

The Cingulate Structures in Autism Spectrum Disorder: Exploring Its Implication on Social Awareness and the Role of CNTNAP2

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Research

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Abstract

Backgrounds Although evidence suggests that the activity of the anterior cingulate cortex involves social cognition, there are inconsistent results regarding the aberrant cingulate gray matter (GM) and scanty evidence about altered cortical thickness and white matter (WM) of cingulate in individuals with autism spectrum disorder (ASD). Evidence supports the association between the autism risk gene *CNTNAP2* variants and altered brain connectivity. This study investigated the cingulate substructure and its association with social awareness deficits and the *CNTNAP2* variants in individuals with ASD.

Methods We assessed 122 individuals with ASD and 118 typically-developing controls (TDC) with MRI and clinical evaluation. The GM, WM volumes and cortical thickness of the cingulate gyrus were compared between ASD and TDC based on fine parcellation. Five SNPs of the *CNTNAP2* linking to ASD and brain structural abnormality were genotyped.

Results ASD individuals showed thinner cortical thickness in bilateral cingulate subregions than TDC but no significant group differences in GM and WM volumes. The WM volume of the right anterior cingulate gyrus was correlated with social awareness deficits in ASD. Two *CNTNAP2* variants demonstrated the main effects on the WM volumes of the right middle cingulate gyrus and the cortical thickness of the right posterior ventral cingulate gyrus. Besides, the *CNTNAP2* variants interacted with ASD status and age on the cortical thickness of the left anterior middle cingulate cortex.

Limitations The *CNTNAP2* variants selected in this study were based on the current literature that repeatedly revealed genetic associations with ASD and brain structures. Future studies may consider fine mapping or sequencing of this gene. Besides, we measured social awareness deficits by caregiver-report questionnaire. Although the observation from caregivers may reflect social function in daily life, a social cognition task may better provide a standardized measurement.

Conclusions Our findings suggest that aberrant cingulate structure in ASD may be associated with the severity of autistic symptoms and genetic variants of the *CNTNAP2*. These novel findings need validation.

Trial registration: ClinicalTrials.gov number, NCT01582256

Introduction

Alterations of the cingulate structure are frequently reported in individuals with autism spectrum disorder (ASD) [1]. A meta-analysis of voxel-based morphometry (VBM) studies suggested that individuals with ASD showed a significant increase of grey matter (GM) volume in the subgenual anterior cingulate cortex (ACC) [1]. However, such associations were not supported by another two meta-analyses [2, 3]. A recent meta-analysis focusing on adults with ASD, instead, reported reduced GM volume in the ACC [4]. These inconsistent findings may be attributable either to different focuses within the cingulate substructures (i.e., subgenual ACC, dorsal/ventral ACC or the whole cingulate cortex) or different age populations

across studies. Most existing studies only involved ACC but ignored other parts of the cingulate structure. Given that the cingulate cortex is anatomically and functionally heterogeneous and consists of distinct cytoarchitectonic zones that differ in cellular structures indicative of functional subdivision [5, 6], whether the subregions of the cingulate structure are altered in ASD in different patterns is worth investigation.

Regarding the function of the cingulate cortex, the ACC has been implicated in a broad range of behaviors and cognitive processes, including social cognition [7–9]. Using functional MRI to assess brain activation when doing the oddball social task, dorsal ACC activation was found to predict the severity of social impairments in a subset of ASD individuals [10]. By contrast, whether the structure of the cingulate cortex can predict the social cognitive impairment in individuals with ASD is largely unknown. A report has displayed that increased thickness in the rostral ACC was associated with more severe social impairment on the Autism Diagnostic Interview-Revised (ADI-R), suggests that cortical thickness of ACC may be correlated with the severity of social deficits in ASD [11]. Although there was evidence implying the role of ACC on regulating social behaviors [7], it is unclear whether the other subregions of cingulate also involves similar functions. New evidence supports that the middle cingulate cortex (MCC), previously noted as the dorsal ACC, may specifically involve in social cognition [12], whereas the posterior cingulate cortex (PCC) may involve in attention and arousal state [13]. The reduced functional coupling between PCC and ventromedial prefrontal cortex was highlighted in a recent meta-analysis that provided an early insight into the multiple dimensions of functioning, including higher-order cognitive and complex social functions [14]. Given that MCC and PCC have been implicated as ASD-associated impairment, such as social cognition [12] and attention [13], whether these structural alterations are associated with social awareness deficits is of particular interest.

Recent studies have shown that brain structures such as total brain volume [15], cortical thickness [16–18], and measures of white matter (WM) integrity derived from diffusion tensor imaging [19] are all under moderate genetic control. Evidence suggests that several genes may play a role in regulating brain development. The contactin-associated protein-like 2 (*CNTNAP2*) gene encodes CASPR2, which is a member of the neurexin superfamily of transmembrane proteins that are responsible for voltage-gated K⁺ channel clustering in juxtaparanodes [20, 21] at the nodes of Ranvier [22]. As a cell adhesion molecule responsible for neuroblast migration and laminar organization [23–25], CASPR2 is suggested to play a role in the brain development. Performing cortical neuron cultures from mouse embryos, Caspr2 was lately demonstrated to play a dose-dependent role in axon growth in vitro [26]. A study of an Amish family reported that a deletion mutation in the *CNTNAP2* gene manifested with many hallmarks of autism including seizures, language difficulties, and social deficits [22]. Other studies suggested that homozygous mutations in exon 22 of the *CNTNAP2* resulted in an ASD diagnosis in 67% of cases [22], while heterozygous mutations were associated with altered brain structure and functional connectivity in otherwise neurotypical subjects [27–29]. On the other hand, mouse models lacking *Cntnap2* presented with autistic behaviors, including repetitive behaviors and impairments in social interactions and communication [30, 31]. Loss of the *CNTNAP2* produces abnormal neuronal migration that was recently highlighted in the neuropathology of ASD [32], decreased numbers of interneurons, and reduced cortical

synchrony among the neuropathological abnormalities [31]. These findings shed light on the functional consequences of *CNTNAP2* disruption at the level of cortical structure.

The *CNTNAP2*, an autism susceptibility gene [23, 24, 33–36], was reported to be associated with GM and WM volume [37–39] and structural disconnectivity [27, 28, 40] in several regions that had already been implicated in ASD, including the cerebellum [39], fusiform gyrus, occipital [37] and frontal cortices [28, 38]. Several common variants of the *CNTNAP2* were reported to be associated with reduced GM volumes (e.g., rs7794745) [37, 39], structural connectivity (e.g., rs2710102 [28], rs2710126, rs759178, and rs2538991 [40]), and functional connectivity (of posterior right temporoparietal junction with rs2710102 [41]). These variants overlap with the SNPs that are associated with the risks for ASD [23, 35], early communicative difficulty [33, 42–44], or social performance [41], particularly the SNPs in intron 13. The genetic effects of these five SNPs of the *CNTNAP2* gene would be tested herein for its role in the cingulate structure.

The current study aimed to investigate the cingulate structures in individuals with ASD and typically developing controls (TDC). With a more delicate method of parcellation (Fig. 1), the subregions of cingulate GM (i.e., ACC, anterior MCC, posterior MCC, ventral and dorsal PCC), WM volumes (rostral ACC, caudal ACC, PCC, and isthmus), and cortical thickness (i.e., ACC, anterior MCC, posterior MCC, ventral and dorsal PCC) were analyzed. We examined whether age, sex and autistic symptoms were significant correlates of cingulate structures. Besides, we analyzed the genetic associations between the cingulate structures and the *CNTNAP2* variants (i.e., rs779475, rs759178, rs2710102, rs2538991, and rs2710126) that were repetitively shown to be associated with ASD and brain structure. We hypothesized that individuals with ASD might have abnormal cingulate structures compared to TDC, and these structural alterations were associated with the severity of social awareness deficits. We also hypothesized that ASD susceptibility gene, *CNTNAP2*, may play a role in modifying cingulate structures of ASD.

Materials And Methods

Participants

We collected brain MRI scans, clinical and genetic data from 118 individuals with ASD (mean 13.1 years, SD 4.6; male 113, 95.8%), and 122 TDC (mean 21.0, SD 9.7; male 75, 61.5%). Individuals with ASD were recruited from National Taiwan University Hospital, Taipei, Taiwan. They were clinically diagnosed as ASD by senior board-certified child psychiatrists based on the Diagnostic and Statistical Manual of Mental Disorders-5th edition (DSM-5) diagnostic criteria of autism spectrum disorder; the diagnosis was further confirmed by a structural interview using the Chinese version of the ADI-R [45, 46]. Participants with major neuropsychiatric disorders or with a full-scale IQ lower than 70 were excluded. The TDC participants were recruited from schools in the same districts of the ASD participants. All the participants went on clinical evaluation and interviews; all their parents were also interviewed by using the Chinese version of the Kiddie epidemiologic version of the Schedule for Affective Disorders (K-SADS-E) interview [47, 48] to exclude any current or lifetime ASD and other major psychiatric disorders including attention-

deficit/hyperactivity disorder, schizophrenia, mood disorders, anxiety disorders, or neurodevelopmental disorders. The details of the psychometric properties of the K-SADS-E and interview training have been described elsewhere [47-50].

Procedure

The Research Ethics Committee approved the study before its implementation (Approval number: 201201006RIB; **ClinicalTrials.gov number**, NCT01582256). After the purposes and procedures of the study were fully explained and confidentiality was assured, written informed consent was obtained from the participants and their parents. All the participants were then assessed with the brain MRI, and the *Wechsler Intelligence Scale for Children (version III)* or the *Wechsler Adult Intelligence Scale (version IV)* for IQ profile according to their ages. The parents (mainly mothers) completed the following clinical measures about the participants.

Measures

The *ADI-R* [46] is a standardized, comprehensive, semi-structured, investigator-based interview for the caregivers of children with a mental age of 18 months into adulthood. It covers most developmental and behavioral aspects of ASD, including qualitative abnormalities in reciprocal social interaction, communication, and restricted, repetitive and stereotyped patterns of behaviors. The Chinese version of the ADI-R was approved by the Western Psychological Services in 2007 and has been widely used in several studies to validate the clinical diagnosis of ASD (e.g., [51-53]). The details of psychometric studies and interviewers training of using the ADI-R in Chinese have been described elsewhere [54, 55].

The *Social Responsiveness Scale (SRS)* [56] is a widely-used quantitative measure of autistic traits in the general population. It includes 65 items to measure the severity of ASD symptoms in natural social settings over the past six months for children and adolescents aged 4–18 years. Items were rated by parents or caregivers on a 4-point Likert scale from “0” (not true) to “3” (almost always true). The SRS has been demonstrated to have good internal consistency, construct validity, inter-rater reliability, test-retest reliability, and discriminative validity in prior research [56]. Its Chinese version demonstrates a four-factor structure (i.e., social communication, stereotyped behaviors/interest, social awareness, and social emotion), but is better conceptualized as a one-factor model [54]. The Chinese SRS has been widely used in ASD research in Taiwan (e.g., [48, 57-59]). High internal consistency was found for the four subscales (Cronbach’s alpha, .94–.95) and the total scale (Cronbach’s alpha, .95). To test the association between cingulate structures and social cognition, we specifically targeted the social awareness deficits subscale in this study.

Genotyping

Single nucleotide polymorphism (SNP) selection and genotyping.

Genomic DNA was prepared from peripheral blood using the Puregene DNA purification system (Gentra Systems Inc. Minneapolis, MI) according to the manufacturer's instructions. Five SNPs of the *CNTNAP2*,

located in the intron 2 (rs779475) [37, 39], intron 13 (rs759178, rs2710102, rs2538991) [28, 40, 41], and intron 15 (rs2710126) [40], were selected for genotyping based on previous imaging genetic studies.

The primers of each SNP were designed by the platform of National Center for Genome Medicine (<http://ncgm.sinica.edu.tw/>), using GenePipe (<http://genepipe.ncgm.sinica.edu.tw/seqtool/pages/getSeq.jsp>) to retrieve SNP flanking sequences. All SNP genotyping was performed by SEQUENOM MassARRAY® System using the method of matrix-assisted laser desorption/ionization-time of flight mass spectrometry. The genotyping technology platform “iPLEX® Gold reaction” provides high throughput, high accuracy, and low cost SNP analysis (http://ncgm.sinica.edu.tw/ncgm_02/snp_platform_e.html). The success rate of genotyping of the five selected SNPs were 99–100%. Genotype frequency is summarized in Supplementary Table S1.

MRI Data Acquisition

Brain images were acquired on a 3T MRI system (Trio, Siemens, Erlangen, Germany). Head movement was restricted with expandable foam cushions and was assessed immediately after image acquisition. High-resolution T1-weighted MR images were acquired covering the whole head with a three-dimensional (3D) magnetization-prepared rapid gradient echo (MPRAGE) sequence, resulting in an isotropic spatial resolution of 1 mm³.

Whole brain segmentation and cortical thickness calculation.

FreeSurfer V5.2.0 (<https://surfer.nmr.mgh.harvard.edu/>) was used on a 64-bit Linux operating system to reconstruct the cortical surface from the MPRAGE images [60]. Whole brain segmentation [61, 62] was performed. The cortical parcellation units of the cortex were automatically identified and labeled according to the Desikan atlas [63] within the FreeSurfer automatic cortical parcellation routine. The cortical thickness was automatically calculated by computing the shortest distance between the WM boundary and the pial surface at each vertex [64]. The reliability of the cortical thickness calculated by FreeSurfer has been validated [65]. The automatic reconstruction and calculation were reprocessed after manually correcting the detected erroneous part. The automatic parcellation of cortical regions derived 74 brain regions in each hemisphere, and the thickness of each region was then calculated. This study focused on the cingulate structure, in which GM was divided into five subregions, i.e., ACC, anterior and posterior parts of MCC, and dorsal and ventral parts of PCC, while WM was divided into four subregions, i.e., the rostral ACC, caudal ACC, PCC, and isthmus (Figure 1).

Statistical Analysis

We used SAS v. 9.4 (SAS Institute Inc, Cary NC, USA) to perform the statistical analyses. Age and IQ profiles were compared by analysis of variance, while SRS subscores were compared between the ASD and TDC groups by the generalized linear model controlling for sex and age. In an age- and sex-compatible subsample (88 ASD & 51 TDC), we compared the volume of the GM and WM, and cortical thickness of cingulate structures for each subregion between ASD and TDC, controlling for sex, age, full-

scale IQ, and handedness. The relationships between cingulate structures and social awareness deficits were examined by Pearson's correlation analyses in the ASD group, partial out the effects of age, sex, and full-scale IQ. The genetic effects of the *CNTNAP2* variants on the cingulate structures were firstly examined by the main effect of each SNP on cingulate structures (i.e., GM and WM volumes, and cortical thickness of each subregion). Then, we tested the interactions between the *CNTNAP2* variants and age or diagnosis on each cingulate structure, controlling for age, sex, and full-scale IQ, as well as the main effects of each SNP and diagnosis. False discovery rate (FDR) was applied to correct for multiple comparisons. FDR q-value < 0.05 was set as statistical significance. As for sensitivity power analysis, with a total sample size of 240, the statistical tests had a power 0.8 to detect a difference with the effect size of 0.27.

Results

The IQ profiles of the whole sample were within the normal range, while the ASD group had significantly lower full-scale IQ (100.8 ± 19.9) compared to the TDC (116 ± 10.9) (Table 1). At the most severe period (age of 4–5 years), the ADI-R subscores of the ASD group passed the cut-off of a diagnosis of autism: Social Reciprocal Interaction, 19.68 ± 6.98 ; Verbal Communication, 14.69 ± 5.03 ; Non-verbal Communication, 7.84 ± 3.43 ; Repetitive/Stereotyped Behavior/Interests, 7.26 ± 2.67 (Table 1). The social awareness deficits measured by SRS were significantly more severe in the ASD group compared to the TDC group (Table 1).

Table 1

Demographic data and autistic symptoms measured on the Social Responsiveness Scale and Autism Diagnostic Interview-Revised

| | ASD (N = 118) | | TDC (N = 122) | | F | p |
|----------------------------------------------|------------------|--------------|------------------|--------------|-------|---------|
| | Mean or N | SD or (%) | Mean or N | SD or (%) | | |
| Age | 13.1 | 4.6 | 20.9 | 9.7 | 63.27 | < .0001 |
| Male (%) | N = 113 | (95.8) | N = 75 | (61.5) | 41.55 | < .0001 |
| Handedness | | | | | | |
| Verbal IQ | 100.8 | 19.9 | 111.6 | 10.9 | 28.51 | < .0001 |
| Performance IQ | 99.7 | 21.1 | 111.5 | 13.3 | 26.99 | < .0001 |
| Full-scale IQ | 100.1 | 20.1 | 112.3 | 11.7 | 33.31 | < .0001 |
| Autism Diagnostic Interview-Revised | | | | | | |
| Most severe at 4–5 years | | | | | | |
| Social reciprocal interaction | 19.68 | 6.98 | | | | |
| Communication: verbal | 14.68 | 5.03 | | | | |
| nonverbal | 7.84 | 3.43 | | | | |
| Repetitive/stereotyped behavior/interests | 7.26 | 2.67 | | | | |
| Current | | | | | | |
| Social reciprocal interaction | 10.29 | 4.71 | | | | |
| Repetitive/stereotyped behavior/interests | 5.27 | 2.52 | | | | |
| Social Responsiveness Scale | | | | | | |
| Social awareness deficits | 20.57 | 5.29 | 12.50 | 5.95 | 78.05 | < .0001 |

Group comparison in cingulate structures

There were no statistically significant group differences in GM and WM volumes of each cingulate subregion except a larger WM volume in the isthmus part of the cingulate in individuals with ASD than TDC (Table 2). By contrast, ASD individuals had thinner cortical thickness than TDC in the following subregions: the right ACC, right anterior MCC, bilateral post MCC, and bilateral ventral and dorsal PCC (Table 2). The most significant difference was found in the right posterior MCC thickness (Cohen's $d = -0.578$).

Table 2
Group comparison of cortical thickness, controlling for sex, age, full-scale IQ and handedness

| | ASD (N = 88) | | TD (N = 51) | | | | |
|------------------------------|----------------|-----------|----------------|-----------|-------|--------|------------------|
| | Mean or N | SD or (%) | Mean | SD or (%) | F | P | |
| Age (age range) | 12.7 (8–20) | 3.1 | 13.8 (8–20) | 3.6 | 3.24 | 0.074 | |
| Male | N = 84 | (95.5) | N = 45 | (88.2) | 2.52 | 0.112 | |
| Left-handedness | N = 80 | (98.9) | N = 48 | (94.1) | 0.46 | 0.499 | |
| Full-scale IQ | 104 | 15.6 | 113 | 12.5 | 13.51 | 0.0003 | |
| Mean thickness | | | | | | | |
| R. hemisphere | 3.101 | 0.169 | 3.157 | 0.100 | 7.47 | 0.007 | |
| L. hemisphere | 3.108 | 0.165 | 3.158 | 0.107 | 6.73 | 0.011 | |
| White matter volumes | | | | | | | Cohen's d |
| R. caudal anterior cingulum | 3092 | 431 | 3124 | 513 | 0.02 | 0.891 | – |
| R. isthmus of cingulum | 3488 | 500 | 3358 | 400 | 9.97 | 0.002* | 0.287 |
| R. rostral anterior cingulum | 2155 | 303 | 2215 | 339 | 0.73 | 0.395 | – |
| R. posterior cingulum | 4466 | 565 | 4456 | 489 | 0.41 | 0.524 | – |
| L. caudal anterior cingulum | 2933 | 472 | 2949 | 483 | 0.04 | 0.849 | – |
| L. isthmus of cingulum | 3871 | 600 | 3689 | 457 | 8.79 | 0.004* | 0.341 |
| L. rostral anterior cingulum | 2697 | 392 | 2670 | 364 | 1.48 | 0.227 | – |
| L. posterior cingulum | 4454 | 542 | 4323 | 501 | 2.02 | 0.157 | – |
| Cortical thickness | | | | | | | Cohen's d |
| R. ACC | 3.45 | 0.27 | 3.55 | 0.21 | 6.93 | 0.010* | -0.426 |

Note. *False Discovery Rate q-value < 0.05; L, left; R, right; ACC, anterior cingulate cortex; MCC, middle cingulate cortex; PCC, posterior cingulate cortex Cohen's d were presented for those with False Discovery Rate q-value < 0.05.

| | ASD (N = 88) | | TD (N = 51) | | | | |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------|------|-------------|------|-------|--------|--------|
| R. anterior MCC | 3.52 | 0.23 | 3.58 | 0.22 | 5.53 | 0.020* | -0.264 |
| R. posterior MCC | 3.38 | 0.22 | 3.50 | 0.17 | 14.46 | 0.000* | -0.578 |
| R. dorsal PCC | 3.71 | 0.24 | 3.81 | 0.20 | 6.79 | 0.010* | -0.442 |
| R. ventral PCC | 3.38 | 0.37 | 3.52 | 0.31 | 5.54 | 0.020* | -0.410 |
| L. ACC | 3.50 | 0.26 | 3.55 | 0.19 | 2.59 | 0.110 | - |
| L. anterior MCC | 3.49 | 0.26 | 3.56 | 0.20 | 3.88 | 0.051 | - |
| L. posterior MCC | 3.37 | 0.24 | 3.46 | 0.18 | 8.70 | 0.004* | -0.396 |
| L. dorsal PCC | 3.72 | 0.27 | 3.82 | 0.19 | 6.57 | 0.012* | -0.398 |
| L. ventral PCC | 2.90 | 0.41 | 3.06 | 0.37 | 5.50 | 0.021* | -0.404 |
| Cortical volume | | | | | | | |
| R. ACC | 6983 | 934 | 6803 | 814 | 1.48 | 0.226 | - |
| R. anterior MCC | 3837 | 586 | 3880 | 492 | 0.09 | 0.770 | - |
| R. posterior MCC | 3696 | 527 | 3679 | 584 | 0.03 | 0.870 | - |
| R. dorsal PCC | 1957 | 399 | 1888 | 335 | 2.72 | 0.102 | - |
| R. ventral PCC | 870 | 218 | 949 | 222 | 2.56 | 0.112 | - |
| L. ACC | 6029 | 951 | 5899 | 649 | 0.68 | 0.411 | - |
| L. anterior MCC | 3478 | 551 | 3519 | 600 | 0.35 | 0.554 | - |
| L. posterior MCC | 3259 | 407 | 3256 | 422 | 0.01 | 0.909 | - |
| L. dorsal PCC | 2063 | 470 | 1995 | 373 | 0.31 | 0.577 | - |
| L. ventral PCC | 865 | 220 | 879 | 224 | 0.01 | 0.936 | - |
| Note. *False Discovery Rate q-value < 0.05; L, left; R, right; ACC, anterior cingulate cortex; MCC, middle cingulate cortex; PCC, posterior cingulate cortex Cohen's d were presented for those with False Discovery Rate q-value < 0.05. | | | | | | | |

Correlations between cingulate structures and autistic symptoms in ASD

Correlation analyses between cingulate structures and social awareness deficits revealed a significant negative correlation between the WM volume of the right caudal ACC and social awareness deficits on SRS ($r = -0.313$, $p = 0.001$) (Fig. 2). There were no significant correlations between social awareness and GM volumes or cortical thickness.

CNTNAP2 genetic association with cingulate structures

Two SNPs, rs759178 and rs2538991, were significantly associated with the WM volume of the right caudal anterior cingulate [rs759178: GG (n = 78) 3187.5 ± 531.7 , GT (n = 127) 2951.3 ± 422.7 , TT (n = 31) 3197.3 ± 559.4 (F = 6.76, p = 0.0014); rs2538991: GG (n = 79) 3188.7 ± 528.4 , GT (n = 127) 2950.5 ± 422.1 , TT (n = 31) 3197.3 ± 559.4 (F = 6.75, p = 0.0014); post-hoc analysis, GG, TT > GT, Bonferroni correction p < 0.05] (Fig. 3a, 3b). However, the heterozygotes (GT), but not the homozygotes (GG and TT), of both SNPs showed the smallest WM volume. Given that the two SNPs had quite similar genotype frequency, they were suspected in linkage disequilibrium. Meanwhile, the SNP rs779475 was associated with the cortical thickness of the right ventral PCC, showing that participants who carried TT had thinner cortex at the right ventral PCC compared to those carrying AA or AT [AA + AT (n = 35), 3.49 ± 0.33 ; TT (n = 201), 3.34 ± 0.34 ; F = 7.13, p = 0.0081] (Fig. 3c).

We further conducted multiple regression analyses separately including each with the main effect of each SNP, two-way interactions (SNP × age, SNP × diagnosis) and three-way interaction (SNP × age × diagnosis) were tested for each SNP. For the model containing both two-way interactions and three-way interaction, the same SNPs rs759178 (Supplementary Figure S1a), rs2538991 (Supplementary Figure S1b), and rs2710102 (Supplementary Figure S1c) interacted with age and diagnosis group on the cortical thickness of the same region, i.e., the left anterior MCC (Supplementary Table S2).

Discussion

Our work is one of the first studies to report delicate parcellation on the cingulate structures in individuals with ASD, including GM and WM volumes and cortical thickness and is also the first to explore the relationships between cingulate structures and the *CNTNAP2* gene. The major findings are three folds. First, individuals with ASD showed thinner cortical thickness of bilateral cingulate subregions, greater WM volume of the isthmus, and no difference of the cingulate GM volume. Second, decreasing WM volumes of the right caudal anterior cingulate gyrus was associated with increasing social awareness deficits. Third, the *CNTNAP2* variants were associated with the WM volumes of the right caudal anterior cingulate gyrus and cortical thickness of the right ventral PCC, and interacted with ASD status on the cortical thickness of the left anterior MCC.

Our finding that ASD youths did show a greater WM volume of isthmus than TDC that has not been reported in previous studies. Given the cingulate body connected to the parahippocampal gyrus via the isthmus, whether the greater WM volume of isthmus here reflects impaired limbic connectivity like previously suggested [67] is worth further investigation. Meanwhile, although there was no significant difference in cingulate GM volumes that was consistent with some previous studies [2, 3], cortical thickness of most subregions was significantly thinner in ASD compared to TDC. This finding was consistent with a recent study with ASD adults that showed thinner caudal ACC thickness in both the discovery (n = 301) and the replication sample (n = 61) [68], yet contradictory to previous studies showing increased cortical thickness of the left cingulate in 19 adults with high-functioning autism [69], bilateral PCC in 15 young adult males with autism [70], and the left MCC in 22 children with ASD (mean age 9.2 ± 2.1 years) [71]. Our results were based on a comparison between 88 ASD and 51 TDC with careful adjustment on sex, age, full-scale IQ, and handedness. Notably, age was a significant covariate for

cortical thickness in most cingulate subregions in our study (except PCC), in support of a previous argument that ASD is characterized by complex alterations in lifetime trajectories of several brain regions that underpin social-cognitive function [72]. Whether the cortical thinning in the cingulate gyrus was abnormal in the ASD population, specifically during adolescence, warrants more research. In contrast to previous findings of reduced cingulate GM volume in adults with ASD [4, 66], in our sample of youths aged 8–20, we did not find group differences in the cingulate GM volume. Whether different age groups of the study samples explained the inconsistent findings warrants further investigation.

Our finding that the WM volumes of the caudal anterior cingulate gyrus (MCC) was negatively correlated with social awareness deficits warrants more research. Although the function of cingulate gyrus has implicated in social cognition, only a few studies investigated the relationship between cingulate structure and autistic symptoms. One study reported that increased thickness in the rostral ACC was associated with poorer social scores [11]. Another study reported that social awareness deficits were correlated with increased functional connectivity between the dorsal ACC (namely MCC in this study) and right superior temporal gyrus, and decreased functional connectivity between the dorsal ACC and right putamen and thalamus [73]. Combining with our findings that ASD youths had a thinner cingulate cortex and that smaller right MCC WM volume was correlated with more severe social awareness deficits, such quantitative trait of social cognition may be associated with cingulate structural variations linked to ASD. These findings, if verified, implied a potential role of the cingulate gyrus in modulating the core symptom of ASD.

As the first study to focus on the genetic association between the *CNTNAP2* variants and cingulate structures, we found that the *CNTNAP2* variants were associated with the right MCC WM volume (rs759178 and rs2538991) and right ventral PCC thickness (rs779475). The common variant of the *CNTNAP2* at intron 2, rs7794745 (risk allele T), has been shown to be associated with reduced GM volume in the occipital gyrus, fusiform gyrus, and frontal cortices [37, 39]. Our finding suggests that this SNP (TT genotype) may also associate with thinner ventral PCC independent of diagnostic status. Besides, several studies support that the *CNTNAP2* plays a role in regulating brain connectivity. Children carrying the autism risk allele (C) of rs2710102 showed reduced long-range functional connectivity (i.e., fronto-occipital) in the prefrontal cortex but increased short-range functional connectivity [28]. Another study used whole-brain tractography and graph metrics to compare structural connectivity between genotypes of this SNP; the result also supported the association between this SNP and the reduced long-range brain connectivity [27]. A neighbor SNP, rs2710126, was also reported to associate with structural connectivity of the uncinate fasciculus [40]. Several SNPs located in intron 13 (rs2710102, rs759178, and rs2538991) were found nominally associated with axial diffusivity of the dorsal cingulum bundle [40]. Our result provided new evidence to support an association between the two SNPs in intron 13 (rs759178 and rs2538991) and MCC WM volume, implying that the *CNTNAP2* variants may possibly in linkage disequilibrium to the genetic markers that regulate cingulate structure. The role of intron 13 of the *CNTNAP2* on the neural development of cingulate WM warrants further research. Intriguingly, the heterozygote genotypes of these two SNPs had the smallest MCC WM volume. Evidence has shown that heterozygous mutations of *CNTNAP2* were associated with altered brain structure and functional

connectivity in neurotypical subjects [27–29]. Most disruptions of the *CNTNAP2* are heterozygous, suggesting that loss of a single allele could be sufficient to cause a disorder [74]. The mechanism of heterozygote of common variants warrants further investigation.

Given the distinct developmental trajectories in ASD brain particularly the GM volume of the MCC [72], we specifically tested whether the *CNTNAP2* variants interacted with age on the cortical thickness of two components of MCC, the anterior and posterior MCC, separately. We found a group × age × SNP interaction on the left anterior MCC only for the three SNPs in intron 13 of the *CNTNAP2* (i.e., rs2538991, rs2710102, and rs759178), which might suggest the effect of intron 13 on cortical thinning differs between ASD and TDC. The finding that ASD participants who carried TT genotype showed a faster cortical thinning with age than TDC counterparts with the same genotype (Fig. 3) needs to be replicated in a larger sample with an adequate number of minor alleles. Also, as a cross-sectional study, age effect can hardly be addressed by the developmental approach. A longitudinal study may further help to elucidate the effect of the allele on cortical thinning.

Limitations

This study had several limitations. First, the ASD participants of the whole sample were male predominant and older than controls. Therefore, cingulate substructures were further compared in a subsample whereby the sex and age were compatible between the ASD and TDC. Second, we measured social awareness deficits by caregiver-report questionnaire rather than a social cognition task. Although the observation from caregivers may better reflect the social function of participants with ASD, particularly in their youth stage, in daily life, a social cognition task that targets the specific social deficit of ASD (e.g., [75]) may provide a standard objective measure and thus should be considered. Third, although the number of our sample is one of the large-scale imaging genetic studies in ASD, the sample size of the current study may not have adequate power to detect small effect differences in the scale of a genetic study. A larger independent sample is needed to verify our results. Third, the candidate SNPs selected in this study were based on previous imaging genetic studies of the *CNTNAP2*. Future studies may consider fine mapping or sequencing of this gene.

Conclusions

The findings of this study may suggest that youths with ASD showed comparable GM and WM volume but reduced the cortical thickness of bilateral cingulate subregions compared to TDC. Besides, the cingulate structure may have clinical implications on moderating the severity of social awareness deficits, particularly the right cingulate WM volume. A delicate parcellation of the cingulate gyrus may benefit from dissecting the relationships between cingulate substructures and the specific symptom domain. Furthermore, as one of the first studies to provide evidence supporting the relationship between autism risk gene *CNTNAP2* and cingulate structure, our findings extend the current knowledge of *CNTNAP2* on long-range connectivity by showing that the *CNTNAP2* gene may also play a role in the

development of GM and WM of specific parts of the cingulate gyrus. The underlying mechanism is worth further research.

List Of Abbreviations

ACC, anterior cingulate cortex; ADI-R, the Autism Diagnostic Interview-Revised; ASD, autism spectrum disorder; CNTNAP2, the contactin-associated protein-like 2 gene; FDR, false discovery rate; GM, gray matter; K-SADS-E, the Kiddie epidemiologic version of the Schedule for Affective Disorders; MCC, middle cingulate cortex; PCC, posterior cingulate cortex; SNP, single nucleotide polymorphism; SRS, Social Responsiveness Scale; TDC, typically developing controls; WM, white matter

Declarations

Ethics approval and consent to participate

This work has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The Research Ethics Committee approved the study before its implementation (Approval number: 201201006RIB).

Consent for publication

Not applicable

Availability of data and material

The datasets generated and/or analyzed during the current study are not publicly available due to the research ethics regulation in Taiwan but are available from the corresponding author on reasonable request.

Competing interests

The authors declare no conflict of interests related to this work.

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Authors' contributions

YLC and SSG designed the study. SSG collected the clinical, image, and genetic data. YCC analysed the brain image data. YLC did the statistical analysis. YLC and SSG interpreted the results. YLC drafted the manuscript, and SSG did rigorous revision on the manuscript. All authors read and approved the final manuscript.

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Figures

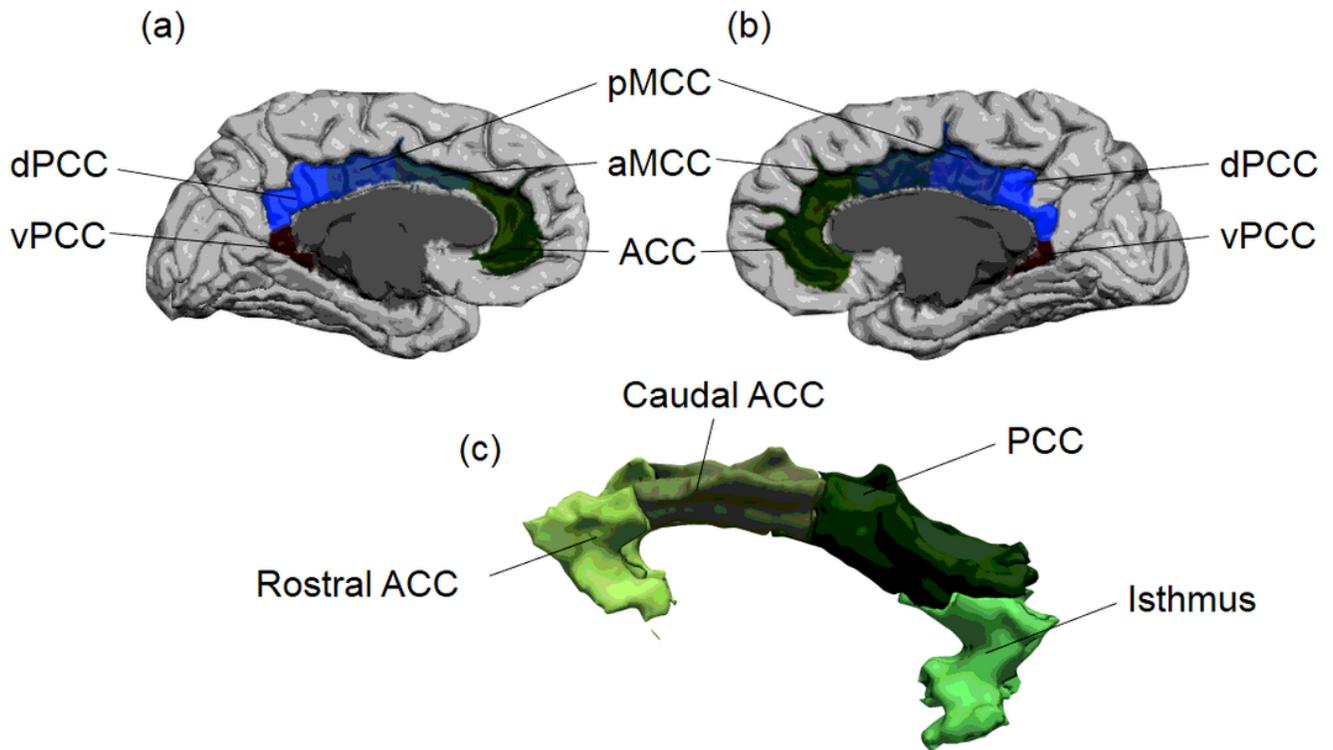


Figure 1

Cingulate substructures on FreeSurfer. (a) the left cingulate gyrus, (b) the right cingulate gyrus, (c) the cingulate substructures. [Abbreviations. ACC: anterior cingulate cortex; PCC: posterior cingulate cortex; aMCC: anterior part of middle cingulate cortex; pMCC: posterior part of middle cingulate cortex; dPCC: dorsal part of posterior cingulate cortex; vPCC: ventral part of posterior cingulate cortex]

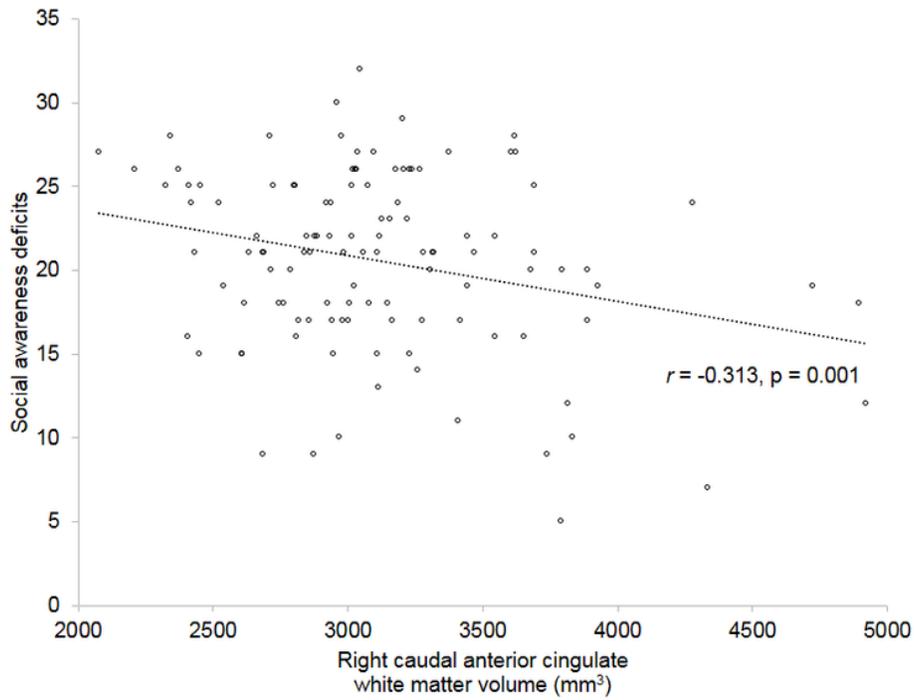


Figure 2

Correlation between cingulate structure and social awareness deficits in individuals with autism spectrum disorder. Right anterior cingulate white matter volume was negatively correlated with social awareness deficits ($r = -0.313, p = 0.001$).

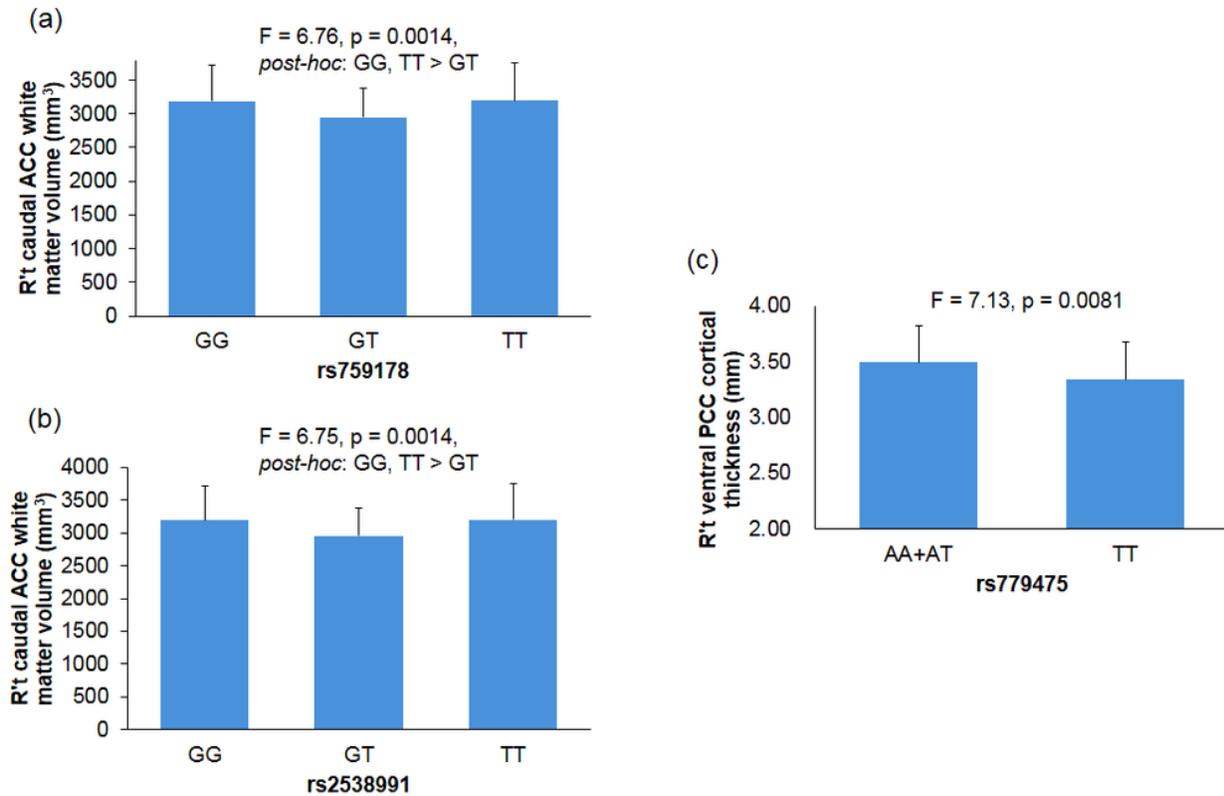


Figure 3

Associations between CNTNAP2 variants and cingulate structure (a) rs759178 vs. right caudal anterior cingulate white matter volume: GG (n = 78) 3187.5 ± 531.7 , GT (n = 127) 2951.3 ± 422.7 , TT (n = 31) 3197.3 ± 559.4 ($F = 6.76, p = 0.0014$); post-hoc analysis, GG, TT > GT (b) rs2538991 vs. right caudal anterior cingulate white matter volume: GG (n = 79) 3188.7 ± 528.4 , GT (n = 127) 2950.5 ± 422.1 , TT (n = 31) 3197.3 ± 559.4 ($F = 6.75, p = 0.0014$); post-hoc analysis, GG, TT > GT (c) rs779475 vs. right ventral posterior cingulate cortical thickness: AA+AT (n = 35), 3.49 ± 0.33 ; TT (n = 201), 3.34 ± 0.34 ; $F = 7.13, p = 0.0081$

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [CNTNAP2ASDCTCingulateSupplementaryTables.docx](#)