisone. *P* values reflect main effects tests (nonzero β). No. (%) are for overall sample of n = 166 with valid estimated IQ scores.
Abbreviations: IQ, intelligence quotient; PS, processing speed; WM, working memory.

**Table A2.** Top 10 Loci Whose Genotypes Are Associated With IQ Score

| snpid | chr.pos | A1 | A2 | β | P | MAF | Gene | Consequence | Adjacent Gene |
|---|---|---|---|---|---|---|---|---|---|
| rs12172900 | chr2:23501161 | A | G | −16.34 | 7.67E-07 | 0.06686 | — | Intergenic | APOB,−,(2234K); KLHL29,+,(107K) |
| rs10503174 | chr8:2163403 | G | A | −16.92 | 1.36E-06 | 0.05523 | — | Intergenic | MYOM2,+,(70K); CSMD1,−,(629K) |
| rs9841725 | chr3:144860888 | T | C | −8.339 | 1.71E-06 | 0.3459 | — | Intergenic | C3orf58,+,(1149K); PLOD2,−926K) |
| rs34881703 | chr18:50236279 | C | T | −11.88 | 1.93E-06 | 0.1105 | DCC | Intron | MEX3C,−,(1512K); MBD2,−,(1441K) |
| rs12626052 | chr20:4299309 | G | A | −13.56 | 2.01E-06 | 0.08092 | — | Intergenic | ADRA1D,−,(69K); PRNP,+,(367K) |
| rs4630424 | chr13:67397239 | T | C | −7.105 | 5.64E-06 | 0.4138 | PCDH9 | Intron | PCDH20,−,(5407K); KLHL1,−,(2877K) |
| rs139686757 | chr20:4300544 | A | C | −12.71 | 6.32E-06 | 0.0843 | — | Intergenic | ADRA1D,−,(70K); PRNP,+,(366K) |
| rs790346 | chr11:83656830 | G | T | 7.839 | 7.38E-06 | 0.3227 | DLG2 | Intron | DLG2,−,(263K); TMEM126B,+,(1682K) |
| rs17728992 | chr4:67286584 | C | T | −19.64 | 7.39E-06 | 0.03448 | — | Intergenic | EPHA5,−,(750K); CENPC1,−,(1051K) |
| rs12307556 | chr12:129243930 | A | T | −7.123 | 8.62E-06 | 0.3517 | — | Intergenic | TMEM132C,+,(51K); SLC15A4,−,(33K) |

Abbreviations: IQ, intelligence quotient; MAF, minor allele frequency.

**Table A3.** Top 10 Loci Whose Genotypes Were Associated With PS

| snpid | chr.pos | A1 | A2 | β | P | MAF | Symbol | Consequence | Adjacent Gene |
|---|---|---|---|---|---|---|---|---|---|
| rs9529837 | chr13:71623913 | G | T | −7.753 | 2.91E-07 | 0.4397 | LINC00348 | Intron | KLHL1,−,(941K); DACH1,−,(388K) |
| rs181359038 | chr4:92546924 | T | C | −29.91 | 1.55E-06 | 0.02616 | — | Regulatory_ region | CCSER1,+,(23K); GRID2,+,(678K) |
| rs10096173 | chr8:112767343 | T | G | 8.786 | 3.28E-06 | 0.2762 | LOC105375707 | Intron | KCNV1,−,(1780K); CSMD3,−,(467K) |
| rs803786 | chr13:71621124 | G | A | −7.083 | 4.33E-06 | 0.3879 | LINC00348 | Intron | KLHL1,−,(938K); DACH1,−,(390K) |
| rs2179621 | chr20:50572145 | A | G | 11.68 | 5.52E-06 | 0.125 | — | Intergenic | SALL4,−,(153K); ZFP64,−,(128K) |
| rs10510004 | chr10:116224579 | A | G | −7.197 | 5.86E-06 | 0.3736 | ABLIM1 | Intron | AFAP1L2,−,(60K); FAM160B1,+,(356K) |
| rs10476853 | chr5:143435722 | C | A | −23.95 | 6.55E-06 | 0.02616 | — | Intergenic | HMHB1,+,(235K); YIPF5,−,(102K) |
| rs13188793 | chr5:30102291 | A | T | −7.496 | 7.4E-06 | 0.4884 | LOC105374707 | Non_coding_ transcript_ exon | CDH9,−,(3063K); CDH6,+,(1091K) |
| rs7903720 | chr10:116226399 | A | G | −7.125 | 7.48E-06 | 0.3837 | ABLIM1 | Intron | AFAP1L2,−,(61K); FAM160B1,+,(355K) |
| rs9310717 | chr3:2400576 | T | C | −7.997 | 7.7E-06 | 0.2936 | CNTN4 | Intron | CNTN6,+,(955K); CNTN4,+,(533K) |
| rs6962537 | chr7:37480974 | T | C | −7.329 | 8.08E-06 | 0.3793 | ELMO1 | Intron | ELMO1,−,(87K); GPR141,+,(299K) |

Abbreviations: MAF, minor allele frequency; PS, processing speed.

**Table A4.** Association Between Genotype and IQ/PS Scores for Previously Reported Polymorphisms

| Previously Published Variants | | | | | | Results From AALL06N1 (N = 172) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | IQ | | PS | |
| Gene | Alias | rsID | Allele | Published Risk Allele | Associated With Phenotype in Publication | Allele Associated With Coefficient | Coefficient*** | *P* | Coefficient | *P* |
| APOE | Cys112Arg | rs429358 | C>T | T | Higher attention problems[15] | NA | Not interrogated** | | Not interrogated | |
| COMT | Val158Met | rs4680 | G>A | A | Hyperactivity/inattention[26] | A | 1.19 | .69 | −2.28 | .21 |
| GSTP1 | G313A | rs1695 | G>A | A | Problems with attentiveness*[15] | A | −0.082 | .96 | −0.98 | .61 |
| GSTP1 | C341T | rs1138272 | C>T | T | Lower digit span scores[26]; problems with attentiveness*[15] | NA | Not interrogated | | interrogated | |
| MAOA | T1460C | rs1137070 | T>C | C | Problems with variability[15] | C | 2.7 | .16 | −1.46 | .47 |
| MS | A2756G | rs1805087 | A>G | G | Problems with attentiveness and response speed[15] | G | −0.55 | .77 | −0.137 | .95 |
| MTFR | A1298C | rs1801131 | A>C | C | Problems with attentiveness[28]; lower TMTB test[27] | C | 0.156 | .93 | 0.334 | .85 |
| | GC677T | rs1801133 | G>T | T | Studied but not signficant in[28] | T | −2.993 | .077 | −2.41 | .17 |
| SLCO2A1 | | rs7625035 | A>G | G | Lower IQ and lower digit span[26] | G | 0.58 | .76 | 1.65 | .23 |
| NOS3 | | rs1799983 | G>T | T | Lower IQ and lower vocabulary[26] | NA | Not interrogated | | Not interrogated | |

Abbreviations: IQ, intelligence quotient; PS, processing speed; TMTB, Trail Making Test-B.
*Exact GSTP1 variant not specified.[15]
**Variants not interrogated on Affymetrix SNP6 or Illumina Exome Beadarray, and not imputed based on 1K genome.
***Positive coefficient indicates the allele is associated with higher IQ or PS.

**Table A5.** Top SNPs Associated With IQ/PS Scores in Previously Reported Genes

| Phenotype | SNP | CHR | BP | A1 | A2 | β | STAT | *P* | MAF | Gene | Consequence | Platform |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PS | rs5993882 | 22 | 19937533 | G | T | −5.768 | −2.853 | .004923 | 0.2281 | COMT | Intron_variant | Snp6 |
| PS | rs5993881 | 22 | 19936340 | C | T | −5.504 | −2.746 | .006727 | 0.2297 | COMT | Intron_variant | Imputed |
| PS | rs112314019 | 22 | 19929937 | A | C | −5.292 | −2.636 | .009224 | 0.2326 | COMT | Intron_variant | Imputed |
| PS | rs11703431 | 22 | 19931054 | G | A | −5.292 | −2.636 | .009224 | 0.2326 | COMT | Intron_variant | Imputed |
| PS | rs9306231 | 22 | 19930002 | G | C | −5.292 | −2.636 | .009224 | 0.2326 | COMT | Intron_variant | Imputed |
| PS | rs56121217 | 3 | 133671726 | A | G | −14.68 | −2.376 | .01869 | 0.02326 | SLCO2A1 | Intron_variant | Imputed |
| PS | rs59449467 | 3 | 133673073 | T | C | −14.68 | −2.376 | .01869 | 0.02326 | SLCO2A1 | Intron_variant | Imputed |
| PS | rs737864 | 22 | 19930159 | T | C | 4.745 | 2.358 | .01961 | 0.25 | COMT | Intron_variant | Imputed |
| PS | rs737865 | 22 | 19930121 | G | A | 4.745 | 2.358 | .01961 | 0.25 | COMT | Intron_variant | Imputed |
| PS | rs737866 | 22 | 19930109 | C | T | 4.745 | 2.358 | .01961 | 0.25 | COMT | Intron_variant | Imputed |
| IQ | rs4075873 | 3 | 133660848 | C | T | −7.451 | −3.278 | .001278 | 0.1483 | SLCO2A1 | Intron_variant | Imputed |
| IQ | rs6804798 | 3 | 133681395 | T | C | −7.19 | −3.107 | .00223 | 0.1494 | SLCO2A1 | Intron_variant | Snp6 |
| IQ | rs9866790 | 3 | 133706861 | C | T | −6.446 | −2.858 | .004824 | 0.1483 | SLCO2A1 | Intron_variant | Imputed |
| IQ | rs4241361 | 3 | 133691880 | G | C | −6.164 | −2.74 | .006829 | 0.1657 | SLCO2A1 | Intron_variant | Imputed |
| IQ | rs11714164 | 3 | 133697764 | T | G | 8.427 | 2.7 | .007663 | 0.07471 | SLCO2A1 | Intron_variant | Snp6 |
| IQ | rs174680 | 22 | 19934999 | T | C | −4.886 | −2.61 | .009895 | 0.3488 | COMT | Intron_variant | Imputed |
| IQ | rs9882333 | 3 | 133691165 | G | A | −5.868 | −2.606 | .01003 | 0.1599 | SLCO2A1 | Intron_variant | Imputed |
| IQ | rs9855403 | 3 | 133685946 | T | A | −6.177 | −2.576 | .01089 | 0.1279 | SLCO2A1 | Intron_variant | Imputed |
| IQ | rs34616463 | 3 | 133667507 | C | T | −17.72 | −2.555 | .01155 | 0.01744 | SLCO2A1 | Synonymous_variant | Imputed |
| IQ | rs6804465 | 3 | 133680963 | A | G | −17.72 | −2.555 | .01155 | 0.01744 | SLCO2A1 | Intron_variant | Imputed |

Abbreviations: BP, base pair; CHR, chromosome; IQ, intelligence quotient; MAF, minor allele frequency; PS, processing speed; SNP, single-nucleotide polymorphism; STAT, statistics.