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Maternal Serotonin Levels Are Associated With Cognitive Ability and Core Symptoms in Autism Spectrum Disorder

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Abstract

Background—Biomarker and neuroimaging findings implicate the serotonin (5-HT) system in autism spectrum disorder (ASD). Recent findings in mice indicate that the maternal 5-HT system influences embryonic neurodevelopment.

Methods—Whole blood serotonin (WB5-HT) levels were obtained from 181 children diagnosed with Autism Spectrum Disorder, 99 of their fathers and 119 of their mothers. Standardized assessments were used to evaluate cognitive, behavioral, and language phenotypes.

Results—Linear regression demonstrated a significant positive relationship between maternal WB5-HT and nonverbal IQ ($F_{1,115} = 3.977, p = .049$), and a significant negative relationship with ADI-R Domain B- Nonverbal Communication Algorithm scores, ($F_{1,117} = 7.762, p = .006$), indicating that higher maternal WB5-HT levels tended to be associated with less deficit in these domains. After correcting for proband age, there was also a significant relationship between maternal WB5-HT and overall adaptive function on the Vineland Adaptive Behavior Scales-II ($F_{2,112} = 6.536, p = .002$). Latent class analysis identified a three-class structure in the assessment data, describing children with low, intermediate, and high severity across measures of behavior, cognition, and adaptive function. Mean maternal WB5-HT differed across classes with the lowest maternal WB5-HT levels seen in the highest severity group, (Welch's $F(2, 46.048) = 17.394, p < .00001$).

Conclusion—These findings suggest that the maternal serotonin system may affect neurodevelopment in humans, as it does in mice. Further studies in animal models may be able to reveal the mechanisms underlying these findings.

Introduction

Several serotonergic measures have been reported to be altered in autism spectrum disorder (ASD), but the role of serotonin (5-hydroxytryptamine, 5-HT) in relation to ASD risk and outcome remains poorly understood. Elevated whole blood 5-HT, termed hyperserotonemia, is present in approximately 30% of children with ASD¹ and is not associated with intellectual disability in the absence of ASD². Within the blood, more than 99% of 5-HT is stored in platelets, taken up via the serotonin transporter (SERT)³. Although best known for its role in neurotransmission, 5-HT also acts as a critical signaling molecule during neurodevelopment⁴. In early gestation, prior to the arrival of serotonergic projections from the midbrain raphe nuclei, the developing forebrain relies on peripheral and placental sources of 5-HT^{5,6}. Further developmental effects of 5-HT occur during critical periods in postnatal life, when activity-dependent mechanisms remodel neural circuitry⁷. Despite known roles of 5-HT both during development and in regulation of mood, anxiety, and cognition in adults, the hyperserotonemia biomarker has not been consistently associated with specific phenotypes in ASD⁴.

Variation in whole blood serotonin (WB5-HT) level is largely genetically driven, with a high broad heritability of 0.99^{8,9}. No 5-HT system genes have consistently been identified as harboring rare, *de novo* variants in simplex ASD¹⁰; however, linkage studies in multiplex families implicate the chromosome 17q region harboring the serotonin transporter (SERT) gene *SLC6A4*^{11–14}, in affected males only. Several rare hyper-functional SERT variants, including Gly56Ala, were identified in these linkage families^{11,15}.

Recent findings in a genetic mouse model of the SERT Ala56 variant indicated a surprising maternal genotype effect on fetal neurodevelopment¹⁶. SERT Ala56 knock-in mice show increased SERT activity, with platelet hyperserotonemia, altered central 5-HT function, and abnormal social and repetitive behavior¹⁷. Maternal SERT Ala56 genotype was associated with decreased placental and embryonic forebrain 5-HT levels at mid-gestation, when hindbrain 5-HT projections have not yet reached the forebrain, compared to wild-type controls. In contrast, the genotype of the embryo itself had no effect on placental, forebrain, or hindbrain 5-HT levels¹⁶. Changes in the development of serotonin-sensitive projections from the thalamus to the cortex were also observed in association with maternal but not embryo SERT Ala56 genotype^{18,19}.

In candidate gene association studies, the largest analyses report an association between ASD risk and the short allele of the promoter length polymorphic repeat (5-HTTLPR)²⁰ but with inconsistent findings across studies⁴. One analysis found that ASD risk was separately associated with the long allele in mothers and the short allele in probands²¹, highlighting the potential for maternal genetic variation to impact embryonic neurodevelopment in ASD independently of offspring genotype. Maternal genotype effects have also been reported in mouse models with disruptions in other serotonin system genes. For example, Cote and colleagues described cortical abnormalities in embryos from dams lacking the tryptophan hydroxylase gene (*Tph1*)²², without abnormalities due to the embryo genotype alone. In parallel, dams lacking the serotonin 5-HT_{1A} receptor gene (*Htr1a*) have offspring with increased anxiety-like behavior, regardless of the offspring's own genotype²³. Both effects presumably derive from the action of the maternal 5-HT system during pregnancy.

Based upon emerging evidence pointing to maternal genotype and the serotonergic system as contributors to offspring neurodevelopment and behavior, we sought to examine whether the maternal 5-HT system is associated with phenotypes in children with ASD, using proband and paternal comparisons as internal controls. Multiple common and rare genetic variants have been described in the serotonin system^{20,22,23}, with little indication of how to integrate them to produce a composite measure of 5-HT system function. We therefore assessed the relationship between offspring phenotypes and maternal whole blood 5-HT level as an indicator of maternal serotonergic functioning that likely reflects the impact of multiple genetic loci.

Methods

Collection of samples was approved by the Institutional Review Board at the University of Illinois at Chicago (UIC) and the University of Texas Southwestern Medical Center (UTSW). Included probands had a diagnosis of Autistic Disorder, Asperger's Disorder or

Pervasive Developmental Disorder Not Otherwise Specific based on DSM-IV-TR criteria²⁴, confirmed with both the Autism Diagnostic Observation Schedule (ADOS)²⁵ and the Autism Diagnostic Interview-Revised (ADI-R)^{26,27}. All standardized assessments were administered by raters without knowledge of WB5-HT measurements.

For data obtained from the ADOS²⁵ standardized observations, revised algorithms²⁸ were used to calculate raw scores, then calibrated Severity Scores (CSS) were calculated for the Social Affect (SA) and Restricted and Repetitive Behavior (RRB) domains^{28–32} of the ADOS²⁵, to allow for comparison across the four ADOS modules available for individuals of differing ages and language levels. Calibrated scores were unable to be calculated for 4 participants who received a module administration outside of the age range for which CSS scores can be calculated. Social Affect CSS (SA-CSS) scores range from 1 to 10. The RRB domain has a limited range of possible raw scores, so the Restricted and Repetitive Behavior CSS (RRB-CSS) is mapped onto a 10 point scale to aid interpretation and compatibility with the SA-CSS³⁰. A score of 1 on the RRB-CSS indicates that no repetitive behaviors were observed during the ADOS, values of 2, 3 and 4 do not exist, then scores between 5–10 indicate increasing severity of observed repetitive behaviors³⁰. The abnormal distribution of these scores was not corrected with standard statistical transformations, so scores of 1 (indicating absence of repetitive or restricted behaviors) were recoded as 4 so that the RRB-CSS could be used as a continuous measure in subsequent analyses.

Further data regarding core symptoms of ASD was obtained using the ADI-R, a standardized interview conducted with caregivers of individuals suspected of having ASD^{26,33}. To allow for comparison across the two diagnostic algorithms for children of differing ages³⁴, we included data from the 26 algorithm items that are common to both, omitting algorithm items that are age- or language-dependent. As nonverbal children are not scored on roughly 25% of ADI-R items overall, this approach avoids non-random missing data³². All children received a total non-verbal communication score ($n = 188$) but not a verbal score ($n = 159$), and these scores were strongly correlated ($r_s = 0.88$, $p < 0.001$). Therefore, we used the total non-verbal score to reflect communication ability in subsequent analyses.

Language was examined using the standardized total scores from the Peabody Picture Vocabulary Test (PPVT-4)³⁵. Verbal and nonverbal IQ scores were derived from a developmental hierarchy of cognitive assessments including the Differential Ability Scales – Second Edition (DAS-II, $n = 137$)³⁶, the Mullen Scales of Early Learning ($n = 23$)³⁷, Wechsler Abbreviated Scale of Intelligence (WASI, $n = 19$)³⁸, and the Raven's Progressive Matrices IQ Test ($n = 5$)³⁹. Adaptive function was assessed using the VABS-II⁴⁰ semi-structured interview with parents or caregivers, with the Adaptive Behavior Composite (ABC) standard score providing a measure of overall adaptive function in subsequent analyses. Finally, stereotyped, self-injurious, compulsive, ritualistic, and restricted behaviors were further assessed with the Repetitive Behavior Scale – Revised (RBS-R⁴¹), with the overall total score from Lam and Aman's (2007) validation and factor analysis used in our subsequent analyses⁴².

Fasting whole blood was obtained by venipuncture using BD Vacutainer tubes containing EDTA and stored at -70°C until WB5-HT assay by high-performance liquid chromatography³. WB5-HT is a reliable measure of serotonin because over 99% of 5-HT is stored in the platelet, while the measurement of platelet 5-HT in platelet-rich plasma can be affected by variable and selective platelet yield and potential 5-HT release during centrifugation⁴³. WB5-HT levels were unavailable for 7 included probands, and were available for all included mothers and fathers.

Statistical Analyses

The distributions of whole blood 5-HT levels were assessed with histograms and Pearson's bivariate correlations (two-tailed) were used to assess for within-family associations between mother, father, and child. Multiple linear regression modelling was used to assess the relationships between each standardized measure of proband phenotype and WB5-HT levels for the maternal, paternal, and proband groups. Given previously reported effects of sex and pubertal status on WB5-HT levels in children with ASD^{44,45}, proband age and sex were included as covariates to assess for evidence of confounding and/or effect modification. Using the backward elimination modelling approach, initial maximal models for each outcome included WB5-HT level, age, sex, and the interaction terms WB5-HTxage and WB5-HTxgender. Non-significant interaction terms were individually dropped from the model in succession. Age and sex were retained in the model as confounders if their coefficient was significant or if their removal distorted the study factor (WB-5HT) coefficient by more than 10%. Model diagnostics were performed, and all assumptions underlying multiple linear regression analysis were satisfied.

Finally, latent class analysis (LCA) was conducted using behavioral summary measures as indicators, to assess our hypothesis in a multivariate context without the limitations of multiple testing associated with individual analysis of multiple outcome measures. The patterns of phenotype represented in our data were assumed to be characterized by an underlying latent categorical variable, with the number of classes determined by indices of goodness of fit. LCA yields predicted probabilities of class membership, and cases were assigned to their most likely class based on these probabilities. Mean scores of indicators (cognitive, behavioral, or language measures) were examined, and finally, mean maternal, paternal, and proband WB5-HT levels were compared by class. Positively skewed distributions were corrected with logarithmic transformation prior to analysis using standard one-way ANOVA. Welch's ANOVA was used when heterogeneity of variances was indicated by Levene's test. The type I error rate was set at 0.05. Descriptive, regression and one-way ANOVA analyses were performed using SPSS Version 24, and latent class analysis was performed in Mplus Version 7.4.

Participant Characteristics

Data was available for 221 children with ASD, and 389 biological parents (214 mothers and 175 fathers). Probands, mothers and/or fathers were excluded from analyses if they were taking medications acting on the 5-HT system, including reuptake inhibitors and mixed dopamine and serotonin receptor antagonists ($n = 8$ probands, 36 mothers, and 14 fathers). 22 probands were excluded because ASD diagnosis was not confirmed on both the ADOS-2

and ADI-R. 1 excluded proband had no available WB5-HT level and no included parents. 2 siblings were excluded, and 39 parents (18 fathers and 21 mothers) were excluded due to proband exclusion. 44 fathers and 38 mothers were excluded due to lack of WB5-HT levels. Following these exclusions, the final sample included 188 eligible children with ASD (male = 157, female = 31), and 218 biological parents (119 mothers and 99 fathers) (Table 1). Further descriptive information regarding excluded subjects is available in the supplementary materials, available online.

Mean proband age was 9.7 years (SD = 5.2 years). Mean maternal age at WB5-HT assay was 41.4 years (SD = 6.8 years), and mean paternal age was 43.3 years (SD = 7.5 years). Mean proband WB5-HT level was 260.3 ng/mL (SD = 110.3 ng/mL), mean maternal WB5-HT was 160.8 ng/mL (SD = 71.9 ng/mL) and mean paternal WB5-HT was 141.3 ng/mL (SD = 62.7 ng/mL). The distribution of proband, maternal, and paternal WB5-HT levels [see Figure S1, available online] were positively skewed compared to a normal distribution. Proband outcomes across the standardized measures of phenotype are summarized in Supplementary Table S1, available online.

Results

Bivariate correlation analyses identified a significant association between proband WB5-HT levels and both maternal WB5-HT levels ($r_s = 0.29$, $p = 0.002$), and paternal WB5-HT levels ($r_s = 0.38$, $p < 0.001$). In addition, maternal and paternal WB5-HT levels were significantly correlated ($r = 0.25$, $p = .031$).

Significant relationships were identified between maternal 5-HT and nonverbal IQ ($F_{1,115} = 3.977$, $p = .049$), the VABS-II Overall Adaptive Behavior Composite Score ($F_{1,113} = 6.225$, $p = .014$) and the ADI-R Domain B (Nonverbal Communication) Algorithm Score ($F_{1,117} = 7.762$, $p = .006$) (see Figure S2, available online). Bivariate regression identified no significant relationships between proband or paternal WB5-HT and outcomes of behavior, language or cognition.

Backward elimination modelling identified no evidence of effect modification in any analysis for the maternal, paternal, or proband groups. Final regression models containing significant predictors and/or confounding variables (whose absence distorted the WB5-HT coefficient by >10%) for the maternal group are presented in Table 2.

The final regression analysis demonstrated no confounders for the significant positive relationship between maternal WB5-HT and nonverbal IQ ($F_{1,115} = 3.977$, $p = .049$). After correcting for proband age, there was a significant relationship between maternal WB5-HT and overall adaptive function on the VABS-II ($F_{2,112} = 6.536$, $p = .002$). Maternal WB5-HT was significantly and negatively associated with the ADI-R Domain B- Nonverbal Communication Algorithm score, ($F_{1,117} = 7.762$, $p = .006$), indicating that higher maternal WB5-HT levels tended to be associated with less deficit in this domain. Proband age and sex did not confound this relationship. Corresponding regression coefficients indicate that for every 10ng/mL increase in maternal WB5-HT, the average increase in NVIQ was 0.59 ($p = .018$) and the average increase in total VABS-II Adaptive Behavior Composite score was 0.41

($p = .006$), while the average decrease in ADI-R Nonverbal Communication Algorithm score was 0.11 ($p = .006$). Relationships at the level of a trend were also noted between maternal WB5-HT and scores on the ADOS-2 RRB CSS ($F_{1,115} = 2.829$, $p = .095$), and standard scores on the PPVT-4 ($F_{1,81} = 3.333$, $p = .072$), with no evidence of confounding by proband age or gender.

The final regression models identified no significant relationships between paternal WB5-HT and outcomes of proband phenotype (see table S2, available online). After correcting for proband age, an isolated significant relationship between proband WB5-HT and the ADOS-2 RRB CSS score was noted ($F_{2,174} = 5.235$, $p = .006$). No other significant relationships between proband WB5-HT and phenotype outcome measures were identified (see table S2, available online).

Latent Class Analysis

Latent class analysis was performed on an ‘a priori’ basis to group probands of similar phenotype into categories containing individuals who score similarly to each other across the range of available outcome measures. Indicator variables included in this analysis were Nonverbal IQ, ADI-R Total Social Reciprocal Behavior, ADI-R Total Non-Verbal Communication, ADI-R Total Restricted and Repetitive Behavior, ADOS-II Social Affect CSS and ADOS-2 RRB CSS, and VABS-II Adaptive Behavior Composite. As verbal IQ estimates were unavailable for some nonverbal subjects and correlated strongly with NVIQ ($r(175) = .739$, $p < .001$), we included NVIQ and omitted VIQ to avoid non-random missing data and problematic within-class correlation. The ADI-R Algorithm Domain Scores were included to represent features of ASD phenotype reported on history, and ADOS-2 Social Affect and Restricted, Repetitive Behavior Calibrated Severity Scores were included to represent features of ASD phenotype noted during clinical observation. The RBS-R total score was considered redundant given the inclusion of ADI-R Domain C Algorithm Scores to represent restricted and repetitive behaviors on history. The PPVT-4 total standard score was omitted due to missing data.

Latent class analysis postulates that the association among observed variables is due to an underlying class structure, and assumes local independence within categories of latent classes – i.e. that the relatedness between a set of variables is zero within the categories of latent classes⁴⁶. The objective is to identify the model that best describes the latent class structure within the dataset, starting with a 1-class model and then fitting successive models with increasing numbers of classes. Models are estimated using maximum likelihood techniques, such that there are several solutions around which a model can converge (local maxima). To ensure that global maximum was identified, we ran at least 200 starts and 20 iterations for each solution. The resulting class structures are described using latent class probabilities (the probability of membership to each latent class), prevalence estimates (the proportion of individuals assigned to each class), and the mean variable outcomes by class. Goodness-of-fit statistics aided in selection of the optimal model (Table 3), including the log likelihood ratio, with higher values supporting models of better fit, and the Bayesian Information Criterion (BIC) and Akaike Information Criterion (AIC), with smaller values supporting models of better fit and parsimony⁴⁷. The entropy statistic ranges from 0 to 1 and

measures the classification accuracy of individuals based on posterior probabilities, with values closest to 1 reflecting better classification⁴⁸. Finally, the Lo-Mendell-Rubin Adjusted Likelihood Ratio Test (LMR-LRT) was used to compare models with different numbers of classes⁴⁹, with non-significant values suggesting that a model with one fewer class is a better explanation of the data.

The non-significant Lo-Mendell-Rubin likelihood ratio test⁴⁹ associated with the four-class solution suggested that the three-class solution was a better representation of the data. The lowest BIC and highest entropy values were associated with the three-class solution, and the changes in log likelihood, AIC and sample-size adjusted BIC between the three and four class models were minimal compared to the difference in these values between the two and three class models. Finally, the three-class solution was substantively meaningful, describing a class structure based on mild, moderate, and high severity across the measures of proband phenotype (Figure 1). This structure is supported by previously reported attempts to identify clinical subgroups within ASD using latent class analysis, which have consistently described between two to four classes distinguished by ASD symptom severity and intellectual capacity^{50,51}. The results of the three-class solution are in Supplementary Table 3, available online.

The mean maternal WB5-HT was statistically significantly different by phenotype severity group (Welch's $F(2, 46.048) = 17.394, p < .00001$). Mean maternal WB5-HT decreased from the mildest severity group ($n = 27, M = 181.49, SD = 92.62$), to moderate severity group ($n = 77, M = 164.04, SD = 64.84$), to highest severity group ($n = 15, M = 106.75, SD = 29.32$) [Figure 2]. Games-Howell post hoc analysis indicated that the mean increase in maternal WB5-HT from the highest severity group to the moderate severity group ($\eta^2 = .57.29, 95\% \text{ CI } [31.67-82.91]$) was statistically significant ($p < .00001$), as was the increase in maternal WB5-HT from the highest severity group to the mildest severity group ($\eta^2 = .74.74, 95\% \text{ CI } [27.29-122.18], p = .0001$). Neither mean paternal WB5-HT ($F(2, 96) = .605, p = .548$) nor mean proband WB5-HT ($F(2, 178) = .492, p = .612$) was statistically significantly different by phenotype severity group.

Discussion

Given the risk of Type I error associated with multiple available measures of proband phenotype, latent class analysis allowed us to assess our hypothesis in a multivariate context without the limitations of multiple testing associated with individual analysis of multiple outcomes. Consistent with our hypothesis, we identified a relationship between maternal WB5-HT levels and phenotypes in offspring with ASD, with higher maternal WB5-HT levels associated with milder proband phenotype severity across measures of cognition, adaptive behavior, language, and restricted, repetitive behaviors. Mean maternal WB5-HT levels were significantly different between phenotype severity classes identified using latent class analysis. After correcting for proband age, a significant relationship between proband WB5-HT and restricted and repetitive behavior on the ADOS-2 RRB CSS was noted, which may align with previous inconsistent reports of an association between WB5-HT and repetitive behaviours in children with ASD⁴. However, this was an isolated finding, and significant relationships were not seen on other measures of repetitive behavior (e.g. the

ADI-R Domain C Algorithm score, or total score on the RBS-R). Finally, despite stronger within-family association between paternal WB5-HT and proband WB5-HT levels compared to maternal WB5-HT and proband WB5-HT levels, there were no significant relationships between paternal WB5-HT level and any outcome measure in our sample.

Our findings add to the body of emerging evidence indicating that maternal genotype and the maternal 5-HT system may contribute to offspring neurodevelopment and behavior. The placenta is a vital source of 5-HT for the developing forebrain in early gestation^{4,5}; however, maternal WB5-HT does not cross the placenta into the fetal circulation¹⁶ and does not appear to exert its influence on fetal neurodevelopment directly, at least in rodents. Maternal WB5-HT may impact the function of the placental 5-HT system directly or indirectly, but this biomarker could also be an indicator that some component of the peripheral maternal 5-HT system, such as synthesis, uptake, or release, impacts both WB5-HT and placental function. Further study will be necessary to understand the mechanisms underlying this association.

The surprising directionality of effects in this study – with higher maternal WB5-HT levels associated with less severe proband phenotype – may suggest that the impact of the maternal 5-HT system on ASD risk may differ in subgroups of ASD with different patterns of cognitive and behavioral phenotypes. The lowest maternal WB5-HT values were seen in the most severely affected latent class, in which *de novo*, gene disrupting variants are most likely to be seen^{4,10,52,53}. Our findings could indicate that the influence of the maternal serotonin system in ASD is more likely to be relevant in the ASD subpopulation with greater contribution from polygenic risk and less contribution from *de novo* mutations⁴. Notably, the 5-HT system is largely absent in the list of high confidence ASD genes with *de novo*, gene disrupting mutations¹⁰. Finally, although considerable attention has previously focused on the possibility that serotonin reuptake inhibitors (SRIs) could be relevant to ASD risk^{54–60}, in the absence of a control population, our findings do not allow us to draw clear conclusions about the possible contribution of the maternal serotonin system to ASD risk in the general population.

Further study will be required to replicate and dissect the mechanisms underlying the intriguing finding of a relationship between maternal WB5-HT levels and cognitive, language, and behavioral outcomes in offspring with ASD. In this study, maternal WB5-HT was assessed cross-sectionally at the time of study entry, but a better approach would be prospective collection of WB5-HT during pregnancy, when the impact of the maternal serotonin system would be expected to occur, with longitudinal follow-up to assess cognitive function and behavior, including ASD symptoms. The high broad heritability of 0.99 for WB5-HT levels may suggest relative stability over time^{8,9}, but levels have been shown to vary across pregnancy in mice¹⁶. Since studies in mice identify an impact of the maternal serotonin system on brain structure in embryos and neonates^{16,22}, an ideal study design would incorporate prospective measurement of maternal WB5-HT levels, early postnatal neuroimaging, and later assessment of cognitive, language, and ASD traits in offspring. Further studies in mice could assess interplay between the maternal and developing embryo serotonin systems as well as identify the specific brain regions and mechanisms underlying altered behavior.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1: Mean outcomes on phenotype measures by latent class

Fig. 1: Latent class analysis identified a three-class structure in the assessment data, describing children with low, intermediate, and high severity across measures of behavior, cognition, and adaptive function.

Figure 2: Mean Maternal WB5-HT by Proband Phenotype Severity Group

Fig. 2: Mean maternal WB5-HT (denoted by 'X') was significantly different between phenotype severity groups (Welch's $F(2, 46.048) = 17.394$, $p < .00001$).

Table 1.

Participant Characteristics

	Probands	Mothers	Fathers
<i>n</i>	188	119	99
Sex (<i>n</i> , %)	157 male, (83.5%) 31 female, (16.5%)	-	-
Mean age in years (SD)	9.7 (5.2)	41.4 (6.8)	43.3 (7.5)
Ancestry (<i>n</i>)			
Caucasian (Non-Hispanic)	106 (56.4%)	58 (48.7%)	67 (67.7%)
Caucasian (Hispanic)	35 (18.6%)	27 (22.7 %)	18 (18.2%)
African American	30 (16.0%)	25 (21.0%)	8 (8.1%)
Asian	7 (3.7%)	6 (5.0 %)	4 (4.0%)
Mixed	10 (5.3%)	3 (2.5%)	2 (2.0%)
Available WB5-HT Level (<i>n</i>)	181	119	99
Mean WB5-HT in ng/mL (SD)	260.3 (110.3)	160.8 (71.9)	141.3 (62.7)
Range (ng/mL)	51.8 – 787.8	34.0 – 367.0	27.0 – 322.1

Table 2:

Final Regression Models for Maternal Analyses

Outcome			Regression Coefficient B	Standard Error SE _B	Standardized Coefficient β	p value
Verbal IQ	$F(2, 109) = 2.431$, adj. $R^2 = .025$, $p = .093$	Constant Maternal WB5-HT Proband Age	66.225 .020 .074	6.630 .031 .037	- .062 .190	- .511 .046
NVIQ	$F(1, 115) = 3.977$, adj. $R^2 = .025$, $p = .049$	Constant Maternal WB5-HT	73.731 .059	5.242 .030	- .183	- .049*
VABS-II Adaptive Composite Standard Score	$F(2, 112) = 6.536$, adj. $R^2 = .089$, $p = .002$	Constant Maternal WB5-HT Proband Age	71.428 .041 -.043	3.117 .015 .017	- .251 -.230	- .006* .012
ADI-R Algorithm A - Reciprocal Social Interaction	$F(2, 116) = .318$, adj. $R^2 = -.012$, $p = .729$	Constant Maternal WB5-HT Proband Age	18.204 -.004 .004	1.286 .006 .007	- -.056 .053	- .549 .567
ADI-R Algorithm B - Nonverbal Communication	$F(1, 117) = 7.762$, adj. $R^2 = .054$, $p = .006$	Constant Maternal WB5-HT	11.613 -.011	.720 .004	- -.249	- .006*
ADI-R Algorithm C Restricted, Repetitive or Stereotyped Behaviors	$F(1, 117) = 1.718$, adj. $R^2 = .006$, $p = .193$	Constant Maternal WB5-HT	5.159 -.003	.441 .003	- -.120	- .193
ADOS-2 Social Affect Calibrated Severity Score	$F(1, 115) = 1.042$, adj. $R^2 = .000$, $p = .310$	Constant Maternal WB5-HT	7.992 -.002	.386 .002	- -.095	- .310
ADOS-2 Restricted and Repetitive Behaviors Calibrated Severity Score	$F(1, 115) = 2.829$, adj. $R^2 = .016$, $p = .095$	Constant Maternal WB5-HT	8.228 -.004	.405 .002	- -.155	- .095^
PPVT-4	$F(1, 81) = 3.333$, adj. $R^2 = .028$, $p = .072$	Constant Maternal WB5-HT	68.056 .074	6.713 .040	- .199	- .072^
RBS-R	$F(2, 113) = .309$, adj. $R^2 = -.012$, $p = .735$	Constant Maternal WB5-HT Proband Age	34.868 -.005 -.021	5.373 .025 .029	- -.018 -.070	- .846 .460

Table 3.

Goodness-of-fit statistics

Solution	No. of Free Parameters	Log Likelihood	Akaike Information Criterion (AIC)	Bayesian Information Criterion (BIC)	Sample-size adjusted BIC	Entropy	Lo-Mendell-Rubin Likelihood Ratio Test ^a	
							2 times the log likelihood difference	p value
1-class	14	−3721.070	7470.140	7515.451	7471.106	-	-	-
2-class	22	−3632.942	7309.885	7381.087	7311.402	0.815	176.256	0.0111
3-class	30	−3593.819	7247.639	7344.732	7249.708	0.830	78.246	0.0009
4-class	38	−3579.807	7235.614	7358.599	7238.235	0.827	28.025	0.2208

^aThe Lo-Mendell-Rubin likelihood ratio test (LMR-LRT) is a test of probability that the data was generated from a model with one fewer class⁴⁹.