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RESEARCH ARTICLE

Dissecting the heterogeneous subcortical brain volume of autism spectrum disorder using community detection

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Abstract

Structural brain alterations in autism spectrum disorder (ASD) are heterogeneous, with limited effect sizes overall. In this study, we aimed to identify subgroups in ASD, based on neuroanatomical profiles; we hypothesized that the effect sizes for case/control differences would be increased in the newly defined subgroups. Analyzing a large data set from the ENIGMA-ASD working group ($n = 2661$), we applied exploratory factor analysis (EFA) to seven subcortical volumes of individuals with and without ASD to uncover the underlying organization of subcortical structures. Based on earlier findings and data availability, we focused on three age groups: boys (<=14 years), male adolescents (15–22 years), and adult men (> = 22 years). The resulting factor scores were used in a community detection (CD) analysis to cluster participants into subgroups. Three factors were found in each subsample; the factor structure in adult men differed from that in boys and male adolescents. From these factors, CD uncovered four distinct communities in boys and three communities in adolescents and adult men, irrespective of ASD diagnosis. The effect sizes for case/control comparisons were more pronounced than in the combined sample, for some communities. A significant group difference in ADOS scores between communities was observed in boys and male adolescents with ASD. We succeeded in stratifying participants into more homogeneous subgroups based on subcortical brain volumes. This stratification enhanced our ability to observe case/control differences in subcortical brain volumes in ASD, and may help to explain the heterogeneity of previous findings in ASD.

Lay summary

- Structural brain alterations in ASD are heterogeneous, with overall limited effect sizes. Here we aimed to identify subgroups in ASD based on neuroimaging measures. We tested whether the effect sizes for case/control differences would be increased in the newly defined subgroups.
- Based on neuroanatomical profiles, we succeeded in stratifying our participants into more homogeneous subgroups. The effect sizes of case/control differences were more pronounced in some subgroups than those in the whole sample.

KEYWORDS
ASD, community detection, neuroanatomical heterogeneity, subcortical volume

The ENIGMA-ASD working group and their affiliations appears in Appendix.

INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental disorder, which is characterized by persistent deficits in communication and social–emotional reciprocity combined with repetitive and stereotypical behaviors and interests (APA, 2013). The median worldwide prevalence estimate for ASD was 62/10,000, but variability is wide (Chiarotti & Venerosi, 2020; Elsabbagh et al., 2012). The prevalence rate in males is estimated to 3:1 higher than in females (Loomes et al., 2017).

Structural brain alterations have been reported in ASD for several decades (Amaral et al., 2008), with pervasive alterations observed in the subcortical areas (van Rooij et al., 2018; Wegiel et al., 2014). Existing literature indicates considerable heterogeneity at an individual level regarding the direction and size of subcortical alterations in ASD (Donovan & Basson, 2017; Haar et al., 2014). For example, a number of studies have shown enlargement of the amygdala, especially in children with ASD (Groen et al., 2010; Nordahl et al., 2012), while other studies with varying participants' age ranges reported either no differences (Barnea-Goraly et al., 2014) or volumetric reduction of the amygdala in ASD (Nacewicz et al., 2006). Hippocampal findings reported from cross-sectional studies are also inconsistent. Increased and decreased hippocampal volumes have been found in ASD, irrespective of age (Barnea-Goraly et al., 2014; Groen et al., 2010; Maier et al., 2015). Overall enlargement of the striatum in individuals with ASD has been reported compared with healthy controls (Hollander et al., 2005; Schuetze et al., 2016); however, notable inconsistencies exist in the literature (Haar et al., 2014; Lange et al., 2015). Similarly, discrepant findings exist for the thalamus (Lange et al., 2015; Schuetze et al., 2016). Recently, the ENIGMA-ASD working group conducted a large-scale case/control mega-analysis reporting smaller subcortical volumes in the pallidum, putamen, amygdala, and nucleus accumbens in individuals with ASD (age range: 2–64 years) (van Rooij et al., 2018). However, all effect sizes observed were small.

We expect that these limited effect sizes may be due to the heterogeneity of neuroanatomical profiles within both ASD patients and the general population. Earlier clustering studies have shown the possibility to stratify a population based on their neuroanatomical profiles, which could increase the power to detect case/control differences within subgroups (Fair et al., 2012; Feczko et al., 2018; Feczko et al., 2019). Similarly, our recent findings from the ENIGMA-attention deficit/hyperactivity disorder (ADHD) working group showed distinct subgroups based on subcortical brain patterns in male participants with and without ADHD (Li et al., 2021). Rather than expecting to find consistent neuroanatomical alterations across the entire ASD population, it may be reasonable to first stratify both participants with and without ASD into more homogeneous subgroups based on their neuroanatomical profiles, and subsequently investigate ASD diagnostic differences within subgroups.

Here, using subcortical brain volume data from the ENIGMA-ASD working group, we applied exploratory factor analysis (EFA) and community detection (CD) to explore the existence of more homogeneous subgroups in participants with and without ASD (Newman, 2006; Rubinov & Sporns, 2011). We expected that similar subgroups should be observed within ASD patients and healthy controls; the effect sizes of case/control comparisons would be increased within each subgroup. We also examined whether the brain-based clusterings were related to clinical ASD profiles.

METHODS

Participants and ASD assessment

The analyzed magnetic resonance imaging (MRI) data came from the ENIGMA-ASD working group (http://enigma.ini.usc.edu/ongoing/enigma-asd-working-group). Full details about the ENIGMA-ASD working group sample have been previously described (van Rooij et al., 2018). The working group implemented a data freeze in July 2018, at which point 1353 patients with ASD (age range: 2.5–64 years old) and 1308 healthy controls (1.5–64 years old) were included. Since ENIGMA-ASD cohort consists of distinct existing data samples from different sites, the only admission condition was clinically valid ASD diagnosis and the presence of minimal demographic variables and MRI data (van Rooij et al., 2018). The inclusion and exclusion criteria were not unified before admission into ENIGMA-ASD working group. For each sample site, the clinical diagnosis of ASD was done according to DSM-IV criteria. All subjects were diagnosed by a clinically experienced and certified psychiatrist/psychologist/physician. Information of DSM-IV subtypes of ASD were not collected by the ENIGMA-ASD working group. Local medical ethical approval was acquired at each site.

Based on earlier findings in participants with ADHD, we expected sex differences in subcortical brain organization (Li et al., 2021), and given the limited data availability in females (only 145 girls, 45 female adolescents, and 33 women with ASD), we decided to only focus on male participants in the current study. As in our previous studies, we subdivided the full cohort into three subsamples based on age (<=14 years, 15–22 years, and >= 22 years) (Boedhoe et al., 2020; Hoogman et al., 2017; Li et al., 2021): a subsample comprised of 772 boys with ASD (mean age: 10.5 ± 2.8) and 733 healthy controls (mean age: 10.6 ± 2.5), a subsample of 360 male adolescents with ASD (mean age: 18.0 ± 2.0) and 321 healthy controls (mean age: 17.9 ± 2.0), and a subsample of 221 adult men with ASD (mean age: 31.7 ± 9.4) and 254 healthy controls (mean age: 30.7 ± 8.1). Schematic

workflow of this study was presented in Figure 1. Information on the ENIGMA-ASD cohorts and subsamples in the current study are presented in Table S1.

Clinical information including ASD severity, IQ, medication uses, and the presence of comorbidities has been described in detail in a previous study (van Rooij et al., 2018). ASD severity was measured using the Autism Diagnostic Observation Schedule-Generic (ADOS) in most studies, and ADOS data were available for 654 individuals with ASD (Lord et al., 2000). In the current study, data on IQ were available for 1100 individuals with ASD and 1068 healthy controls. The information about medication use at the time of scanning (i.e., current use of psychiatric treatment for ASD or comorbid conditions) was available for 652 individuals with ASD. The information on the presence/absence of at least one comorbid condition (i.e., ADHD, obsessive–compulsive disorder, depression, anxiety, and/or Tourette's syndrome) was available for 211 individuals with ASD.

Neuroimaging segmentation

Structural T1-weighted brain MRI scans were collected at the various contributing sites. The MRI data were segmented using standardized ENIGMA imaging protocols based on FreeSurfer version 5.3 (http://enigma.ini.usc.edu/protocols/imaging-protocals/). Standardized quality control (QC) relied on the automatic detection of segmentation outliers for each volume. Detailed information on the QC procedure has been described in our previous study (van Rooij et al., 2018). For each participant, seven subcortical volumes were averaged across the two hemispheres. Before running the main analyses, the subcortical volumes of children and the rest of participants in the whole ENIGMA-ASD working group were regressed with age, age^2, intracranial volume (ICV), and cohort sites separately, allowing nonlinear patterns of subcortical brain volumes (the underlying functional or anatomical connection between subcortical volumes) across age. The residuals of the regression were used for subsequent analysis.

Factor analysis

We performed EFA to uncover the latent structure underlying the subcortical brain, and reduce the input variables to a more parsimonious model consisting of fewer factors than the total number of subcortical volumes. Following our previously established analyses pipeline (Li et al., 2021), covariance matrices and squared multiple correlations were built as prior communality estimates for each participant overall subcortical volumes. Subsequently, the maximum likelihood method and oblique rotation were applied to extract factors in the EFA. If the loading on the factor was more than 0.40, a variable would be loaded on one factor. Model fitness was evaluated by Tucker Lewis index (TLI), Bayesian information criterion (BIC), and the root mean square error of approximation (RMSEA). Given the EFA generated differential model outcome in the adult men compared with the boys and male adolescents, Confirmatory factor analysis (CFA) was applied to test whether the factor structure generated in adult men was superior to the factor structure observed in the other two subsamples. This was done by evaluating comparative fit index (CFI), TLI, BIC, and RMSEA between the resulting models. The analyses were conducted in R programming v3.6.2 using the "psych" package.

CD

CD was utilized to identify distinct subgroups of participants in each of the three subsamples (Newman, 2006; Rubinov & Sporns, 2011). Using the normalized factor scores generated by EFA, $n \times n$ weighted, undirected networks were built to obtain distance information among participants. We then used a weight-conserving modularity algorithm to identify distinct communities in each network (Fair et al., 2012; Rubinov & Sporns, 2011). This modularity measures the strength of the division of each network into subgroups, which does not constrain the sizes of subgroups or discard any nodes (Newman, 2006). The algorithm sorts iteratively nodes (participants in this study) into subgroups until the modularity (Q) reaches a maximum to find the optimal partition. The variation of information (VOI) was calculated to assess robustness of the community structure. VOI indicates the variance between the original and perturbed networks over a range of alpha, which ranges between 0 and 1 (Karrer et al., 2008). The CD analyses were performed in Matlab (Rubinov & Sporns, 2011).

Statistical analysis

Descriptive statistics of age and IQ was compared between participants with and without ASD, using independent-samples t test and ANOVA. Within each subsample, chi-square test was used to check whether the distribution between communities differs in ASD patients and healthy controls. t test was used to compare subcortical factor scores and subcortical brain volumes between participants with and without ASD in each subgroup. Multivariate analysis of variance (MANOVAs) was applied to test which kind of grouping (brain-based subgroup or ASD diagnosis group) showed the main effect on subcortical brain volumes in each subsample, in which age, age^2, intracranial volume (ICV), and cohort sites were included as covariates. We assessed whether the clinical presentations differed among communities in each subsample. False discovery rate (FDR) correction at p-value of 0.05 was used for multiple comparisons. All analyses were performed in IBM SPSS Statistics 25.

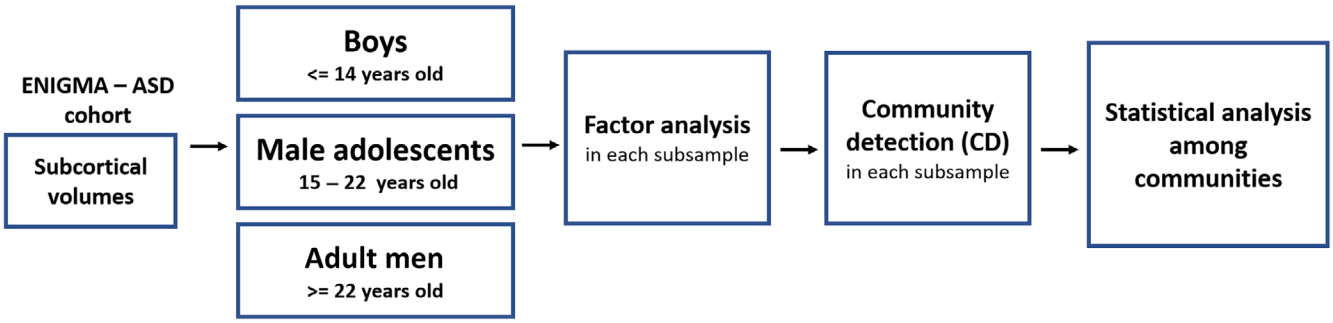

FIGURE 1 Schematic workflow of this study

RESULTS

Participant characteristics

Age and IQ in each subsample were presented in Table 1. There were no case/control differences in age in each subsample after regressing the effect of cohort sites (boys: $t = -1.2$, $p = 0.46$; male adolescents: $t = 0.97$, $p = 0.54$; adult men: $t = 1.29$, $p = 0.42$). Case/control differences in IQ were significant in each subsample (boys: $F = 47.8$, $p = 9.4e{-}10$; male adolescents: $F = 26.6$, $p = 6.3e{-}06$; adult men: $F = 16.6$, $p = 2.8e{-}04$).

EFA on subcortical volumes

EFA in boys

EFA was applied to the residualized subcortical volumes in boys with and without ASD both separately and together, which resulted in similar factor structures. Three eigenvectors were extracted from the covariance matrix (Model fitness: TLI = 0.95, BIC = 1.94, RMSEA = 0.07). The first eigenvector was composed of caudate nucleus, globus pallidus, nucleus accumbens, and putamen. The second eigenvector included the hippocampus and amygdala, and the third eigenvector only included the thalamus. We interpreted them as "basal ganglia," "limbic system," and "thalamus" (Figures 2 and S1). The three eigenvectors accounted for 30%, 16%, and 9% of the total shared variance, respectively.

EFA in male adolescents

EFA was next applied to male adolescents, including both participants with and without ASD. The same three eigenvectors as in the boys subsample were extracted (Model fitness: TLI = 0.94, BIC = −3.72, RMSEA = 0.08) (Figures 2 and S1). The proportion of variance accounted for each eigenvector was 28%, 20%, and 12% of the total shared variance, respectively.

EFA in adult men

In the subsample of adult men, the EFA resulted in a different factor structure than those observed in boys and male adolescents (Model fitness: TLI = 1.01, BIC = −16.99, RMSEA = 0.00). The first eigenvector, named "basal ganglia," included caudate nucleus, globus pallidus, and putamen; The second eigenvector, named "limbic system-accumbens," included the nucleus accumbens, hippocampus, and amygdala; the third eigenvector included the thalamus only (Figures 2 and S1). The three eigenvectors respectively accounted for 28%, 21%, and 12% of the total shared variance.

Given that the EFA showed nucleus accumbens loading on the limbic system, rather than on the basal ganglia in the subsample of adult men, an additional CFA was run to compare the fitness of the two models in adult men. CFA confirmed that the factor structure including the nucleus accumbens loading on the limbic system in adult men was superior compared with the factor structure including the nucleus accumbens loading on the basal ganglia (Model fitness: CFI = 0.70, TLI = 0.47, BIC = 48570.5, RMSEA = 0.24; compared with CFI = 0.59, TLI = 0.28, BIC = 48688.2, RMSEA = 0.28, respectively; chi-square difference = 117.69, $p = 1.2e{-}14$).

CD in each sample based on subcortical factor scores

CD in boys

The CD algorithm was initially performed on the subcortical factor scores in boys (with and without ASD). Four distinct communities were generated, each comprising between 22.9% and 26.7% of the subsample (Figure 3; Table 2). Community 1 was characterized by increased volume of the basal ganglia and limbic system, but smaller thalamic volume compared with the average volume of the whole subsample. Community 2 showed smaller basal ganglia and limbic system, but larger thalamic volumes. Community 3 had a larger volume in the limbic system, but smaller basal ganglia volume,

compared with the average volume. Community 4 had larger basal ganglia, but smaller limbic system and thalamic volumes compared with the average volume of the whole subsample.

CD in male adolescents

CD in male adolescents resulted in three communities. Each community accounted for 27.0% to 44.8% of the subsample. No participants were present in the equivalent of Community 3 from the CD analysis in boys (Figure 3, Table 2). The three remaining communities had quite similar features to the equivalent communities in boys. Community 1 was characterized by increased volumes of the basal ganglia and limbic system above the average volume, but with smaller thalamus. The volume of basal ganglia and limbic system was smaller than average, but the thalamus had larger volume in Community 2. Community 4 showed larger basal ganglia, but smaller limbic system and thalamus than average in the male adolescents.

CD in adult men

In adult men, CD revealed three communities with the proportion of participants from 21.3% to 48.8% of the sample. The equivalent of Community 3 in boys was absent (Figure 3, Table 2). In Community 1, the basal ganglia and limbic system-accumbens had increased volumes compared with the average level overall groups, but the thalamus was smaller. Community 2 had reduced volume of the basal ganglia, but larger thalamus than average. The volume of basal ganglia in Community 4 was increased compared with the average volume, but the limbic system-accumbens and thalamus were smaller than average.

In all three CD analyses, the quality index (Table 2) and VOIs (Figure S2) indicated that these communities significantly differed from random networks, and the networks were robust against chance variation. In this way, the VOI analysis can be viewed as an internal replication method, showing the CD results do not change when random parts of the sample were perturbed. There were no significant differences in the distribution of ASD participants and healthy controls between communities at each age bin (boys: Chi-square = 10.6, $df = 3$, $p = 0.08$; male adolescents: Chi-square = 3.3, $df = 2$, $p = 0.41$; adult men: Chi-square = 2.5, $df = 2$, $p = 0.49$). The distribution of ASD patients and healthy controls in each cohort is presented in Tables S2–S4.

Case/control comparison of subcortical factor scores in ASD

We examined whether participants with ASD showed altered subcortical factor scores from healthy controls, first in each subsample and then in each community separately. The results, as presented in Table 3, Figures 3 and 4, indicate that boys with ASD had smaller basal ganglia than healthy controls in Community 3 ($t = -5.6$, $p = 1.0e{-}06$, $d = -0.63$, 95% CIs [−0.86, −0.41]). For the limbic system, compared with healthy controls, boys with ASD showed increased volume in Community 1 ($t = 3.1$, $p = 0.01$, $d = 0.30$, 95% CIs [0.11, 0.49]), but reduced volumes in Community 2 and 3 (Community 2: $t = -5.9$, $p = 1.5e{-}07$, $d = -0.56$, 95% CIs [−0.75, −0.37]; Community 3: $t = -4.4$, $p = 1.7e{-}04$, $d = -0.50$, 95% CIs [−0.73, −0.27]). In Community 3, boys with ASD had larger thalamic volume compared with healthy controls ($t = 4.5$, $p = 1.4e{-}04$, $d = 0.51$, 95% CIs [0.28, 0.74]). In the sample of male adolescents and adult men, two case/control differences were found, but did not survive FDR correction.

In Table S5–S7, we present case/control comparisons for each individual subcortical brain volume in the whole sample and each Community. We observed several significant case/control differences within the communities: eight case/control comparisons in boys and three in male adolescents survived FDR correction. The effect sizes reaching significance with communities ranged from $d = -0.84$ (95% CIs [−1,07, −0.60]) to −0.42 (95% CIs [−0.69, −0.14]) and from $d = 0.37$ (95% CIs [0.14, 0.59]) to 0.51 (95% CIs [0.28, 0.74]), which were more pronounced than those in the whole subsample with d ranging from $d = -0.29$ (95% CIs [−0.44, −0.13]) to −0.17 (95% CIs [−0.32, −0.02]) and from $d = 0.01$ (95% CIs [−0.09, 0.11]) to 0.04 (95% CIs [−0.14, 0.22]). MANOVAs indicated that the communities accounted for more variance in subcortical brain volumes than ASD diagnosis in each subsample (boys: Communities: $F = 147.8$, $p = 1.2e{-}14$; ASD diagnosis: $F = 0.95$, $p = 0.69$; male adolescents: Communities: $F = 113.7$,

TABLE 1 Information on the three subsamples of the ENIGMA-ASD working group data set

	Boys		Male adolescents		Adult men	
Variables	**Patients**	**Controls**	**Patients**	**Controls**	**Patients**	**Controls**
N	772	733	360	321	221	254
Mean age (*SD*)	10.5 (2.8)	10.6 (2.5)	18.0 (2.0)	17.9 (2.0)	31.7 (9.4)	30.7 (8.1)
Mean IQ (*SD*)	103.9 (19.5)	111.0 (15.5)	105.4 (17.8)	111.8 (12.4)	109.7 (14.9)	115.1 (11.6)

Abbreviations: IQ, intelligence quotient; *SD*, standard deviation.

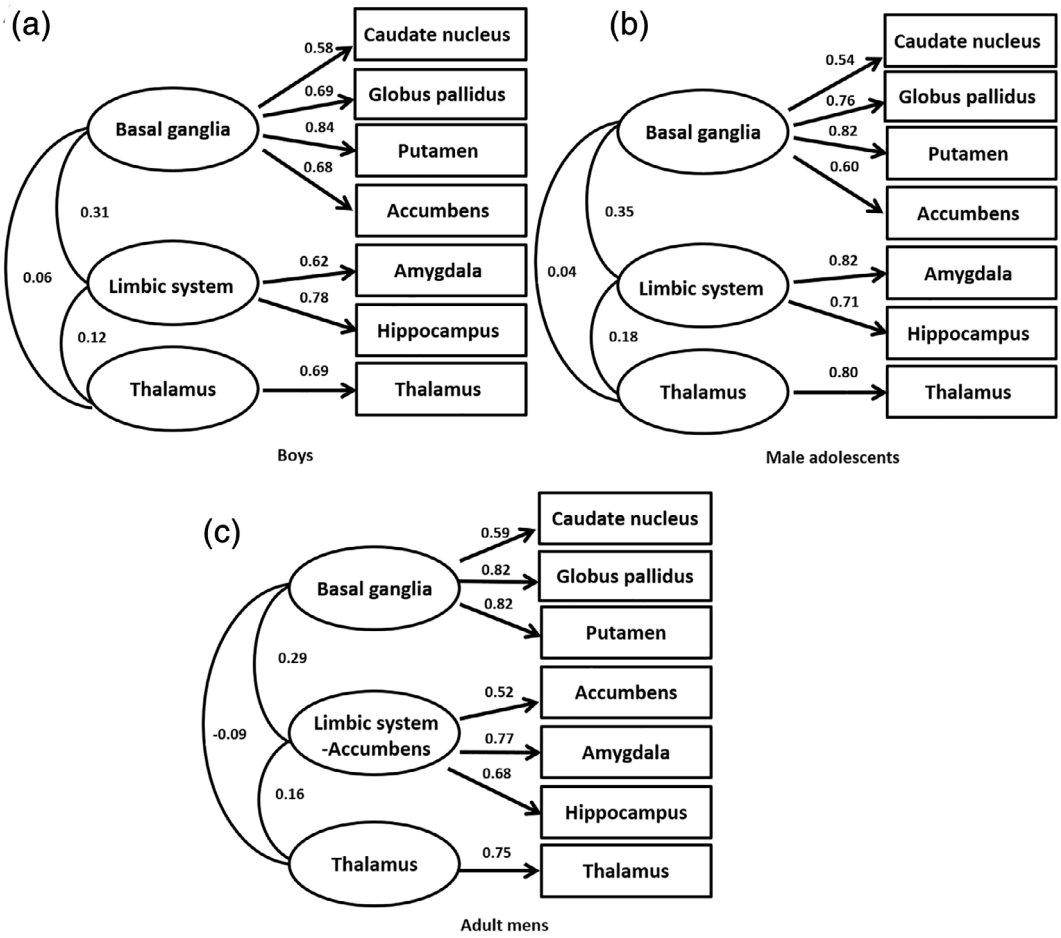

FIGURE 2 The three-factor model that was generated by EFA. (a) Boys. (b) Male adolescents. (c) Adult men

$p = 1.2e{-}14$; ASD diagnosis: $F = 4.38$, $p = 6.9e{-}04$; adult men: Communities: $F = 3.12$, $p = 6.8e{-}04$; ASD diagnosis: $F = 0.83$, $p = 0.75$).

ASD clinical profiles and comorbidities in communities

In current study, we added analyses on the relations between brain-based community and ASD clinical presentation, including IQ, ADOS score, comorbidities, and medication use, which is presented in Table S8. In the subsample of boys with ASD, the ADOS total score was available for $n = 338$ (43.8%), which was used as an estimate of ASD severity (Table S8). After controlling for age and cohort sites, we found significant differences in ADOS scores between communities ($F = 5.24$, $df = 3$, $p = 0.013$). Post hoc analysis indicated Community 1 had a significantly lower ADOS score than Community 2 ($p = 0.003$); Community 4 had significantly lower ADOS score than Community 2 ($p = 0.0002$) and Community 3 ($p = 0.03$). There were no significant differences in ADOS score between the communities in the subsample of male adolescent ($n = 198$ [55.0%], $F = 2.0$, $df = 2$, $p = 0.14$) and adult men ($n = 118$ [53.4%], $F = 0.08$, $df = 2$, $p = 0.98$).

IQ scores were available for 577 (74.7%) boys with ASD, 322 (89.4%) male adolescents with ASD and 201 (91.0%) adult men with ASD (Table S8); no association between IQ and the communities was observed (boys: $F = 0.13$, $df = 3$, $p = 0.99$; male adolescents: $F = 0.61$, $df = 3$, $p = 0.74$; adult men: $F = 0.79$, $df = 3$, $p = 0.46$).

Medication use information was available for $n = 403$ (52.2%) boys, $n = 151$ (41.9%) male adolescents, and $n = 98$ (44.3%) adult men with ASD (Table S8). No significant association between medication use and the communities was found (boys: $\chi^2 = 4.3$, $p = 0.47$; male adolescents: $\chi^2 = 0.84$, $p = 0.82$; adult men: $\chi^2 = 1.3$, $p = 0.74$).

we investigated the presence or absence of common comorbidities in ASD, including ADHD, OCD, Tourette's symptoms, learning disability, depression, and anxiety. Information was available only for a very limited number of participants, that is, 87 (11.3%) boys with ASD, 66 (18.3%) male adolescents with ASD, and 58 (26.2%) adult men. There was no significant association between comorbidities and the communities (boys: $\chi^2 = 4.4$, $p = 0.46$; male adolescents: $\chi^2 = 0.48$, $p = 0.90$; adult men: $\chi^2 = 0.66$, $p = 0.85$).

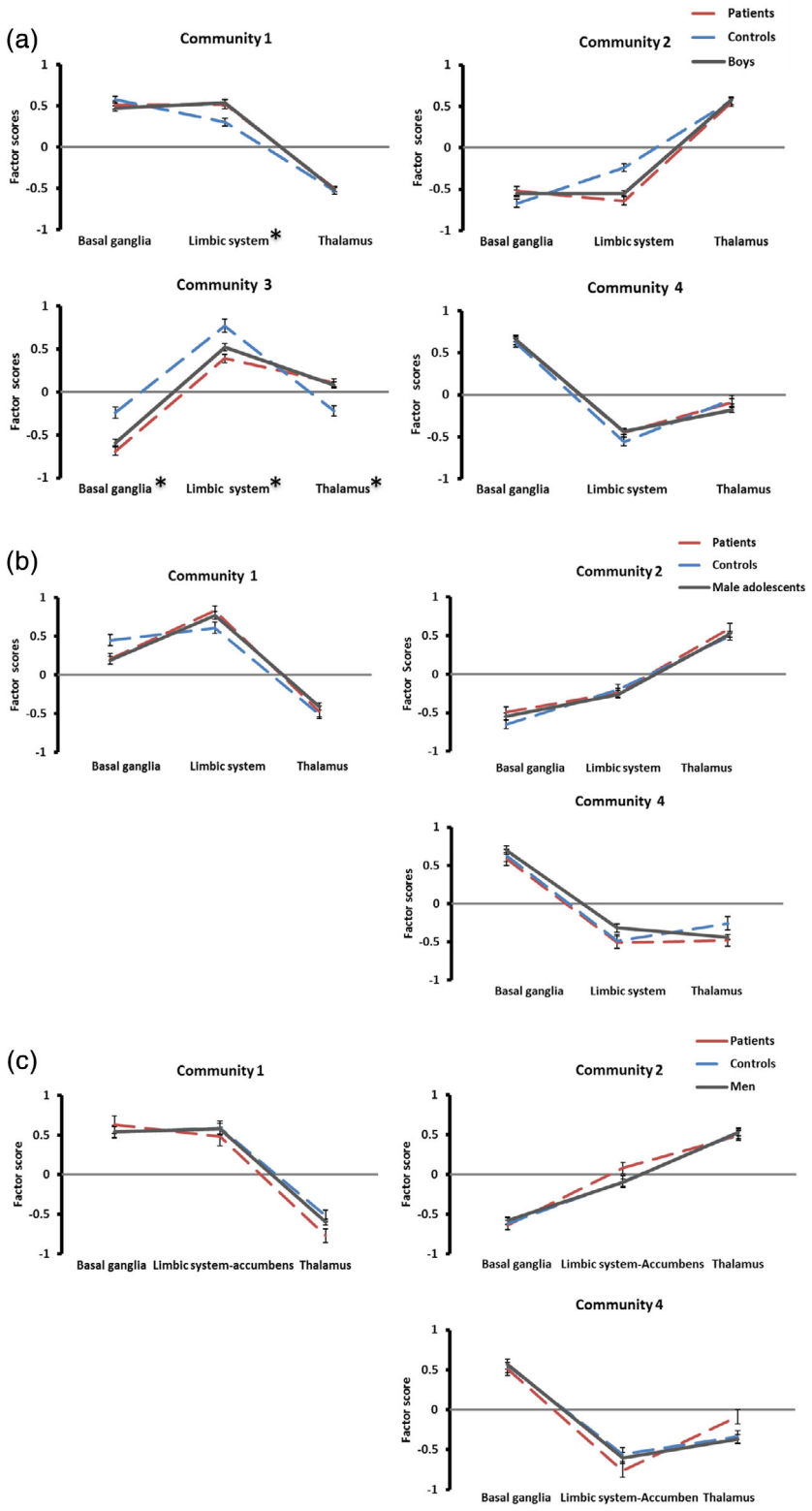

FIGURE 3 Subgroups generated by CD. (a) Boys. (b) Male adolescents. (c) Adult men. *Lines* represent participants in each community from CD. y-axis indicates the mean factor scores for each factor. Error bars: *SEM*. *indicates case/control difference of subcortical factor scores was significant

DISCUSSION

In this study, we aimed to dissociate subgroups of ASD participants based on neuroanatomical profiles of subcortical brain volumes. We hypothesized that the effect sizes of case/control differences would be larger within each subgroup. In our EFA, we found that the latent structure of subcortical volumes was composed of three factors, which remain largely stable across the lifespan and were identical in participants with and without ASD.

TABLE 2 The percentages of participants in each community of the three subsamples

Sample	Total	Patients	Controls
Boys (*N*)	1505	772	733
1	381 (25.3%)	221 (28.6%)	200 (27.3%)
2	402 (26.7%)	204 (26.4%)	240 (32.7%)
3	345 (22.9%)	193 (25.0%)	129 (17.6%)
4	377 (25.0%)	154 (19.9%)	164 (22.4%)
Q values	0.45	0.46	0.43
Male adolescent (*N*)	681	360	321
1	184 (27.0%)	105 (29.2%)	105 (32.7%)
2	305 (44.8%)	159 (44.2%)	143 (44.5%)
4	192 (28.2%)	96 (26.7%)	73 (22.7%)
Q values	0.47	0.48	0.48
Men (*N*)	475	221	254
1	142 (29.9%)	60 (27.1%)	75 (29.5%)
2	232 (48.8%)	104 (47.1%)	119 (46.9%)
4	101 (21.3%)	57 (25.8%)	60 (23.6%)
Q values	0.44	0.47	0.44

Note: Q values: the quality index of modularity.

Among them, we discerned four distinct communities in boys, three in male adolescents, and adult men. Within some communities, the effect sizes of case/control differences in neuroanatomical volume were much stronger than those across the whole sample. Moreover, these communities have potential clinical links with ASD symptom severity as indicated by higher ADOS scores.

In the subsamples of boys and male adolescents, the same three-factor structures—basal ganglia, limbic system, and thalamus were observed based on subcortical brain volume. In adult men, the three-factor structure was slightly different; nucleus accumbens loaded into the second factor, which was named "limbic system-accumbens," instead of the limbic system factor. These structural patterns of subcortical brain volumes were observed regardless of diagnostic status in those with and without ASD, indicating no qualitative differences in subcortical brain organization exist in ASD. The factor structures are largely in line with previous smaller-scale studies on subcortical brain organization. One study using 322 healthy adults (age range 65–85 years) reported three clusters based on cortex and subcortical structures, with one cluster comprising of the basal ganglia (caudate, putamen, and pallidum) and the second cluster including the nucleus accumbens, amygdala, hippocampus, and thalamus; the cortical lobes were in the third cluster (Wen et al., 2016). Another study on 404 healthy adults indicated that subcortical brain volumes could be partitioned into three factors: basal ganglia/thalamus, nucleus accumbens, and limbic factor (Eyler et al., 2011). In recent a study of the ENIGMA-ADHD working group, identical subcortical factor structure as in the

TABLE 3 Case/control comparison of subcortical factor scores in ASD

	Basal ganglia					Limbic system					Thalamus				
	Mean factor scores			Adjusted		Mean factor scores			Adjusted		Mean factor scores			Adjusted	
Community	Patients	Controls	*p*-value	*p*-value	Cohen's d (95% CIs)	Patients	Controls	*p*-value	*p*-value	Cohen's d (95% CIs)	Patients	Controls	*p*-value	*p*-value	Cohen's d (95% CIs)
Boys	−0.03 (0.93)	0.03 (0.91)	0.15	0.37	−0.07 (−0.18–0.03)	−0.01 (0.87)	0.01 (0.85)	0.54	0.74	−0.03 (−0.13–0.07)	0.01 (0.75)	−0.01 (0.78)	0.57	0.74	0.03 (−0.07–0.13)
1	0.51 (0.73)	0.58 (0.58)	0.33	0.53	−0.10 (−0.29–0.10)	0.52 (0.77)	0.30 (0.67)	**2.0e−03**	**0.01**	0.30 (0.11–0.49)	−0.50 (0.64)	−0.53 (0.58)	0.41	0.62	0.05 (−0.14–0.24)
2	−0.52 (0.75)	−0.67 (0.78)	**0.04**	0.15	0.19 (−0.01–0.38)	−0.64 (0.70)	−0.24 (0.71)	**6.6e−09**	**1.4e−07**	−0.56 (−0.75 - −0.37)	0.55 (0.70)	0.57 (0.70)	0.82	1.0	−0.02 (−0.21–0.17)
3	−0.69 (0.68)	−0.24 (0.75)	**5.2e−08**	**1.0e−06**	−0.63 (−0.86 - -0.41)	0.39 (0.69)	0.77 (0.86)	**1.5e−05**	**1.7e−04**	−0.50 (−0.73 - -0.27)	0.11 (0.62)	−0.22 (0.68)	**1.1e−05**	**1.3e−04**	0.51 (0.28–0.74)
4	0.65 (0.69)	0.62 (0.68)	0.67	0.81	0.05 (−0.17–0.27)	−0.45 (0.60)	−0.56 (0.64)	0.26	0.31	0.17 (−0.05–0.40)	−0.09 (0.56)	−0.06 (0.65)	0.64	0.81	−0.05 (−0.27–0.17)
Male adolescents[a]	0.00 (0.91)	0.00 (0.93)	1.0	1.0	5.7e−18 (−0.15–0.15)	0.00 (0.91)	0.00 (0.90)	1.0	1.0	2.2e−16 (−0.15–0.15)	0.00 (0.95)	0.00 (0.82)	1.0	1.0	−6.5e−17 (−0.15–0.15)
1	0.21 (0.68)	0.45 (0.75)	**0.02**	0.09	−0.33 (−0.60 - -0.05)	0.83 (0.66)	0.61 (0.72)	**0.02**	0.10	0.32 (0.04–0.59)	−0.47 (0.77)	−0.51 (0.57)	0.68	0.81	0.06 (−0.21–0.33)
2	−0.49 (0.82)	−0.65 (0.69)	0.07	0.22	0.21 (−0.02–0.44)	−0.24 (0.73)	−0.20 (0.85)	0.06	0.20	−0.06 (−0.28–0.17)	0.60 (0.82)	0.50 (0.72)	0.28	0.48	0.12 (−0.10–0.35)
4	0.58 (0.82)	0.63 (0.76)	0.67	0.81	−0.06 (−0.37–0.24)	−0.51 (0.79)	−0.49 (0.75)	0.89	0.94	−0.03 (−0.33–0.28)	−0.48 (0.74)	−0.26 (0.75)	0.06	0.20	0.29 (−0.60–0.01)
Adult Men[a]	0.00 (0.92)	0.00 (0.93)	1.0	1.0	2.0e−17 (−0.18–0.18)	0.00 (0.89)	0.00 (0.89)	1.0	1.0	−2.7e−16 (−0.18–0.18)	0.00 (0.83)	0.00 (0.82)	1.0	1.0	6.9e−17 (−0.18–0.18)
1	0.63 (0.88)	0.54 (0.71)	0.52	0.72	0.11 (−0.23–0.46)	0.48 (0.95)	0.59 (0.80)	0.49	0.69	−0.12 (−0.46–0.22)	−0.77 (0.66)	0.52 (0.60)	**0.02**	0.10	−0.40 (−0.75–−0.05)
2	−0.64 (0.60)	−0.62 (0.76)	0.81	0.90	−0.03 (−0.30–0.23)	0.08 (0.75)	−0.09 (0.84)	0.11	0.30	0.22 (−0.05–0.48)	0.49 (0.63)	0.52 (0.52)	0.08	0.24	−0.04 (−0.31–0.22)
4	0.51 (0.62)	0.55 (0.65)	0.70	0.83	−0.06 (−0.42–0.29)	−0.66 (0.66)	−0.56 (0.64)	0.40	0.61	−0.15 (−0.52–0.21)	−0.09 (0.68)	−0.39 (0.63)	**0.02**	0.08	0.46 (0.09–0.82)

Note: Adjusted *p*-value: adjusted *p*-value: FDR-correction. Significant difference in bold.

Abbreviation: 95% CIs: 95% Confidence intervals.

[a]Community 3 is absent in male adolescents and adult men, because no healthy controls were loaded using CD.

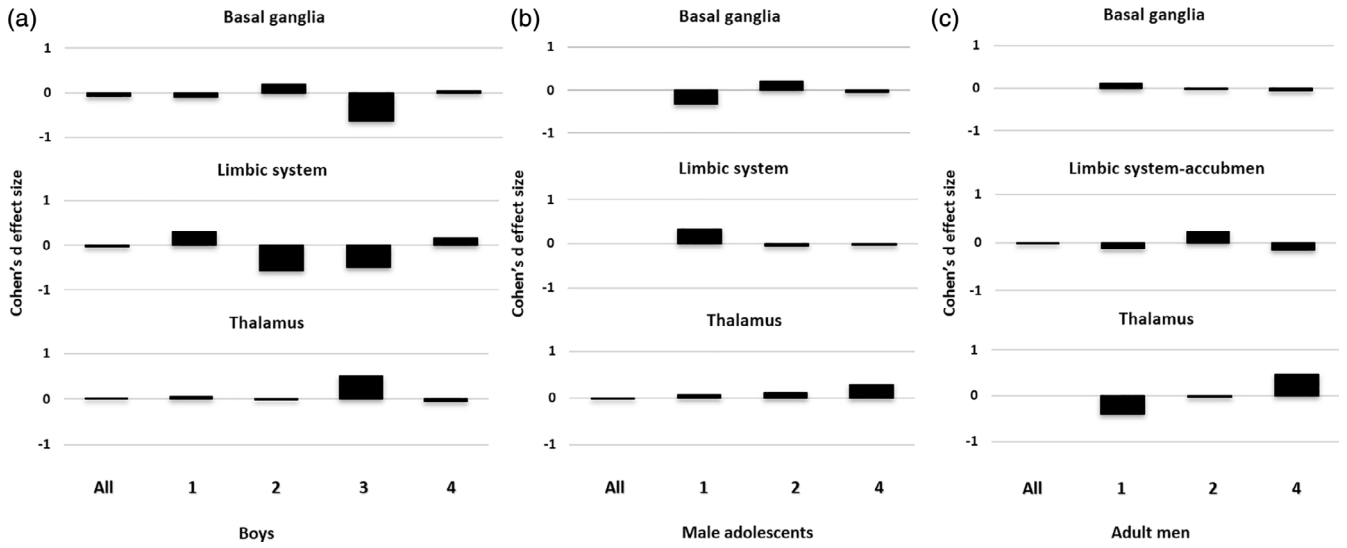

FIGURE 4 Effect sizes of case/control comparison within each community and the whole subsample. (a) Boys. (b) Male adolescents. (c) Adult men. All: The whole subsample, 1: Community 1; 2: Community 2; 3: Community 3; 4: Community 4

current analysis—basal ganglia, limbic system, and thalamus—existed in boys and adult men, which was irrespective of ADHD diagnosis and age (Li et al., 2021). Nucleus accumbens receives direct glutamatergic inputs from the amygdala and hippocampus, and the nucleus accumbens shell may be regarded as a part of the extended amygdala (Salgado & Kaplitt, 2015); this may explain why the nucleus accumbens loads on either the basal ganglia or the limbic system in the current study. Using a lifespan approach, we observed variated factor structure between the three aged subsamples, which may suggest the correlations between subcortical structures change slightly during maturation (Sussman et al., 2016).

Using CD analysis, each subsample could be stratified into similar subgroups with more homogeneous neuroanatomical patterns. Four communities were observed in boys, three were seen in male adolescents and adult men, irrespective of ASD status and age; The CD results indicated that the heterogeneity in subcortical brain volumes is nested within normative variability, with different neuroanatomical communities existing in both patients and healthy controls (Marquand et al., 2016). Importantly, the observed community structure is highly consistent with our findings in the ENIGMA-ADHD working group (Li et al., 2021). With two independent cohorts (different samples of healthy controls), we observe not only identical factor structures, but also similar communities, which greatly supports the robustness of our current analysis. In fact, the CD results in the healthy controls group of the ENIGMA-ADHD cohort can be viewed as an independent, external validation of the current observation. This also allows us to investigate whether participants with ADHD and ASD show differences in their community structure. In the current analysis, Community 3 was not observed in adolescents and men. In the ENIGMA-ADHD analysis, we also observed that Community 3 was absent in healthy men, but not in men with ADHD (Li et al., 2021). This reduction of subgroups from four in the subsample of boys to three in male adolescents and adult men may be related to structural brain maturation over age, leading to less diversity in the organization of subcortical volumes in the population (Coupe et al., 2017).

In the current study, analyzing case/control differences within communities indicated substantially larger effect sizes than those in the entire sample (van Rooij et al., 2018). Interestingly, case/control differences are not consistently present in all subcortical factors of each community. For example, boys with ASD have increased volume of the limbic system in Community 1, but smaller volume in Community 2 and 3 compared with healthy controls. The substantially larger effect sizes suggest that neuroanatomical-based subgroups may exist in the entire population. The results also may explain the subcortical heterogeneity. Previous small studies may have accidentally recruited a disproportionately large number of specific subgroups, which resulted in contradictory subcortical alterations in ASD (Lombardo et al., 2019). In our study, the brain-based ASD subgroups accounted for more variance of subcortical brain volumes than the ASD diagnostic groups.

We further investigated associations between the brain-based communities and clinical presentation, including ADOS scores, IQ, medication use, and comorbidities. Only ADOS scores, indicating the ASD severity, showed significant differences between communities in the subsample of boys with ASD. Specifically, in Community 1, boys with ASD had lower ADOS scores than Community 2; boys with ASD in Community 4 also had lower ADOS scores than those in Communities 2 and

3. No differences between communities were observed in male adolescence and adult men. A previous study on the full ENIGMA-ASD cohort reported significant associations between ADOS scores and subcortical volumes (van Rooij et al., 2018). Although it is hard to directly compare these results due to differences in samples and methodologies used in each ENIGMA-ASD cohort, our findings support the notion that subcortical brain volumes are linked to ASD clinical presentation. Unfortunately, ADOS scores were only available in a small proportion of ENIGMA-ASD cohorts. We cannot entirely exclude the possibility that the identified communities were associated with residual side effects that were not eliminated by the regression analysis. Without detailed clinical information, our interpretations must be treated with caution until the findings have been replicated. Significant difference in IQ was reported between ASD patients and healthy controls, however, we did not observe that the brain-based communities were associated with IQ in ASD patients. This indicates brain-based subtypes are unlikely to reflect IQ differences. We did not find the association between brain-based communities and medication uses or the presence/absence of any comorbidities either. As the spare clinical information has been mentioned before, the lack of significant association may be due to insufficient statistical analysis power. Replication in an independent, large, and enriched data set with deep phenotypes is in need.

This work has to be viewed in light of several strengths and limitations. Using the MRI data set from the ENIGMA-ASD working group, we had a large sample size allowing us to explore underlying structural pattern and subgroups in ASD across the lifespan; the large sample size enabled us to split the whole cohort into three subsamples. By applying EFA and CD independently in each subsample, we were able to observe consistent subgroups across subsamples. Combination of our previous work in ENIGMA-ADHD cohort, in which similar subgroups were reported in a completely independent cohort. It has sufficiently validated the robustness of cluster analysis in this study; However, as previously mentioned, the limited availability of demographic and clinical information restricted our ability to explore how brain-based communities are linked to the clinical presentation of ASD. Related to the limited information, potential variations in clinical assessment across different sample sites increase the difficulty of harmonized data sets. As mentioned before, ASD represents spectra with large heterogeneity in symptomatology, neuropsychology, neurobiology, and comorbidity (Lord, 2019; Masi et al., 2017). Whether the varied presentation may come forth from the same underlying putative mechanism or maybe the result of different mechanisms remains to be clarified. In the future, more diverse, demographically enriched data sets with deep phenotypes are needed to tackle such huge heterogeneity in ASD. Moreover, in this study, we only had sufficient power to analyze male participants. Previous studies have reported sex differences in subcortical brain volumes (Ritchie et al., 2018), and different underlying subcortical organizations were reported in females from the ENIGMA-ADHD working group (Li et al., 2021). Given that sex-based differences in neuroanatomy are central topics in ASD (Ecker et al., 2017; Lai et al., 2017), further analyses including female samples may help to elucidate the association between neuroanatomical organization and the specific etiology of ASD in females. Thirdly, the arbitrariness of employing the modularity algorithm can be a potential limitation. Although it is a widely used technique and consistent approximations were obtained across subsamples and disease diagnoses (Li et al., 2021), this unsupervised approach might be influenced by unknown effects. We also cannot exclude the possibility that other classification methodologies may result in different subgroups. Further analysis could leverage hybrid approaches, such as normative modeling (Marquand et al., 2016), surrogate variable analysis (Leek & Storey, 2007) to parse and understand the complex, heterogeneous nature of ASD. An additional alternative could be to apply a priori subtyping based on a more biologically informed phenotype framework, like the hierarchical taxonomy of psychopathology (HiTOP) framework (Kotov et al., 2017). In conclusion, using subcortical brain volume data from the ENIGMA-ASD working group, we were able to stratify participants with and without ASD into more homogeneous subgroups based on the underlying neuroanatomical organization. Our results indicate that this stratification may enhance our capability to observe case/control differences and may help to explain the contradictory results observed in previous studies on brain structure in ASD.

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CONFLICT OF INTEREST

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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