Altered Functional Connectivity of the Amygdala and Sex Differences in Functional Dyspepsia

Fang Zeng, MD, PhD[1], Ruirui Sun, MD, PhD[1], Zhaoxuan He, MD[1], Yuan Chen, MD, PhD[1], Du Lei, MD, PhD[2], Tao Yin, MD[1], Xiaoyan Liu, MD[1], Yue Yang, MD[3], Peihong Ma, MD[1], Yuzhu Qu, MD[1], Danhua Zhang, MD[4], Courtney Lang, BA[5], Joel Park, BA[5], Jin Lu, MD[1], Lei Lan, MD, PhD[1], Zhengjie Li, MD,PhD[1], Qiyong Gong, PhD[2], Fanrong Liang, MD, MS[1] and Jian Kong, MD, MS, MPH[5,6]

INTRODUCTION: **The influence of sex on the prevalence and clinical manifestations of functional dyspepsia (FD) has recently been a topic of increasing interest. However, brain MRI pathology based on sexual dimorphism in FD has not yet been investigated. The amygdala, which plays a vital role in processing gastrointestinal signals, may be associated with the sex-related pathophysiology of FD.**

METHODS: **We investigated the resting-state functional connectivity (rsFC) of amygdala subregions in patients with FD and healthy subjects as well as the sex differences between male and female FD patients.**

RESULTS: **The results showed that FD patients manifested altered rsFC in the basolateral amygdala (BLA) and centromedial amygdala subregions compared with HS and that female FD patients showed increased BLA rsFC with the insula (INS) and decreased BLA rsFC with the medial prefrontal cortex and dorsal lateral prefrontal cortex compared with male FD patients and female HS.**

DISCUSSION: **Our findings suggest that FD females tend to have more severe dysfunction of cognitive-affective processing among the brain regions associated with the salience network, central executive network, and default mode network.**

SUPPLEMENTARY MATERIAL accompanies this paper at http://links.lww.com/CTG/A45

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INTRODUCTION

Functional dyspepsia (FD), one of the most common functional gastrointestinal disorders (FGIDs), is defined as the presence of early satiation, postprandial fullness, epigastric pain, or epigastric burning in the absence of an organic, systemic, or metabolic disease that could explain the symptoms according to the Rome III consensus (1). With a high prevalence rate ranging from 8% to 40% (2–4), FD has significant influence on quality of life of the patient and high medical cost (5). It has long been noticed that women have a higher prevalence than men (6) and tend to have more delayed gastric emptying (7), higher anxiety and depression symptoms (8), and lower tolerance of somatic pain (9). Besides symptom presentation, accumulating evidence has also suggested that sex differences of patients with FD exist in pathophysiologic mechanisms and treatment response (10–12). For instance, female patients with FD have increased bacterial load, lower mRNA expressions of nerve growth factor, and transient receptor potential vanilloid receptor 1 (8) compared with male patients. Hence, exploring the relationships between sex-related pathophysiological differences and clinical manifestations will help us better understand the sex-specific disease and facilitate development of sex-specific diagnoses and treatments.

In the past decades, neuroimaging techniques have been widely used to explore the central pathogenesis of FGIDs in cerebral activity based on the theory of brain-gut interaction. Present studies have identified significant functional and anatomical alterations in multiple brain regions, which are involved in the default mode network (DMN), salience network (SN), etc. in patients with FD (13–19). Among these altered brain regions, the amygdala is believed to be one of the key regions in the central pathogenesis of FD (15–17,20) because of its wide involvement in processing interoceptive signals of fullness and satiety (21), food intake (22), endogenous pain inhibition (23),

[1]Brain Research Center, Acupuncture and Tuina School/The 3rd Teaching Hospital, Chengdu University of Traditional Chinese Medicine, Chengdu, Sichuan, China; [2]Departments of Radiology, Huaxi MR Research Center (HMRRC), West China Hospital of Sichuan University, Chengdu, Sichuan, China; [3]Department of Radiology, Sichuan Academy of Medical Sciences and Sichuan Provincial People's Hospital, Chengdu, Sichuan, China; [4]Department of Neurological Rehabilitation of the 181st Chinese People's Liberation Army Hospital, Guilin, Guangxi, China; [5]Department of Psychiatry, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts, USA; [6]Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Charlestown, Massachusetts, USA.
Correspondence: Fanrong Liang, MD. E-mail: acuresearch@126.com. Jian Kong, MD. E-mail: JKONG2@mgh.harvard.edu.
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and emotion expression and regulation (24,25). In addition, the role of the amygdala in sex-related difference in cerebral activity has also been identified in brain imaging studies performed on healthy subjects (HS) (23,32,33), patients with irritable bowel syndrome (IBS) (30,31), and patients with chronic visceral pain (23). However, whether the amygdala is involved in the sex-related difference in the central nervous system in processing gastrointestinal (GI) signals of patients with FD is still uncertain.

As a complex brain structure, the amygdala consists of several subregions, each with different functions (26–29). The basolateral subregion is a major sensory input region of the amygdala, whereas the centromedial subregion relates to visceral responses and provides much of the descending output of the amygdala (27–29). With the aid of resting-state functional connectivity (rsFC) analysis, this study aimed at (i) investigating abnormalities in the rsFC of amygdala subregions in patients with FD compared with HS; (ii) analyzing whether the alterations of the rsFC of amygdala subregions have sex-related differences in patients with FD; and (iii) exploring the possible associations between sex differences in the rsFC of amygdala subregions, dyspepsia symptoms, and fasting plasma ghrelin concentrations so as to deepen our understanding of FD's pathogenesis and provide a new approach for treatment development.

METHODS

Participants

Patients with FD. A total of 100 patients diagnosed with FD were recruited from the First Teaching Hospital of Chengdu University of Traditional Chinese Medicine and Chengdu University of Traditional Chinese Medicine from March 2014 through December 2017.

After being evaluated by 2 gastroenterologists and undergoing physical and laboratory examinations, patients with FD were enrolled if they fulfilled the following inclusion criteria: (i) matched the Rome III criteria for FD; (ii) were right handed and aged 18–45 years; (iii) did not take any gastroenteric dynamic drugs in the 2 weeks before enrollment; and (iv) were not taking part in any other clinical trials. Patients were excluded if they (i) were pregnant or lactating; (ii) had a history of psychiatric and neurological disorders or head trauma with loss of consciousness; (iii) had organic diseases; or (iv) had any contraindications to fMRI scanning, such as a cardiac pacemaker, defibrillator, metal stent or electronic implant, intraocular metal foreign body, claustrophobia, and hyperpyrexia.

Healthy subjects. One hundred HS with no history of GI, neurological, or psychiatric disorders were recruited. They underwent a basic evaluation including a review of medical history, a physical examination, electrocardiogram, upper abdominal ultrasound, and GI endoscopy.

Symptom measurement

The Nepean Dyspepsia Symptom Index (NDSI), a dyspepsia-specific index for measuring the frequency, intensity, and level of discomfort of 15 upper GI symptoms as well as quality of life over the prior 14 days (30), was used to evaluate dyspepsia symptoms of the patients with FD. In addition, the Zung Self-Rating Anxiety Scale (SAS) (31) and Zung Self-Rating Depression Scale (SDS) (32) were applied to evaluate the psychological condition of all participants.

Ghrelin concentration measurement

In this study, half of the patients with FD were randomly selected to have their plasma ghrelin concentrations measured. Ghrelin, an important brain-gut peptide, has been found to participate in the pathology of FD (11). Because ghrelin levels can be altered by food intake, blood samples were obtained in the morning after an overnight fast ≥10 hours and then were immediately transferred to chilled polypropylene tubes containing ethylenediaminetetraacetic acid disodium salt (Na_2EDTA) and aprotinin. The samples were centrifuged at 2000*g* for 20 minutes at 4 °C, and the plasma was stored at −80 °C for further measurement. Ghrelin concentrations were detected using enzyme-linked immunosorbent assay kits (Human Ghrelin EIA Kit, Catalog No. EIA-GHR-1; RayBiotech, Norcross, GA) according to the manufacturer's instructions. Intraassay and interassay coefficients of variation reported by the manufacturer were <10% and 15%, respectively.

fMRI scan

All participants underwent MRI scans in a 3.0T magnetic resonance scanner (Siemens, Munich, Germany) at Huaxi Magnetic Resonance Research Center, West China Hospital of Sichuan University, Chengdu, China. During the MRI scans, each participant was asked to keep their eyes closed and underwent a high-resolution 3-dimensional T1-weighted and blood oxygenation level-dependent (BOLD)-fMRI sequence. Three-dimensional anatomical image parameters were as follows: repetition time/echo time = 1,900 ms/2.26 ms, slices = 176; matrix size = 256 × 256, field of view = 256 × 256 mm^2, slice thickness = 1 mm. The BOLD images were acquired using the following parameters: repetition time/echo time = 2,000 ms/30 ms, flip angle = 90°, slices = 30; matrix size = 64 × 64 mm^2, field of view = 240 × 240, slice thickness = 5 mm, total volume = 180, and the functional scan lasted 360 seconds.

For female participants, scanning took place during the week after a menstrual period to avoid the influence of possible symptoms such as dysmenorrhea, breast distention, and premenstrual tension on brain activity.

Statistics

fMRI data.

Data preprocessing. The fMRI data were preprocessed and analyzed using statistical parametric mapping (SPM12; Wellcome Department of Imaging Neuroscience, London, UK; http://www.fil.ion.ucl.ac.uk/spm/) and the Functional Connectivity Toolbox (http://www.nitrc.org/projects/conn) in MATLAB 8.6 (MathWorks, Natick, MA). The basic preprocessing steps included discarding the first 10 time points, slice-timing correction, head motion estimation, realignment, smoothing with a Gaussian kernel of 6 mm^3 full-width at half maximum, and band-pass filtering (0.009–0.08 Hz). The Artifact Detection Tools (http://www.nitrc.org/projects/artifact_detect) was used to detect outlier head motion parameters and artifacts. For each scan, time points were taken as outliers if the global mean intensity exceeded 3 SDs from the mean or if scan-to-scan motion

deviation was greater than 0.5 mm. The motion parameters and outliers were included as regressors in the first-level general linear model (33).

The data of 6 participants (3 FD males and 3 HS males) were excluded because of defects in functional images and significant head motion. Hence, 97 patients with FD (70 females + 27 males) and 97 HS (70 females + 27 males) were included in the final data analysis.

Regions of interest. The amygdala subregions were divided based on the stereotaxic, probabilistic maps of cytoarchitectonic boundaries developed by Amunts et al. (34). The regions of interest (ROIs) in the amygdala subregions were extracted based on the FMRIB Software Library's Juelich histological atlas. To provide the maximum probabilistic map of each amygdala subregion (35), we created the ROIs in standard space, including voxels with a probability of at least 45% of belonging to each subdivision, including (i) the basolateral subdivision (left: 196 mm^3, right: 218 mm^3); (ii) the centromedial subdivision (left: 56 mm^3, right: 47 mm^3); and (iii) the superficial subdivision (left: 111 mm^3, right: 113 mm^3).

Seed-based functional connectivity. After preprocessing, the average BOLD time series from each amygdala ROI was extracted, and Pearson correlation coefficients were computed between that time course and the time courses of all other voxels in each subject. Then, the correlation coefficients were converted to normally distributed Z-scores using the Fisher transformation to allow for second-level analysis.

For each amygdala subregion rsFC, the analysis of covariance (ANCOVA) test was used to test the interaction between FD and sex so as to determine the differences in sex associated with disease (FD [female − male] − HS [female − male]) and the differences in disease related to sex (female [HS − FD] − male [HS − FD]). If there was interaction (both disease and sex influence rsFC) then 4 *a priori* contrasts would be tested: female FD vs male FD, female HS vs male HS, female FD vs female HS, and male FD vs male HS. Age, SAS, and SDS were included in the above analyses as covariates. A threshold of voxel-wise $P < 0.001$ uncorrected and familywise error (FWE)-corrected $P < 0.05$ at the cluster level were applied in the group rsFC analysis.

Clinical data analysis

Differences in demographic and clinical variables were analyzed using 2 × 2 mixed analysis of variance (ANOVA) in SPSS version 22. Regression analysis was performed to investigate the association between the Fisher transformed z value extracted from the BLA rsFC of 35 FD females and their NDSI scores and ghrelin concentrations. Age, SAS, and SDS were included in the analysis as covariates of noninterest.

RESULTS

Demographic and clinical characteristics

There were significant differences in the SAS score and SDS score between the patients with FD and HS, the FD females and HS females, and the FD males and HS males ($P < 0.05$). None reported a history of abuse during childhood (Table 1; see Table 1, Supplementary Digital Content 1, http://links.lww.com/CTG/A45).

Table 1. Baseline characteristics

	Patients with FD		HS		Group effect			Sex effect			Disease effect			
	Females (n = 70) Mean ± SD	Males (n = 27) Mean ± SD	Females (n = 70) Mean ± SD	Males (n = 27) Mean ± SD	Patients with FD vs HS		(FD + HS) females vs males	FD females vs FD males		HS females vs HS males	FD females vs HS females		FD males vs HS males	
					F^A	*P*	F	T^B	*P*	T	T	*P*	T	*P*
Age (y)	21.96 ± 2.20	22.89 ± 1.80	22.21 ± 1.52	22.33 ± 1.98	0.243	0.623	10.757	0.084	1.962	0.053	0.317	0.752	−0.805	0.422	1.077	0.286
BMI (kg/m^2)	19.78 ± 2.61	20.10 ± 2.22	19.44 ± 2.05	20.57 ± 1.62	0.036	0.849	4.102	0.044*	−0.572	0.569	−2.562	0.012*	0.843	0.400	0.054	0.380
Education (y)	15.34 ± 2.26	15.78 ± 1.95	15.76 ± 1.89	15.74 ± 1.75	0.338	0.561	0.416	0.519	0.941	0.351	−0.041	0.968	−1.175	0.242	0.074	0.942
College students (N)	65/70	23/27	64/70	26/27	—	—	—	—	—	—	—	—	—	—	—	—
NDSI scores	43.70 ± 13.82	47.63 ± 15.17	—	—	—	—	—	1.222	0.225	—	—	—	—	—	—	—
Duration (mo)	37.77 ± 26.55	39.15 ± 27.48	—	—	—	—	—	−0.227	0.821	—	—	—	—	—	—	—
SAS scores	43.36 ± 7.82	38.98 ± 7.69	34.94 ± 5.30	35.95 ± 5.07	29.044	0.000**	2.517	0.114	2.482	0.015*	−0.848	0.399	7.458	0.000**	1.712	0.094
SDS scores	43.84 ± 8.56	41.99 ± 12.17	34.71 ± 4.64	36.60 ± 5.95	34.540	0.000**	0.000	0.988	0.724	0.474	−1.654	0.102	7.846	0.000**	2.070	0.045*

A, ANOVA test; B, 2-sample *t* test; BMI, body mass index; Groups: FD, functional dyspepsia; HS, healthy subjects; N, number; NDSI, Nepean Dyspepsia Symptom Index; SAS, the Zung Self-Rating Anxiety Scale; SDS, the Zung Self-Rating Depression Scale.
* $P < 0.05$; ** $P < 0.001$.

Abnormal rsFC of amygdala subregions in patients with FD compared with HS

Abnormal BLA rsFC. Compared with HS, patients with FD showed significantly increased BLA rsFC with the bilateral supplementary motor cortex (SMC)/middle cingulate cortex, central/parietal operculum, left precentral gyrus (PrG), postcentral gyrus (PoG), supramarginal gyrus (SMG), right anterior insula (INS), planum temporale (PT), and superior temporal gyrus (STG). Patients also showed significantly decreased BLA rsFC with the bilateral cerebellum, brain stem, left medial prefrontal cortex (mPFC [prefrontal cortex]), posterior cingulate cortex (PCC), dorsal lateral prefrontal cortex (dlPFC), angular gyrus, middle temporal gyrus (MTG), and right frontal pole (Figure 1; see Table 2, Supplementary Digital Content 1, http://links.lww.com/CTG/A45).

Abnormal centromedial amygdala rsFC. Compared with HS, patients with FD showed significantly increased centromedial amygdala (CMA) rsFC with the left dlPFC, angular gyrus, and SMG and significantly decreased CMA rsFC with the bilateral occipital fusiform gyrus, PrG/PoG, SMC, lingual gyrus, right precuneus/cuneus, and anterior INS (Figure 1; see Table 2, Supplementary Digital Content 1, http://links.lww.com/CTG/A45).

Abnormal superficial amygdala rsFC. No significant differences in superficial amygdala (SFA) rsFC were found between patients with FD and HS.

Sex differences in the rsFC of amygdala subregions in patients with FD

Sex differences in BLA rsFC. Significant interaction was found when examining the differences between males and females related to disease (FD [F-M] − HS [F-M]). It showed increased BLA rsFC with the right STG, temporal pole, posterior INS, and planum polare and decreased BLA rsFC with the bilateral mPFC and right dlPFC.

Compared with male patients with FD, female patients with FD showed increased BLA rsFC with the left STG/temporal parietal junction, MTG, and posterior and anterior

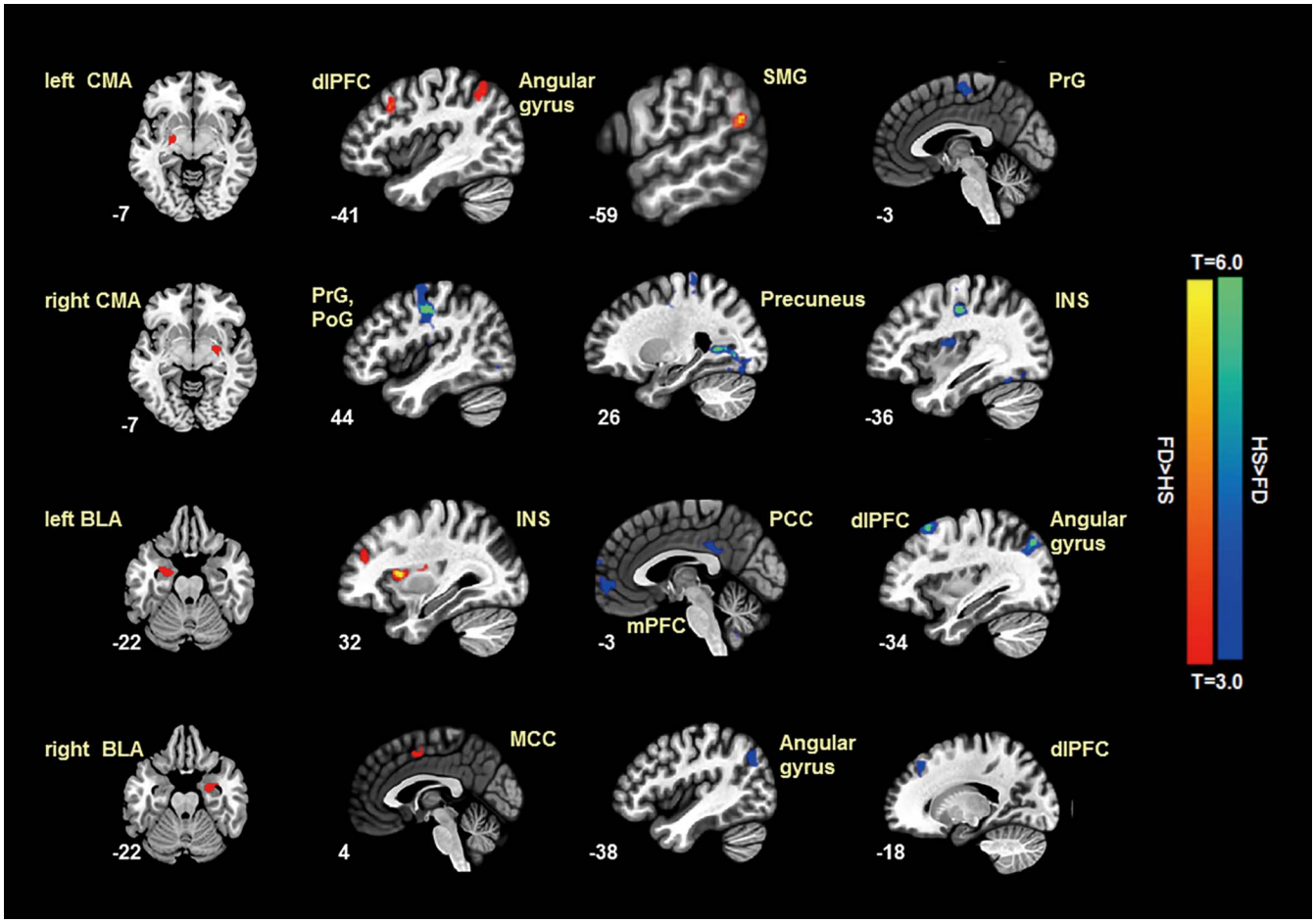

Figure 1. Abnormal rsFC between amygdala subregions and the whole brain in 97 patients with FD compared with 97 HS. Compared with HS, patients with FD showed (**a**) significantly increased left CMA rsFC with left dlPFC, angular gyrus, and SMG and significantly decreased left CMA rsFC with left PrG; (**b**) significantly decreased right CMA rsFC with right PrG/PoG, right precuneus, and INS; (**c**) significantly increased left BLA rsFC with right INS and significantly decreased left BLA rsFC with left mPFC, PCC, dlPFC, and angular gyrus; (**d**) significantly increased right BLA rsFC with right MCC and significantly decreased rsFC with left angular gyrus and dlPFC. Participants: FD, functional dyspepsia; HS, healthy subjects. Seed: BLA, basolateral amygdala; CMA, centromedial amygdala. Brain regions: dlPFC, dorsal lateral prefrontal cortex; SMG, supramarginal gyrus; PrG, precentral gyrus; PoG, postcentral gyrus; INS, insular cortex; mPFC, medial prefrontal cortex; PCC, posterior cingulate cortex; MCC, middle cingulate cortex. Cluster level, FWE $P < 0.05$; voxel level, $P < 0.001$; cluster size >20 voxels; with age, SAS, and SDS as covariates. FWE, Familywise error (FWE); PFC, prefrontal cortex; rsFC, resting-state functional connectivity; SAS, Self-Rating Anxiety Scale; SDS, Self-Rating Depression Scale.

Table 2. Sex differences of functional connectivity between amygdala subregions and the whole brain in patients with FD

Contrast	ROI/seed	Cluster size	rsFC regions	L/R	BA	MNI			*F*
						x	y	z	
[(Female FD − male FD) > (female HS − male HS)]/[(female FD − female HS) > (male FD − male HS)]	Left BLA	144	PP/STG/TP	R	21	48	−2	−12	4.53
		139	Posterior INS	R	13	40	−22	2	4.33
	Right CMA	285	Cerebellum	R		20	−50	−22	4.63
	Left SFA	159	STG/S2	R	40	66	−28	10	4.44
[(Male FD − female FD) > (male HS − female HS)]/[(male FD − male HS) > (female FD − female HS)]	Left BLA	184	dlPFC	R		16	48	42	3.97
	Right BLA	251	mPFC	Bilateral	10	4	50	−6	4.59
	Right SFA	486	SPL/angular gyrus	R	40	32	−62	58	5.49

The ANCOVA test was used. Cluster level, FWE $P < 0.05$; voxel level, $P < 0.001$; cluster size >20 voxels; with age, SAS, and SDS as covariates.
[(Female FD − male FD) > (female HS − male HS)]/[(male FD − female FD) > (male HS − female HS)]: significant differences between males and females related to disease.
[(Female FD − female HS) > (male FD − male HS)]/[(male FD − male HS) > (female FD − female HS)]: significant differences between FD and HS related to sex.
ANCOVA, analysis of covariance; BLA, basolateral amygdala; CMA, centromedial amygdala; dlPFC, dorsal lateral prefrontal cortex; FD, functional dyspepsia; FWE, Familywise error (FWE); HS, healthy subjects; INS, insular cortex; mPFC, medial prefrontal cortex; PFC, prefrontal cortex; PP, planum polare; ROI, regions of interest; rsFC, resting-state functional connectivity; SFA, superficial amygdala; SPL, superior parietal lobule; STG, superior temporal gyrus; TP, temporal pole.

INS and decreased BLA rsFC with the bilateral mPFC and right dlPFC.

Compared with female HS, female patients with FD showed increased BLA rsFC with the bilateral superior frontal gyrus, INS, SMC, right central/frontal operculum, STG, PT, SMG, and middle frontal gyrus and decreased BLA rsFC with the bilateral dlPFC, angular gyrus, cerebellum, left MTG, mPFC, PCC/precuneus, right middle frontal cortex, frontal pole, and brain stem.

No significant difference was found in male FD vs male HS and in female HS vs male HS (Table 2 and Figure 2; see Tables 3 and 4, Supplementary Digital Content 1, http://links.lww.com/CTG/A45).

Sex differences in CMA rsFC. Significant interaction was found in the interaction contrasts (FD [F-M] − HS [F-M]), which showed increased CMA rsFC with the right cerebellum.

Compared with male patients with FD, female patients with FD showed increased CMA rsFC with the left putamen.

Compared with female HS, female patients with FD showed decreased CMA rsFC with the bilateral medial PrG, right SMC/PoG, superior frontal gyrus, occipital fusiform gyrus, and lingual gyrus.

Compared with male HS, male patients with FD showed decreased CMA rsFC with the bilateral PrG/PoG, left SMC, middle cingulate cortex, right cerebellum, anterior INS, putamen, and central operculum.

Compared with male HS, female HS showed decreased CMA rsFC with the right cerebellum (Table 2, Figure 2; see Tables 3 and 4, Supplementary Digital Content 1, http://links.lww.com/CTG/A45).

Sex differences in SFA rsFC. Significant interaction was found in the interaction contrasts (FD [F − M] − HS [F − M]), which showed increased SFA rsFC with the right STG and SMG and decreased rsFC with the right superior parietal lobule (SPL) and angular gyrus.

Compared with male patients with FD, female patients with FD showed increased SFA rsFC with the bilateral putamen and left caudate.

Compared with male HS, female HS showed increased SFA rsFC with the right SPL, accumbens area, posterior orbital gyrus, and putamen and decreased SFA rsFC with the right PT and STG.

Compared with female HS, female patients with FD showed decreased SFA rsFC with the right SPL.

Compared with male HS, male patients with FD showed increased SFA rsFC with the right SPL and angular gyrus and decreased SFA rsFC with the bilateral PrG/PoG (Table 2, Figure 2; see Tables 3 and 4, Supplementary Digital Content 1, http://links.lww.com/CTG/A45).

Correlations of altered BLA rsFC with fasting plasma ghrelin concentrations and NDSI scores in 35 FD females

Because of the significant differences found in female patients with FD compared with both male patients with FD and female HS and because ghrelin concentration was measured in only half of the female patients with FD, we performed a correlation analysis between the z values of BLA-INS, BLA-dlPFC, and BLA-mPFC rsFC in the interaction contrast and NDSI scores and ghrelin concentrations of 35 female patients with FD.

The results showed that (i) NDSI scores were negatively correlated with left BLA-right INS rsFC ($r = -0.348$, false discovery rate (FDR)-corrected $P = 0.041$), whereas ghrelin concentrations were positively correlated ($r = 0.394$, FDR-corrected $P = 0.033$); (ii) NDSI scores were positively correlated with left BLA-right dlPFC rsFC ($r = 0.361$, FDR-corrected $P = 0.041$), whereas ghrelin concentrations were negatively correlated ($r = -0.376$, FDR-corrected $P = 0.033$); and (iii) NDSI scores were negatively correlated with right BLA-bilateral mPFC rsFC ($r = -0.367$, FDR-corrected $P = 0.041$), whereas ghrelin concentrations were

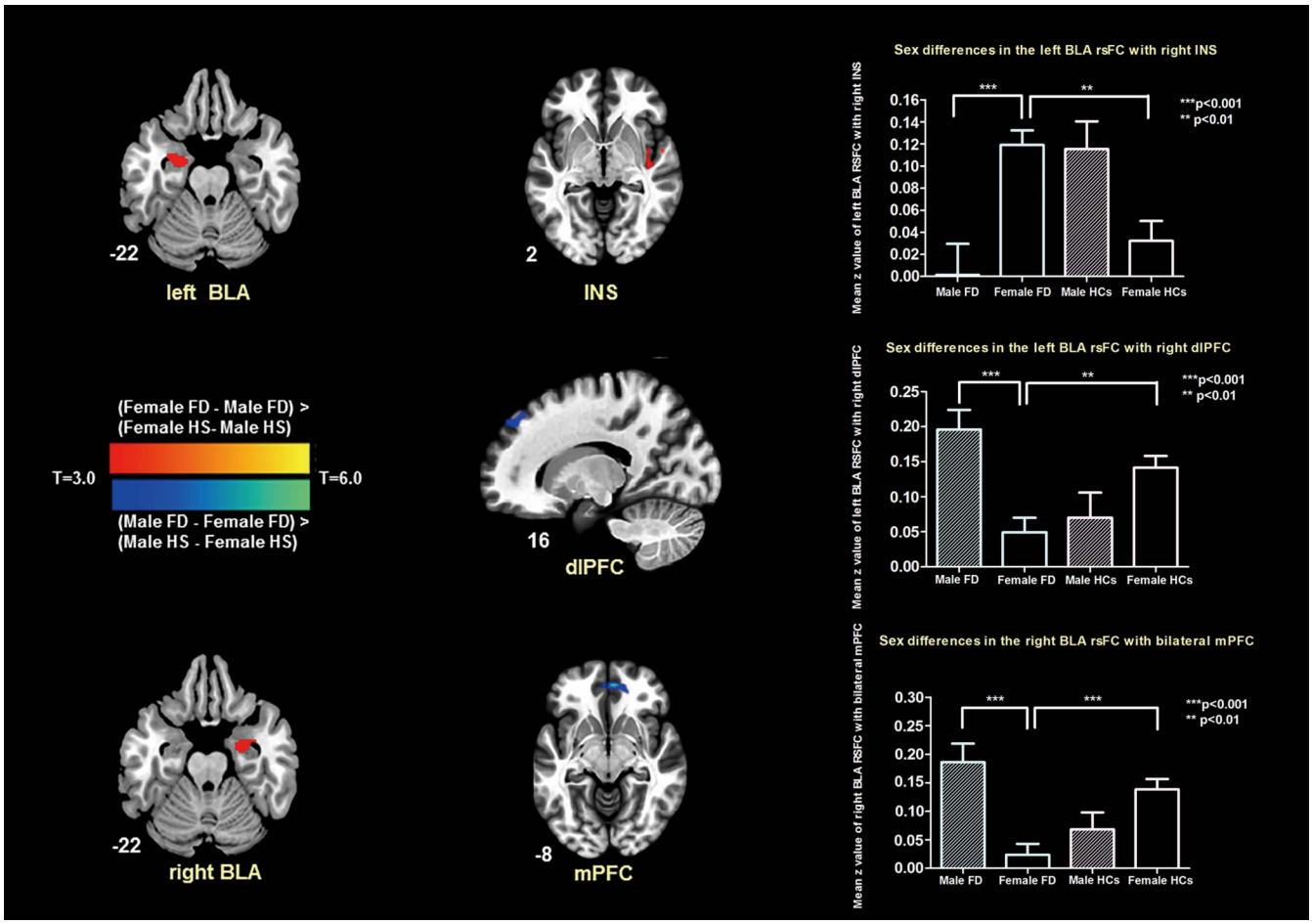

Figure 2. Sex differences in the rsFC between amygdala subregions and the whole brain in patients with FD. Interaction contrasts examining the differences between males and females related to disease (FD [female-male] − HS [female-male]) showed increased left BLA rsFC with right INS, decreased left BLA rsFC with right dlPFC, and decreased right BLA rsFC with bilateral mPFC. Participants: FD, functional dyspepsia; HS, healthy subjects. Interaction of sex × disease to compare sex differences in FD vs HS: ([female FD − male FD] − [female HS − male HS])/([female FD − female HS] − [male FD − male HS]) or ([male FD − female FD] − [male HS − female HS])/([female HS − female FD] − [male HS − male FD]). Seeds: BLA, basolateral amygdala. Brain regions: INS, insular cortex; mPFC, medial prefrontal cortex; dlPFC, dorsal lateral prefrontal cortex. The ANCOVA test was used. Cluster level, FWE $P < 0.05$; voxel level, $P < 0.001$; cluster size >20 voxels; with age, SAS, and SDS as covariates. $^{**}P < 0.01$; $^{***}P < 0.001$. ANCOVA, Analysis of Covariance; FWE, Familywise error (FWE); PFC, prefrontal cortex; rsFC, resting-state functional connectivity; SAS, Self-Rating Anxiety Scale; SDS, Self-Rating Depression Scale.

positively correlated ($r = 0.361$, FDR-corrected $P = 0.033$) (Figure 3).

DISCUSSION

The current study has demonstrated the abnormal rsFC of amygdala subregions in patients with FD and found more significant abnormalities in FD females for the first time. The results suggest not only functional abnormalities existing within the SN but also the imbalance of multiple brain networks in FD. This study provides a new approach for exploring sex differences in FGIDs.

The altered amygdala rsFC and potential imbalance of multiple networks in patients with FD

The abnormal amygdala rsFC in patients with FD. The amygdala not only influences multiple cortical regions (e.g., PFC and INS) to process chronic visceral pain/discomfort within the "pain matrix" but also participates in the descending pain inhibitory pathway that modulates GI function (36). Both positron emission tomography-computed tomography and fMRI studies have found abnormal activity of the amygdala in patients with FD (13,37). In this study, the amygdala showed altered rsFC mainly in its subregions BLA and CMA. The BLA, CMA, and SFA are 3 main subregions of the amygdala, which are involved in a variety of different functions. The CMA is regarded as the visceral and somatomotor output of the amygdala (28), whereas the BLA and SFA are regarded as the sensory input. Specifically, the SFA mainly receives olfactory sensation from higher-order olfactory cortical areas, and the BLA receives nonolfactory sensations. This may be the reason why the CMA and BLA, but not the SFA, showed significantly abnormal rsFC with the fronto-temporo-insular cortical areas in patients with FD compared with HS in this study. Furthermore, many studies have demonstrated that the BLA and CMA serve as the main amygdala subregions involved in processing food intake and visceral sensation (21,22,38,39). Thus, the altered BLA and CMA rsFC in this study may suggest abnormal visceral sensory input and output connections of the amygdala in FD.

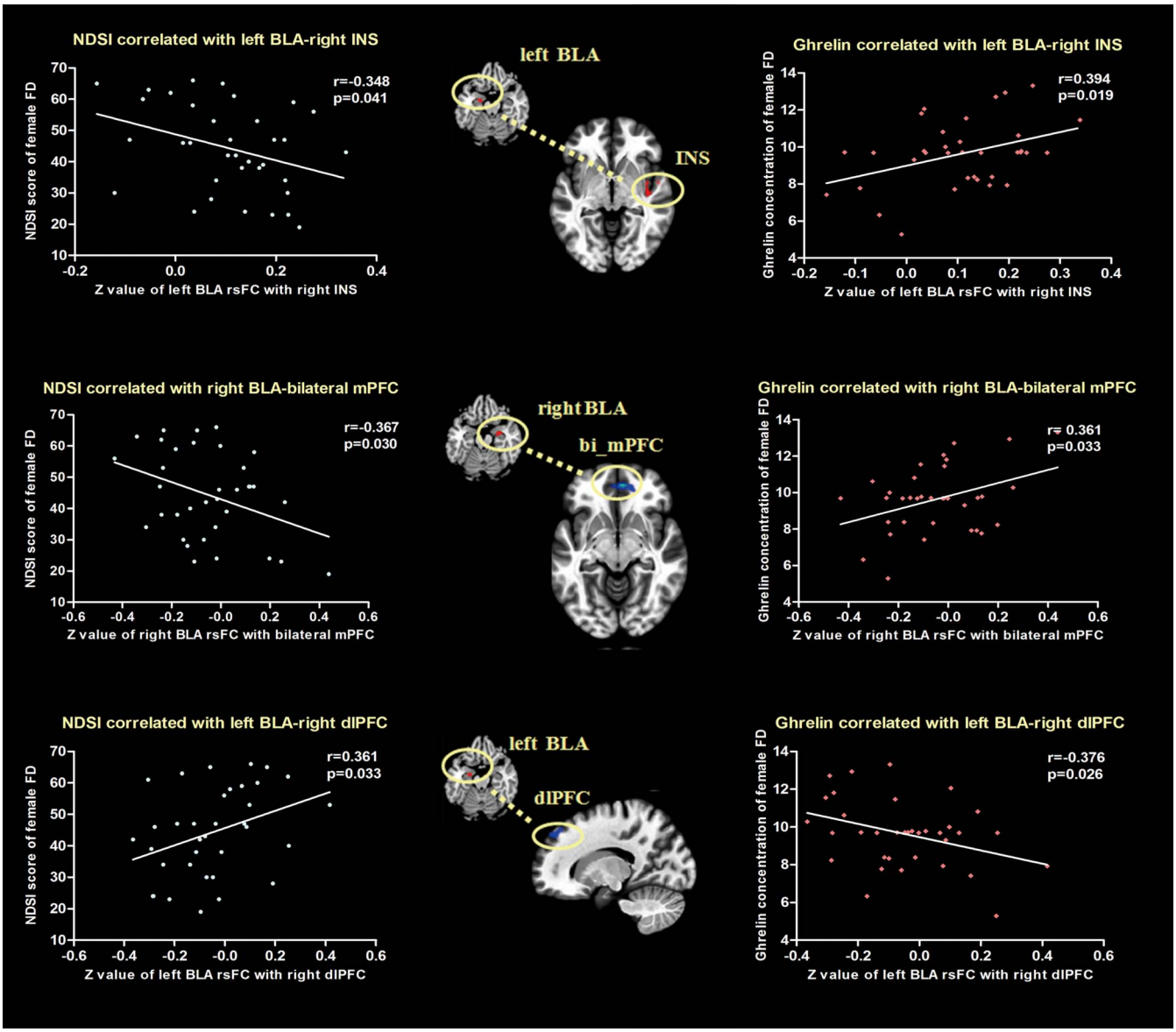

Figure 3. Correlations between BLA rsFC with Ghrelin concentrations and NDSI scores in female patients with FD. In female patients with FD: (i) left BLA rsFC with right INS showed a negative correlation with NDSI scores and a positive correlation with ghrelin concentrations; (ii) left BLA rsFC with right dlFPC showed a positive correlation with NDSI scores and a negative correlation with ghrelin concentrations; and (iii) right BLA rsFC with bilateral mFPC showed a negative correlation with NDSI scores and a positive correlation with ghrelin concentrations (FDR-corrected *P* value). BLA, basolateral amygdala; dlPFC, dorsal lateral prefrontal cortex; FD, functional dyspepsia; rsFC, resting-state functional connectivity; NDSI, Nepean Dyspepsia Symptom Index.

The functional abnormality of the SN in patients with FD. The SN is a network participating in monitoring and organizing sensory and visceral afferents (40). It is sensitive to the homeostatic response to internal and external stimuli in the brain. The amygdala and INS are main cortical and subcortical regions in the SN. The amygdala is linked with the INS by the tract uncinate fasciculus and receives visceral afferents and transmits the affective-motivational component to the INS. The INS is reported to be sensitive to internal signals (41) and is closely related to visceral sensation (13) and pain modulation (42). The pathways for communicating between the INS and amygdala for visceral motion allow integration of salient signals to guide behavior.

Our previous studies have shown functional activity changes of main regions in the SN including the anterior cingulate cortex, INS, and amygdala in patients with FD compared with HS (13–19). In this study, amygdala subregions showed altered rsFC with the INS in patients with FD compared with HS, which demonstrates functional abnormality within the SN. It may suggest disrupted function within the SN in saliency detection and organization. The abnormal salience signals during chronic pain/gastric discomfort may help us understand why patients with FD have symptoms such as satiety, disgust, and fullness. Meanwhile, the BLA (sensory input) showed increased rsFC with the INS, whereas the CMA (sensory output) showed decreased rsFC with the INS. This result is consistent with other FGID studies showing that the evaluation, integration, and response to salient stimuli were altered in IBS (43,44). A study on chronic lower back pain found that the rsFC of the BLA and the CMA with

the SN presented different changes (increase vs decrease) (27). These results indicate that the dysfunction within the SN and the abnormal sensory processing within the amygdala exist in patients with visceral pain/discomfort or nonvisceral pain disorders.

The possible functional imbalance of networks between the SN, DMN, and central executive network. Some studies hold that the SN acts as a dynamic switch between the DMN and central executive network (CEN) (40,45). The DMN is responsible for internal mentalization and is deactivated in certain goal-oriented tasks. The CEN is crucial to working memory and cognitive control of thought, emotion, and behavior. The SN monitors the CEN regions to maintain cognitive set and manipulate information in working memory while suppressing the DMN to keep attention focused on task-relevant goals.

Interestingly, the amygdala, a key region in the SN, showed altered rsFC with the main regions of the DMN and CEN. Specifically, both the BLA and CMA showed decreased mPFC and PCC/precuneus in patients with FD compared with HS. The mPFC and PCC/precuneus are 2 primary DMN hubs (46). Our previous study also demonstrated significant functional alterations in the DMN in FD using independent component analysis (47). In this study, the altered rsFC between the amygdala and the DMN hubs may suggest disruption of the SN and DMN. In patients with FD relative to HS, the BLA showed decreased rsFC with the dlPFC, whereas the CMA showed increased rsFC with the dlPFC. The dlPFC is the core of the CEN (40). The altered rsFC between the amygdala and dlPFC in this study may suggest an altered interaction between the signal output/input and cognitive-affective processing toward chronic visceral pain/discomfort in FD. As a whole, altered rsFC between the amygdala and INS (main regions within the SN), mPFC/PCC (core hubs in the DMN), and dlPFC (a core hub in the CEN) may suggest a failure of the INS to keep balance between the CEN and DMN in modulating visceral afferents. Although no more abnormal amygdala rsFC regions were found in the DMN and CEN and the imbalance between networks needs further investigation, this study may provide insight into developing a new approach for exploring the interaction between whole brain networks.

Female patients with FD have more increased BLA-INS rsFC and decreased BLA-mPFC/dlPFC rsFC compared with male patients with FD and female HS

An increasing number of studies have reported differences in the function (26,48), volume (49), and size (50) of amygdala subregions between males and females. For example, a study found that females had increased BLA rsFC with emotion-related brain regions compared with males. This finding might explain why women were more commonly afflicted with negative affect than men (51). A study on patients with IBS also found that females showed greater activation in the amygdala and mPFC and lower activation in the dlPFC than males when exposed to a visceral stressor (52). In this study, we found that the BLA showed significant sex differences in the resting state. Specifically, female patients with FD presented increased BLA-INS rsFC and decreased BLA-mPFC/dlPFC rsFC compared with both male patients with FD and female HS even after emotional factors (anxiety and depression) were controlled. The results provide new evidence that female and male patients with FD differ in visceral sensory processing.

Altered BLA-INS rsFC in FD females. Together with the INS, the BLA plays a vital role in processing motivational salience (53) and negative emotion (54). Meanwhile, the pathways between the BLA and INS influence the integration of salient signals that are associated with visceral motion. A study on participants with rectal distension found sex differences in the cerebral activity of INS (55). In this study, the increased BLA-INS rsFC in female patients with FD indicates that the visceral afferents are amplified, and negative emotion is enhanced compared with FD males and HS females. The results shed light on the phenomenon that women are more sensitive to pain and more frequently report their GI pain/discomfort than men.

Altered BLA-mPFC/dlPFC rsFC in FD females. It has been reported that the amygdala shares reciprocal inhibitory connections with the PFC (56), and altered functional connectivity of the amygdala-mPFC neurocircuitry has been found in many sex-specific disorders such as schizophrenia, bipolar disorder, and mood disorders, suggesting disrupted emotional and cognitive ability (26). The mPFC belongs to the "gastric sensation neuromatrix" (37) and occupies the highest position in the visceral sensory network (13,57,58). It receives and processes sensory, affective, motivational, and cognitive information related to visceral sensation directly from the amygdala (58) and mediates the connections between the dlPFC and amygdala. The dlPFC is implicated in cognitive control, affective modulation, sensory processing, and (visceral) pain inhibition (59) and has direct anatomical connections with the amygdala. The deactivation of the dlPFC was found in HS during gastric distention and patients with FD (47). In this study, decreased BLA rsFC with the mPFC/dlPFC might indicate severe dysfunctions in PFC modulation of the amygdala in visceral processing, mediation, and pain inhibition in female patients with FD. This result may help explain why women have a lower tolerance to somatic pain and more delayed gastric emptying than men.

The altered BLA-INS/mPFC/dlPFC rsFC also suggests that FD females have more severe dysfunction of cognitive-affective processing with disrupted balance between the SN, CEN, and DMN.

Altered rsFC correlated with ghrelin concentration. To further explore the physiopathology of sex differences in FD, we performed a correlation analysis of the fasting plasma ghrelin concentration and the abnormal rsFC of the amygdala with the INS, mPFC, and dlPFC. Ghrelin is a peripheral appetite-regulatory peptide involved in regulating food intake, promoting GI motility, emptying, acid secretion, etc. *via* the GI tract (60). Ghrelin abnormality has been implicated in the pathophysiology of FD (61,62). In a previous study, we also found that patients with FD presented decreased ghrelin levels compared with HS (63). However, the involvement of ghrelin in sex-related differences in FGIDs is uncertain. A study showed that significantly lower levels of plasma acyl ghrelin were only found in FD males compared with HS, but no significant differences were found between FD females and males (8). In the current study, although no significant sex differences in ghrelin concentrations were found, the remarkable correlation between ghrelin concentrations, NDSI scores, and the abnormal rsFC of BLA in FD females indicates that uncomfortable peripheral GI signals can transmit to the central nervous system and have reflection on cerebral changes. The closer connection between

brain networks, hormone levels, and GI symptoms suggests a significant abnormality in the brain-gut axis in female patients. Nevertheless, ghrelin has been proven to play a vital role in processing peripheral GI signals (62) and transmitting visceral discomfort within the BLA (23). The association between ghrelin and the BLA in FD females suggests an intimate peripheral-central connection.

In summary, this study demonstrated altered amygdala subregion rsFC in FD compared with HS and differences in BLA-INS rsFC and BLA-mPFC/dlPFC rsFC between FD males and females. Furthermore, altered rsFC in female patients with FD was associated with ghrelin concentrations and dyspepsia symptoms. Moreover, this study suggests that female patients with FD tend to have more severe dysfunction of cognitive-affective processing among brain regions associated with the SN, CEN, and DMN.

CONFLICTS OF INTEREST

Guarantor of the article: Fang Zeng, MD, PhD.

Specific author contributions: The first three authors contributed equally to this work. F.Z., R.S., Z.H., F.L., and J.K.: study design. F.Z., R.S., and Z.H.: manuscript drafting. Y.C., Y.Y., D.Z., L.L., and Z.L.: participants' recruitment and clinical measurement. D.L. and Q.G.: fMRI scan design and images collection. T.Y., X.L., P.M., Y.Q., C.L., J.P., and J.L.: clinical data collection. F.Z. and R.S.: data analysis. All authors discussed, read, and revised the manuscript, and all approved the publication of this protocol.

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Study Highlights

WHAT IS KNOWN

- ✓ There are sex-related differences in the prevalence and clinical manifestations of FD.
- ✓ The amygdala, a key region in GI signal processing, plays an important role in the pathophysiology of FD.

WHAT IS NEW HERE

- ✓ FD is associated with altered amygdala subregion functional connectivity.
- ✓ Altered amygdala rsFC with brain regions associated with the SN, DMN, and CEN indicates possible functional imbalance among multiple networks in FD.
- ✓ FD females are associated with more severe dysfunction of cognitive-affective processing among multiple networks.

TRANSLATIONAL IMPACT

- ✓ Elucidating sex-related differences in FD may shed light on the development of sex-specific diagnoses and treatment for FD.

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