Citation: Transl Psychiatry (2012) 2, e153; doi:10.1038/tp.2012.78

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Reduction of *N*-acetylaspartate in the medial prefrontal cortex correlated with symptom severity in obsessive-compulsive disorder: meta-analyses of ^{1}H-MRS studies

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Structural and functional neuroimaging findings suggest that disturbance of the cortico–striato–thalamo–cortical (CSTC) circuits may underlie obsessive-compulsive disorder (OCD). However, some studies with ^{1}H-magnetic resonance spectroscopy (^{1}H-MRS) reported altered level of *N*-acetylaspartate (NAA), they yielded inconsistency in direction and location of abnormality within CSTC circuits. We conducted a comprehensive literature search and a meta-analysis of ^{1}H-MRS studies in OCD. Seventeen met the inclusion criteria for a meta-analysis. Data were separated by frontal cortex region: medial prefrontal cortex (mPFC), dorsolateral prefrontal cortex, orbitofrontal cortex, basal ganglia and thalamus. The mean and s.d. of the NAA measure were calculated for each region. A random effects model integrating 16 separate datasets with 225 OCD patients and 233 healthy comparison subjects demonstrated that OCD patients exhibit decreased NAA levels in the frontal cortex ($P = 0.025$), but no significant changes in the basal ganglia ($P = 0.770$) or thalamus ($P = 0.466$). Sensitivity analysis in an anatomically specified subgroup consisting of datasets examining the mPFC demonstrated marginally significant reduction of NAA ($P = 0.061$). Meta-regression revealed that NAA reduction in the mPFC was positively correlated with symptom severity measured by Yale–Brown Obsessive Compulsive Scale ($P = 0.011$). The specific reduction of NAA in the mPFC and significant relationship between neurochemical alteration in the mPFC and symptom severity indicate that the mPFC is one of the brain regions that directly related to abnormal behavior in the pathophysiology of OCD. The current meta-analysis indicates that cortices and sub-cortices contribute in different ways to the etiology of OCD.
Translational Psychiatry (2012) **2,** e153; doi:10.1038/tp.2012.78; published online 14 August 2012

Introduction

Frontal–subcortical circuits are effector mechanisms that allow an organism to act in its environment.[1] Three cortico–striato–thalamo–cortical (CSTC) circuits originating from three prefrontal regions are particularly important for the manifestation of neuropsychiatric behavior. These circuits include the dorsolateral prefrontal cortex (DLPFC) circuit, which allows the organization of information to facilitate a response, the medial prefrontal cortex (mPFC) circuit, which is required for motivation-related behavior, and the orbito-frontal cortex (OFC) circuit, which allows the integration of limbic and emotional information into behavioral responses.[1]

Previous structural and functional neuroimaging studies have indicated that dysfunction of CSTC circuits, and imbalance between these circuits may be the neural basis of the symptoms of obsessive-compulsive disorder (OCD).[2] Positron emission tomography studies using resting and symptom-provoking designs revealed significantly elevated metabolic rates of glucose in the cerebral cortex and basal ganglia in patients with OCD.[3–6] These studies confirmed abnormal metabolism in the frontal cortex, including the OFC[3,6] and mPFC.[3–6] Recently, functional magnetic resonance imaging (MRI) studies have also reported different pattern of activation of brain regions within CSTC circuits in OCD patients. Although some studies reported altered activation in the frontal cortex including the OFC[7–9] and mPFC,[7,8,10] others demonstrated abnormalities in the basal ganglia, including the striatum[10–12] and thalamus.[4]

Evidence from structural MRI studies also indicates volumetric differences between OCD patients and healthy comparison (HC) subjects in CSTC circuits.[13] Interestingly, recent meta-analyses of voxel-based morphometry studies and structural MRI studies in OCD patients indicated volume reductions in the frontal cortex, but not in the basal ganglia or thalamus, suggesting that underlying etiologies differ in the frontal cortex and subcortical areas.[14,15] Although functional disturbance and structural abnormality of the CSTC circuit are possible bases for the etiology of OCD, recent studies have suggested that the frontal cortex and subcortical areas have different roles in the symptoms of OCD.[14,15,16]

^{1}H-magnetic resonance spectroscopy (^{1}H-MRS) is a non-invasive neuroimaging technique that estimates specific chemical metabolite measures *in vivo*.[17] *N*-acetylaspartate (NAA) is a metabolite that can be accurately measured with a 1.5-tesla scanner[18] and has been widely studied in the field of

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Keywords: cortico–striato–thalamo–cortical circuity; MRS; *N*-acetylaspartate (NAA); obsessive-compulsive disorder (OCD); systematic review
Received 21 May 2012; revised 9 July 2012; accepted 14 July 2012

neuroscience.[19] Although NAA is widely recognized as a marker of neuron density,[20] recent experimental studies reported that NAA reflects the functional role of neurochemical alterations.[21] Previous studies have used ^{1}H-MRS to examine OCD patients, reporting decreased,[22] unchanged,[23] or increased[24] NAA levels in OCD patients compared with HC subjects.

To our knowledge, neither a systematic review nor a meta-analysis of ^{1}H-MRS studies in OCD patients has been previously reported. The current systematic review and meta-analysis were designed to investigate the neurochemical background of abnormal activity in CSTC circuits, and to identify direction and location of neurochemical alteration that relates to abnormal behavior in OCD patients. In the current study, to examine in which part of CSTC circuit NAA levels alter, at first we conduct three meta-analyses with the datasets from the frontal cortex, thalamus and basal ganglia. Then, to investigate which CSTC circuit among three circuits mentioned above has a key role in the pathophysiology of abnormal behavior of OCD, we separate datasets from the frontal cortex into three subgroups based on anatomical location of volume of interests (VOIs), such as mPFC, DLPFC and OFC, and perform three meta-analyses.

Materials and methods

Data sources. ^{1}H-MRS studies that examined metabolite measures in the brains of individuals with OCD and HC subjects were obtained through the digital MEDLINE and EMBASE databases. A comprehensive literature search was performed using the terms 'obsessive compulsive disorder' combined with 'magnetic resonance spectroscopy'. The titles and abstracts of the studies were examined to determine whether or not they should be included. The reference lists of the included articles were also examined to search for additional relevant studies to be included.

Selection of studies. Studies were included in our database if (1) they were peer-reviewed brain ^{1}H-MRS studies published between 1980 and December 2011, (2) they examined patients with OCD in comparison with a HC group, and (3) NAA levels were measured. Further, studies were included in the meta-analysis, if (4) they reported sufficient data to estimate their effect sizes, and (5) they located VOIs at least one region among the frontal cortex, basal ganglia or thalamus. The literature search was performed without language restriction. If the study did not provide sufficient data, we emailed the corresponding author to obtain more data. In cases where the author did not respond, we excluded the study. Two of the authors (YA and AA) independently performed the study screening.

Data extraction. To perform the meta-analyses, we defined a standardized mean difference as effect size, which is calculated as the difference between the mean of the experimental group and the mean of the comparison group, divided by the pooled s.d.

In the current meta-analyses, the mean NAA level in individuals with OCD was subtracted from that in the HC group in each VOI, and divided by the pooled s.d. of these VOIs.

Identification of VOIs. The data were separated by the brain region (frontal cortex, thalamus and basal ganglia). The data assigned to frontal cortex were divided into three subgroups, the DLPFC, mPFC and OFC, based on the location of VOI. In addition to an original classification of VOI placement, the classification was evaluated by two independent reviewers who were blind to an original classification (AA and HS). In case of discrepancy, the agreement was achieved by discussion with the third reviewer (YA). All the cases were considered their classification in this process. In the case of a study reporting measures from more than one subregion in one area (for example, left and right anterior cingulate cortex), we calculated the mean of effect sizes from all the VOIs from one area and integrated it into the analysis.[25] In the case of studies reporting more than two kinds of measures of metabolites, we adopted the different priority for extraction depending on the method of cerebrospinal fluid (CSF) ratio correction. In studies which conducted tissue segmentation within VOIs, we determined the priority for extraction as the absolute NAA level and then its ratio to creatine (Cre) levels. Whereas in studies which did not implement tissue segmentation within VOIs, we determined the priority for extraction as the ratio of NAA level to Cre and then its absolute levels. In the case of longitudinal or interventional studies, we determined the NAA levels at baseline, that is, before treatment.[22] Three independent datasets located in the white matter in the frontal lobe were excluded from the analysis of the frontal cortex.[22,26,27] VOIs in HC subjects who were compared with more than two OCD groups were identified[28] and divided into the appropriate number of comparison subgroups to avoid duplication. Two authors (YA and AA) independently performed all of the data extractions and computations of effect sizes to minimize errors. The Epidemiology guidelines for meta-analyses of observational studies were followed.[29]

Meta-analysis. All meta-analyses were performed using Comprehensive Meta-analysis version 2.0 from Biostat (Englewood, NJ, USA). A random effects model was adapted for the current meta-analysis to control for potential heterogeneity, such as variations in the location of the VOIs, the implementation of the tissue segmentation within the VOIs, the use of single- vs multi-voxel spectroscopy, the echo time, the volumes of the VOIs and the types of metabolite measure. Meta-analysis was performed only when there were more than three datasets from more than two independent studies. A standardized mean difference was calculated and used as effect sizes. The significance level was set at $P<0.05$.

Sensitivity analyses. The robustness of significant findings from meta-analysis was further tested using a sensitivity analysis in specified subgroups, excluding studies with potential confounds. These potential confounds included pharmacological status, psychiatric comorbidity, type of metabolite measures, strength of magnetic field and implementation of tissue segmentation within VOIs. The significance level was set at $P<0.05$.

Table 1 Summary of included studies

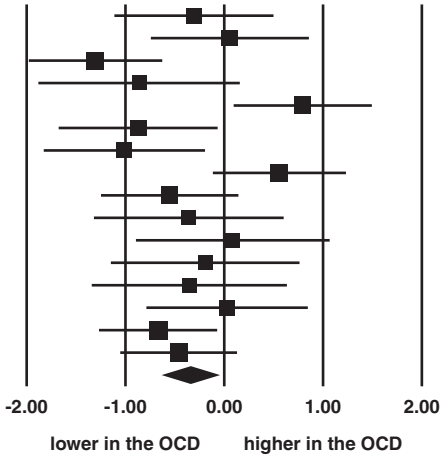
Author	Mean age (year)	N of OCD[a]	N of male OCD[a]	N of HC[a]	N of male NHC[a]	Diagnostic tool	Duration of illness (year)	Psychiatric comorbidity	Medication	Y-BOCS[b]			Magnetic field strength (tesla)	Segmentation	MRS sequence	Single- vs multi- voxel	Types of measure	TE (ms)	TR (ms)	NAA	Cre	Cho	ml	Glx	VOI location	VOI size (ml)	Results
										Total	Obsession	Compulsion														
Atmaca *et al.*[51]	28.1	18	6	18	6	DSM-IV	5.5	No	No	17.2	NA	NA	1.5	Undone	NA	Single	Cre ratio	15.6	2000	○	○	○	×	×	bl.hippocampus	4.7	NAA↓
Bartha *et al.*[50]	34.9	13	7	13	7	DSM-IV	NA	No	No	25.9	11.6	14.3	1.5	Undone	NA	Single	Absolute	20	1500	○	○	○	○	○	lt.corpus striatum	4.5	NAA↓
Beédard *et al.*[43]	40.54	13	4	12	4	DSM-IV	12.77	No	Medicated	26.5	12.92	13.62	1.5	Undone	PROBE	Single	Cre ratio	102	3100	○	×	○	○	○	rt.OFA	8	NS
																				○	×	○	○	○	lt.OFA	8	NS
																				○	×	○	○	○	rt.MTL	8	NS
																				○	×	○	○	○	lt.MTL	8	NS
																				○	×	○	○	○	rt.thalamus	9	NS
																				○	×	○	○	○	lt.thalamus	9	NS
																				○	×	○	○	○	ACC	9.6	NS
Besiroglu *et al.*[42]	32.1	30	12	15	6	DSM-IV	6.35	No	No	24.15	13.1	11.05	1.5	Undone	PRESS	Single	Absolute Cre ratio	135	1500	○	○	○	×	×	rt.ACC	8	NAA↓
																				○	○	○	×	×	rt.AHC	4.096	NAA↓
Ebert *et al.*[34]	27	12	8	6	5	DSM-IV	11	No	No	28	NA	NA	2	Undone	PRESS	Single	Cre ratio	30	1500	○	×	○	○	○	ACC	8	NAA↓
																				○	×	○	○	○	rt.striatum	8	NAA↓
																				○	×	○	○	○	rt.PL	8	NS
Fan *et al.*[24]	25.62	21	12	19	13	DSM-IV	4.45	No	No	22.52	NA	NA	1.5	Undone	PRESS	Single	Cre ratio	35	1500	○	×	○	○	×	MPFC	8	NAA↓
Jang *et al.*[22]	27.77	13	8	13	8	DSM-IV	7.23	Two: depression	No	30.23	15.31	14.92	1.5	Done	PRESS	Multi	Cre ratio	272	2000	○	×	×	×	×	ACC	1.125	NAA↓
																				○	×	×	×	×	FWM	1.125	NAA↓
																				○	×	×	×	×	Prefrontal cortex	1.125	NAA↓
																				○	×	×	×	×	PC	1.125	NS
																				○	×	×	×	×	PWM	1.125	NS
																				○	×	×	×	×	Posterior cingulate	1.125	NS
Kitamura *et al.*[26]	22.7	12	3	32	10	DSM-IV	6.3	No	Medicated	20.4	NA	NA	3	Undone	PRESS	Single	Cre ratio	24	6000	○	×	○	×	×	ACC	3	NS
																				○	×	○	×	×	lt.basal ganglia	6.75	NS
																				○	×	○	×	×	lt.thalamus	3.375	NS
																				○	×	○	×	×	lt.FWM	3.375	NS
																				○	×	○	×	×	lt.PWM	3.375	NS
Mirza *et al.*[38]	10.31	27	13	18	NA	DSM-IV	2.63	No	No	26.8	NA	NA	1.5	Undone	NA	Single	Absolute	272	2300	○	○	○	×	×	lt.thalamus	0.8	NS
																				○	○	○	×	×	rt.thalamus	0.8	NS
Mohamed *et al.*[28]	36.7	10	4	10	4	DSM-IV	23.5	No	Medicated	14.3	NA	NA	1.5	Undone	NA	Single	Cre ratio	280	2650	○	×	○	×	×	lt.thalamus	1.1	NS
																				○	×	○	×	×	rt.thalamus	1.1	NAA↓
																				○	×	○	×	×	lt.basal ganglia	1.1	NS
																				○	×	○	×	×	rt. basal ganglia	1.1	NS
Ohara *et al.*[35]	24.5	12	10	12	10	DSM-IV	5.6	No	Medicated	29.5	NA	NA	1.5	Undone	PROBE	Single	Cre ratio	40	1500	○	×	○	×	×	lt.lenticular nucleus	8	NS
																				○	×	○	×	×	rt.lenticular nucleus	8	NS
Rosenberg *et al.*[23]	11.39	20	9	14	5	DSM-IV	3.87	Two: anxiety	No	25.8	NA	NA	1.5	Undone	PRESS	Single	Absolute	272	2300	○	○	○	○	○	ACC	3	NS
Rosenberg *et al.*[37]	11.19	11	4	11	4	DSM-IV	2.56	Yes: anxiety	No	26.55	NA	NA	1.5	Undone	PRESS	Single	Absolute	272	2300	○	○	○	×	×	lt.medial thalamus	NA	NS
																				○	○	○	×	×	rt.medial thalamus	NA	NS
																				○	○	○	×	×	lt.lateral thalamus	NA	NS
																				○	○	○	×	×	rt.lateral thalamus	NA	NS
Rosenberg *et al.*[36]	11	11	4	11	4	K-SADS-PL[c]	1.2	Yes: anxiety	No	30.36	15.27	15.09	1.5	Undone	PRESS	Single	Absolute	272	2300	○	○	○	○	○	lt.caudate	0.7	NS
																				○	○	○	○	○	Occipital gray matter	8	NS
Scherk *et al.*[40]	NA	17	NA	16	NA	DSM-IV	NA	No	Medicated	NA	NA	NA	1.5	Undone	NA	Single	Cre ratio	30	1500	○	×	○	○	○	DLPFC	3.5	NS
																				○	×	○	○	○	PMFC	3.6	NS
																				○	×	○	○	○	Putamen	3.3	NS
Starck *et al.*[41]	32.6	9	6	15	11	DSM-IV	19.4	Yes: depression	Medicated	22.9	11.9	11	1.5	Undone	PRESS	Single	Absolute	30	2000	○	○	○	○	○	rt.caudate nucleus	1.5	NA
																				○	○	○	○	○	Anterior cingulate gyrus	3.6	NA
																				○	○	○	○	○	OC	4	NA
Sumitani *et al.*[27]	29.99	20	13	26	10	DSM-IV	NA	NA	Medicated	23.79	NA	NA	1.5	Done	STEAM	Single	Absolute	18	5000	○	○	○	×	×	ACC	4.335	NAA↓
																				○	○	○	×	×	lt.basal ganglia	4.335	NS
																				○	○	○	×	×	lt.FL	4.335	NS
Whiteside *et al.*[39]	41.2	15	9	15	9	DSM-IV	23.09	Yes: depression	Medicated	24.13	11.73	12.4	1.5	Undone	PROBE	Single	Cre ratio	35	2000	○	×	○	○	○	lt.head of caudate	4	NS
																				○	×	○	○	○	rt.head of caudate	4	NS
																				○	×	○	○	○	lt.OFWM	4	NS
																				○	×	○	○	○	rt.OFWM	4	NAA↓
Yucel *et al.*[31]	34.4	20	10	26	13	DSM-IV	13.4	NA	No/stable	19.55	9.35	10.2	3	Done	PRESS	Single	Absolute	30	3000	○	○	○	○	○	rt.dACC	6.5	NS
																				○	○	○	○	○	rt.rACC	6.5	NS
																				○	○	○	○	○	lt.dACC	6.5	NS
																				○	○	○	○	○	lt.rACC	6.5	NS

Abbreviations: ACC, anterior cingulate cortex; AHC, amygdala hippocampus complex; bl., bilateral; Cre,creatine; dAC, dorsal anterior cingulate; dACC, dorsal anterior cingulate cortex; DLPFC, dorsolateral prefrontal cortex; FL, frontal lobe; FWM, frontal white matter; HC, healthy control; lt., left; MPFC, medial prefrontal cortex; MRS, magnetic resonance spectroscopy; MTL, medial temporal lobe; NA, not applicable; NAA, *N*-acetylaspartate; NHC, non-healthy control; NS, nonsignificant; OC, occipital cortex; OCD, obsessive-compulsive disorder; OFA, orbitofrontal area; OFWM, orbitofrontal white matter; PC, parietal cortex; PMFC, posterior frontal cortex; PWM, parietal white matter; PL, parietal lobe; rACC, rostal anterior cingulate cortex; rt., right; TE, echo time; TR, repetition time; VOI, volume of interest.
[a]Number. [b]Yale–Brown Obsessive Compulsive Scale. [c]The schedule for affective disorders abd schizophrenia for school-age children—present and lifetime versions.

a

Meta-analysis of NAA in the frontal cortex

Study name	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Bedard et al. 2011. ACC.	-0.307	0.411	0.169	-1.112	0.498	-0.747	0.455
Bedard et al. 2011. OFA.	0.057	0.408	0.167	-0.743	0.857	0.140	0.889
Besiroglu et al. 2011.	-1.303	0.345	0.119	-1.979	-0.627	-3.779	0.000
Ebert et al. 1997.	-0.863	0.520	0.271	-1.882	0.157	-1.658	0.097
Fan et al. 2010.	0.794	0.357	0.127	0.095	1.494	2.226	0.026
Jang et al. 2006. ACC.	-0.872	0.410	0.168	-1.676	-0.067	-2.123	0.034
Jang et al. 2006. DLPFC.	-1.010	0.417	0.173	-1.827	-0.194	-2.426	0.015
Kitamura et al. 2006.	0.558	0.344	0.118	-0.116	1.231	1.623	0.105
Rosenberg et al. 2004.	-0.552	0.355	0.126	-1.247	0.143	-1.556	0.120
Scherk et al. 2009. group 1. DLPFC.	-0.359	0.490	0.240	-1.319	0.601	-0.734	0.463
Scherk et al. 2009. group 2. DLPFC.	0.086	0.500	0.250	-0.894	1.066	0.172	0.863
Scherk et al. 2009. group 1. mPFC.	-0.194	0.487	0.237	-1.148	0.761	-0.398	0.691
Scherk et al. 2009. group 2. mPFC.	-0.353	0.504	0.254	-1.341	0.635	-0.701	0.484
Starck et al. 2007.	0.029	0.417	0.174	-0.788	0.846	0.069	0.945
Sumitani et al. 2007.	-0.670	0.306	0.093	-1.269	-0.071	-2.192	0.028
Yucel et al. 2008.	-0.462	0.301	0.091	-1.052	0.129	-1.533	0.125
	-0.338	0.151	0.023	-0.634	-0.042	-2.240	0.025

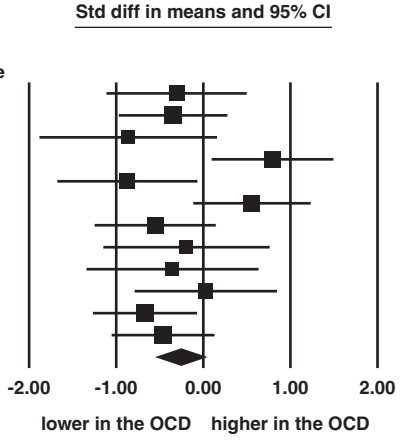


The meta-analysis demonstrated significant NAA reduction in the frontal cortex in OCD patients.

b

Meta-analysis of NAA in the mPFC

Study name	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Bedard et al. 2011.	-0.307	0.411	0.169	-1.112	0.498	-0.747	0.455
Besiroglu et al. 2011.	-0.347	0.318	0.101	-0.971	0.277	-1.091	0.275
Ebert et al. 1997.	-0.863	0.520	0.271	-1.882	0.157	-1.658	0.097
Fan et al. 2010.	0.794	0.357	0.127	0.095	1.494	2.226	0.026
Jang et al. 2006.	-0.872	0.410	0.168	-1.676	-0.067	-2.123	0.034
Kitamura et al. 2006.	0.558	0.344	0.118	-0.116	1.231	1.623	0.105
Rosenberg et al. 2004.	-0.552	0.355	0.126	-1.247	0.143	-1.556	0.120
Scherk et al. 2009. group 1.	-0.194	0.487	0.237	-1.148	0.761	-0.398	0.691
Scherk et al. 2009. group 2.	-0.353	0.504	0.254	-1.341	0.635	-0.701	0.484
Starck et al. 2007.	0.029	0.417	0.174	-0.788	0.846	0.069	0.945
Sumitani et al. 2007.	-0.670	0.306	0.093	-1.269	-0.071	-2.192	0.028
Yucel et al. 2008.	-0.462	0.301	0.091	-1.052	0.129	-1.533	0.125
	-0.253	0.154	0.024	-0.555	0.049	-1.642	0.101



The meta-analysis demonstrated no significant difference in NAA level in the mPFC between OCD patients and HC.

Figure 1 Forest plot of *N*-acetylaspartate (NAA) measure by regions. (**a**) Standardized mean differences for NAA levels between obsessive-compulsive disorder patients and healthy comparisons within the frontal cortex, (**b**) standardized mean differences for NAA levels between obsessive-compulsive disorder patients and healthy comparisons within the medial prefrontal cortex, thalamus. Regarding with *P*-value for Besiroglu *et al*, in Figure 1a, *P*-value was <0.001. ACC, anterior cingulate cortex; CI, confidence interval; DLPFC, dorsolateral prefrontal cortex; OCD, obsessive-compulsive disorder; OFA, orbitofrontal area; VOI, volume of interest.

Meta-regression. To investigate the effect of neurochemical alteration on behavioral abnormality, we conducted meta-regression analyses to examine the relationship between the total, obsession and compulsion scores of the subject on the Yale–Brown Obsessive Compulsive Scale (Y-BOCS) and the effect size for the NAA levels in the mPFC where there was enough number of datasets to perform regression analysis. Further, to explore the effects of various parameters on neurochemical abnormalities, we performed meta-regression analyses to test the relationship between the percentage of OCD patients with diagnosis of depression and anxiety disorder, the mean duration of the illness, the mean age of the subject and size of VOI with the effect size for the NAA levels. Regression was examined using comprehensive meta-analysis and the significance level was set at $P<0.05$.

Assessing between-study heterogeneity. The current meta-analyses included studies with considerable differences in many factors, such as prescribed medications, types of MRS measures (for example, absolute measures or ratios to Cre), segmentation within the VOI and the volume of the VOI. I^2 statistics was employed to assess between-study heterogeneity. Thresholds for the interpretation of I^2 were

Table 2 Meta-analyses of NAA levels by region

Region	*Separate dataset*	*No. of OCD*	*No. of HC*	*Z-value*	P-*value*	I^2(%)	*Publication bias*
Frontal cortex	16	225	233	−2.240	0.025	56.2	0.92
mPFC	15	183	192	−1.871	0.061	63.3	0.99
DLPFC	3	30	29	−0.819	0.413	63.7	NA
Thalamus	4	63	73	−0.729	0.466	0	NA
Basal ganglia	10	105	115	−0.292	0.770	42.5	0.36

Abbreviations: DLPFC, dorsolateral prefrontal cortex; HC, healthy control; mPFC, medial prefrontal cortex; NA, not applicable; NAA, *N*-acetylaspartate; OCD, obsessive-compulsive disorder.

Table 3 Results from sensitivity analyses in NAA levels in the frontal cortex

Specified criteria	*No. of OCD*	*No. of HC*	*No. of studies*	*No. of datasets*	*95% CI lower*	*95% CI upper*	Z-*value*	P-*value*
No medication	106	77	5	6	−1.268	0.026	−1.881	0.060
No psychiatric comorbidity	130	125	6	10	−0.613	0.273	−0.753	0.452
Cre ratio	156	151	7	12	−0.536	0.135	−1.173	0.241
1.5-Tesla scanner	193	175	9	14	−0.713	−0.092	−2.538	0.011
Segmented within VOIs	66	78	3	4	−1.035	−0.357	−4.023	<0.001

Abbreviations: CI, confidence interval; VOI, volume of interest; mPFC, medial prefrontal cortex; NAA, *N*-acetylaspartate; OCD, obsessive-compulsive disorder; HC, healthy control.

based on previous studies suggesting that 0–50% represents mild heterogeneity, 50–75% moderate heterogeneity and 75–100% considerable heterogeneity.[30]

Publication bias. Publication bias was assessed qualitatively by visual inspection of funnel plots, and quantitatively by linear regression analysis for each group and each brain region. On the basis of previous literature, this calculation was tested with datasets of at least 10 data.[30] The significance level was defined as $P<0.10$ to conclude existence of publication bias.[30]

Data synthesis. Twenty-seven demographic, clinical and methodological variants were extracted from the included studies, as shown in Table 1. These included the number of participants, the number of male participants, the mean age of the participants, diagnostic tools, the duration of the illness, psychiatric comorbidity, pharmacological status, total, obsessive, and compulsive scores on the Y-BOCS, the sequence used for MRS acquisition, single- vs multi-voxel analysis, whether segmentation within VOIs was used, the strength of magnetic field (Tesla), the echo time, the repetition time, the types of MRS measurements (absolute measure or ratio to Cre), the location of the VOI, and the size of the VOI. The total number of participants, datasets, and mean differences, the effect sizes, *P*-values, I^2 scores and the significance of the linear regression analysis of the symmetry of the funnel plots calculated from each meta-analysis are shown in Table 2.

Results

Study selection. The literature search described above yielded 214 articles, of which 26 were identified as potential candidates for the meta-analysis.[22–24,26–28,31–51] Of these, three studies were excluded because they did not compare metabolites between OCD patients and HC subjects.[44–46] Another study was excluded because it was a review article.[47] In addition, three studies were discarded because of overlapping data with other studies from the same research group.[32,48,49] Thus, 19 studies were included in the database (Table 1). From this database, one study was excluded because they did not provide sufficient data to calculate effect sizes.[50] Another study was excluded from the analysis because they located their VOI only in the hippocampus,[51] and the hippocampus is not related to the current study. Thus, 17 studies were included in the final meta-analysis.[22–24,26–28,31,34–43]

Characteristics of included studies. The 17 studies included in the meta-analysis were published between 1997 and 2011, and examined a total of 273 OCD patients and 271 HC subjects. The mean age of OCD patients ranged from 10 to 41. Fifteen studies reported the duration of illness[22–24,26,28,31,34–39,41–43] with a range from 1.2 years[36] to 23.5 years.[28] Seven studies involved OCD patients who were not currently treated with medication or had never been treated with medication for OCD.[22–24,34,36,37,42,43] Sixteen studies reported the total scores on the Y-BOCS;[22–24,26–28,31,34–39,41–43] the highest mean score was 30.36 ref.[36] and the lowest was 14.3.[28] Two studies utilized a 3-tesla MRI scanner,[26,32] one study utilized a 2-tesla MRI scanner,[34] and 14 studies utilized a 1.5-Tesla MRI scanner.[22–24,27,28,35–43] Three studies implemented tissue segmentation within the VOI.[22,27,31] Seven studies reported absolute NAA levels,[23,27,31,36–38,42] whereas nine studies reported NAA levels as a ratio of Cre levels.[22,24,26,28,34,35,39,40,43] Sixteen studies utilized single-voxel MRS.[23–24,26–28,31,34–43]

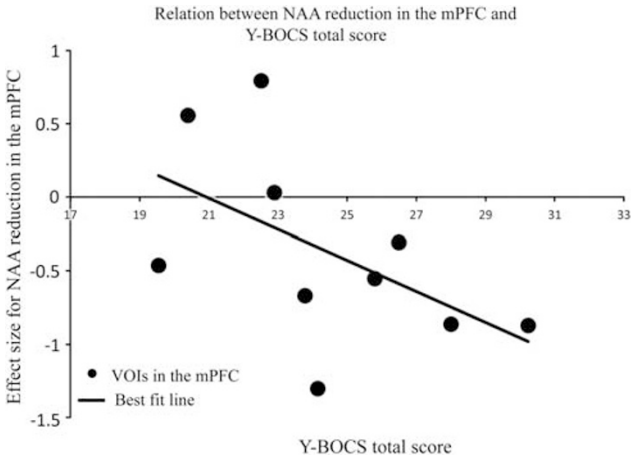


Figure 2 Meta-regression of *N*-acetylaspartate (NAA) reduction in the mPFC and Yale–Brown Obsessive Compulsive Scale (Y-BOCS) total score. The relationship between effect sizes for reduced NAA and Y-BOCS total score. Effect sizes from each comparison are plotted by the mean Y-BOCS total scores of participants with obsessive-compulsive disorder of the study. The line of best fit shows a substantial decrease in NAA reduction. mPFC, medial prefrontal cortex; VOI, volume of interest.

Meta-analysis for NAA measures by region

Frontal cortex. In the frontal cortex, 16 separate datasets from 11 independent studies were included.[22–24,26,27,31,34,40–43] These datasets included 225 individuals with OCD and 233 control subjects. The meta-analysis demonstrated significant reductions in NAA levels in OCD patients compared with HC subjects ($P=0.025$). Although publication bias was not present, moderate heterogeneity was found ($I^2=56.2\%$) (Figure 1 and Table 2).

Thalamus. In the thalamus, four datasets from 63 individuals with OCD and 73 HC subjects were integrated into the meta-analysis, and revealed no significant differences in NAA levels between individuals with OCD compared with HCs (Table 2). Four studies were included in the analysis.[23,26,38,42]

Basal ganglia. Ten datasets from eight studies were included in the meta-analysis of the basal ganglia.[27,28,34–36,39–41] The meta-analysis included 105 individuals with OCD and 115 HC subjects, and revealed no significant difference in NAA levels. No significant heterogeneity or significant publication bias were present (Table 2).

Medial prefrontal cortex. Among 16 separate datasets in the frontal lobe, 12 examined the mPFC in 11 independent studies.[22–24,26,27,31,34,40–43] The meta-analysis with 183 OCDs and 192 HCs revealed that NAA was marginally significantly reduced in OCD patients ($P=0.061$) with moderate heterogeneity ($I^2=63.3\%$) and no publication bias (Figure 1 and Table 2).

Dorsolateral prefrontal cortex. Only three separate datasets from two independent studies investigated NAA levels in the DLPFC.[22,40] These studies enrolled 30 OCD patients and 29 HC subjects, and the meta-analysis reported no significant difference in NAA level between OCD and HC individuals ($P=0.413$) (Table 2).

Orbitofrontal cortex. Only one study[43] reported NAA levels in the OFC. As a sufficient number of studies were not included, we did not conduct a meta-analysis.

Sensitivity analysis. Sensitivity analysis to test the robustness of the significance of NAA decrease in the frontal cortex was conducted. Sensitivity analyses performed in the specified subgroups with studies whose participants were not medicated ($P=0.060$), that have implemented tissue segmentation ($P<0.001$), and that utilized 1.5-tesla scanner ($P=0.011$) preserved the significance, whereas sensitivity analyses with specified-subgroups with studies whose participants do not have comorbid psychiatric disorder and that adopted Cre ratio did not preserve the significance (Table 3).

Meta-regression. To investigate the effects of NAA reductions in the mPFC on abnormal behavior of OCD, we performed meta-regression analyses with the total, obsession and compulsion of scores of the Y-BOCS. Meta-regression demonstrated significant effect of the reduction of NAA levels in the mPFC on the Y-BOCS total score ($P=0.011$) but no significant effect on the Y-BOCS obsession score ($P=0.245$) or compulsion score ($P=0.797$) (Figure 2 and Table 4). Further meta-regression analyses revealed no significant effects of the percentage of OCD patients with diagnosis of depression ($P=0.259$), anxiety disorder ($P=0.696$), the duration of illness of the patients ($P=0.742$), the age of the OCD patients ($P=0.297$) and size of VOI ($P=0.783$) on the reduction of NAA levels in the mPFC (Table 4).

Table 4 Meta-regression of NAA levels in the mPFC

Modifier	*Separate dataset*	*Intercept*	*Slope*	P-*value*
Y-BOCS				
Total	10	1.94	−0.10	0.011
Obsession	5	−0.51	−0.09	0.245
Compulsion	5	−0.33	−0.02	0.797
% Of patients with depression	10	−0.20	−2.00	0.259
% Of patients with anxiety	10	−0.24	−1.36	0.696
Duration of illness	9	−0.22	−0.01	0.742
Age (years)	10	0.11	−0.02	0.297
Size of VOI	10	−0.28	−0.01	0.783

Abbreviations: mPFC, medial prefrontal cortex; NAA, *N*-acetylaspartate; VOI, volume of interest;Y-BOCS, Yale–Brown Obsessive Compulsive Scale.

Discussion

Summary. To our knowledge, this is the first systematic review and meta-analysis of ^{1}H-MRS studies in people with OCD. There were three main findings. First, the meta-analysis demonstrated a significant reduction in NAA levels

in the frontal cortex of OCD patients compared with HC subjects, but no significant difference in subcortical areas such as the basal ganglia or thalamus. The second finding is that sensitivity analysis in anatomically specified subgroup consisting of datasets examining the mPFC demonstrated marginally significant NAA reductions in OCD patients compared with HC. The third, meta-regression revealed significant positive correlation between NAA reduction and symptom severity of OCD, which indicates that neurochemical alteration in the mPFC is directly related to abnormal behavior of OCDs. The systematic review revealed obvious methodological heterogeneities across studies, including differences in pharmacological status, strength of magnetic field and utilization of segmentation within VOIs. However, the sensitivity analyses, which excluded the potential effects of these confounds, further emphasized the robustness of current findings, regarding the reduction of NAA levels in the frontal cortex in OCD patients.

Methodological considerations. Although we utilized a random effects model to account for between-study heterogeneity, and none of the analyses revealed a severe degree of heterogeneity in the current meta-analyses (Table 2), there is inherent between-study heterogeneity in data acquisition, and subject characteristics between ^{1}H-MRS studies. Thus, the current results should be interpreted cautiously.

Regarding data acquisition, correcting the CSF ratio within VOIs is required in ^{1}H-MRS studies, because there is no metabolite in the CSF. Two methods are commonly used to correct for this problem: The first is to report the level of the metabolite of interest in comparison with the other metabolite. The other is to implement tissue segmentation within VOIs to exclude the CSF component. In the current analysis, some studies reported NAA level to Cre ratio, whereas others reported absolute NAA levels. But as all the studies except two[27,31] included into the meta-analysis reported absolute NAA level without implementing tissue segmentation within VOIs (Table 1), absolute level of NAA does not reflect CSF ratio within VOI and what extent gray and white matter were included. This is the reason why we adopted the different priority for extraction depending on implementation of tissue segmentation within VOIs.

In the current work, the sensitivity analysis, which was conducted by subgrouping studies that implemented tissue segmentation, demonstrated significant NAA reductions in the frontal cortex in OCD patients, whereas those that reported NAA level ratio to Cre did not preserve the significance. The potential explanation for disappearance of significance is decreased number of integrated studies and increased weight of outlier in the meta-analysis. Although supplemental meta-analysis of absolute Cre levels in the frontal cortex demonstrated no significant difference between OCD and HC subjects (data not shown), it should be noted that, for example, decrease of the NAA/Cre could reflect decreased NAA, increased Cre or a combined effect of alterations in the levels of both metabolites.

Several clinical factors may have also affected the current results. Two important clinical characteristics that can affect NAA levels are comorbid psychiatric disorder and treatment. For example, previous studies have demonstrated that other psychiatric disorders, such as schizophrenia and autism, also alter NAA levels.[52,53] Although in the current study, the sensitivity analysis with subgrouping to exclude studies that recruited medicated patients demonstrated marginally significant NAA reduction in the frontal cortex, those that recruited patient with comorbid psychiatric disorder did not reach the significance. However, meta-regression showed no significant relation between percentages of patients with concurrent diagnosis of depression and anxiety disorder and NAA reduction in the mPFC, some studies recruited OCD patients with psychological symptoms, such as subclinical depression and anxiety. Although one meta-analysis demonstrated no significant alteration of NAA in the frontal lobe in patients with depression,[54] recent studies have suggested that baseline NAA is decreased among patients with depression[19,55] and recovers after treatment.[19] Thus, although meta-regression analysis demonstrated significant correlation between NAA reduction in the mPFC and symptom severity, it should be noted that the NAA reduction in the frontal cortex and mPFC may not only have been caused purely by symptoms of OCD, but also by comorbid depression or anxiety of OCD. Regarding the treatment of OCD, some of the included studies recruited OCD patients with current or prior treatment. Although sensitivity analysis with non-medicated patients showed marginally significant NAA reduction in the mPFC, treatment of OCD may also affect NAA levels measured by ^{1}H-MRS. Two of the studies we included conducted longitudinal assessments of the effects of medication. Jang *et al*.,[22] examined thirteen drug-naive OCD patients before and after treatment with medication and found significant increases in NAA in the prefrontal cortex and anterior cingulate. Besiroglu *et al*.,[42] also found increased NAA in the anterior cingulate cortex. These results suggest that examining OCD patients who have previously undergone treatment with medication does not cause underestimation of NAA levels, which indirectly supports the robustness of NAA reductions in OCD patients.

Context of findings. It is currently unclear exactly what is reflected by the measurement of NAA using ^{1}H-MRS. As NAA is synthesized from aspartate and acetyl-coenzyme A in the mitochondria of neurons, it has been proposed as a marker of neuron density[20] and mitochondrial activity.[56] Recent studies have revealed that astrocytes and oligodendrocytes were involved in the metabolism of NAA.[21] As such, not only increased synthesis of NAA, but also decreased metabolism of NAA may result in increased NAA levels.[57] For example, 'NAA trapping theory' suggests that NAA may increase even in cases of dysfunctional metabolism of the astrocytes or oligodendrocytes.[58] For example, patients with Canavan's disease, caused by mutations in the gene that codes for the enzyme of aspartoacylase, which is necessary for NAA metabolism, have reported increased NAA without increased neuron density.[57] One experimental study reported that intravenous ethanol infusion enhances the activity of acetyl-aspartylase, a glial enzyme that degrades NAA, and dynamically decreases cortical NAA levels measured by ^{1}H-MRS.[59] These studies suggested that NAA reflects dynamic processes rather than structural effect.[60] Thus, some types of pathophysiology may cause NAA reduction by decreasing NAA synthesis (via decreased mitochondrial

function in neurons or decreased neuron density), whereas other types may decrease NAA by increasing NAA metabolism (via excessive function of glial cells).

Correlation between NAA reduction in the mPFC and symptom severity. Alteration of function of the mPFC has been recognized in a variety of functional neuroimaging studies in OCD, including resting state studies,[6] interference and error processing,[61,62] performance monitoring,[63] and symptom provocation studies.[9] Further, the importance of the mPFC in individuals with OCD has been highly recognized in the field of neurosurgery. Numerous studies have reported the effectiveness of anterior cingulotomy for OCD[64] and the mPFC is recognized as a promising target region for deep brain stimulation for OCD.[65]

Structural abnormalities of the mPFC have been reported and a meta-analysis of voxel-based morphometry studies of OCD[15] and a meta-analysis of the brain volume studies[16] demonstrated robust gray matter volume reductions in the mPFC.

No significant difference in NAA level in basal ganglia and thalamus. Although previous studies strongly demonstrated the critical role of basal ganglia and thalamus in the symptoms of OCD and there is an indirect evidence for basal ganglia involvement in OCD from findings that patients who suffer focal lesions often then exhibit striking obsessive-compulsive behaviors,[5,11–13,66] the current meta-analyses showed no significant difference in NAA level in these areas between OCD patients and HC. Interestingly, the finding is also concordant with previous meta-analyses of the brain volume studies that reported no reduction of gray matter volume.[15,16] Potential explanation for these negative findings that there were no significant differences in NAA levels in basal ganglia and thalamus between OCD patients and HC may be that the basal ganglia and thalamus were included into other CSTC circuits that may have different role in the etiology of OCD. Recent researches revealed that one potential etiology of OCD is an imbalance of inhibition and disinhibition between CSTC loops.[3] As more than two circuits include different part of basal ganglia and thalamus, for example, mPFC circuit includes ventral caudate nucleus, on the other hand DLPFC circuit contains dorsal caudate nucleus, it is assumed that some parts of basal ganglia and thalamus are disinhibited by mPFC circuit and others are inhibited by DLPFC circuit, vice versa.[3] Thus, we assume that diversity of location of relatively large VOIs of integrated studies, which may include projections from several circuits, resulted in no significant differences in NAA level between OCD patients and HC. With regard to other CSTC circuits, such as OFC and DLPFC, the current meta-analysis could not provide sufficient data to discuss. Although the current meta-analysis demonstrated significant correlation between NAA reduction in the mPFC and symptom severity, the result does not reduce the importance of other areas in the etiology of OCD.

Limitations. Several limitations of the present study should be considered. First, because of the nature of a meta-analysis, we can only provide statistical analyses at the level of whole studies. The combination of results from different studies into a single analysis might cloud individual differences within studies. Second, because too few studies have examined certain brain areas, we could not assess the role of NAA levels in the OFC, and only two studies were included in the meta-analysis of the DLPFC, even though we recognize that previous neuroimaging studies have reported these areas to be important in OCD.[67,68] Third, although NAA reduction in the mPFC was robustly correlated with scores of symptom severity, and meta-regression showed no significant relation between percentage of comorbid diagnosis of depression and anxiety disorder and NAA reduction, there still remains the possibility that NAA reduction may be due to subclinical depression or anxiety, as sensitivity analysis that excluded patients with comorbid psychiatric disorder did not demonstrate significant NAA reduction and other anxiety disorders also demonstrated NAA reduction in the mPFC.[69] Forth, although we focused on three CSTC circuits and demonstrated marginally significant NAA reduction in one of the three, we could not conduct network analysis. Fifth, although we categorized the locations of the VOIs into three brain areas, this classification scheme might be criticized as being over-simplified. Though we recognize the functional variability within subregions of these areas, we could not divide VOIs into subarea such as anterior cingulate cortex accurately and objectively only based on Figures and legend supplied in the included studies. Finally, the current meta-analysis included both pediatric and adult studies. Although we investigated the effect of age, the effect size was calculated from two groups of similar ages. Thus, given the striking developmental changes that occur in these regions, we could not assess the underlying developmental trajectory of NAA levels in OCD.

Conclusion

In conclusion, the current meta-analysis revealed a significant NAA reduction in the frontal cortex in OCD patients, while no significant differences were present in the thalamus and basal ganglia. Sensitivity analysis in an anatomically specified subgroup consisting of datasets examining the mPFC demonstrated marginal significant reduction of NAA. The NAA reduction in the mPFC was correlated to symptom severity of OCD. These findings suggest that neurochemical alteration in the mPFC is one of the abnormal neural bases that directly relate to behavioral abnormality.

Conflict of Interest

The authors declare no conflicts of interest.

Acknowledgements. We thank all of the authors of the included studies and especially Dr Jang and Dr Carlsson and their colleagues for kindly sharing their unpublished data for inclusion in this meta-analysis.

Author contributions

YA and AA performed study screenings independently. In the case of discrepancies, consensus was reached by means of discussion with the third author (HS). YA performed all of the data extractions and computations of effect size twice to avoid

mistakes. AA further performed the data extractions and computations of the effect sizes independently. YA wrote the paper.

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