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WHOLE BLOOD SEROTONIN AND PLATELET 5-HT_{2A} BINDING

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Abstract

Elevated whole blood serotonin (WB5-HT) is a well-replicated biomarker in autism spectrum disorder (ASD). Decreased platelet serotonin receptor 5-HT_{2A} binding has been reported in ASD. WB5-HT levels and platelet 5-HT_{2A} specific binding were obtained from 110 individuals with ASD and 18 controls. Individuals with ASD had significantly higher WB5-HT levels than controls. There was no difference in the platelet 5-HT_{2A} specific binding between groups. Multiple regression analyses revealed that platelet 5-HT_{2A} binding significantly predicted WB5-HT in the control sample but not in the ASD sample. These results indicate that the relationship between WB5-HT and platelet 5-HT_{2A} binding differs depending on ASD diagnosis, suggesting differences in platelet 5-HT system regulation in ASD.

Keywords

autism spectrum disorder; biomarker; serotonin; hyperserotonemia; receptor in Autism Spectrum Disorder

The etiology and genetics of autism spectrum disorder (ASD) are incompletely understood. While there are some known genetic and environmental risk factors for ASD, these only account for the minority of the population. Some of these factors include advanced maternal and paternal age, prenatal exposure to valproic acid, short inter-pregnancy interval, fragile X syndrome, and tuberous sclerosis (Lord et al. 2018). Research on the genetics of ASD has explored the role of various neurotransmitters, including serotonin. Over 25% of individuals with autism spectrum disorder (ASD) have hyperserotonemia, or elevated whole blood serotonin (5-hydroxytryptamine, 5-HT) levels (Anderson et al. 1990; Gabriele et al. 2014). Despite decades of study, the mechanisms underlying this well-replicated biomarker and the contributions of the serotonin system in ASD remain unclear (Muller et al. 2016). Nearly all whole blood 5-HT is located in the platelet (Anderson et al. 1987), and various mechanisms could explain elevated platelet 5-HT, including 5-HT synthesis in the intestine, 5-HT uptake into the platelet as it passes through the enteric circulation, or differences in the release of 5-HT, potentially related to changes in 5-HT receptor expression or function (Cook et al. 1993; Oblak et al. 2013; Muller et al. 2016). The 5-HT receptor with the best characterized role in platelet function is the serotonin 5-HT_{2A} receptor. This receptor enhances platelet functions induced by adenosine diphosphate (ADP) signaling, such as phosphatidylserine (PS) exposure and fibrinogen receptor activation (Lin et al. 2014). Overall, the 5-HT_{2A} receptor enhances platelet aggregation (Oliver et al. 2016). While the mechanisms connecting platelet 5-HT levels and platelet 5-HT_{2A} receptor expression are incompletely known, previous reports of correlations between the two suggest some level of reciprocal regulation (Cook et al. 1993; Goldberg et al. 2009).

Serotonin receptor 5-HT₂ binding has been studied extensively in ASD. In the current paper, “5-HT₂” is used to indicate binding studies with methods that do not allow for differentiation between the 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors. In contrast, “5-HT_{2A}” is used to indicate binding studies in which the use of a ligand or a combination of ligands allows for specific implication of the 5-HT_{2A} receptor. Findings **about the 5-HT₂ receptor in ASD** have not been consistent in platelets, with McBride et al. reporting that children with autism had lower densities of 5-HT₂ receptors (by (¹²⁵I)-2-iodo-lysergic acid diethylamide binding) in comparison to controls, and Perry et al. concluding that the density of 5-HT₂ receptors (by ¹²⁵Iodospiroperidol binding) did not differ between children with autism, their first-degree relatives, or controls (McBride et al. 1989; Perry et al. 1991). In one study, hyperserotonemic first-degree relatives of children with autism had lower densities of 5-HT₂ receptors (by [3H]-LSD binding) in their platelets in comparison to normoserotonemic relatives (Cook et al. 1993). Further, 5-HT₂ receptor densities in the platelet were negatively correlated with whole blood 5-HT levels in these first-degree relatives (Cook et al. 1993).

Previous work has also studied 5-HT₂ receptor activity in the brain. In a PET study, individuals with autism had lower density of 5-HT_{2A} receptors (by DOI binding) in the cingulate cortex and fusiform gyrus than unaffected individuals (Oblak et al. 2013). In a SPECT study, adults with Asperger’s syndrome had lower cortical 5-HT_{2A} binding (by (¹²³I)-5-I-R91150 binding) than unaffected adults (Murphy et al. 2006). Further, a PET study found that parents of two or more individuals with ASD had lower 5-HT₂ density (by

[¹⁸F]setoperone binding) in the cortex in comparison to controls, and that these cortical 5-HT₂ receptor densities were negatively correlated with platelet 5-HT levels (Goldberg et al. 2009).

In addition to previous work suggesting differences in 5-HT_{2A} receptor activity in the platelet and the brain in ASD, the 5-HT_{2A} receptor has also been implicated in the treatment of ASD. 5-HT_{2A} antagonists are often used in ASD treatment. For example, risperidone acts as a dopamine D₂ and 5-HT_{2A} receptor antagonist and aripiprazole acts as a partial agonist of dopamine D₂ and 5-HT_{1A} receptors and as an antagonist of 5-HT_{2A} receptors. Both medications have been shown to improve irritability and stereotyped behaviors in ASD (Fung et al. 2016). Some work in animal models related to ASD also focuses on the 5-HT system, including the finding that 5-HT_{2A} receptor antagonists reduce repetitive grooming in the BTBR mice, a behavioral mouse model with social deficits and repetitive behavior (Amodeo et al. 2016). It is unclear how to reconcile response to 5-HT_{2A} receptor antagonists with the finding that individuals with ASD have lower densities of 5-HT₂ receptor binding (McBride et al. 1989).

Despite decades of study, the role of the 5-HT_{2A} receptor in relation to hyperserotonemia in ASD remains incompletely understood. Previous work evaluating the 5-HT_{2A} receptor in the platelet has been inconsistent, and few studies have evaluated the relationship between 5-HT_{2A} receptor binding and WB5-HT levels. The current study aims to further elucidate the 5-HT₂ receptor functions in ASD by evaluating the relationship between platelet 5-HT_{2A} specific binding and whole blood 5-HT levels in a sample of individuals with ASD in comparison to a sample of control individuals. We hypothesized that the relationship between platelet 5-HT_{2A} specific binding and whole blood 5-HT levels in individuals with ASD would be significantly different than the relationship in control individuals.

Methods

Data collection was approved by the Institutional Review Board at the University of Illinois at Chicago (UIC) and the University of Texas Southwestern Medical Center (UTSW), in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Participants were recruited through advertising and reaching out to patients applying for services in UIC clinics and affiliates of the Midwest Autism Consortium (MAC) about participating in **a larger study** at the UIC Autism Center of Excellence (ACE). The larger study evaluated the relationship between WB5-HT, ASD symptoms, and brain activation patterns during specific tasks (Chen et al. 2017; Francis et al. 2016; Levin-Decanini et al. 2013; Shuffrey et al. 2017). The inclusion criteria for individuals to participate in this larger study were: aged 3 to 55 years; a diagnosis of ASD or suspected ASD from an initial screening; nonverbal IQ ≥ 35; individual with ASD not taking selective serotonin reuptake inhibitors, antidepressants, stimulants, atypical antipsychotics, or antipsychotics; biological parent(s) willing and able to participate; no history of significant medical or neurologic disorder, including specifically an absence of a known metabolic or genetic disorder. Fragile X DNA testing was performed for all participants and led to exclusion at screening. Informed consent was obtained from all individual participants included in the study.

Participants were administered the Autism Diagnostic Observation Schedule (ADOS) (Lord et al. 1999; Gotham et al. 2007; Gotham et al. 2009; Hus and Lord 2014) and/or the Autism Diagnostic Interview-Revised (ADI-R) (Rutter et al. 2003; Risi et al. 2006). All but nine of the included ASD participants met criteria for ASD on both the ADOS and the ADI-R. Five of these nine participants met on the ADI-R but not on the ADOS, two of the participants met on the ADOS but not on the ADI-R, and two adult participants met on the ADOS but the ADI-R was not completed because their primary caregivers from childhood were not available. All included participants in the ASD sample had a best estimate clinical diagnosis of Autism Spectrum Disorder (Autistic Disorder, Asperger's Disorder or Pervasive Developmental Disorder Not Otherwise Specific based on DSM-IV-TR criteria) (American Psychiatric Association 2000) based on all clinical information. Included participants in the Control sample were assessed using the ADOS (Lord et al. 1999) or the ADI-R (Rutter et al. 2003; Risi et al. 2006) in order to confirm that they did not meet diagnostic criteria for autism spectrum disorder. The control participants had no known psychiatric problems or pervasive developmental disorders in self, 1st or 2nd degree family members. All standardized assessments were administered by experienced raters without knowledge of blood measurements. Both ASD and control participants were excluded from the analysis if they were taking any medication affecting the serotonin system, including serotonin reuptake inhibitors, stimulants, and atypical antipsychotic medications. Eight participants were excluded due to medication use, four participants were excluded due to lack of diagnostic confirmation with the ADI-R or ADOS, and two older siblings with ASD were excluded because their younger sibling with ASD was included. Nine participants did not have whole blood 5-HT (WB5-HT) values, and 100 participants did not have platelet 5-HT_{2A} specific binding values. Following exclusions, a total of 110 participants with ASD were included in the analysis, and a total of 18 controls.

Full Scale IQ was assessed for the participants with ASD using the following measures: the Differential Abilities Scale, Second Edition (DAS-II, N = 75) (Elliot 2007), the Wechsler Abbreviated Scale of Intelligence (WASI, N = 17) (Wechsler 1999), the Mullen Scales of Early Learning (MSEL, N = 5) (Mullen 1995), and the Wechsler Adult Intelligence Scale (WAIS, N = 2) (David Wechsler 1955). No IQ data was collected for the control individuals. Eleven participants with ASD did not have Full Scale IQ scores, and none of the control individuals had Full Scale IQ scores.

Pubertal status was determined by Tanner staging (Marshall and Tanner 1969, 1970) or chronological age. Pre-pubertal status was defined by a Tanner stage of I or II, or in cases where Tanner stage was unavailable (n= 29), pre-pubertal status was defined by chronological age of less than 144 months. Post-pubertal status was defined by a Tanner stage of III or higher, or chronological age of 144 months and higher if Tanner stage was unavailable (n= 13).

Whole Blood 5-HT Assay

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 chromatography (Anderson et al. 1987). WB5-HT is a reliable measure of serotonin in platelets within a

volume of blood because over 99% of 5-HT is stored in the platelet; whereas 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 centrifugation (Anderson et al. 1987).

Platelet 5-HT_{2A} Receptor Binding

Platelets were isolated from the blood (10 ml) by standard centrifugation techniques and were kept frozen at -80°C until assayed. Briefly, blood was spun at 210 *g* for 10 min at 4°C . Platelet-rich plasma thus obtained was centrifuged at 4000 *g* for 10 min at 4°C . [^{125}I]LSD binding to 5-HT_{2A} receptors in platelet membranes was carried out according to the method described by Pandey et al. with modification (Pandey et al. 1990). Briefly, the receptor binding was carried out in duplicate tubes that contained incubation buffer, [^{125}I]LSD (1.8 nM), and 40 μl of platelet membrane suspension with or without 1 μM ketanserin in a total volume of 100 μl . It was incubated at 37°C for 3 h. The incubation was terminated by rapid filtration over Brandel Cell Harvester GF/B filters (Biomedical Research and Development Laboratories, Inc., Gaithersburg, Md., USA) and filters were washed three times with 5.0 ml cold 50 mM TRIS buffer (pH 7.7), which contained 0.01% bovine serum albumin. Filters were dried and then counted in an analytic gamma counter. Specific binding was defined as the difference between binding observed in the presence and absence of 1 μM of ketanserin. The protein in platelet membranes was determined by the method of Lowry et al. (Lowry et al. 1951).

Statistics

IBM SPSS Statistics 24 was used to conduct all statistical analyses. Fisher's exact test was used to assess whether there was a difference in the proportions of ancestral background and sex between the sample of control individuals and the sample of individuals with ASD. Independent samples t-test was used to determine whether there were significant differences in the ages of the individuals with ASD and control individuals. There was one outlier in the age distribution data, as determined by running the Grubb's test on any values flagged as potential outliers in the box plots. The analysis was run with and without the outlier, and since the presence of the outlier did not change the interpretation of significance, the outlier was kept in the analyses. The age distributions for the control and ASD populations were normally distributed. The assumption of homogeneity of variances was violated according to Levene's test for equality of variances, $p = 0.036$. Thus, the test statistics were assessed with equal variances not assumed.

An independent samples t-test was used to assess whether there were significant differences in the log-transformed 5-HT_{2A} specific binding values between the ASD and control samples. There was one outlier in the data, identified by the box plots and confirmed using Grubb's test. The analysis was run with and without the outlier, and since the presence of the outlier did not change the interpretation of significance, the outlier was kept in the analyses. The log-transformed 5-HT_{2A} specific binding values for the control and ASD populations were normally distributed and there was homogeneity of variances (Levene's test for equality of variances, $p = 0.591$). An independent samples t-test was also used to assess whether there were differences in the log-transformed WB5-HT values between the

individuals with ASD and the control individuals. There were no outliers in the data, identified by the box plots and confirmed using Grubb's test. The log-transformed WB5-HT values for the control and ASD populations were normally distributed and there was homogeneity of variances (Levene's test for equality of variances, $p = 0.945$).

Multiple linear regression modelling was used to assess the relationship between 5-HT_{2A} specific binding and WB5-HT levels in the entire sample and in the Caucasian subsample (as a sensitivity analysis), including sex, age, pubertal status, and diagnosis as covariates (see Table 1 for correlation matrix) [Table 1]. The nominal variables were assigned values in order to perform the multiple regression (Sex: 0 = Female, 1 = Male; Diagnosis: 0 = Control, 1 = ASD; Pubertal status: 0 = Post-pubertal, 1 = Pre-pubertal). Log transformation of 5-HT_{2A} and WB5-HT resulted in normal distributions in both cases. Using the backward elimination modelling approach and log₁₀_WB5-HT as the dependent variable, initial maximal models for each outcome included log₁₀_5-HT_{2A} specific binding, sex, age, pubertal status, diagnosis, and the interaction terms log₁₀_5-HT_{2A} x Sex, log₁₀_5-HT_{2A} x Age, log₁₀_5-HT_{2A} x Pubertal status, and log₁₀_5-HT_{2A} x Diagnosis. Non-significant interaction terms were individually dropped from the model in succession. Sex, age, pubertal status, and diagnosis were retained in the model as confounders if their coefficient was significant or if their removal distorted the study factor (5-HT_{2A} specific binding) coefficient by more than 10%.

The interaction term log₁₀_5-HT_{2A} x Diagnosis was significant in the entire sample (Table 2) and in the Caucasian subsample (Online Resource 1) [Table 2]. Due to the presence of effect modification based on diagnosis, multiple linear regression modeling was used in the separate diagnostic samples to assess the relationship between 5-HT_{2A} binding and WB5-HT in the sample of individuals with and separately in the control individuals. Sex, age, and pubertal status were included as covariates. The same backward elimination modelling approach described above was used. Model diagnostics were performed on the four final models, and all assumptions underlying multiple linear regression analysis were satisfied.

Results

The sample consisted of 110 individuals with ASD (80.9% male) and 18 control individuals (77.8% male) (Fisher's exact test $p = 0.75$). The ASD group was significantly younger (mean 140.7 months, standard deviation [SD] 73.9 months) than the control group (mean 201.3 months, SD 100.7 months; $t = -2.45$, $p = 0.024$). To account for this difference, age was included in the base model for the multiple regression analysis. There was no significant difference in ancestry background (Fisher's exact test $p = 0.343$). The ASD group had a mean Full Scale IQ score of 84.3 (SD = 21.7) and a mean ADOS Calibrated Severity Score (CSS) of 7.1 (SD = 2.2) (Table 3) [Table 3].

The individuals with ASD had a mean platelet 5-HT_{2A} specific binding value of 24.5 fmoles/mg/protein (SD = 10.6) and the control individuals had a mean platelet 5-HT_{2A} specific binding value of 26.2 fmoles/mg/protein (SD = 9.2). The distribution of platelet 5-HT_{2A} specific binding values for individuals with ASD were positively skewed and kurtosed compared to a normal distribution. A logarithmic transformation of the 5-HT_{2A} specific

binding values normalized the distribution for individuals with ASD (skewness and kurtosis < 2 and > -2). The distributions for both raw and logarithmically transformed 5-HT_{2A} specific binding values were normal for control individuals, so the logarithmic transformation of 5-HT_{2A} specific binding values was used for analyses. An independent samples t-test indicated that there was not a significant difference in log-transformed 5-HT_{2A} specific binding values between the individuals with ASD and the control individuals, $t(126) = -0.864$, $p = 0.390$.

The individuals with ASD had a mean WB5-HT level of 253.9 ng/mL (SD = 101.5) and the control individuals had a mean WB5-HT level of 169.0 ng/mL (SD = 66.6). The distributions for both raw and logarithmically transformed WB5-HT scores were normal for ASD and control individuals. The WB5-HT logarithmic transformation distributions had skewness and kurtosis values that were closer to 0, so logarithmic transformation of WB5-HT was used for analyses. The individuals with ASD had significantly higher log-transformed WB5-HT values than control individuals, $t(126) = 3.724$, $p < 0.001$.

In order to evaluate the relationship between platelet 5-HT_{2A} specific binding and WB5-HT levels, a multiple regression model was run in the entire sample with sex, age, pubertal status, and diagnosis as covariates. The final regression model for the entire sample accounted for 22.7% of the variance in WB5-HT ($F(5,122) = 8.452$, $p < .001$, $R = 0.507$, adj $R^2 = 0.227$). The interaction between 5-HT_{2A} specific binding and diagnosis was an effect modifier in the final regression models for the entire sample (Table 2) and the Caucasian subsample (Online Resource 1). This interaction indicates that the correlation between 5-HT_{2A} specific binding and WB5-HT levels was significantly different between the ASD and control groups. Thus, multiple regression analyses were performed for the ASD and control groups separately in both samples.

The final regression model (Table 4) for the ASD sample accounted for 8.3% of the variance in WB5-HT ($F(2,107) = 5.955$, $p = 0.004$, $R = 0.316$, adj $R^2 = 0.083$), with age the only significant covariate [Table 4]. Platelet 5-HT_{2A} specific binding did not significantly predict WB5-HT after correcting for age ($t = 1.024$, $p = 0.308$) in the ASD sample. However, age did significantly independently predict WB5-HT ($t = -3.280$, $p = 0.001$) in the ASD sample [Figure 1]. The final regression model for the ASD sample within the Caucasian subsample (Online Resource 2) yielded results that were not substantially different ($F(3,78) = 4.667$, $p = 0.005$, $R = 0.390$, adj $R^2 = 0.120$). The final regression model for the control sample accounted for 63.6% of the variance in WB5-HT ($F(3,14) = 10.917$, $p = 0.001$, $R = 0.837$, adj $R^2 = 0.636$), with both sex and age as significant covariates. Platelet 5-HT_{2A} specific binding significantly predicted WB5-HT after correcting for sex and age ($t = -2.981$, $p = 0.010$) in the control sample. The final regression model for the Caucasian control subsample yielded results that were not substantially different ($F(3,13) = 13.804$, $p < 0.001$, $R = 0.872$, adj $R^2 = 0.706$).

Discussion

Elevated whole blood 5-HT is the most well-replicated biomarker finding in ASD. Previous research has also revealed differences in 5-HT₂ receptor binding between individuals with

autism spectrum disorder or their first-degree relatives and controls (Murphy et al. 2006; McBride et al. 1989; Oblak et al. 2013; Goldberg et al. 2009). Less is known about the relationship between 5-HT₂ receptor binding and WB5-HT within ASD. Our results indicate that the relationship between WB5-HT and platelet 5-HT_{2A} specific binding is significantly different depending on ASD diagnosis. In the control participants, platelet 5-HT_{2A} specific binding independently predicted WB5-HT levels; whereas in the ASD participants, platelet 5-HT_{2A} specific binding did not predict WB5-HT levels.

The predictive relationship between 5-HT_{2A} specific binding and WB5-HT levels in the control sample mirrors previous platelet findings in first-degree relatives of children with autism or in the cortex of parents of two or more individuals with ASD (Cook et al. 1993; Goldberg et al. 2009). The lack of a relationship with 5-HT_{2A} binding in the ASD sample suggests that WB5-HT levels result from a different set of factors or contribute differently to downstream alterations in the 5-HT system in people with ASD than in the general population.

The current study replicated the well-known finding of increased WB5-HT levels in ASD in comparison to controls. However, the current study did not replicate the previous studies that found differences in platelet 5-HT₂ binding in ASD (Murphy et al. 2006; McBride et al. 1989; Oblak et al. 2013); although one previous study also did not find a difference in comparison to controls (Perry et al. 1991). Importantly, we had limited power to assess a difference in platelet 5-HT_{2A} binding in our population, with an ability to detect only an effect size greater than 0.92 with 95% confidence. Our data indicate a likely difference in the impact of WB5-HT on 5-HT₂ binding or the impact of 5-HT₂ binding on WB5-HT in ASD; although it is also possible that a common factor differs in its impact on both, such as the microbiome, a potential source of variation in WB5-HT levels (Yano et al. 2015).

While the current study provides important information about the role of the 5-HT_{2A} receptor in autism, there are some important limitations. As noted, our power was limited, with an ability to detect only a large effect size, particularly for group comparisons. Importantly, we conducted a single-point binding experiment, rather than a full Scatchard plot analysis, which would allow us to also estimate the dissociation constant, K_d, as well as the maximum binding (B_{max}). We have measures of WB5-HT and 5-HT₂ binding from ASD probands and controls but not from first-degree relatives. We do not have measures of other components of the peripheral 5-HT system, such as enterochromaffin cells or the microbiome. Further, we do not have measures of central nervous system 5-HT synthesis or receptor binding to connect our peripheral findings with the presumed substrate for ASD symptoms in the brain. Finally, the sample of control individuals was small, and the sample was predominantly Caucasian, which limits the generalizability of our findings to other populations.

In summary, the current study reveals that platelet 5-HT_{2A} specific binding significantly predicts WB5-HT levels in control individuals but does not predict WB5-HT levels in individuals with ASD. It is important for future studies to explore other possible variables that could impact WB5-HT in ASD, such as the microbiome or enterochromaffin cells. By investigating these variables in addition to those included in the current study, future work

will strengthen our understanding of what accounts for the variability in WB5-HT in ASD. While further work is needed to unravel the underlying mechanisms, this study provides further evidence of disruptions in the function of the serotonin system in children with autism spectrum disorder.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

5-HT_{2A} x WB5-HT in individuals with ASD vs. control individuals. Pre-pubertal males are depicted by empty blue circles; pre-pubertal females are depicted by empty green circles; post-pubertal males are depicted by filled blue circles; post-pubertal females are depicted by filled green circles.

Table 1.

Correlations

	Log10_WB5-HT	Log10_5-HT _{2A} Specific Binding	Pubertal Status	Diagnosis	Sex	Age
Log10_WB5-HT						
Log10_5-HT _{2A} Specific Binding	-.012					
Pubertal Status	.278 **	.086				
Diagnosis	.315 **	-.077	.104			
Sex	.174 *	-.010	.025	.027		
Age	-.372 **	.016	-.808 **	-.262 **	.002	

** Correlation is significant at the 0.01 level

* Correlation Is significant at the 0.05 level

Table 2.

Initial WB5-HT Regression Model

	Regression Coefficient B	Standard Error SE _B	Standardized Coefficient β	95% Confidence Interval for β	p-value
Constant	3.138	0.367	-		-
Log10_5-HT _{2A} Specific Binding	-0.608	0.258	-0.568	-1.044 to -0.092	0.02*
Sex	0.066	0.038	0.137	-0.020 to 0.293	0.09
Age	-0.001	0.000	-0.307	-0.467 to -0.147	<0.001*
Diagnosis	-0.840	0.381	-1.535	-2.914 to -0.156	0.03*
Log10_5-HT _{2A} Specific Binding $\times$ Diagnosis	0.697	0.273	1.828	0.409 to 3.246	0.01*

N = 128; Degrees of Freedom: 5, 122

Table 3.

Participant Characteristics

	Individuals with ASD	Control Individuals
<i>n</i>	110	18
Sex (<i>n</i> , %)	89 male (80.9%) 21 female (19.1%)	14 male (77.8%) 4 female (22.2%)
Mean age in months (SD)	140.7 (73.9)	201.3 (100.7)
Ancestry (<i>n</i> , %)		
Caucasian (Non-Hispanic)	64 (58.2%)	14 (77.8%)
Caucasian (Hispanic)	18 (16.4%)	3 (16.7%)
African American	17 (15.5%)	0 (0.0%)
Asian	3 (2.7%)	0 (0.0%)
Mixed	8 (7.3%)	1 (5.6%)
Mean WB5-HT in ng/mL (SD)	253.9 (101.5)	169.0 (66.6)
Range (ng/mL)	69.1 – 665.0	83.0 – 307.0
Mean platelet 5-HT _{2A} specific binding in fmoles/mg/protein (SD)	24.5 (10.6)	26.2 (9.2)
Range	6.4 – 90.6	9.8 – 49.1

Table 4.

Final WB5-HT Regression Models

<i>Autism Spectrum Disorder Sample</i>					
	Regression Coefficient B	Standard Error SE_B	Standardized Coefficient β	95% Confidence Interval for β	p-value
Constant	2.345	0.131	-	-	-
Log10_5-HT _{2A} Specific Binding	0.095	0.093	0.094	-0.088 to 0.276	0.308
Age	-0.001	0.000	-0.301	-0.483 to -0.119	0.001*
N = 110; Degrees of Freedom: 2, 107					

<i>Control Sample</i>					
	Regression Coefficient B	Standard Error SE_B	Standardized Coefficient β	95% Confidence Interval for β	p-value
Constant	2.938	0.259	-	-	-
Log10_5-HT _{2A} Specific Binding	-0.508	0.170	-0.477	-0.820 to -0.134	0.010*
Sex	0.161	0.065	0.405	0.054 to 0.756	0.027*
Age	-0.001	0.000	-0.470	-0.795 to -0.146	0.008*
N = 18; Degrees of Freedom: 3, 14					