Original Article
Neurochemical abnormalities in anterior cingulate cortex on betel quid dependence: a 2D ^{1}H MRS investigation

Tao Liu[1,2*], Jianjun Li[3*], Shixiong Huang[2], Zhongyan Zhao[2], Guoshuai Yang[1], Mengjie Pan[3], Changqing Li[3], Feng Chen[3], Suyue Pan[1]

[1]Department of Neurology, Nanfang Hospital, Southern Medical University, Guangzhou 510515, China; Departments of [2]Neurology, [3]Radiology, People's Hospital of Hainan Province, Haikou 570311, China. *Equal contributors.

Abstract: The effects of betel quid dependence (BQD) on biochemical changes remain largely unknown. Individuals with impaired cognitive control of behavior often reveal altered neurochemicals in Magnetic Resonance Spectroscopy Imaging (MRSI) and those changes are usually earlier than structural alteration. Here, we examined BQD individuals (n = 33) and age-, sex-, and education-matched healthy control participants (n = 32) in an 2D ^{1}H-MRS study to observe brain biochemical alterations in the anterior cingulated cortex (ACC) associated with the severity of BQD and duration of BQD. In the bilateral ACC, our study found NAA/Cr were lower in BQD individuals compared to the healthy controls, Cho/Cr and Glx/Cr were higher in individuals with BQD compared to the healthy group, but increase was noted for mI/Cr in BQD individuals only in the left ACC. NAA/Cr ratios of the right ACC negatively correlated with BQDS and duration, NAA/Cr ratios of the left ACC negatively correlated with duration, Glx/Cr ratios of the right ACC positively correlated with BQDS. The findings of the study support previous analyses of a role for ACC area in the mediation of BQ addiction and mechanistically explain past observations of reduced ACC grey matter in BQD patients. These data jointly point to state related abnormalities of BQ effect and provide a novel strategy of therapeutic intervention designed to normalize Glu transmission and function during treating BQ addiction.

Keywords: Betel quid, substance dependence, magnetic resonance spectroscopy, anterior cingulated cortex, N-acetylaspartate, myo-inositol, creatine, choline, glutamate

Introduction

Areca nut (AN) can be chewed wrapping in a betel leaf or together with tobacco (betel quid, BQ), and its composition differs among diverse populations and areas [1]. In Hainan, China, BQ is the combination of fresh areca nut and slaked lime such as aqueous calcium hydroxide paste winded by betel leaves with no tobacco or any ingredients. BQ is broadly used by people of all ages around the globe, south-east Asia specifically. It ranked among the most broadly consumed psychoactive substances worldwide following only nicotine, ethanol and caffeine, and its consumer population accounts for almost 10% of the world. The IARC review gave the conclusion that AN can be carcinogenic to people and may lead to cancers of the pharynx, oral cavity, esophagus, the uterus as well as liver and biliary tracts [2].

The alkaloid arecoline contains parasympathomimetic properties which stimulate the muscarinic and nicotinic receptors, therefore the AN primarily takes effects on the central and the autonomic nervous systems [3]. Frequent consumers indicate euphoria, which is a sense of warmth, happiness, salivation, elevated alertness, anti-migraine, palpitation and increased working capabilities [4]. BQ consumption is related to a dependency syndrome, which consists of mild euphoria, enhanced concentration, postprandial satisfaction, relaxation and a withdrawal syndrome related to mood swings, insomnia, anxiety and irritability, and its severity is similar to that of amphetamine [5]. The first instrument measuring BQ dependence: the Betel Quid Dependence Scale (BQDS) was developed and given initial validation by Lee recently [6]. However, most studies regarding Betel Quid chewing have been confined to epi-

demiological and biological investigations so far [7, 8]. Researchers have conducted limited research to figure out the psychological and behavioral factors that cause people to start and/or maintain BQ consumption. To date, why people develop BQ chewing habit still remains unknown.

As a non-invasive imaging technique, Proton magnetic resonance spectroscopy (¹H MRS) measures specific compounds or metabolites within the tissue of interest. A number of biochemical cerebral alterations had been detected by drug abuse studies using ¹H MRS. Reduced N-acetylaspartate (NAA) and elevated myo-inositol (mI) were the most consistent changes across drug class, while alterations in creatine, choline and amino acid transmitters were also significant [9]. The research discussed herein indicates that abused drugs may affect an individual's energy metabolism and maintenance, neuronal health, inflammatory processes, neurotransmission and cell membrane turnover, and these alterations may underlie the neuropathology within cerebral tissue that leads to the behavioral and cognitive impairments related to drug addiction subsequently [9, 10]. However, rather than merely identifying the cerebral consequences caused by drug abuse, ¹H MRS may track the progression of disease and/or treatment.

The role of the anterior cingulate cortex (ACC) has led to its inclusion in many major theories of addiction, where it is believed to form part of an inhibitory system that exercises control over reward-related behavior [11, 12]. As far as we know, no magnetic resonance spectroscopy (MRS) studies in individuals with BQD have been reported. However, based on findings in individuals with other drug abuse, we anticipated that patients with BQD would have similar deficits related to the ACC to those which have been observed in patients with alcohol abuse [13-15] or opiates abuse [16, 17]. The present study assessed the ACC in individuals with BQD using MRS aim to find the biochemical alterations, which underlie the neuropathology within cerebral tissue that subsequently leads to dependency syndrome such as withdrawal, tolerance, desire, concentration, and reduced management of BQ addiction as well as persistent chewing despite convincing indications of harmful influences.

Materials and methods

Ethics statement

The present study was approved by our research ethics review board of People's hospital of Hainan Province, Haikou, China according to the Declaration of Helsinki. The consent form has been read and signed by each subject before being included in the study.

Inclusion and exclusion criteria

Native peoples that take BQs exclusively in Wanning City, Hainan province were included. Persons using Betel Quid with no tobacco more than 5 years were regarded as "individuals using BQ without tobacco". The criteria for BQD group inclusion were as follows: (1) age: 18~60 years; (2) persons with usage of BQ; (3) BQ chewer with BQD, the diagnosis was made according to BQD Scale (BQDS) by the BQDS > 4; (4) persons without usage of different forms of tobacco at least for the last 1 years in order to exclude the influence of nicotine; (5) not taking antidepressant drugs or psychotropic drugs; (6) persons without self-claimed systemic illnesses (e.g. diabetes mellitus, cardiovascular disease, neurological disorder, thyroid, renal disorders and epilepsy; (7) persons without present or recent history of any Axis I psychiatric and/or substance use diseases; (8) No abnormal manifestation on conventional MRI; (9) left-handers; and (10) can read and write Chinese.

The "control individuals" were persons without using all forms of BQ, areca nut and tobacco. The exclusion criteria for control group were as follows: (1) tobacco consumers; (2) persons using diverse forms of tobacco with no smoke such as gutka and/or paan masala; (3) persons with self-claimed systemic illnesses e.g. cardiovascular disease, neurological disorder, epilepsy, diabetes mellitus, renal disorders and thyroid; (4) persons that have present or recent history of any Axis I psychiatric and/or substance use diseases; (5) currently using psychotropic drugs; (6) left-handers; and (7) cannot read and write Chinese.

Study participants

Finally, we included 33 BQD individuals and 32 individuals as the control group selected from Wanning City of Hainan province, China.
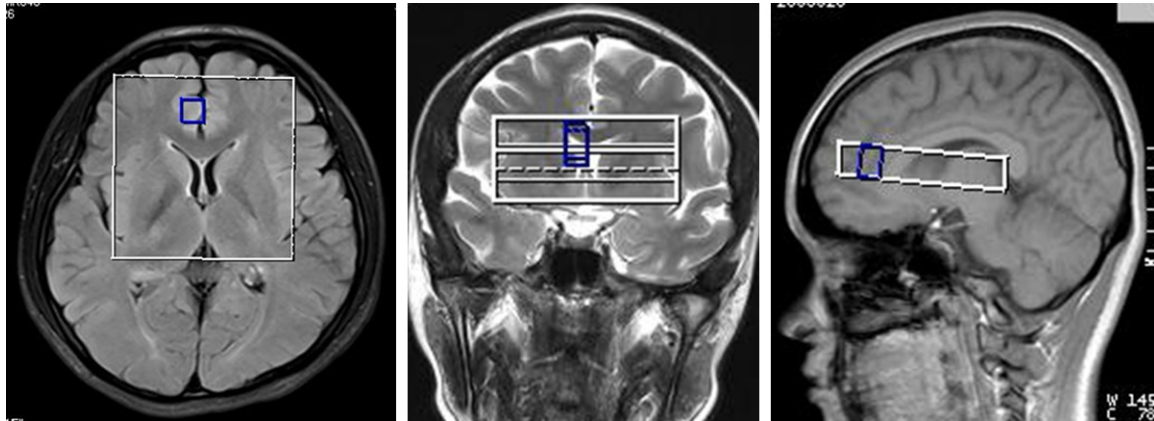
Figure 1. Placing region of interesting in right ACC and measuring the metabolite ratio.

Questionnaire

We distributed questionnaires regarding information of age and sex, educational status, monthly income, daily dosage of BQ, duration of BQ chewing habit and duration time of quid placement in mouth in simple Chinese to all participants (n = 65). Since wine plays a significant part in Chinese society, alcohol use in the past 30 days of the participants were also required, as well as the number of drinks per occasion and average frequency of drinking.

MRI data acquisition

Structural MR and ^{1}H-MRS were performed using a Siemens Verio3T MRI scanner and a 6 channel Sense head coil. We conducted a routine structure MR scan to rule out gross cerebral pathology. Anatomical images were acquired with a high-resolution T1-weighted MPRAGE sequence for the acquisition of 3D volume images. The parameters were: repetition time = 2300 ms, echo time = 2.9 ms, TI = 900 ms, field of view = 256 × 256 mm^2, flip angle = 9°, in-plane matrix = 256 × 256, slice thickness = 1 mm, no gap, and voxel dimension = 1 × 1 × 1.33 mm^3. All ^{1}H-MRS were acquired with a single slice 2D ^{1}H MRS technique with short echo time. We placed a 15 mm axial slice along the AC-PC line to cover ACC as shown in **Figure 1**. We acquired spectroscopic data from this slice with a nominal voxel size of 10 × 10 × 15 mm^3, TR/TE = 1700 ms/30 ms, flip angle = 90°, FOV = 16 cm × 16 cm, and 3 repetitions were obtained in 6 minutes 53 seconds.

MRS data processing and analysis

MRS data were post processed with Syngo spectroscopy software (Siemens Healthcare, Erlangen, Germany). The postprocessing included the following steps: frequency shift correction by using the residual water signal, removal of residual water signal, filtering the data by a Hanning low-pass filter with a width of 400 ms, zero-filling the data to 2048 points from 1024 points, Fourier transformation, automatic baseline correction, automatic phase correction (manual adjustment were done if automatic correction did not work well for some cases) and curve fitting of the signal peak at around 2.0 ppm as NAA, 2.4 ppm as Glx, 3.0 ppm as Cr, 3.2 ppm as Choline, and 3.60 ppm as Myo-inositol respectively using Gaussian line-shape.

Statistical analysis

All data were compared between the two groups by using SPSS software (version 16.0; SPSS, Inc., Chicago, IL). The data are presented as means ± standard deviations. An independent two-sample t test was taken as continuous variables such as age, years of education, monthly income, SDS, alcohol last 30 days, SAS, and the ratios between the metabolites (NAA/Cr, NAA/Cho, MI/Cr and Glx/Cr) after homogeneity of variance test, and a Chi-square test was performed for proportions. *P* values < 0.05 was regarded as statistically significant.

Pearson's or Spearman's correlation analysis was performed between the Cho/Cr, NAA/Cr,

Table 1. Demographics and clinical characteristics of participants

	Betel Quid Dependence (BQD)	Healthy control (HC)	t-value or x^2	P value
Mean Age, y	46.7 ± 9.4	45.8 ± 9.3	0.446	0.657[b]
Male to female, no	24/9	20/12	0.777	0.378[a]
Years of education	12.3 ± 2.7	12.6 ± 2.4	0.512	0.610[b]
Income (rmb/m)	2668.2 ± 464.5	2602.3 ± 460.0	0.799	0.427
BQDS scores	10 ± 3.4	N/A		
BQ dosage (g/day)	342 ± 106	N/A		
Time of placement of BQ in the mouth (min)	7.6 ± 2.4	N/A		
Duration of BQD (years)	20.6 ± 6.9	N/A		
Wine intake last 30 days (g)	200.2 ± 34.8	189.0 ± 33.4	1.025	0.309[b]
Right handedness to left handedness	33/0	32/0		> 0.99[a]
SAS scores	27.2 ± 5.6	28.3 ± 6.1	0.759	0.451[b]
SDS scores	28.6 ± 6.6	32.8 ± 7.5	2.385	0.020[b]

Note: unless otherwise indicated, data are means ± standard deviations. N/A = not applicable. [a]The *P* value between the two groups was obtained by chi-square test. [b]The *P* value between the two groups was obtained by independent-samples *t* test.

Table 2. Means (standard deviations) for neurochemicals of the BQD and health controls

Metabolite ratio	The left ACC				The right ACC			
	BQD	HC	*t* value	*P* value	BQD	HC	*t* value	*P* value
NAA/Cr	1.43 ± 0.23	1.59 ± 0.28	2.543	0.013*	1.45 ± 0.19	1.61 ± 0.23	2.908	0.005**
Cho/Cr	0.85 ± 0.18	0.74 ± 0.18	-2.263	0.021*	0.87 ± 0.25	0.74 ± 0.21	-2.267	0.027*
MI/Cr	0.51 ± 0.12	0.44 ± 0.12	-2.399	0.019*	0.45 ± 0.12	0.40 ± 0.11	-1.951	0.055
Glx/Cr	0.33 ± 0.11	0.24 ± 0.10	-3.227	0.002**	0.33 ± 0.14	0.25 ± 0.10	-2.524	0.014*

*P < 0.05, **P < 0.01.

MI/Cr, and Glx/Cr values of the ROIs and the BQDS, duration of the patients to evaluate their relation according to the normal test results.

Results

Demographics and clinical characteristics

65 subjects (33 BQD users and 32 controls) were included in the final data analysis. The BQD individuals and control groups had no differences in sex, age. High comorbidity of addiction along with mental diseases were demonstrated in patient samples with cross-sectional, with affective disorders (e.g. depression), anxiety disorders (generalized anxiety disorder, social anxiety disorder) in particular. SAS and SDS were used to evaluate all participants were evaluated for the purpose of eliminating the interference of depression and anxiety levels, and the outcomes didn't reach cut-off for clinical significance on average although the SDS scores in BQD group were significant lower than those in controls. It has been reported that the extract of AN has potential for the treatment of depression, so the SDS scores difference may be implicated in those anti-depression effect. The alcohol dosage in the past 30 days in BQD and HC group were 200.2 ± 34.8 g and 189.0 ± 33.4 g respectively, which showed that both groups were not alcohol addicts, and therefore alcohol would not cause any effect on BQD usage. Most participates are rubber tappers in state farm, they have a better cultural and economic status than volunteers in other addiction study. No significant differences were observed in education levels, alcohol last 30 days, monthly income and SAS between the two groups (*P* values > 0.05). Participants expressed that they had maintained BQ chewing with dependency syndrome for an average BQDS of 10 ± 3.4 (range 5 to 16), an average time of 20.6 ± 6.9 years (range 7 to 31 years) and consumed an average of 342 ± 106 g/day BQ (range 200 to 500 g/day) daily. Before spitting-out the remnants, BQ consumers kept BQ in their mouth for an average of 7.6 ± 2.4 minutes (range 3 to 12). **Table 1** summarizes the demographics of BQD and healthy control participants.
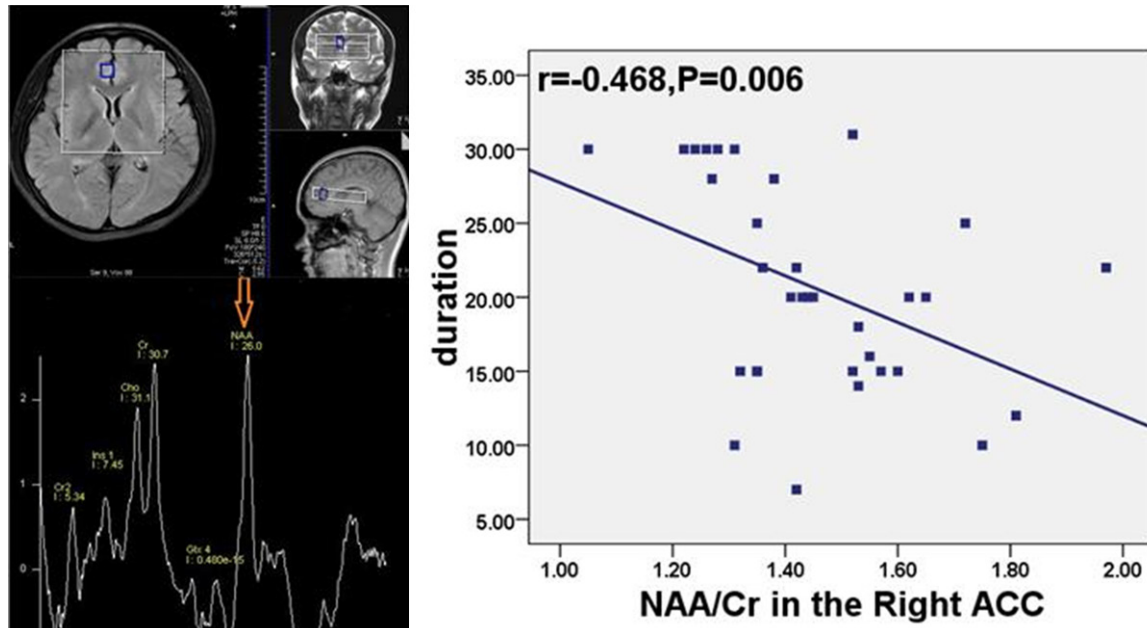
Figure 2. Scatter-plots of the correlation between duration and NAA/Cr in the right ACC. See text in the Results section for further explanations of the correlations.
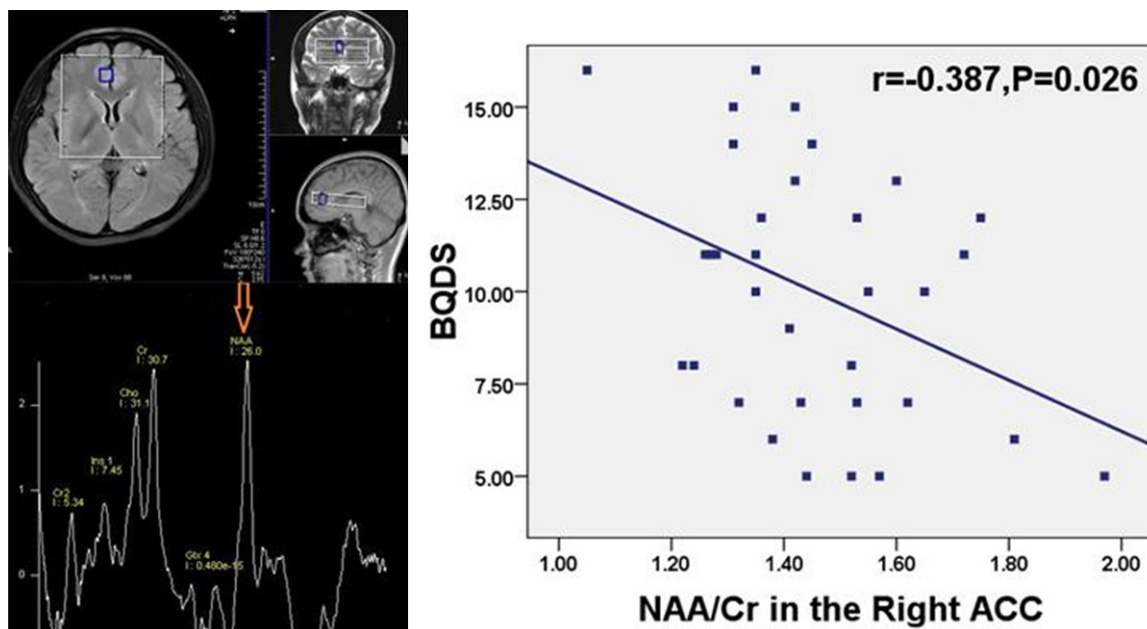

Figure 3. Scatter-plots of the correlation between BQDS and NAA/Cr in the right ACC. See text in the Results section for further explanations of the correlations.

Metabolic changes between the BQD and control groups

NAA/Cr: NAA/Cr ratios of the bilateral anterior cingulate cortex (L: 1.43 ± 0.23 and R: 1.45 ± 0.19, respectively) in the BQD group were significantly lower than those of the control group

(L: 1.59 ± 0.28, R: 1.61 ± 0.23, respectively) (P < 0.05) (**Table 2**).

Cho/Cr: Cho/Cr ratios of the bilateral anterior cingulate cortex (L: 0.85 ± 0.18 and R: 0.87 ± 0.25, respectively) in the BQD group were significantly higher than those in the control group
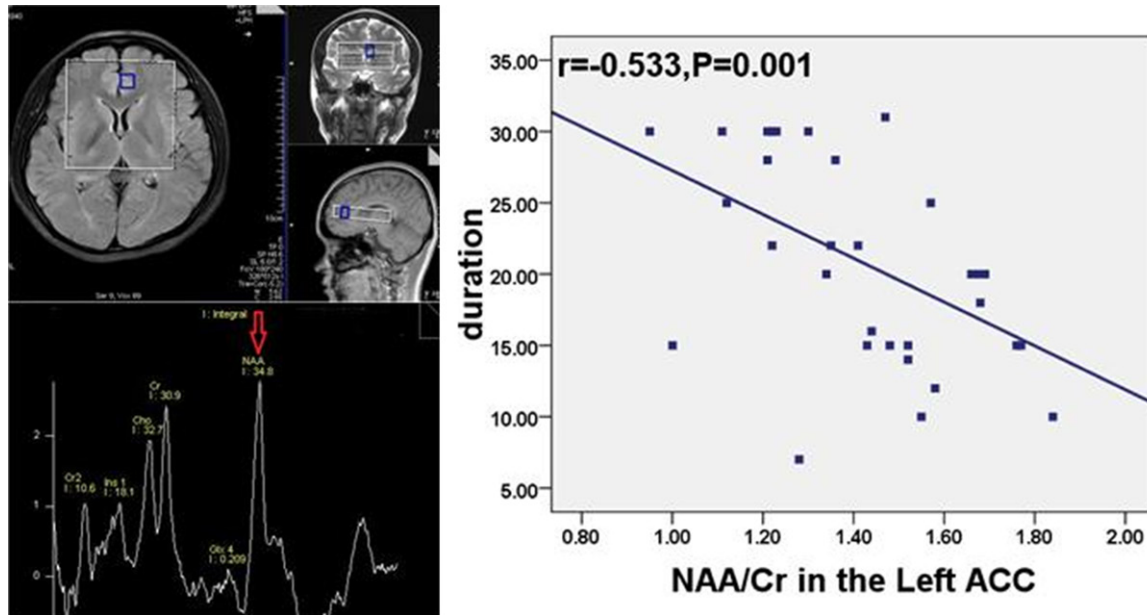
Figure 4. Scatter-plots of the correlation between duration and NAA/Cr in the left ACC. See text in the Results section for further explanations of the correlations.
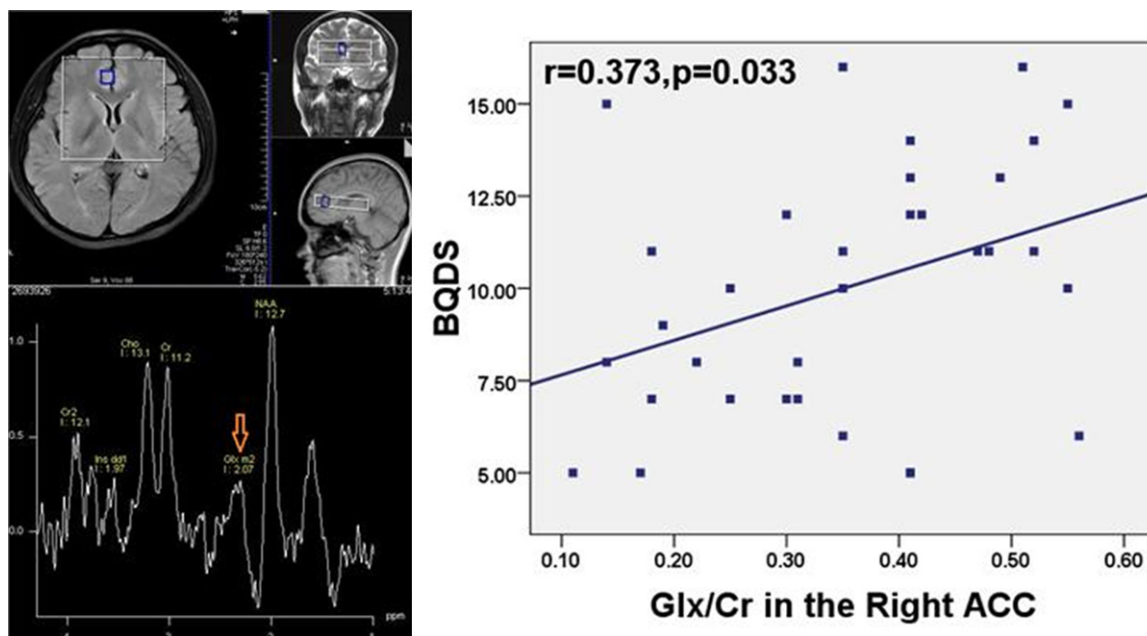

Figure 5. Scatter-plots of the correlation between BQDS and Glx/Cr in the right ACC. See text in the Results section for further explanations of the correlations.

(L: 0.74 ± 0.18 and R: 0.74 ± 0.21, respectively) (P < 0.05) (**Table 2**).

MI/Cr: MI/Cr ratios of the left anterior cingulate cortex (0.51 ± 0.12) in the BQD group were significantly higher than those of the control group (0.44 ± 0.12) (P < 0.05) (**Table 2**). MI/Cr ratios

of the right anterior cingulate cortex had no statistical differences between the two groups (0.45 ± 0.12 v.s 0.40 ± 0.11) (P > 0.05).

Glx/Cr: Glx/Cr ratios of bilateral anterior cingulate cortex (L: 0.33 ± 0.11 and R: 0.33 ± 0.14, respectively) in the BQD group were significant-

ly higher than those of the control group (L: 0.24 ± 0.10, and R: 0.25 ± 0.10, respectively) (P < 0.05) (**Table 2**).

Correlations between clinical characteristics and metabolites

As shown in **Figures 2**, **3**, in BQD individuals, NAA/Cr ratios of the right ACC negatively correlated with BQDS and BQD duration (r1 = -0.387, P1 = 0.026, Pearson's correlation analyses; r2 = -0.468, P2 = 0.006, Spearman's correlation analyses), NAA/Cr ratios of the left ACC showed a negative correlation with duration (r4 = -0.533, P4 = 0.001, Spearman's correlation analyses)(see **Figure 4**), Glx/Cr ratios of the right ACC showed a positive correlation with BQDS (r3 = 0.373, P3 = 0.033, Pearson's correlation analyses) (see **Figure 5**). We did not observe significant correlation in other metabolite ratios with BQDS and duration.

Discussion

As far as we know, this study was the first to investigate the local brain metabolic changes and focused on ACC in individuals with BQD using ^{1}H-MRS in vivo. The spectrum results were stable and reliable because the data collection area were located in the central region of brain, and with a short TE (TE = 30 ms) and 2D multi-voxel MRS method, mI and glutamic acid metabolites can better be measured. Cr has an equal distribution in the brain with grey matter contains higher concentrations [18]. The Cr peaks (at 3.03 and 3.94 ppm) are more accurately regarded as total Cr (tCr), and tCr levels usually maintain relatively stable, except in stroke, trauma, Cr deficiency syndromes and tumor [19]. As a result, Cr is often referred to as a putative internal standard and the other metabolites can be compared to it [20]. In the bilateral ACC, our study found NAA/Cr were lower in BQD individuals compared to the healthy controls, Cho/Cr and Glx/Cr were higher in individuals with BQD compared to the healthy group, but increase was noted for mI/Cr in BQD individuals only in the left ACC. These results indicate a neurotransmitter/metabolic dysregulation related to BQD and provide a feasible therapeutic intervention strategy designed to normalize the Glu transmission and function in treating BQD.

The non-specific reduction in NAA is the most frequently mentioned change in metabolite co-

ncentration. For instance, NAA was reduced to a similar extent in both the dorsal anterior cingulate [17] and frontal gray matter [16] of opiate-dependent individuals who were maintained on opioid replacement therapy. In contrast, when comparing smokers with nonsmokers, the null result was detected in the ACC [21]. Compared with glia, NAA is localized almost simply to neuronal tissue, thus rendering NAA a neuronal marker. Researchers have often interpreted NAA reductions as the representation of neuronal damage and/or loss [22-25], or markers for reduction in synaptic density, as well as dysregulated neuronal function and/or neurotransmission [26]. It's true that there has been hypothesis that NAA reductions indicate the cerebral hypoxic-ischemic events related to drug abuse more generally [16]. The reductions of NAA level in the dACC in BQD individuals are in accordance with a preclinical work indicating that a major alkaloid of the areca nut, a.k.a. arecoline, leads to neurotoxicity with enhanced oxidative stress and suppressed antioxidant protective system [27]. From the perspective that NAA serves as a marker for neuronal viability and/or synaptic density [26], the reductive outcome in the ACC provides evidence for our previous study showing reduced GM volume in the right rostral ACC within that cerebral region of BDQ individuals in comparison with controls [28].

The ratio of Cho/Cr in the ACC in BDQ group is significantly higher than that of the control group, suggesting that high intracellular Cho in ACC neurons may be the pathological phenomenon or compensatory response to BDQ. The cerebral distribution of Cho is regionally-dependent [18]; due to this, the resonance of Cho may differ a lot across disease states, especially when turnover of membrane has been referred to in the etiology [29, 30]. Therefore, the high Cho peak of the ACC may be related to nerve cell membrane phospholipid metabolism abnormalities and intracellular signal transduction abnormalities in the area.

The research results show that the mI/Cr ratios of the right ACC in BQD group are significantly higher than those of the control group. A previous study by Schweinsburg showed that levels of mI were elevated in the ACC in patients with alcoholism, especially during short-term temperance [13]. It has been indicated that mI is an osmoregulator [31] or that it contributes to glucose storage [32]. Most frequently however,

mI serves as a marker for glial content [33]. Therefore, the elevated mI level indicates a temporarily increased glial activation or, in a state of drug-induced osmotic stress, the attempt to regulate cell volume [13].

Except for these metabolic changes, alterations in glutamatergic neurotransmission have also been suggested in the BQD etiology. Researchers have developed a variety of methods to circumvent the complicated spectral patterns arising from their overlapping resonances [34], but the peak (at 2.4 ppm) is mainly due to resonances which arise from Glu and Gln and is regarded as "Glx". In accordance with chronic GABAA receptor abnormalities induced by alcohol and the following glutamatergic hyperactivity observed during withdrawal [35, 36], Glx and Glu/Cr were increased in the basal ganglia [37] and the anterior cingulate [15] of detoxified alcoholics, respectively, while GABA was decreased by about 30% with no other metabolic alterations within the occipital cortex [38]. It has been demonstrated that opiate dependence is related to a decrease in Glx within the dorsal anterior cingulate [17]. Although we still don't know whether the following neuronal excitability reduction is conducive to the processes accompanying and/or underling BQD, the Glx increase has the potential to be the representation of abnormalities in decision making and reward-based learning, or in the modulation of dopaminergic neurotransmission [17]. Gln, GABA, and Glu not only keep an ideal balance between cerebral inhibition and excitation, but they also work jointly in the regulation of neuronal energy metabolism [38]. These data jointly provide a novel strategy of therapeutic intervention designed to normalize Glu transmission and function during treating BQ addiction [39-41].

The increased levels of Glx in the ACC were also correlated with severity of BQD as measured by BQDS. Finally, the reduction in NAA in the ACC was shown to have correlation not only with severity of BQD, but also with longer BQ use duration. Together, these findings suggest that the duration of use may influence metabolite degrees and subsequent neuron damage, and varying severity of dependence from BQ use may impact reported metabolites values. But the more sophisticated metabolite-behavior relationships should been demonstrated in the future.

A few technical and methodological limitations should be taken into account when regarding the ^{1}H MRS findings as a whole. First, the study relied on self-report of retrospective BQ use. The fact that an individual cannot randomly infer a causative relationship between drug intake and hypothetic neurobiological consequences remains a problem. Secondly, although ^{1}H MRS has proven to be an amazing research tool, several limitations still exist in terms of the spectra quantification, acquisition, and interpretation. Finally, various statistical testing corrections were not performed because of the limitation of subject population.

Conclusions

In conclusion, our study indicated metabolic changes in the ACC of human BQD individuals with a modified MRS acquisition and analysis technique, providing past research a role for ACC area in the mediation of BQD and a mechanistic interpretation relating to past observations of decreased ACC grey matter in BQD individuals. These data jointly provide a novel strategy of therapeutic intervention designed to normalize Glu transmission and function during treating BQ addiction.

Acknowledgments

Address correspondence to: Chen Feng, Department of Radiology, People's Hospital of Hainan Province, Xiuhua Road 19, Xiuying District, Haikou 57-0311, China; E-mail: fenger0802@163.com; Suyue Pan, Department of Neurology, Nanfang Hospital, Southern Medical University, Guangzhou 510515, China; E-mail: pansuyue82@126.com

References

[1] Gupta PC, Warnakulasuriya S. Global epidemiology of areca nut usage. Addict Biol 2002; 7: 77-83.

[2] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Betel-quid and areca-nut chewing and some areca-nut derived nitrosamines. IARC Monogr Eval Carcinog Risks Hum 2004; 85: 1-334.

[3] Garg A, Chaturvedi P, Gupta PC. A review of the systemic adverse effects of areca nut or betel nut. Indian J Med Paediatr Oncol 2014; 35: 3-9.

[4] Chu NS. Neurological aspects of areca and betel chewing. Addict Biol 2002; 7: 111-114.

[5] Giri S, Idle JR, Chen C, Zabriskie TM, Krausz KW, Gonzalez FJ. A metabolomic approach to the metabolism of the areca nut alkaloids arecoline and arecaidine in the mouse. Chem Res Toxicol 2006; 19: 818-827.

[6] Lee CY, Chang CS, Shieh TY, Chang YY. Development and validation of a self-rating scale for betel quid chewers based on a male-prisoner population in Taiwan: the Betel Quid Dependence Scale. Drug Alcohol Depend 2012; 121: 18-22.

[7] Ghani WM, Razak IA, Yang YH, Talib NA, Ikeda N, Axell T, Gupta PC, Handa Y, Abdullah N, Zain RB. Factors affecting commencement and cessation of betel quid chewing behaviour in Malaysian adults. BMC Public Health 2011; 11: 82.

[8] Lee CH, Ko AM, Warnakulasuriya S, Ling TY, Sunarjo, Rajapakse PS, Zain RB, Ibrahim SO, Zhang SS, Wu HJ, Liu L, Kuntoro, Utomo B, Warusavithana SA, Razak IA, Abdullah N, Shrestha P, Shieh TY, Yen CF, Ko YC. Population burden of betel quid abuse and its relation to oral premalignant disorders in South, Southeast, and East Asia: an Asian Betel-quid Consortium Study. Am J Public Health 2012; 102: e17-e24.

[9] Licata SC, Renshaw PF. Neurochemistry of drug action: insights from proton magnetic resonance spectroscopic imaging and their relevance to addiction. Ann N Y Acad Sci 2010; 1187: 148-71.

[10] Gruber SA, Silveri MM, Yurgelun-Todd DA. Neuropsychological consequences of opiate use. Neuropsychol Rev 2007; 17: 299-315.

[11] Koob GF. The neurobiology of addiction: a neuroadaptational view relevant for diagnosis. Addiction 2006; 101: 23-30.

[12] Volkow ND, Wang GJ, Fowler JS, Tomasi D. Addiction circuitry in the human brain. Annu Rev Pharmacol Toxicol 2012; 52: 321-336.

[13] Schweinsburg BC, Taylor MJ, Videen JS, Alhassoon OM, Patterson TL, Grant I. Elevated myo-inositol in gray matter of recently detoxified but not long-term abstinent alcoholics: a preliminary MR spectroscopy study. Alcohol Clin Exp Res 2000; 24: 699-705.

[14] Bartsch AJ, Homola G, Biller A, Smith SM, Weijers HG, Wiesbeck GA, Jenkinson M, De Stefano N, Solymosi L, Bendszus M. Manifestations of early brain recovery associated with abstinence from alcoholism. Brain 2007; 130: 36-47.

[15] Lee E, Jang DP, Kim JJ, An SK, Park S, Kim IY, Kim SI, Yoon KJ, Namkoong K. Alteration of brain metabolites in young alcoholics without structural changes. Neuroreport 2007; 18: 1511-1514.

[16] Haselhorst R, Dürsteler-MacFarland KM, Scheffler K, Ladewig D, Müller-Spahn F, Stohler R, Seelig J, Seifritz E. Frontocortical N-acetylaspartate reduction associated with long-term i.v. heroin use. Neurology 2002; 58: 305-7.

[17] Yücel M, Lubman DI, Harrison BJ, Fornito A, Allen NB, Wellard RM, Roffel K, Clarke K, Wood SJ, Forman SD, Pantelis C. A combined spectroscopic and functional MRI investigation of the dorsal anterior cingulate region in opiate addiction. Mol Psychiatry 2007; 12: 611, 691-702.

[18] Pouwels PJ, Frahm J. Regional metabolite concentrations in human brain as determined by quantitative localized proton MRS. Magn Reson Med 1998; 39: 53-60.

[19] Alkan A, Sharifov R, Akkoyunlu ME, Kiliçarslan R, Toprak H, Aralasmak A, Kart L. MR spectroscopy features of brain in patients with mild and severe obstructive sleep apnea syndrome. Clin Imaging 2013; 37: 989-992.

[20] Cecil KM, Jones BV. Magnetic resonance spectroscopy of the pediatric brain. Top Magn Reson Imaging 2001; 12: 435-452.

[21] Gallinat J, Lang UE, Jacobsen LK, Bajbouj M, Kalus P, von Haebler D, Seifert F, Schubert F. Abnormal hippocampal neurochemistry in smokers: evidence from proton magnetic resonance spectroscopy at 3 T. J Clin Psychopharmacol 2007; 27: 80-84.

[22] Simone IL, Tortorella C, Federico F. The contribution of (1)H-magnetic resonance spectroscopy in defining the pathophysiology of multiple sclerosis. Ital J Neurol Sci 1999; 20: S241-S245.

[23] Chen JG, Charles HC, Barboriak DP, Doraiswamy PM. Magnetic resonance spectroscopy in Alzheimer's disease: focus on N-acetylaspartate. Acta Neurol Scand Suppl 2000; 176: 20-26.

[24] Brooks WM, Friedman SD, Gasparovic C. Magnetic resonance spectroscopy in traumatic brain injury. J Head Trauma Rehabil 2001; 16: 149-164.

[25] Demougeot C, Marie C, Giroud M, Beley A. N-acetylaspartate: a literature review of animal

research on brain ischaemia. J Neurochem 2004; 90: 776-783.

[26] Li SJ, Wang Y, Pankiewicz J, Stein EA. Neurochemical adaptation to cocaine abuse: reduction of N-acetyl aspartate in thalamus of human cocaine abusers. Biol Psychiatry 1999; 45: 1481-1487.

[27] Shih YT, Chen PS, Wu CH, Tseng YT, Wu YC, Lo YC. Arecoline, a major alkaloid of the areca nut, causes neurotoxicity through enhancement of oxidative stress and suppression of the antioxidant protective system. Free Radic Biol Med 2010; 49: 1471-1479.

[28] Chen F, Zhong Y, Zhang Z, Xu Q, Liu T, Pan M, Li J, Lu G. Gray matter abnormalities associated with betel quid dependence: a voxel-based morphometry study. Am J Transl Res 2015; 7: 364-374.

[29] Miller BL. A review of chemical issues in 1H NMR spectroscopy: N-acetyl-L-aspartate, creatine and choline. NMR Biomed 1991; 4: 47-52.

[30] Gonzalez-Toledo E, Kelley RE, Minagar A. Role of magnetic resonance spectroscopy in diagnosis and management of multiple sclerosis. Neurol Res 2006; 28: 280-283.

[31] Nakanishi T, Turner RJ, Burg MB. Osmoregulatory changes in myo-inositol transport by renal cells. Proc Natl Acad Sci U S A 1989; 86: 6002-6006.

[32] Ross BD. Biochemical considerations in 1H spectroscopy. Glutamate and glutamine; myo-inositol and related metabolites. NMR Biomed 1991; 4: 59-63.

[33] Brand A, Richter-Landsberg C, Leibfritz D. Multinuclear NMR studies on the energy metabolism of glial and neuronal cells. Dev Neurosci 1993; 15: 289-298.

[34] Mullins PG, Chen H, Xu J, Caprihan A, Gasparovic C. Comparative reliability of proton spectroscopy techniques designed to improve detection of J-coupled metabolites. Magn Reson Med 2008; 60: 964-969.

[35] Hoffman PL, Tabakoff B. Alcohol dependence: a commentary on mechanisms. Alcohol Alcohol 1996; 31: 333-340.

[36] Tsai GE, Ragan P, Chang R, Chen S, Linnoila VM, Coyle JT. Increased glutamatergic neurotransmission and oxidative stress after alcohol withdrawal. Am J Psychiatry 1998; 155: 726-732.

[37] Miese F, Kircheis G, Wittsack HJ, Wenserski F, Hemker J, Mödder U, Häussinger D, Cohnen M. 1H-MR spectroscopy, magnetization transfer, and diffusion-weighted imaging in alcoholic and nonalcoholic patients with cirrhosis with hepatic encephalopathy. AJNR Am J Neuroradiol 2006; 27: 1019-1026.

[38] Behar KL, Rothman DL, Petersen KF, Hooten M, Delaney R, Petroff OA, Shulman GI, Navarro V, Petrakis IL, Charney DS, Krystal JH. Preliminary evidence of low cortical GABA levels in localized 1H-MR spectra of alcohol-dependent and hepatic encephalopathy patients. Am J Psychiatry 1999; 156: 952-954.

[39] Baker DA, McFarland K, Lake RW, Shen H, Tang XC, Toda S, Kalivas PW. Neuroadaptations in cystine-glutamate exchange underlie cocaine relapse. Nat Neurosci 2003; 6: 743-749.

[40] Kalivas PW. Neurobiology of cocaine addiction: implications for new pharmacotherapy. Am J Addict 2007; 16: 71-78.

[41] Mardikian PN, LaRowe SD, Hedden S, Kalivas PW, Malcolm RJ. An open-label trial of N-acetylcysteine for the treatment of cocaine dependence: a pilot study. Prog Neuropsychopharmacol Biol Psychiatry 2007; 31: 389-394.