A Magnetic Resonance Spectroscopy Study of the Anterior Cingulate Cortex In Youth with Emotional Dysregulation

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Abstract

Background—The main aim of this study was to use proton Magnetic Resonance Spectroscopy (MRS) to identify brain biomarkers for emotional dysregulation in youth as measured by subscales of the Child Behavior Checklist (CBCL).

Methods—We measured glutamate (Glu) concentrations in the anterior cingulated cortex (ACC) of 37 pediatric subjects (aged 6-17 years) using high field (4.0 Tesla) proton Magnetic Resonance Spectroscopy (MRS). Subjects were grouped based on combined T scores on three subscales (Anxiety/Depression, Aggression and Attention) of the CBCL previously associated with deficits in the regulation of emotion. Subjects were stratified into those with high (>180) (N=10) and low (<180) (N=27) scores.

Limitations—Limitations include small sample size, wide age range studied, focus on Anterior Cingulate Cortex (ACC) only, and that some subjects received psychopharmacological treatments.

Results—We found a statistically significant correlation between Glu levels in the ACC and CBCL dysregulation profile scores among subjects with high dysregulation profile scores.

Conclusions—These results suggest that glutamatergic dysregulation in the ACC may represent a useful biomarker of emotional dysregulation in youth. Further investigation into the causality, time line and utility as a predictive metric is warranted.

Introduction

Despite ongoing controversy on how to best categorize emotional volatility in the young, there is no debate that a sizeable minority of youth is affected with various forms of emotional regulation deficits which are associated with high levels of morbidity and

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disability (1-8). Recent efforts at operationalizing emotional regulation deficits have relied on the Child Behavior Checklist (CBCL), a paper and pencil empirically derived scale with excellent psychometric properties (9-18).

Recent work by our group and others have documented that a profile consisting of marked (>2SD) elevations of three of the CBCL clinical scales (Anxiety/Depression, Aggression and Attention [A-A-A profile]) was associated with very severe morbidity and dysfunction including suicidality and need for hospitalization, regardless of diagnosis, hence termed by some the "dysregulation profile" (6, 19-28). The same profile has also been associated with increased likelihood to satisfy diagnostic criteria on structured diagnostic interview for bipolar disorder (29, 30) and hence termed the CBCL-Juvenile bipolar profile. More recent work has linked an intermediate profile characterized by moderate scores (>1 SD) on the same CBCL scales with deficient emotional self regulation (DESR). However, whether deficits in emotional regulation are associated with unique biomarkers remains unknown.

One approach non-invasively to identify brain bio-markers is magnetic resonance imaging neuroimaging methodology. Proton Magnetic Resonance Spectroscopy (1HMRS) examines brain biochemistry in various regions of the brain and allows in vivo quantification of metabolic changes including those related to glutamate (Glu), the most abundant neurotransmitter in the brain. However, Glu analysis via 1HMRS is challenging due to its J-coupled multiple resonance patterns and overlapping resonances from other metabolites primarily glutamine (Gln). Although separating glutamate from glutamine levels can help in understanding the pathophysiology of various psychopathological states, field strengths less than 2.0 Tesla do not allow to resolve the resonances of Glu and Gln. Thus, often times the composite peak (Glx) is reported rather than the individual Glu and Gln levels (31).

A number of prior studies have linked abnormalities in Glx to mood disorders. In bipolar disorder, almost all studies report elevated Glx independent of disease state (32-39). However, as the majority of these previous 1HMRS results come from 1.5 Tesla strength imagers, few studies have quantified glutamate and glutamine separately. Furthermore, children and adolescents have been relatively understudied in general as well as with high field strength magnets.

The main aim of this study was to use 1HMRS to identify biomarkers of emotional regulation deficits in youth using a high field scanner capable of differentiating Glu from other metabolites. To this end, we conducted a 4.0 T proton Magnetic Resonance Spectroscopy study focusing on the anterior cingulate cortex (ACC) in 37 youth with high (>1SD) and low (<1SD) score on the CBCL A-A-A profile. The ACC was chosen because of its importance in cognitive and emotional regulation and because previous studies have reported neurometabolite abnormalities in mood disordered youth in the ACC (40-42). We hypothesized that Glu may represent a useful biomarker of emotional dysregulation in youth and that higher Glu levels would predict more emotional regulation deficits as indicated by higher CBCL scores. To the best of our knowledge this is the first examination of biomarkers of emotional regulation deficits in youth using a high field 1HMRS scan.

Methods

Participants

The 37 participants were ages 6-17 years old and either probands (N=24) or controls (N=13) from a high risk offspring study of youth (6-24 years) recruited based on having a parent with bipolar disorder or in the case of controls, without a family history of mood disorder or personal history of mood disorder or major psychiatric disorder. Controls were recruited to match the age and sex of the high risk sample. For this high risk offspring study 91 potential participants were screened, 61 met the inclusion and exclusion criteria, and of this group 37 were aged 6-17 years and had a completed CBCL. Participants were recruited from advertisements to the public in the local media as well from the Massachusetts General Hospital Pediatric Psychopharmacology Clinic and Research Program. Exclusion criteria included clinically significant chronic medical conditions, organic brain disorders, documented mental retardation, phobia of small spaces, contraindication to MRI including presence of metal or surgical devices, and pregnancy. Female participants of child bearing potential received a urine pregnancy test prior to scanning.

Procedures

Prior to enrollment, participants were screened by phone to describe study procedures and evaluate study eligibility. Study procedures were approved by the MGH and McLean Hospital human subjects Internal Review Boards (IRBs). Consent was obtained from a parent and the child provided written assent. Participants were compensated for their participation. Only anonymous de-identified data are presented.

Prior to scanning, all subjects were assessed diagnostically using the Kiddie Schedule for Affective Disorders and Schizophrenia, Epidemiologic Version for DSM-IV (K-SADS-E) (43). In addition to a diagnostic interview, participants were assessed using clinician-administered measures of mania and depression: the Young Mania Rating Scale (YMRS) (44) and the Child Depression Rating Scale (CDRS) (45). These measures were administered by board-certified child and adult psychiatrists who had been trained to reliability. Socioeconomic Status (SES) was assessed using the Hollingshead Socioeconomic Status scale. IQ was assessed using the Wechsler Abbreviated Scale of Intelligence Scale (WASI) (46) Vocabulary and Matrix Reasoning subtests.

Parents (usually the mother) completed the Child Behavior Checklist (CBCL) (9). T scores from subscales of interest included the Anxiety/Depression, Aggression and Attention subscales (CBCL A-A-A). Due to small sample size, 37 subjects (N=13 healthy comparison participants and N=24 high-risk offspring) were grouped into two groups based on their T-scores on the CBCL A-A-A profile: high score group (>180) (N=10) and low score group (<180) (N=27). The 10 subjects in the high score group included only high risk offspring. The 27 subjects in the low score group comprised all 13 healthy controls and 14 high risk offspring. A T-score of 60 is one standard deviation from normal based on well established norms for the CBCL. The high score group (>180) reflects subjects whose scores on the three subscales on average are at least one standard deviation from normal. A T-score of 60

or greater is considered to be of clinical concern and thus the high score group comprises subjects with a clinical picture generally meeting standards for psychiatric intervention.

Imaging Procedures

Data acquisition was performed on a 4.0 T Varian Unity/Inova whole body MR scanner (Varian NMR Instruments, Palo Alto, CA) equipped with proton volumetric head coil. The MR protocol consisted of anatomical and spectral data acquisitions. Anatomical MR images were used for patient positioning, voxel localization and tissue segmentation. Spectral data were acquired from a 2cm×2cm×2cm voxel localized on the ACC using PRESS (point-resolved spectroscopic sequence) (TR=2000ms, TE=30ms, number of averages=128, acquisition time<5 minutes). Manual shimming within the voxel produced unsuppressed water signal linewidths of less than 11 Hz. A systematic approach to voxel positioning was used in all subjects. Voxels were placed on the ACC on midsagittal T1-weighted images, anterior to genu of the corpus callosum, and positioned on the midline on axial images.

MRS Data Processing

All MRS processing was conducted blinded to diagnosis and group assignment. The automated spectral-fitting package LC Model (version 6.2-1F) and the standard vendor-supplied simulated basis set were used for quantification of metabolite concentrations (Figure 1).

The basis set included alanine, aspartate, creatine, phosphocreatine, gamma-aminobutyric acid, glucose, glutamine, glutamate, glycerophosphocholine, phosphocholine, myo-inositol, lactate, n-acetylaspartate, n-acetylaspartylglutamate, syllo-inositol and taurine. Data and fitting quality were visually verified and further assessed by the percent standard deviation of the estimated concentration of each metabolite (CRLB), linewidth (FWHM) and signal-to-noise (SNR), all calculated by LCModel. The results were presented in institutional units (I.U.) and no attempt was made to convert IU to absolute concentrations due to the lack of knowledge about the Glu T1 and T2 relaxation times. Glu levels were corrected for the cerebrospinal fluid (CSF) and gray matter (GM) fraction. Tissue-segmentation of T1-weighted images into GM, white matter (WM), and CSF was automatically done using an open source software, "NVM" (freely available from Neuromorphometrics, Inc. at http://neuromorphometrics.org:8080/nvm/).

Statistical Analysis

Statistical analysis was performed using the IBM SPSS software (version 19.0.0.1 for Macintosh). Chi-Squared tests (for categorical variables) and t-tests (for continuous variables) were used to compare demographic and clinical characteristics across groups (low score and high score). Correlation between the clinical index and metabolite levels was carried out with Pearson bivariate correlation as well as partial correlation controlling for age, sex, and medication status (on/off). All tests were two-tailed, except for correlation analysis. Since a directional prior hypothesis had been made, the correlations were evaluated with one-tailed tests. A p-value of < 0.05 was considered statistically significant.

Results

Subject characteristics are summarized in Table 1. Groups were comparable with respect to age, gender, IQ and socioeconomic status. YMRS, CDRS and CBCL A-A-A scores were statistically significantly higher in the high CBCL score group than the low CBCL score group.

Four subjects (15%) in the low CBCL score group and seven subjects (70%) in the high CBCL score group (all high risk offspring subjects) were taking one or more types of medication at the time of scans. The medication class rates are shown in Table 1.

Good quality MRS data were obtained with low CRLB, high SNR and low FWHM from both groups as shown in Table 2. There were no between group differences in any of these measures. Unobstructed clear Glu peak is demonstrated in Figure 1.

Within the low dysregulation profile score group, Glu levels were increased in high-risk offspring subjects (n=14; mean CBCL score=161.29±10.49; mean Glu level=6.00±1.95) when compared with the healthy controls (n=13; mean CBCL score=154.38±7.37; mean Glu level=4.88±2.07) but did not reach statistical significance (two sample t-test; t = 1.963, d.f. = 25, p = 0.06 (2-tailed)). Hence control and high-risk offspring subjects in the low dysregulation profile score group have been combined into a single group and compared with the high dysregulation profile group.

Despite absence of statistically significant differences in Glu levels between the low and high dysregulation profile groups (Table 2), there was a positive correlation between glutamate levels with the CBCL dysregulation profile scores in the high score group (Pearson correlation=0.659, p=0.019 (1-tailed)) (Figure 2). This finding held true when partial correlation controlling for age, sex, and medication status (on/off) was carried out (correlation=0.759, p=0.024 (1-tailed), df=5). The CBCL-Glu correlation was not significant in the low score group (p=0.111 (1-tailed)) or in the total (low+ high score groups) dataset (p=0.170 (1-tailed)).

Glu levels were increased in youth with high dysregulation profile scores (n=10; mean CBCL score=207.40±15.51; mean Glu level=5.45±1.77) when compared with just the healthy controls from the low dysregulation profile score group (n=13; mean CBCL score=154.38±7.37; mean Glu level=4.88±2.07) (two sample t-test; t = 10.88, d.f. = 21, p < 0.001 (2-tailed)).

Discussion

This study found a positive correlation between emotional dysregulation as measured by CBCL A-A-A scores (>180) and glutamate concentrations in the ACC in youth at high risk for bipolar disorder. Although in need of confirmation in larger studies, these findings suggest that glutaminergic dysregulation could represent a biomarker for emotional dysregulation in youth at risk for bipolar disorder.

Our finding of higher Glu levels in mood disordered youth with high CBCL dysregulation profile score is consistent with previously reported glutamatergic abnormalities in bipolar disorder and with a literature that suggests that glutamatergic abnormalities are a prominent feature of mood disorders (47). In major depressive disorder and bipolar disorder, serum, plasma and ACC levels of glutamate have been found to be altered (47-49). Glutamate level in the frontal cortex has been reported to be elevated in postmortem brains of patients with bipolar disorder and major depression (50). Glutamate is thought to be a marker of glial cell functioning and glial cell number and density reduction has been consistently demonstrated in mood disorders in the ACC in postmortem studies (51, 52). In bipolar disorder, almost all MRS studies report elevated Glx independent of disease state (31, 36, 38, 48, 53-57), making it a most consistent finding in the MRS literature.

Our 1HMRS ACC findings are also consistent with the literature that has previously found the ACC to be the site of neurometabolite abnormalities in mood-disordered youth. Davanzo et al. found significantly higher myo-inositol/creatine-phosphocreatine and mI levels in the ACC in bipolar youth versus healthy subjects or those with intermittent explosive disorder (40, 41). Cecil et al. (42) also found ACC abnormalities in mood disordered children, while Auer et al. reported ACC abnormalities in mood disordered adults (55).

On the other hand, our findings are discrepant with those of Singh et al. (58) who reported that high-risk offspring for bipolar disorder with subsyndromal symptoms of mania did not exhibit differences in Glu or Gln. They are also discrepant with findings by Moore and colleagues who reported that unmedicated youth with bipolar disorder had significantly lower Glx/Cr levels than healthy comparison subjects and medicated subjects with bipolar disorder (39, 59). More work is needed to reconcile these discrepant findings.

However, despite the positive correlation between Glu levels with the CBCL dysregulation profile score, there were no statistically significant Glu differences between the low versus high CBCL dysregulation profile groups. There could be several possible explanations for this finding: First, the low dysregulation profile group comprised a mixture of offspring of controls and bipolar disorder parents. It is possible that Glu is elevated only among youth at risk for bipolar disorder who also exhibit emotional dysregulation. This possibility is supported by our finding that there was a significant difference between high-risk offspring and healthy controls among the high emotional dysregulation group, but not the low dysregulation group. Thus, it would be important for subsequent studies to examine whether emotional dysregulation, as indexed by the CBCL represents a marker of risk or an endophenotype in these offspring at risk for bipolar disorder.

While no previous study has specifically correlated HMRS findings with the CBCL dysregulation profile, our 1HMRS results are also consistent with those from several studies that have connected the CBCL A-A-A dysregulation profile to genetic and other biomarkers. Althoff et al. have demonstrated in a very large sample that this CBCL profile is heritable, using latent class analysis (60). Doyle et al. in a genetic linkage study of 154 families estimated the heritability of this CBCL profile at 0.71 (61). Boomsma et al. (62) examined longitudinal data on Dutch mono- and dizygotic twin pairs (N = 8013 pairs) and found that

80% of the stability in childhood CBCL-Dysregulation profile was a result of additive genetic effects.

Zepf et al. (63) linked the profile to brain chemistry and reaction time. These authors used a placebo-controlled double-blind within-subject crossover design to compare the reaction times of high and low scorers on this CBCL-Dysregulation profile after a rapid tryptophan depletion test (RTD) (which lowers the central-nervous system 5-HT synthesis rate). Subjects with a high CBCL-Dysregulation score showed a slower reaction time under RTD compared to patients with low CBCL-Dysregulation profile. Another study found endocrino-logical correlates to the CBCL-Dysregulation profile. Basal serum TSH was measured in 114 children and adolescents with (N=53) and without (N=61) the CBCL-Dysregulation profile; TSH was elevated in those with the CBCL-Dysregulation profile compared to controls (64). Ducharme et al. reported on 193 healthy children aged 6-18 and found the Aggressive Behavior CBCL subscale alone to be correlated with bilateral striatal volumes and right ACC cortical thickness (65). Taken together, these studies all provide evidence that the CBCL A-A-A profile may be uniquely useful in the search for biomarkers of emotional dysregulation in the young.

This study has important strengths. Our definition of deficits in emotional regulation was anchored on a unique profile of the CBCL, an empirically derived scale with excellent psychometric properties, previously shown to discriminate youth with deficits in emotional regulation. By using a high field MRI scanner, the size of brain tissue volumes from which chemical information was obtained, was decreased which was an important consideration for acquiring MRS data from young children who have smaller brain volumes than adults. In addition, the improved signal to noise ratio at high field increased the metabolite signal enabling more accurate quantification including differentiation of Glu and Gln.

On the other hand, results of this study must be considered in light of some limitations. Our sample size was relatively small, resulting in very small cell sizes limiting the power of the study and increasing the possibility of spurious findings. Thus, our findings must be considered as preliminary until replicated with larger samples. To facilitate recruitment, this study included youth with a wide age range 6-17 years providing an additional confounding factor. Little is known about neurodevelopmental changes occurring during these years in the functioning of the ACC among typically developing youth. Such disparate ages would likely provide a confounding factor making a significant finding less likely. It is all the more remarkable that a correlation between CBCL scores and glutamate was noted. In addition, age was not statistically different between our two groups of interest, low and high scorers. Nonetheless, future confirmatory studies would benefit from examination of this brain region in youth of a narrower age range to remove any effects occurring during normal maturation. Although our focus on the ACC was well grounded on previous studies and theoretical considerations, future studies should examine other brain regions as well. Some of the subjects received pharmacologic treatment, which may have confounded the findings. In fact, that 70% of the high score group were taking one or more types of psychotropic medications at the time of the scan and that these medications were varied is a significant weakness of the study. Future studies would benefit from study of either treatment naïve or treatment free subjects.

Despite these considerations, our findings suggest that, among youth at risk for bipolar disorder, there is a relationship between emotional regulation deficits and neurometabolite glutamate in the ACC. Although additional work is needed to replicate these findings and further examine the implications of glutaminergic dysregulation in the ACC on the development of emotional dysregulation, our findings may have important scientific and clinical implications. Biomarkers of risk for emotional dysregulation may allow the identification of subjects at risk for this serious clinical problem as well as increase our understanding of the neural and biochemical bases of emotional dysregulation in youth. In addition, the construct of emotional dysregulation is consistent with the NIMH Research Domain initiative and may provide a fruitful area of scientific inquiry in the quest for biomarkers of psychopathological dysfunction.

Acknowledgments

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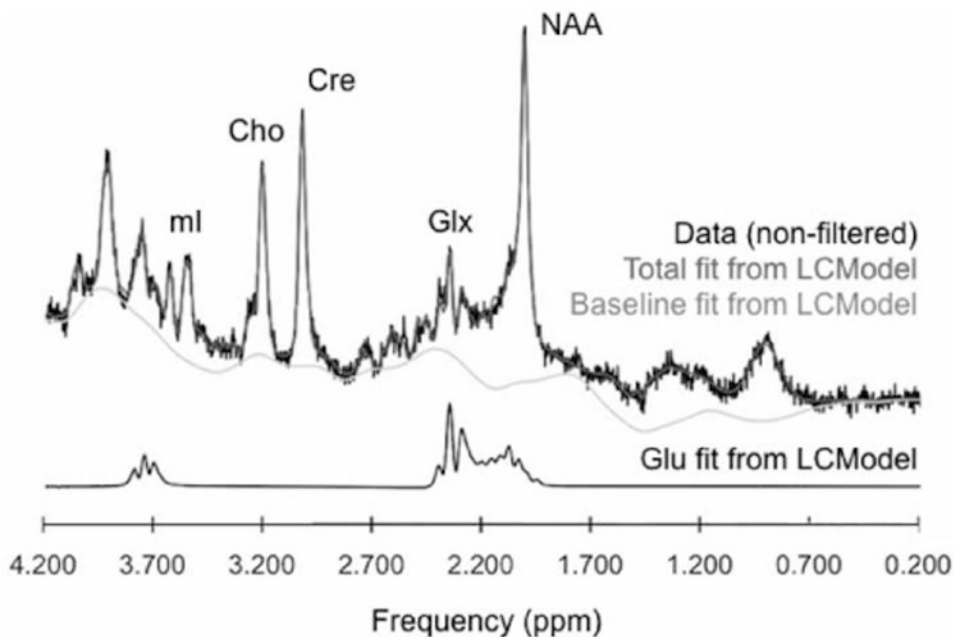
Figure 1.
Representative proton magnetic resonance spectrum of the anterior cingulate cortex at 4 Tesla collected at TE=30ms with a point-resolved spectroscopy sequence along with spectra of Glu. The real part of the frequency-domain data (phased and referenced FFT of raw input data with no smoothing) is plotted as the black curve. The red curve is the LCModel fit to this data. Also plotted as the gray curve is the baseline. Below is the fiting line for Glu only. Cho = Choline; Cr = Creatine; Glx = Glutamine + Glutamate; Glu = Glutamate; NAA = N-Acetyl Aspartate; mI = myo-inositol; ppm = parts per million.
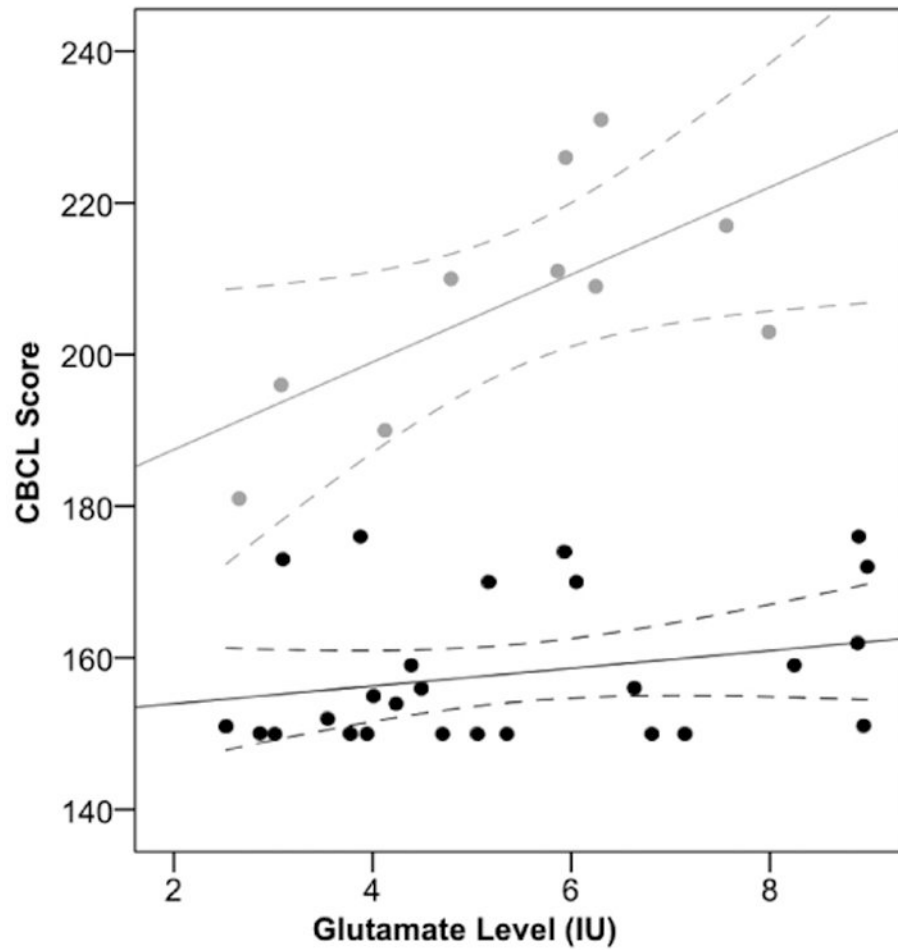
Figure 2. Study Participant CBCL A-A-A Scores (Low and High) versus ACC Glutamate Levels
Solid lines represent the linear fits to the low score group data (black) and high score group
data (grey). Dashed lines represent 95% confidence intervals. CBCL, Child Behavior
Checklist; A-A-A, Anxiety/Depression, Aggression, Attention subscale; ACC, Anterior
Cingulate Cortex.

Table 1

Demographic and Clinical Characteristics of Study Participants in Low versus High CBCL Score Groups

Subgroups	Low Score Group mean ± SD			High Score Group mean ± SD		Comparison (Low Score-Combined vs High Score Group)
	Controls (n=13)	High Risk Offspring (n=14)	Combined (n=27)	High Risk Offspring (n=10)	
Age (years)	11.50 ± 3.90	12.04 ± 3.11	11.78 ± 3.56	11.50 ± 3.50	NS: t = 0.212, d.f. = 35, p = 0.833
Males (N; %)	9; 69	8; 57	17; 63	8; 80	NS: d.f. = 1, χ2 = 0.967, p = 0.326
iQ	103.00 ± 17.08	104.43 ± 12.66	10374 ± 14.51	106.44 ± 12.80	NS: t = 0.497, d.f. = 34, p = 0.622
SES	1.83 ± 0.58	2.23 ± 0.941	2.04 ± 0.81	1.90 ± 0.74	NS: d.f. = 3, χ2 = 0.437, p = 0.932
YMRS	0.17 ± 0.39	5.50 ± 7.47	2.96 ± 5.98	14.01 ± 11.03	t = 3.006, d.f. = 11.025, p = 0.012
CDRS	1775 ± 1.36	22.63 ± 8.42	20.30 ± 6.73	38.41 ± 12.25	t = 4.432, d.f. = 11.077, p = 0.001
CBCL (T score A-A-A)	154.38 ± 7.37	161.29 ± 10.49	157.96 ± 9.61	207.40 ± 15.51	t = 11.688, d.f. =35, p < 0.001
Diagnoses (N)					
Bipolar Disorder	0	5	5	8	
Major Depression	0	3	3	7	
Generalized Anxiety Disorder	0	0	0	5	
Oppositional Defiant Disorder	0	2	2	7	
Conduct Disorder	0	1	1	3	
Attention Deficit Hyperactivity Disorder	0	2	2	6	
Medication Classes (N)					
A typical Antipsychotics	0	3	3	3	
Antidepressants	0	4	4	4	
Stimulants	0	0	0	1	
Mood Stabilizers	0	0	0	2	
other	0	0	0	4	

Continuous variables expressed as mean ± SD. YMRS, Young Mania Rating Scale; CDRS, Children's Depression Ratio Scale; CBCL, Child Behavior Checklist; SES, Socio-economic status; iQ, intelligence Quotient; NS, non-significant.

Table 2

Magnetic Resonance Spectroscopy Data of Study Participants in Low versus High CBCL score groups

	Low Score Control Group (n=13)	Low Score At Risk Group (n=14)	Low Score Group Combined (n=27)	High Score Group (n=10)	Comparison
SNR			1237 ± 5.02	11.60 ± 5.70	NS: t = 0.400, d.f. = 35, p = 0.692
FWHM (ppm)			0.05 ± 0.01	0.05 ± 0.02	NS: t = 0.514, d.f. = 35, p = 0.610
Glu CRLB (%)			10.04 ± 3.11	10.11 ± 2.89	NS: t = 0.063, d.f. = 35, p = 0.950
Mean Glu (i.U.)	4.88 ± 2.07	6.00 ± 1.95	5.47 ± 2.05	5.45 ± 1.77	NS: t = 0.016, d.f. = 35, p = 0.987

All variables expressed as mean ± SD. SNR, Signal to Noise Ratio; FWHM, Full Width at Half Max; CRLB, Cramer-Rao Lower Bound; Glu, Glutamate; NS, non-significant.