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Archival Report

Examining Associations Between Amygdala Volumes and Anxiety Symptoms in Autism Spectrum Disorder

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ABSTRACT

BACKGROUND: Anxiety is one of the most common co-occurring conditions in people with autism spectrum disorder. The amygdala has been identified as being associated with anxiety in populations with and without autism, yet associations in autism were based on relatively small or developmentally constrained samples, leaving questions as to whether these results hold at different developmental ages and in a larger, more robust sample.

METHODS: Structural neuroimaging and parent report of anxiety symptoms of children ages 5–13 years with (n = 123) and without (n = 171) a diagnosis of autism were collected from the University of Maryland and three sites from the Autism Brain Imaging Data Exchange. Standardized residuals for bilateral amygdala volumes were computed adjusting for site, hemispheric volumes, and covariates (age, sex, Full Scale IQ).

RESULTS: Clinically significant anxiety symptoms did not differentiate amygdala volumes between groups (i.e., autism and anxiety, autism without anxiety, without autism or anxiety). No significant association between left or right amygdala volumes and anxiety scores was observed among the sample of individuals with autism. Meta-analytic and Bayes factor estimations provided additional support for the null hypothesis. Age, sex, and autism severity did not moderate associations between anxiety and amygdala volumes.

CONCLUSIONS: No relation between amygdala volumes and anxiety symptoms in children with autism was observed in the largest sample to investigate this question. We discuss directions for future research to determine whether additional factors including age or method of assessment may contribute to this lack of association.

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Autism spectrum disorder (ASD) is marked by difficulties in social interaction and communication and restricted interests/repetitive behaviors (1) and frequently co-occurs with other conditions (2). Anxiety is one of the most common co-occurring conditions with ASD, with more than 50% diagnosed with at least one anxiety disorder (3). Conceptual and empirical work has identified the amygdala as a potential key player in the etiology, development, and maintenance of anxiety symptoms (4–9) and is part of a larger network of brain regions (e.g., prefrontal cortex, bed nucleus of stria terminalis) (10,11) associated with emotion processing and regulation. The amygdala, as compared with other regions within this larger network, has been identified as being associated with co-occurring anxiety symptoms in autism samples (12,13) and differentiating between those with autism alone, those with autism with anxiety, or typical comparison participants (14). Yet, given the small sample sizes of previous studies (12,14) and limited developmental timeframe assessed (13), it is unclear how anxiety is associated with amygdala volumes in autism.

To date, three studies have assessed associations between amygdala volume and anxiety symptoms in autism (12–14), and only one study (14) assessed anxiety symptoms in a group without ASD as well as with ASD. Herrington *et al.*'s (14) study of children and adolescents ages 6–17 years with ASD, those with ASD and anxiety, and nonautistic comparison participants indicated that children with ASD and clinically diagnosed anxiety (i.e., based on a clinical interview) demonstrated smaller amygdala volumes relative to children with ASD alone or typical comparison participants. When anxiety symptoms were assessed dimensionally using the total anxiety score from the Screen for Child Anxiety Related Emotional Disorders, parent report version (15), a trend between smaller right amygdala volume and higher levels of symptoms was observed ($r = -0.21$, $p = .09$) in the ASD group (both with and without a categorical diagnosis of anxiety). Only the Panic/Somatic Symptoms subscale of the Screen for Child Anxiety Related Emotional Disorders, parent report version, was significantly associated with right amygdala volumes ($r = -0.30$, $p = .02$). Alternatively, using a solely dimensional approach (12), one study found that increased total amygdala volumes (sum of left and right) and right amygdala volumes were associated with higher levels of anxiety symptoms as measured using the Anxious/Depressed subscale of the Child Behavior Checklist (CBCL) parent-report form (16) in a sample of 3- to 14-year-olds with ASD and anxiety symptoms (but not

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considering explicit diagnosis). Finally, in one study (13) of 2- to 5-year-old children with ASD, larger right amygdala volume was associated with greater internalizing symptoms assessed using the broadband Internalizing Scale on the CBCL, although this effect was only seen in females. Therefore, preliminary evidence suggests that when using a dimensional approach to examine associations between amygdala volumes and anxiety in ASD samples, larger amygdala volumes are associated with higher levels of symptoms. Alternatively, when assessing the presence of anxiety using a categorical or diagnostic approach, children diagnosed with both anxiety and ASD were found to have smaller amygdala volumes relative to those with ASD alone and a typical comparison. However, given the small sample sizes (n <50 for ASD participants) in two of the studies and opposite findings among the three published studies assessing these associations, questions remain as to whether associations between amygdala volumes and anxiety exist in a larger sample of children and adolescents with ASD. Furthermore, the specific measure used to assess anxiety symptoms has varied, including a clinical diagnostic interview, a broadband internalizing scale, an anxiety specific scale, or subscales within anxiety measures, making it unclear whether there are true associations between amygdala volumes and anxiety symptoms specifically.

Potential Moderators of Amygdala-Anxiety Associations

Factors that may account for discrepancies between studies examining associations between anxiety symptoms and amygdala volumes in autism samples include age, sex, and autism symptoms.

Age. The growth trajectory of amygdala volumes in autism samples may be different from samples without autism, suggesting that examining the effect of age is necessary to investigate associations between amygdala volumes and anxiety symptoms (17–19). Specifically, a period of overgrowth has been observed starting prior to age 3 in autism (8,9,19), followed by a period of slowed growth. In typical development, the amygdala continues to increase in volume from middle childhood through adolescence (17). In studies of older children and adolescents with autism, amygdala volumes are similar to or smaller in size than typically developing children (17,20).

The developmental trajectory of anxiety symptoms also changes with age. Children as young as infants and toddlers can experience anxiety (i.e., separation anxiety symptoms), with increasing rates of anxiety occurring during adolescence in both children with and without autism (21,22).

Studies investigating associations among age, anxiety, and amygdala volumes have not yielded consistent developmental time periods in which associations between amygdala volumes and anxiety are observed. For example, in samples without ASD, associations between anxiety symptoms and amygdala volumes have been observed, but some associations were observed only when age was included as a moderator (23), and others when age was not included as a moderator (24). Among the three samples with autism examining associations between amygdala volumes and anxiety, none examined moderation by age (12–14). Therefore, work is still needed to determine whether age is a moderator of associations between anxiety and amygdala volumes.

Sex. Males are diagnosed with autism much more frequently than females (25), and sex differences associated with amygdala volumes in samples with and without autism are mixed. Enlarged amygdala volumes in males relative to females have been observed in some studies of those without autism, including meta-analyses (26,27), whereas other studies have found no difference (28). Similar discrepancies have been observed in autism samples, with some finding larger amygdala volumes in females relative to males (9) and others pointing to larger amygdala volumes in males (29,30) compared with females. Furthermore, sex differences in amygdala-internalizing associations in children with autism were only observed in females and not males (6). Thus, sex should be examined as a potential moderator of associations between amygdala and anxiety symptoms.

Autism Symptoms. Amygdala volumes have also been associated with several characteristics and symptoms of ASD, although the direction of the associations are varied. Specifically, larger amygdala volumes have been associated with increased joint attention (8), mentalizing ability (31), and autism severity in males but not females (9); smaller amygdala volumes have been associated with restricted and repetitive behaviors (32); and severity of social and communication difficulties has been associated with both larger (33) and smaller (34) amygdala volumes. Thus, investigating autism symptoms as a potential moderator of associations between anxiety symptoms and amygdala volumes is necessary.

Current Study

Given the few studies investigating associations between amygdala volumes and anxiety symptoms in samples of children and adolescents with autism, as well as the open questions related to whether sex, age, and autism symptoms moderate these associations, the current study had several goals. The first goal was to examine group differences in amygdala volumes in a large sample of 1) children with autism without anxiety symptoms, 2) children with autism and clinically significant levels of anxiety symptoms, and 3) a non-autistic comparison sample without anxiety symptoms. Second, we tested for continuous associations between amygdala volumes and anxiety symptoms within the autism group. We hypothesized that higher symptoms of anxiety would be associated with amygdala volumes, although the direction of the association is unclear based on mixed results and the potential for age, sex, and autism severity to moderate these associations. We also examined whether amygdala volumes were associated with internalizing symptoms more broadly as well as additional measures of anxiety/depression (i.e., Withdrawn/Depressed, Anxious/Depressed, Somatic Symptoms). Finally, we assessed whether age, sex, and/or autism severity moderated associations between amygdala volume and anxiety symptoms in autism.

To accomplish these aims, we leveraged data from a large sample of children and adolescents diagnosed with ASD—

Autism Brain Imaging Data Exchange (ABIDE) (35)—with available phenotypic data regarding the presence of anxiety symptoms, details regarding autism severity, as well as a typically developing comparison sample with data regarding anxiety symptoms.

METHODS AND MATERIALS

Participants

Participants included 294 (206 male) children ages 5.32–13.80 (mean = 10.02, SD = 1.81) from the University of Maryland (UMD), as well as three sites in ABIDE-II that had phenotypic data related to anxiety or internalizing symptoms using the CBCL (16,36): Kennedy Krieger Institute, New York University (NYU), and Georgetown University. Participants included in the current project were either diagnosed with ASD or had no diagnosis of ASD, had usable T1 structural magnetic resonance imaging (MRI) images, and had phenotypic data related to anxiety or internalizing symptoms as assessed using the CBCL (16,36) available. One participant in the nonautism group was within the clinically significant range on the DSM-5 Anxiety Problems Scale and, therefore, was removed from analyses. Demographic information for the current sample is presented in Table 1 and Table S1. A full list of participant identification numbers used in the current study from ABIDE II is provided in Table S2.

Clinical Assessments

Autism Diagnosis/Severity. Methods to determine autism diagnosis varied across sites; however, all used some form of gold standard assessment of autism such as the Autism Diagnostic Observation Schedule, Second Edition (37), or the Autism Diagnostic Interview, Revised (38), in addition to clinical judgment. See Di Martino *et al.* (35) for more information regarding the ABIDE II sites' diagnostic evaluations. Children from UMD with an autism diagnosis had to have a previous diagnosis of ASD and meet criteria within the autism spectrum on the Autism Diagnostic Observation Schedule, Second Edition, which was administered and coded by a research-reliable clinician. Autism severity was based on the Autism Diagnostic Observation Schedule, Second Edition, Comparison score, which indicates the level of autism-related symptoms.

Parent-Reported Anxiety and Internalizing Symptoms. Anxiety and internalizing symptoms were assessed based on parent-report forms of the CBCL for ages 1.5–5 years (39) and 6–18 years (36), depending on the child's age. Five subscales were used to assess anxiety and internalizing symptoms. Specifically, the DSM-5 Anxiety Problems Scale; the broadband Internalizing Scale; and the Withdrawn/Depressed, Anxious/Depressed, and Somatic Symptoms subscales of the Internalizing Scale. Individual item scores were not provided from the ABIDE-II dataset; therefore, we could not assess internal consistency. However, the CBCL has shown acceptable internal consistency (36). Anxiety subgroups were defined based on *t* scores on the DSM-5 Anxiety Problems Scale: 1) the autism and anxiety subgroup had *t* scores greater than 70 (scores $\geq$ 70 are considered clinically significant), 2) the autism without anxiety subgroup had *t* scores of 69 or below, and 3) the without autism or anxiety subgroup had *t* scores of 69 or below. The NYU sample did not have *t* scores available for the DSM-5 Anxiety Problems subscale and was therefore not included in the analyses assessing associations with DSM-5 Anxiety Problems.

Full Scale IQ. Each site used various measures to assess participants' verbal, nonverbal, and Full Scale IQ (FSIQ) (35), which are noted in Table 2. At UMD, children were eligible to participate in the larger study if they had an FSIQ of 80 or above.

Magnetic Resonance Imaging

Acquisition. For the Kennedy Krieger Institute sample, T1-weighted images were acquired using a 3.0T scanner

Table 1. Demographic Characteristics by Group

Characteristic	ASD, *n* = 123	Nonautistic Comparison Group, *n* = 171	Test of Difference
Sex, Female/Male, *n*	21/102	67/104	$\chi^2_{1,294} = 16.67$, $p < .01$
Age at Scan, Years	9.51 (2.02)	10.40 (1.55)	$t_{292} = 4.28$, $p < .01$
Full Scale IQ	108.99 (17.50)	115.57 (11.91)	$t_{289} = 3.82$, $p < .01$
Verbal IQ	110.56 (18.71)	117.81 (12.74)	$t_{290} = 3.94$, $p < .01$
CBCL Internalizing	63.07 (9.28)	45.97 (8.82)	$t_{292} = -16.05$, $p < .01$
CBCL DSM-5 Anxiety	63.45 (9.84)	52.22 (4.14)	$t_{233} = -12.29$, $p < .01$
CBCL Withdrawn/Depressed	64.80 (8.86)	52.32 (4.17)	$t_{292} = -16.12$, $p < .01$
CBCL Anxious/Depressed	62.14 (9.69)	52.39 (4.23)	$t_{292} = -11.71$, $p < .01$
CBCL Somatic Symptoms	58.46 (8.40)	52.78 (4.20)	$t_{292} = -7.62$, $p < .01$
ADOS-2 Severity	6.95 (1.77)	–	–
Left Amygdala Volume, mm^3	1775.28 (212.46)	1751.06 (187.53)	$t_{292} = -1.03$, $p = .30$
Right Amygdala Volume, mm^3	1976.52 (252.94)	1939.90 (213.63)	$t_{292} = -1.34$, $p = .18$
Total Gray Matter Volume, mm^3	715,110.12 (75,894.23)	682,265.15 (62,874.13)	$t_{292} = -4.05$, $p < .01$
Harmonized Left Amygdala Volume, mm^3	1763.43 (194.66)	1756.99 (196.52)	$t_{292} = -0.28$, $p = .78$
Harmonized Right Amygdala Volume, mm^3	1964.21 (236.14)	1939.16 (224.80)	$t_{292} = -0.92$, $p = .36$

All values are presented as mean (SD) except those for sex. Harmonized amygdala volumes are amygdala volumes after adjusting for site effects using ComBat (43,44).

ADOS, Autism Diagnostic Observation Schedule; ASD, autism spectrum disorder; CBCL, Child Behavior Checklist.

Table 2. Demographic and Phenotypic Characteristics of Groups With the CBCL DSM-5 Anxiety Subscale Included

Measure	No Autism or Anxiety, *n* = 157	Autism No Anxiety, *n* = 40	Autism and Anxiety, *n* = 38
Sex, Female/Male, *n*	66/91	5/35	11/27
Age, Years	10.47 (1.49)	10.46 (1.83)	9.98 (1.78)
Full Scale IQ	115.32 (11.63)	112.97 (16.63)	111.76 (15.54)
Verbal IQ	117.65 (12.66)	116.73 (18.30)	113.34 (17.28)
ADOS-2 Severity	–	7.04 (1.43)	6.93 (1.61)
CBCL DSM-5 Anxiety	52.22 (4.14)	55.13 (4.42)	72.21 (5.23)
Left Amygdala Volume, mm^3	1742.86 (185.63)	1774.35 (159.51)	1741.26 (197.98)
Right Amygdala Volume, mm^3	1932.63 (214.02)	1977.10 (207.67)	1969.45 (228.58)
Total Gray Matter Volume, mm^3	679,807.89 (63,606.81)	709,253.85 (70,478.93)	711,307.89 (60,540.85)
Harmonized Left Amygdala Volume, mm^3	1753.13 (199.71)	1777.76 (1981.51)	1746.49 (205.59)
Harmonized Right Amygdala Volume, mm^3	1935.34 (229.38)	1981.51 (220.73)	1968.98 (236.57)

Values are presented as mean (SD) except those for sex. Harmonized amygdala volumes are amygdala volumes after adjusting for site effects using ComBat (43,44). IQ was assessed using the Wechsler Intelligence Scale for Children, 4th edition (51), the Wechsler Intelligence Scale for Children, Fifth edition (52), Wechsler Abbreviated Scale of Intelligence (53), Differential Abilities Scales-II Early Years or School Age (54), or the Kaufman Brief Intelligence Test, Second edition (55).

ADOS, Autism Diagnostic Observation Schedule; CBCL, Child Behavior Checklist.

(Achieva, Philips Healthcare). Data from 41 children were collected using a 32-channel head coil, and data from 80 children were collected using an 8-channel head coil. For the Georgetown University sample, T1-weighted images were acquired using a 3.0T scanner (MAGNETOM Trio Tim System, Siemens Medical Solutions) and a 12-channel head coil. For the NYU sample, T1-weighted images were acquired using a 3.0T scanner (MAGNETOM Allegra, Siemens Medical Solutions) and an 8-channel head coil. For the UMD sample, T1-weighted images were acquired using a Siemens 3.0T scanner (MAGNETOM Trio Tim System, Siemens Medical Solutions) and a 32-channel head coil. Structural scans from UMD consisted of collecting 192 contiguous sagittal slices, voxel size = 0.45 × 0.45 × 0.90 mm, repetition time = 1900 ms, echo time = 2.32 ms, flip angle = 9°, and pixel matrix = 512 × 512. Additional information regarding scan parameters and site-specific information for ABIDE II can be found in Di Martino *et al.* (35), as well as at http://fcon_1000.projects.nitrc.org/indi/abide/.

MRI Data Segmentation and Quality Control. First, raw data files were downloaded for all participants and converted to analyze format using DtiStudio software (40). The anonymized and defaced MRIs were uploaded to MRICloud (41) and segmented into anatomically defined regions based on a multiatlas approach using the fully automated MRICloud T1-Segmentation pipeline version 10A, which is based on the Diffeomorphic Multi-Atlas Likelihood Fusion algorithm. The Pediatric 8- to 19-year-old (287 labels, 37 atlases) multiatlas library was used. The whole-brain segmentation output for each subject was downloaded, and all images were visually inspected for segmentation quality. See Figure S1 for an example of segmentation. In addition to amygdala volumes, volumes for left and right hemispheres were extracted and used when controlling for left and right amygdala volumes. This pipeline has been used in a recent study of amygdala volume with high reliability and validity (13).

Several rounds of data quality checking were completed. First, visual inspection of all possible participants from each site was conducted to assess data quality. A second round of data quality checking was completed after running MRI segmentation. A total of 149 participants were excluded because of poor segmentation or data image quality. Scans were excluded if pia mater or cerebral spinal fluid was classified as gray matter on several slices, portions of gray matter were not included in the gray matter mask for several slices, or there was significant motion such that significant portions of the brain were unable to be segmented. There were no significant differences in age, sex, autism severity, FSIQ, or internalizing *t* score (*p*s > .05) between participants whose data remained in the current sample and those whose data were excluded. However, there was a marginal difference (*p* = .08) on the DSM-5 Anxiety Problems subscale between those included and excluded. Specifically, participants who were included in the study had an average anxiety *t* score of 55.34 (SD = 7.77), and those who were excluded had an average anxiety *t* score of 57.11 (SD = 9.04). However, when investigating this difference within each group (i.e., ASD group vs. nonautistic comparison group), there were no significant differences within the ASD group (*p* = .81) between those whose data were included (mean = 62.56, SD = 9.27) and those whose data were excluded (mean = 62.12, SD = 9.66). Similarly, there was no significant difference within the nonautistic comparison group (*p* = .85) regarding those whose data were included in the study (mean = 52.02, SD = 3.73) and those whose data were excluded (mean = 51.91, SD = 4.12).

Data Analysis

SPSS version 26.0 was used for most analyses. Moderation analyses were completed using the PROCESS add-on package (42). Meta-analyses were performed using the metafor package in R (R Foundation for Statistical Computing). Independent samples *t* tests and χ^2 tests were conducted to assess whether there were differences between groups among demographic variables, anxiety symptoms, and bilateral amygdala volumes. Next, one-way analyses of variance were used to examine differences among sites on demographic

Table 3. Left and Right Amygdala Volume Associations With DSM-5 Anxiety, Internalizing Symptoms, and Internalizing Symptoms Subscales

	Left Amygdala			Right Amygdala		
Subscale	*r*	*p* Value	Bayes Factor	*r*	*p* Value	Bayes Factor
Internalizing	−0.03	.72	13.08	0.04	.69	12.83
DSM-5 Anxiety	−0.04	.73	10.51	0.06	.63	9.92
Withdrawn/Depressed	−0.17	.07	2.70	−0.04	.70	12.88
Anxious/Depressed	−0.03	.77	13.33	0.01	.95	13.87
Somatic Symptoms	0.02	.84	13.62	0.03	.78	13.38

Pearson correlations are reported using standardized residuals of left and right amygdala volumes controlling for age, sex, and Full Scale IQ after adjusting for site effects on left and right amygdala volumes and left and right hemispheric volumes using ComBat (43,44).

variables, anxiety symptoms, and amygdala volumes within the autism sample.

To examine associations between amygdala volumes and anxiety, first, we adjusted for site effects using ComBat (43,44) on bilateral amygdala volumes and hemispheric volumes, and then, we calculated standardized residuals using the harmonized values and linear regression adjusting for covariates (age, sex, FSIQ, and respective harmonized values of hemispheric volumes).

Standardized residuals of bilateral amygdala volumes were then submitted to separate one-way analyses of variance to examine the effect of group (i.e., autism, autism and anxiety, no autism or anxiety). Next, Pearson correlations of standardized residuals with each of the CBCL scales (DSM-5 Anxiety, Internalizing, Anxious/Depressed, Withdrawn/Depressed, Somatic Symptoms) were computed. A random-effects meta-analysis tested for the overall effect by site. Separate tests examined moderation by sex, age (linearly and as a quadratic), FSIQ, and autism severity on the relation between anxiety symptoms and standardized residuals. Finally, we used standardized residuals of bilateral amygdala volumes correcting for all covariates except site (not harmonized using ComBat) to have site as the grouping variable. Specifically, Pearson correlations between anxiety symptoms and left and right amygdala volumes from each site were Fisher's *z*-transformed, confidence intervals were

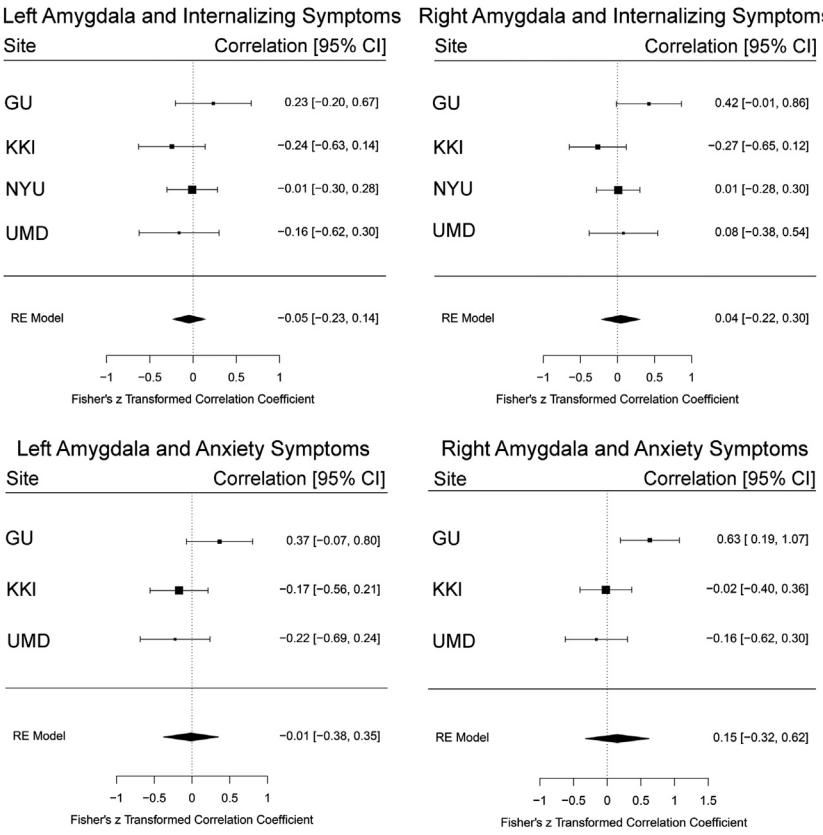

Figure 1. Effect sizes (*r* to Fisher's *z*-transformed) for left and right amygdala volume associations with internalizing and DSM-5 anxiety symptoms. GU, Georgetown University; KKI, Kennedy Krieger Institute; NYU, New York University; RE, random effects; UMD, University of Maryland.

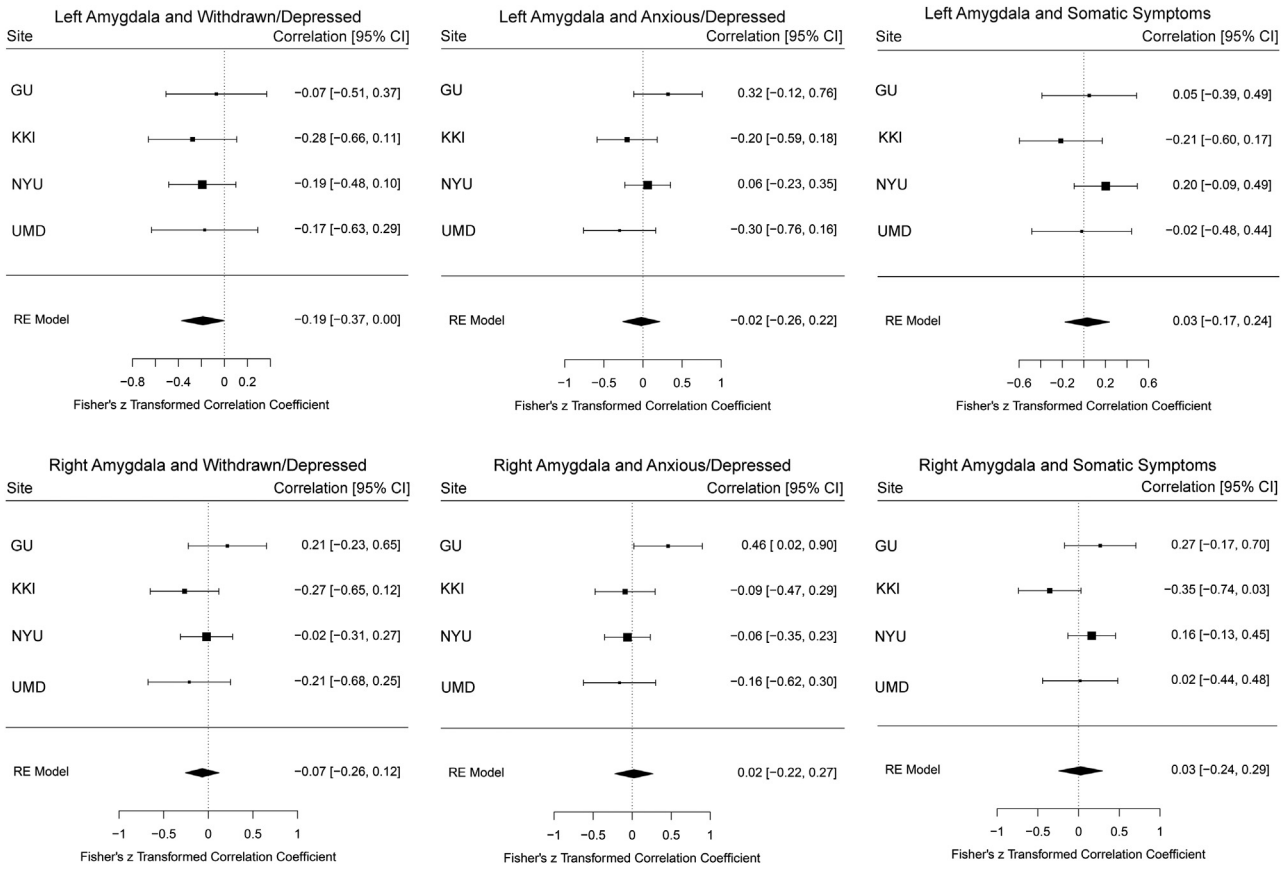

Figure 2. Effect sizes (*r* to Fisher's *z*-transformed) for left and right amygdala volume associations with subscales of the Internalizing Scale. GU, Georgetown University; KKI, Kennedy Krieger Institute; NYU, New York University; RE, random effects; UMD, University of Maryland.

computed, and the final effect was transformed back to a correlation.

RESULTS

Group Differences in Amygdala Volume

There were no statistically significant differences between groups (i.e., autism with anxiety, autism without anxiety, without autism or anxiety) for left ($F_{2,230}$ = 2.22, p = .11) or right ($F_{2,230}$ = 0.68, p = .51) amygdala volumes. See Table 2 and Figure S2.

Individual Differences in Amygdala Volumes in Autism Related to Anxiety

There were no associations between bilateral amygdala volumes and anxiety symptoms using any of the subscales. Post hoc data analyses using Zellnor-Siow's Bayes factor (BF_{01}) was computed to assess the likelihood that the null hypothesis (i.e., no association) was true for each correlation (45). As the BF_{01} increases, there is more support that the null hypothesis is true. Results of BF_{01} ranged from 2.70 to 13.87, indicating that there is moderate to strong evidence for the null hypothesis (i.e., no association between left or right amygdala volumes and anxiety symptoms) (45). Or, stated differently, the observed data are 2–13 times more likely under H_0 than H_1. See Table 3 for associations between amygdala volumes and anxiety symptoms, as well as BFs for each correlation.

Overall, meta-analytic results largely support these findings, such that the overall effects for bilateral amygdala volumes and internalizing, DSM-5 Anxiety, Anxious/Depressed, and Somatic Symptoms are not statistically significant. However, the overall effect for associations between the left amygdala and the Withdrawn/Depressed subscale was statistically significant (r = −0.19, 95% CI −0.37 to 0.00). See Figures 1 and 2 for the results of the random-effects models and Figure 3 for a scatterplot of amygdala and anxiety symptoms by site (controlling for site effects using ComBat).

Potential Moderators of Associations Between Amygdala Volumes and Anxiety Symptoms in Autism

Age (linear and quadratic) and sex did not moderate associations between DSM-5 anxiety and left or right amygdala volumes. However, a trend was observed regarding autism severity as a predictor but not moderator of left amygdala volumes (autism severity: β = 1.06, p = .10; interaction: β = −0.01, p = .16) and right amygdala volumes (autism severity: β = 1.20, p = .07; interaction: β = −0.02, p = .11).

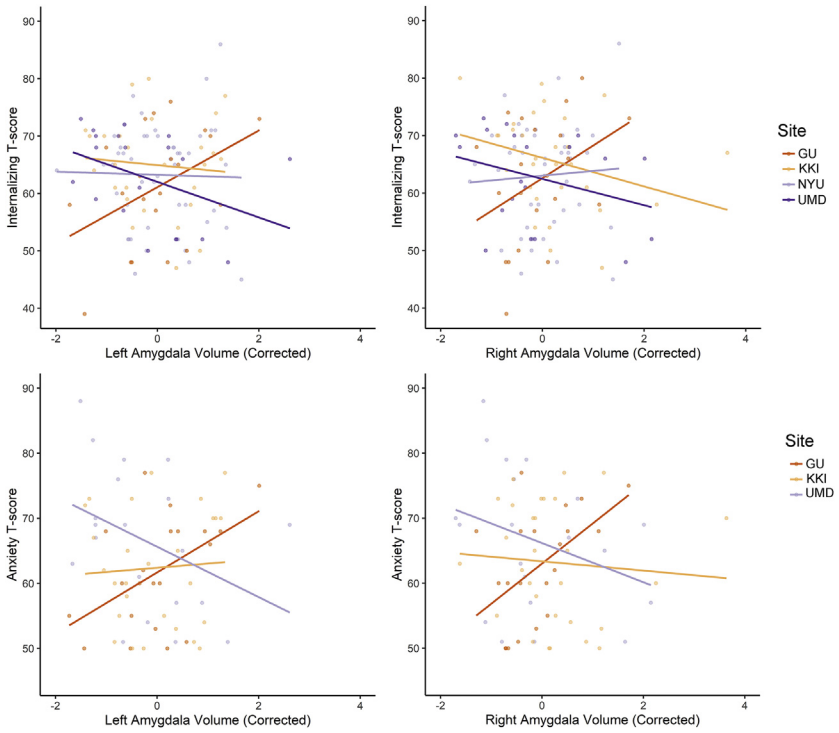

Figure 3. Scatterplot of anxiety and internalizing symptom associations with left and right amygdala volumes by site. Standardized residuals of left and right amygdala volumes were adjusted for age, sex, and Full Scale IQ after adjusting for site effects on left and right amygdala volumes and left and right hemispheric volumes using ComBat (43,44). GU, Georgetown University; KKI, Kennedy Krieger Institute; NYU, New York University; UMD, University of Maryland.

DISCUSSION

The current study aimed to examine whether amygdala volumes in a sample of children ages 5–13 years with and without autism were associated with the presence of parent-reported anxiety symptoms in a large, multisite sample. Results did not support our hypothesis that there would be group differences in amygdala volumes among children with autism, those with autism and anxiety symptoms, and a sample without autism or anxiety symptoms. In addition, when examining only children with autism, we found no association between amygdala volumes and anxiety or internalizing symptoms. These data held both when pooling the full sample and through a random-effects meta-analysis. Furthermore, there remained a lack of association between amygdala volumes and the Anxious/Depressed and Somatic Symptoms subscales of the CBCL, despite significant associations in previous studies (12). In contrast to previous results, we observed a significant association between the left amygdala and Withdrawn/Depressed symptoms when assessing the overall effect size using the meta-analytic approach but not when evaluating the association with the full sample adjusting for site using ComBat. However, this was without controlling for multiple comparisons.

Overall, our findings largely support the null hypothesis that there is no association between amygdala volume and anxiety or internalizing symptoms within a sample of children and adolescents diagnosed with autism. Although this may seem surprising given the previous studies demonstrating such associations, there are several reasons why this result is plausible. First, our results may be explained by the type of anxiety that is being measured. Recent work has suggested that people with autism experience high levels of distinct anxiety, which is theorized to be different from those captured by measures and interviews assessing more traditional DSM-5 anxiety disorders (46). Distinct anxiety includes idiosyncratic fears (e.g., fear of glasses), social fears related to things such as social confusion, and fears of change and is not captured by traditional parent-report forms or even gold standard diagnostic interviews assessing anxiety. Second, these data lack a comparison group with clinically significant levels of anxiety, without autism, which does not allow for examination of whether relations between amygdala and anxiety are different for those with and without co-occurring autism. Therefore, future studies should assess the presence of distinct anxiety in autism samples as well as a sample of children diagnosed with DSM-5 anxiety disorders and no autism. In support of these potential issues, Andrews *et al.* (47) demonstrate different associations between amygdala volumes and anxiety based on whether one is measuring distinct anxiety versus DSM-defined anxiety. Furthermore, the lack of valid assessments that characterize anxiety in autism make it difficult to probe the heterogeneity within our sample related to the amygdala-anxiety association. For example, the direction of the association differed across sites, and some showed a trend for a significant association (e.g., positive right amygdala volume and DSM-5 anxiety symptoms for Georgetown University and negative association for UMD). These differences across sites highlight the possibility that small sample sizes may lead to spurious effects, underscoring the need for large sample sizes. Differences in scanning and other potential demographic variables (e.g., sex) that we had access to were controlled for in each analysis, yet it is possible that there are other factors that

may be contributing to the discrepancies in the direction and strength of the associations at each site that should continue to be investigated. For example, given the high rate of anxiety and depression reported in people with autism (45), it may be hard to assess whether anxiety alone contributes to amygdala-anxiety associations.

Apart from the limitations discussed earlier, there are several additional limitations worth noting. Although we used a large sample of children and adolescents with and without autism, there were still several whose neuroimaging data could not be used due to the quality of the images. Future research investigating whether amygdala volumes are associated with anxiety in autism samples should improve data quality during MRI acquisition, although this is difficult to achieve in samples of children and adolescents with neurodevelopmental disorders (48). Furthermore, we were not able to assess associations between amygdala volumes and anxiety in children younger than age 5, where an association has been previously reported (13). Therefore, assessing whether an association exists at different ages will be important. This study was also limited by not having a wide distribution of children with lower IQ scores. Not only does this restricted range limit the generalizability of our study, but lower IQ scores also make it harder to assess the presence of anxiety (22). Another important limitation of this study is the lack of information regarding race or ethnicity from the ABIDE II dataset, which limits our ability to clearly articulate the generalizability of our findings to populations that are often diagnosed later in life (49). Finally, the amygdala is part of a larger network associated with emotion processing and has been implicated in processing anxiety and fear especially. It is possible that the amygdala-anxiety associations are present when examining amygdala connectivity with other areas of the network (e.g., prefrontal cortex, bed nucleus of stria terminalis) (10,11) rather than the structure of the amygdala itself. Relatedly, we are looking at the macrostructure, but there may be microstructural differences that underlie anxiety that cannot be detected with MRI (e.g., bed nucleus of stria terminalis, central amygdala) (50). Future research should continue to probe these potential associations within other areas of the network, as well as connectivity among brain regions.

Overall, our findings provide evidence that there is no association between amygdala volumes and anxiety symptoms in children with autism using parent-based measures of DSM related anxiety. If there is an association, it may be present in more complex ways, such that age, autism severity, or sex may be playing a moderating role that was not identified in this current sample.

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