Neurosci Bull February 1, 2013, 29(1): 4–15. http://www.neurosci.cn
DOI: 10.1007/s12264-013-1299-x

·Original Article·

The long rather than the short allele of 5-HTTLPR predisposes Han Chinese to anxiety and reduced connectivity between prefrontal cortex and amygdala

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ABSTRACT

The short allele of the serotonin-transporter gene is associated with higher risk for anxiety and depression in Caucasians, but this association is still unclear in Asians. Here, we addressed this issue using behavioral and multi-modal MRI approaches in a large group of healthy Han Chinese participants (n = 233). In contrast to findings in Caucasians, we found that long-allele (L) carriers had higher anxiety scores. In another group (n = 64) experiencing significant levels of depression or anxiety, the L-allele frequency was also significantly higher. In healthy participants, L-carriers had reduced functional and anatomical connectivity between the amygdala and prefrontal cortex (PFC), which was correlated with anxiety or depression scores. Our findings demonstrated that in Chinese Han participants, in contrast to Caucasians, the L-allele confers vulnerability to anxiety or depression and weakens top-down emotional control between the PFC and amygdala. Therefore, ethnic background should be taken into account in gene-related studies and their potential clinical applications.

Keywords: 5-HTTLPR; functional and anatomical connectivity; amygdala; prefrontal cortex; Han Chinese

INTRODUCTION

Emotional disorders are among the leading causes of disability worldwide and place significant mental and economic burdens on patients and their families[1]. Numerous studies have indicated that functional impairment of the brain serotoninergic (5-HT) system is involved in the pathogenesis of emotional disorders[2,3]. The serotonin transporter-linked polymorphic region (5-HTTLPR) is in its promoter region and comprises short (S) and long (L) variants[4]. Many studies have shown that the 5-HTTLPR is associated with the etiology of anxiety and depression[4,5], while the presence of the S-allele is associated with increased anxiety-related traits[6] and a higher risk for depression following exposure to stressful life events[7] in Caucasian populations. However, this association is unclear in Asian populations, in which investigations of either associations with mood disorders or responses to treatment with selective serotonin-reuptake inhibitors, have mainly indicated that the pattern of the as-

sociation with S and L variants may be opposite to that of Caucasians[8-11]. Moreover, a neuroimaging study[12] in Korean participants further supports such an inverse association by demonstrating that L- rather than S-carriers have higher activation in the amygdala.

The amygdala is particularly involved in emotional processing[13] and is also implicated in some mood disorders[14]. It is considered to be the core region in the systems mediating emotion perception and responses in humans[15], and the prefrontal cortex (PFC) in this network is particularly important in exerting top-down regulation of emotional responses through its control over the amygdala; this control is impaired in mood disorders[16]. So the amygdala–PFC pathway seems to be a sensitive and suitable intermediate phenotype for linking genes with mood disorders. Indeed, studies in Caucasian participants have shown that 5-HTTLPR variation affects amygdala–PFC functional connectivity during tasks with emotional stimuli[17-19] and another study in an ethnically heterogeneous group of participants showed an association of 5-HTTLPR variants with fronto–limbic white matter[20]. However, whether 5-HTTLPR variants influence the amygdala–PFC pathway in the Han Chinese population is currently unknown.

Our hypothesis was that the L- rather than the S-variant of the 5-HTTLPR may be associated with anxiety/depression and weakened connectivity between the amygdala and PFC in Han Chinese. In this study, we first systematically investigated the association between 5-HTTLPR variants and scores on a range of anxiety and depression questionnaires in a large cohort of healthy Han Chinese participants. Then we used both resting-state functional magnetic resonance imaging (fMRI) and diffusion tensor imaging (DTI) in the same participants to investigate the impact of the L and S 5-HTTLPR variants on the functional and anatomical connectivity of the amygdala–prefrontal pathway. A correlation analysis was also carried out to evaluate the association between altered connectivity in the amygdala–PFC pathway and anxiety/depression-related scores, to establish whether altered coupling may be a promising endophenotype linking the 5-HTTLPR genotype to behavior.

PARTICIPANTS AND METHODS

Participants

Three hundred and twenty-three young right-handed people were enrolled in this study. To avoid stratification artifacts, we only included those of Han Chinese ancestry. Subjects were recruited by advertisement and all gave written informed consent in accordance with the requirements of the Medical Research Ethics Committee of Tianjin Medical University. We used the Spielberger State-Trait Anxiety Inventory (STAI)[21] and the Self-Rating Anxiety Scale (SAS) to measure anxiety and the Beck Depression Inventory-II (BDI)[22,23] to measure depression in each individual. To reduce the potential effects of non-genetic factors, we carefully screened all participants to ensure a healthy phenotype. A total of 19 who did not complete all the anxiety/depression questionnaires were excluded. We next identified those who had scores indicating absent or minimal levels of depression or anxiety[21-23]. For the BDI this is defined as scores ≤13, for SAS ≤49, and for STAI-Trait and State ≤54. Of the 304 remaining participants, 58 had higher BDI scores and 7 had higher SAS, STAI-Trait or STAI-State anxiety scores and so were excluded from the main group of healthy participants. Thus a total of 239 participants met our healthy criterion and 65 did not. Details of participant numbers and inclusions/exclusions are given in Fig. 1.

Genotyping

We extracted genomic DNA from whole blood using the EZgene™ Blood gDNA Miniprep Kit (Biomiga Inc., San Diego, CA). Then we genotyped the 5-HTTLPR polymorphism in all the participants using the PCR and ligation detection reaction method[24, 25]. Six participants whose genotype was not successfully tested were excluded from further analysis, and the number remaining was 233 (101 males and 132 females).

MRI Data Acquisition

All participants were scanned on the same 3.0 Tesla GE scanner (Signa HTX 3.0 T scanner; GE Healthcare; Milwaukee, WI). During scanning, foam padding was used to reduce head motion and earplugs to reduce scanning noise. All participants received a high-resolution T1-weighted brain volume 3D MRI sequence for obtaining T1 images [repetition time (TR), 8.1 ms; echo time (TE), 3.1 ms; 176 sagittal slices; flip angle, 13°; voxel size, 1 mm × 1 mm × 1 mm]. After structural imaging, functional imaging during the resting-state took place with a single-shot, gradient-echo, echo-planar-imaging sequence sensitive to BOLD contrast.

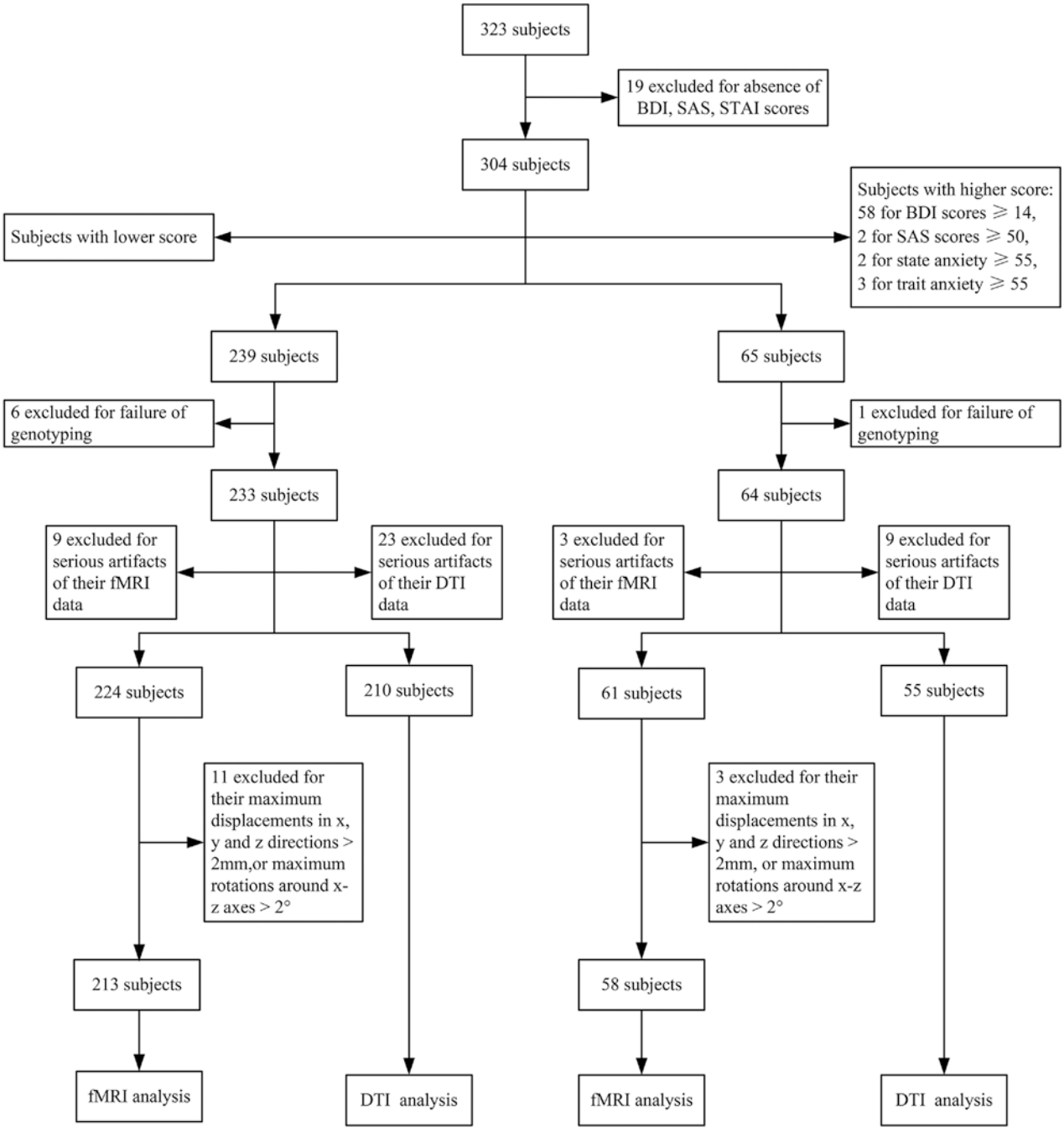

Fig. 1. Flow-chart of participant selection.

The parameters were as follows: 40 slices; 180 volumes; TR, 2 000 ms; TE, 30 ms; no gap; voxel size, 3.75 mm × 3.75 mm × 4.0 mm; FOV, 240 × 240 mm; matrix, 64 × 64; flip angle, 90°. During resting-state fMRI, all participants were instructed to lie still with their eyes closed and to move as little as possible but without falling asleep. Then a single-shot, spin-echo, echo-planar-imaging sequence was used to collect DTI data for each participant, with the following parameters: TR/TE, 10 000 ms/64.2 ms; no gap; B_0, 1 000; gradient direction, 55; 45 slices; voxel size, 2 mm ×

2 mm × 3 mm; FOV, 256 × 256 mm; matrix, 128 × 128; flip angle, 90°.

Functional Magnetic Resonance Imaging Analysis

First, two board-certified neuroradiologists blinded for genotype independently inspected the raw fMRI data for all participants, and excluded nine because their data contained serious inter-slice motion artifacts or did not cover the whole brain. We used conventional image data preprocessing steps for fMRI data and all the steps were completed using statistical parametric mapping (SPM8, http://www.fil.ion.ucl.ac.uk/spm) and DPARSFA (Data Processing Assistant for Resting-State fMRI Advanced Edition, http://www.restfmri.net/forum/DPARSF). The first 10 volumes of each participant's functional images were discarded in order to avoid the effects of scanning noise on participants and to maintain signal equilibrium. The detailed preprocessing steps for the remaining images were as follows: (1) slice timing; (2) head-motion correction; (3) normalization to the Montreal Neurological Institute (MNI) standard space with resampling to 2 × 2 × 2 mm^3; (4) smoothing with a Gaussian kernel of 4 mm full-width at half maximum; (5) linear regression to reduce the effects of confounding factors, including head motion, linear trends, average white-matter signal, average cerebrospinal fluid signal and average whole-brain signal (Note: although there are arguments about whether or not to regress the global signal, we think this is a valid and common preprocessing maneuver to correct for physiological noise and improve the anatomical specificity for resting-state functional connectivity and the anticorrelation is not just an artifact induced by global signal regression, as previous studies have done[19,26-28]); and (6) temporal band-pass filtering between 0.01 Hz and 0.08 Hz. After checking the head-motion parameters for all participants, we further excluded 11 whose maximum displacements in the x, y, and z directions were >2 mm, or whose maximum rotations around the x-z axes were >2°. Finally, after all these preprocessing steps a total of 213 participants from the healthy group were included in the subsequent functional connectivity analysis. Specifically, the L-carrier group included 90 participants (LL: 15; LS: 75) and the S group 123.

The left and right amygdala used as seed regions were extracted from the cytoarchitectonic probabilistic maps developed by Amunts *et al.*[29] in FSL (http://www.fmrib.ox.ac.uk/fsl/) by combining three subdivisions separately for the left and right amygdala, and the threshold for each subdivision was 50% probability[27]. The average time-series for each seed region was created by averaging the time-series of all voxels in it; termed left/right amygdala seed time-series.

We calculated correlation coefficients between the right amygdala seed time-series and the time-series for all other voxels, resulting in a correlation map for each participant. Then a Fisher r-to-z transformation was used to transform the correlation coefficient to Z values to improve normality. A one-sample *t* test was carried out on the resulting Z maps of all participants. At a threshold of P <0.05, with family-wise-error (FWE) rate correction, we found the clusters which showed significant functional connectivity with the right amygdala. The same analysis was performed for the left amygdala.

In order to investigate the effect of the 5-HTTLPR variants on the functional connectivity between amygdala and PFC, we used two-sample *t* tests to compare the resulting Z maps separately for left and right amygdala between the two genotype groups and with small volume correction restricted to the PFC, created by combining BA9, BA10, BA11, BA25 and BA46[30]. Moreover, in view of the significant impact of 5-HTTLPR variants on BA32 reported in a previous study[19], we also included BA32 in our identified PFC regions. Results are reported at a threshold of P <0.05 with FWE correction. In the functional connectivity analysis, the age and gender of each participant were considered as covariates of no interest.

Diffusion Tensor Imaging Analysis

The raw DTI data for all participants were checked by two experienced radiologists blinded for genotype, and 23 participants were excluded due to serious motion artifacts and signal loss in the raw data. So the number of remaining participants for DTI analysis was 210, including 87 L-carriers (LL: 14; LS: 73) and 123 S-homozygotes. The preprocessing of DTI data was all completed in FSL. First, eddy-current correction was carried out to correct for distortion. Then we ran "*dtifit*" in FSL to fit the diffusion tensor model at each voxel and the fractional anisotropy (FA) image was calculated for each participant. Next, the B_0 image (without gradient direction) of each participant was normalized to the EPI template in MNI space and the parameters of this

transformation were applied to each participant's FA image with resampling to 2 × 2 × 2 mm^3. For assessing the effect of the 5-HTTLPR variants on anatomical connectivity along the amygdala–PFC pathway, we focused on the uncinate fasciculus (UF). Thus we calculated the mean FA for each participant in a probabilistic tract of the UF in FSL. This method is similar to that reported in a previous study[20].

Statistical Analysis

We used a Pearson χ^2 test to assess gender differences and a two-sample *t* test to assess differences in age, BDI score, SAS score, STAI-State score, STAI-Trait score and mean FA value separately for left and right UF between the two genotype groups. Then, we extracted regions of interest which survived under the two-sample *t* test on functional connectivity and averaged the Z scores in each region for each participant as a measure of altered functional connectivity. For investigating the association between altered amygdala–PFC pathway coupling and anxiety/depression scores, Pearson's correlation test was used to calculate the correlation between altered functional or anatomical connectivity and the four scores (BDI, SAS, STAI-State and STAI-Trait), with age and gender as nuisance covariates. Results were considered significant at a threshold of $P < 0.05$, and all statistical analyses were performed in SPSS (SPSS Inc., Chicago, IL).

RESULTS

Demographics of the Genotype Groups

Demographic information for the 233 healthy participants included in the final analysis is given in Table 1. Genotyping yielded three 5-HTTLPR groups: long/long (L/L), 15 participants; long/short (L/S), 85; short/short (S/S), 133. The allelic distribution of 5-HTTLPR was in Hardy–Weinberg equilibrium ($P = 0.775$). Consistent with previous Asian population studies[9,12], we combined the L/L and L/S genotypes into the L-carrier group ($n = 100$) and compared them with the S-homozygote group ($n = 133$).

There were no significant differences in age and gender between the two groups. The L group showed significantly higher STAI-State scores ($P = 0.032$) than the S group. In addition, the STAI-Trait scores of the L group tended to be higher than those of the S group ($P = 0.063$).

We also analyzed the genotypes of the 64 participants with high depression and anxiety scores who were excluded from the healthy group due to potential pathology. Genotyping revealed a significantly different distribution of genotypes in these participants (LL, 10; LS, 26; SS, 28) compared to the healthy participants ($\chi^2 = 6.911$, df = 2, $P = 0.032$) and a significant difference in the distribution of L *versus* S allelic frequencies ($\chi^2 = 6.443$, df = 1, $P = 0.011$). There was also a trend towards an increased overall proportion of L-carriers in the high-score group ($\chi^2 = 3.595$, df = 1, $P = 0.058$) (Table 2). Overall, this showed that in the group with high depression and anxiety scores, there was a stronger representation of L-alleles and a reduced representation of S-alleles.

Table 1. Demographics of genotype groups

	L-carriers ($n = 100$[a])		S-homozygotes ($n = 133$)		*P*-value
	Mean	SD	Mean	SD	
Male/female	39/61		62/71		0.246[b]
Age (years)	22.79	2.41	22.73	2.38	0.848[c]
BDI	5.36	3.79	4.87	3.81	0.307[d]
SAS	28.92	4.55	28.64	4.85	0.629[d]
STAI-State	31.94	6.06	30.05	6.27	0.032[d]
STAI-Trait	35.67	6.48	33.93	6.28	0.063[d]

[a]L-carriers included all participants carrying the L allele (L/L, 15 individuals; L/S, 85 individuals). [b]*P* value, Pearson χ^2 test. [c]*P* value, two-sample *t*-test. [d]*P* value, two-sample *t*-test, with age and gender as nuisance covariates. BDI: Beck Depression Inventory-II scores; SAS: Self-Rating Anxiety Scale scores; STAI-State: State-Trait Anxiety Inventory-State; STAI-Trait: State-Trait Anxiety Inventory-Trait. SD: standard deviation.

The Effect of 5-HTTLPR Variants on the Functional Connectivity between Amygdala and PFC

In our study, the amygdala showed significant positively correlated functional links with medial PFC regions, such as medial orbital frontal cortex and anterior cingulate cortex, as well as with bilateral precentral/postcentral gyrus, and middle and superior temporal gyrus. There were also positively correlated links with some subcortical regions such as the putamen, caudate and thalamus. On the other hand, significant negatively correlated links existed between the

Table 2. 5-HTTLPR genotype and allele frequencies in lower and higher score groups

Group	*n*	Genotype distribution[a] (%)			Genotype distribution[b] (%)		Allele frequency[c] (%)	
		LL	LS	SS	L carriers	SS	L	S
Lower score group	233	15	85	133	100	133	115	351
		(6.4)	(36.5)	(57.1)	(42.9)	(57.1)	(24.7)	(75.3)
Higher score group	64	10	26	28	36	28	46	82
		(15.6)	(40.6)	(43.8)	(56.2)	(43.8)	(35.9)	(64.1)

[a]Comparison of genotype distribution (LL, LS, SS), lower score group *versus* higher score group: χ^2 = 6.911, df = 2, P = 0.032. [b]Comparison of genotype distribution (L-carriers, SS), lower score group *versus* higher score group: χ^2 = 3.595, df = 1, P = 0.058. [c]Comparison of allele frequency distribution (L, S), lower score group *versus* higher score group: χ^2 = 6.443, df = 1, P = 0.011.

amygdala and the lateral and dorsal prefrontal cortices, including the middle and superior frontal gyrus, the precuneus and the angular gyrus. The left and right amygdala exhibited similar patterns of functional connectivity (Fig. 2).

Functional connectivity between the right amygdala and the PFC was significantly different between the two genotype groups (P = 0.016, after FWE correction). The L group showed significantly reduced functional connectivity between the right amygdala and right frontal pole compared with the S group (Fig. 3A, B; Table 3).

The Effect of 5-HTTLPR Variants on Anatomical Connectivity between Amygdala and PFC

The UF is an important white matter tract that connects the amygdala to the PFC[31,32] (Fig. 4A). We extracted the UF from the probabilistic UF at a threshold of 30% probability

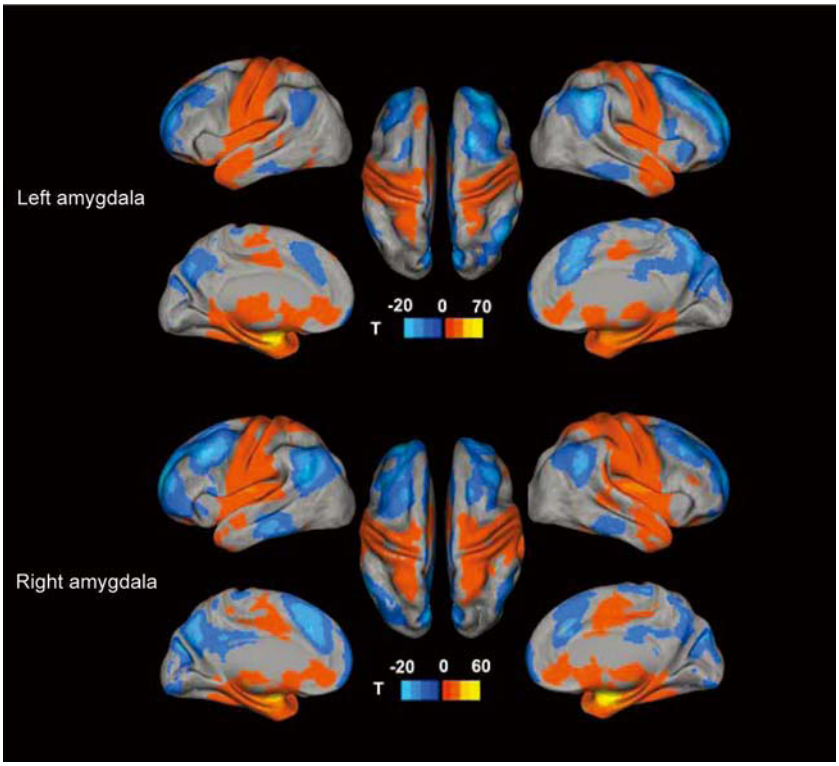

Fig. 2. Regions showing significant functional connectivity with the left and right amygdala. The pseudocolor bar indicates T-scores. Warm colors indicate positive connectivity and cool colors indicate negative connectivity with the amygdala.

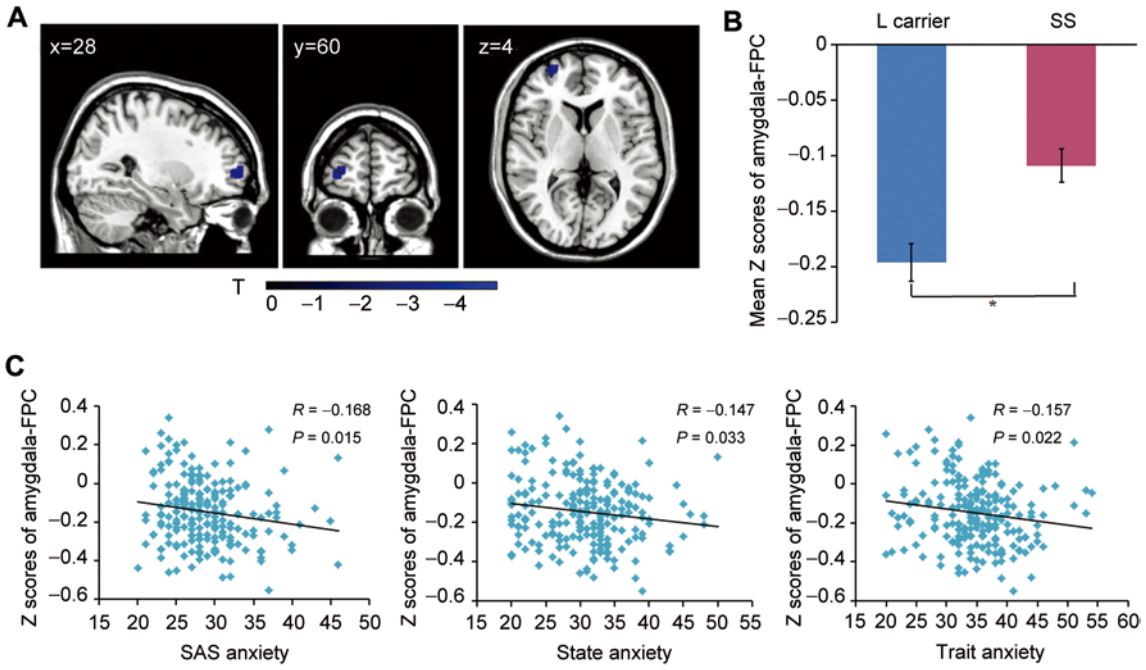

Fig. 3. Functional connectivity. A: Right frontal pole (FPC) in prefrontal cortex (PFC) showed significantly decreased coupling with the right amygdala in L-carriers compared with S-homozygotes. B: Mean ± SEM functional connectivity of the amygdala–PFC pathway in the 5-HTTLPR genotype groups. *P <0.05 (after FWE correction) between the groups. C: Regression lines showing that the functional connectivity of the amygdala–PFC pathway is negatively correlated with the anxiety-related scores on SAS, state anxiety and trait anxiety.

and projected it onto a standard FA map, and found that the FA value of the right UF was significantly decreased in L-carriers compared with the S group (P = 0.035, Fig. 4B).

Correlation between Altered Functional and Anatomical Connectivity and Levels of Depression and Anxiety

The functional connectivity between the amygdala and frontal pole was negatively correlated with the anxiety-related scores SAS (r = −0.168, P = 0.015), STAI-State (r = −0.147, P = 0.033) and STAI-Trait (r = −0.157, P = 0.022) (Fig. 3C) but not with depression-related scores (BDI: r = −0.047, P = 0.500) (Table 4). On the other hand, amygdala–PFC anatomical connectivity, measured by the mean FA value of the UF, was negatively correlated with depression scores (BDI: r = −0.149, P = 0.032; Fig. 4C) but not with anxiety scores (SAS: r = 0.037, P = 0.592; STAI-State: r = 0.051, P = 0.465; STAI-Trait: r = 0.041, P = 0.558).

Additional Results of the Analysis in the Higher Score Group

We also carried out a separate analysis on the 64 subjects excluded from the main study due to having high BDI-II or anxiety scores indicative of potential pathology as that in the healthy subjects. There were no significant differences in age, gender and four depression/anxiety scores (BDI-II score, SAS score, state and trait anxiety score) between the two genotypes (Table 5). Meanwhile, the functional connectivity analysis found no impact of 5-HTTLPR on the amygdala–PFC coupling at the threshold of P <0.05, FWE correction. And the bilateral UF did not show any significant difference between the two genotypes (Table 5).

DISCUSSION

In this study, we systematically investigated the association between 5-HTTLPR variants and levels of depression and anxiety in a large sample of healthy Han Chinese participants. We found that L-carriers had significantly higher levels of state anxiety than SS-homozygotes with a trend (P <0.07) towards higher levels of trait anxiety as well. Furthermore, in a separate group of 64 participants exhibiting higher and potentially pathological levels of depression

Table 3. Right prefrontal cortex shows significantly decreased functional connectivity with right amygdala in L-carriers

Brain region	K	*t* value	Z score	*P*[a]	MNI coordinates			L-carriers		S-homozygotes	
					x	y	z	Mean	SD	Mean	SD
R. FPC	115	4.74	4.62	<0.001	32	58	2	–0.196	0.156	–0.109	0.166

[a]*P* value, uncorrected. The region in the table was significant at the threshold of *P* <0.05, FWE correction restricted in prefrontal cortex. FPC, frontal pole; K, cluster size (number of voxels); R, right; SD, standard deviation.

Table 4. Correlations between altered connectivity and anxiety/depression scores

	BDI *r* (*P* value)	SAS *r* (*P* value)	STAI-State *r* (*P* value)	STAI-Trait *r* (*P* value)
Z scores of right amygdala–FPC	−0.047 (0.500)	−0.168 (0.015)	−0.147 (0.033)	−0.157 (0.022)
Mean FA of R. UF	−0.149 (0.032)	0.037 (0.592)	0.051 (0.465)	0.041 (0.558)

Pearson's correlation coefficients and corresponding *P* values. BDI, Beck Depression Inventory-II scores; FA, fractional anisotropy; FPC, frontal pole; R, right; SAS, Self-Rating Anxiety Scale scores; STAI-State, State-Trait Anxiety Inventory-State; STAI-Trait, State-Trait Anxiety Inventory-Trait; UF, uncinate fasciculus.

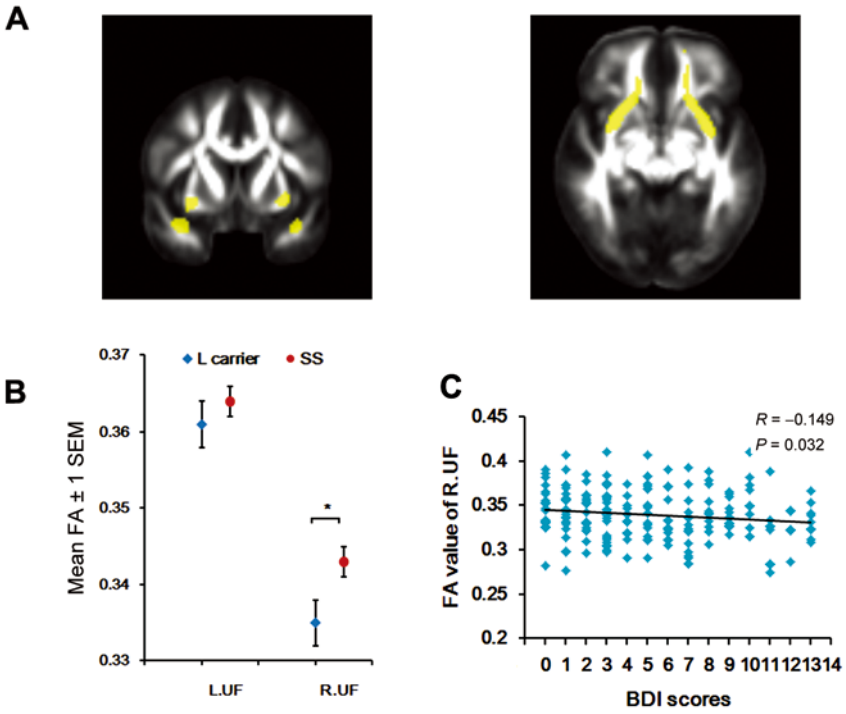

Fig. 4. Anatomical connectivity. A: The bilateral uncinate fasciculus (UF) (in yellow) overlaid on a mean fractional anisotropy (FA) map in standard space in coronal (left image) and axial (right) sections. B: FA values of the amygdala–prefrontal cortex (PFC) pathway in the 5-HTTLPR genotype groups (L-carriers and S-homozygotes) for the left and right UF (mean ± SEM). *P <0.05 between groups. C: Regression line showing that anatomical connectivity (FA value) of the amygdala–PFC (UF) pathway is significantly negatively correlated with Beck Depression Inventory-II (BDI) scores. L, left; R, right.

Table 5. Demographics of genotype groups in higher score group

	L-carriers (n = 36[a])		S-homozygotes (n = 28)		P-value
	Mean	SD	Mean	SD	
Male/female	27/9		15/13		0.073[b]
Age (years)	22.86	2.36	22.57	3.11	0.673[c]
BDI	18.42	7.37	16.04	5.78	0.194[d]
SAS	36.47	8.87	35.25	7.78	0.454[d]
STAI-State	39.67	11.00	40.18	10.25	0.836[d]
STAI-Trait	45.47	10.05	44.25	9.42	0.360[d]
L.UF	0.37	0.03	0.36	0.02	0.834[d]
R.UF	0.34	0.03	0.34	0.03	0.177[d]

[a]L carriers included all participants carrying the L-allele (L/L, 10 individuals; L/S, 26 individuals). [b]P value, Pearson χ^2 test. [c]P value, two-sample t-test. [d]P value, two-sample t-test, with age and gender as nuisance covariates. BDI, Beck Depression Inventory-II scores; L.UF: left uncinate fasciculus; R.UF: right uncinate fasciculus; SAS, Self-Rating Anxiety Scale scores; SD, standard deviation; STAI-State, State-Trait Anxiety Inventory-State; STAI-Trait, State-Trait Anxiety Inventory-Trait.

and anxiety, the frequency of L-allele expression was significantly higher than in the healthy group. Functional connectivity analysis of the healthy group demonstrated that L-allele carriers had significantly reduced coupling between the amygdala and frontal pole compared with S-allele homozygotes. The FA value of the right UF, which links the amygdala and prefrontal cortex, was also significantly decreased in the L-carriers. Importantly, both functional and anatomical connectivity were significantly negatively correlated either with anxiety or depression scores in healthy participants. Thus, converging behavioral and neuroimaging evidence indicated that, in contrast to Caucasian populations, the presence of the L- rather than the S-allele of 5-HTTLPR may predispose healthy Chinese Han people to anxiety or depression.

We found that the L-allele in Han Chinese participants was associated with a higher level of anxiety, consistent with some other studies of Asian participants[8-11], and contrary to those on Caucasians that report associations with the S-allele[6,7,33-35]. The distribution of the two 5-HTTLPR variants was also significantly different between Caucasian and Asian populations. The frequency of SS-homozygotes is 12–24% in Caucasian samples, which is much lower than that reported in East Asians (49–74%)[36], and the distribution of the S-allele is also significantly different[34]. Interestingly, we also found that, in the sub-group excluded from the healthy group due to higher depression and anxiety scores, indicative of potential pathology, there was a significant shift in distribution towards a higher frequency of L-alleles (35.9% *vs* 24.7%) and a reduced frequency of S-alleles (64.1% *vs* 75.3%). The participants in this sub-group mainly had high BDI scores in a range indicative of mild to moderate levels of depression. This finding therefore provides evidence for an association between the L-allele in Chinese Han participants and the actual development of significant symptoms of depression.

To investigate the neural basis of the behavioral effects of 5-HTTLPR variants in Han Chinese participants, we also used functional and structural neuroimaging to establish potential effects on the amygdala–PFC pathway. This pathway is well known to play an important role in emotion regulation, with the PFC imposing top-down regulation on amygdala responses[37-40]. There is also evidence that functional connectivity in this pathway is weakened in patients with mood disorders[16]. Resting-state fMRI analysis confirmed that the Chinese Han L-carriers did indeed have significantly reduced functional connectivity between amygdala and PFC, especially the frontal pole. The frontal pole is in the anterior part of the prefrontal cortex and plays an important role in processing internal rather than external information[41,42], which could explain its recruitment during resting-state conditions when no salient external stimuli are present. The frontal pole is also implicated in the process of updating existing emotional memories[43], and its connection with the amygdala is associated with the self-regulation of negative emotion through reappraisal[44]. Thus Chinese Han L-carriers may have a reduced ability both to regulate responses to negative emotions through cognitive appraisal and to re-shape existing emotional memories in the light of new experiences.

Previous studies of Caucasian or heterogeneous populations have also found different effects of 5-HTTLPR variants on amygdala–frontal cortex connectivity[17-19]. Functional connectivity studies in healthy Caucasians during tasks involving medial frontal cortex regions and the amygdala have reported increased coupling associated with the S- rather than the L-allele[17-19], but these prefrontal regions dif-

fer from that in our current study. Indeed, our finding is also in contrast to some evidence for decreased coupling between the amygdala and prefrontal cortical region in S-allele carriers[19]. Further support for the differential importance of the S- and L-alleles in the amygdala and PFC in Caucasian and Asian populations is provided by studies investigating associations with amygdala activation. Thus, whereas there is a link between the S-allele and higher amygdala activation in response to emotional stimuli in Caucasian/heterogeneous populations[45-48], in Asian populations amygdala hyperactivation is associated with the L-allele during both task[12] and resting-state[49] conditions.

Our DTI analysis also provided anatomical support for reduced functional connectivity between the amygdala and PFC in the L-carrier group, at least in terms of a reduced FA value for the UF. The UF is the main white matter tract connecting the amygdala and the frontal lobe[31,32] and has been shown to have reduced FA in both generalized social anxiety disorder[50] and depression[51]. Our finding is once again in complete contrast to a previous study in an ethnically heterogeneous, although primarily Caucasian, population showing associations with the S-allele[20] and reduced FA values in the UF. This provides yet further support for ethnic differences on the influence of 5-HTTLPR variants on the amygdala–PFC pathway.

Importantly, we also found that coupling between the amygdala and PFC was significantly negatively correlated with anxiety (functional) and depression (structural) scores in healthy participants. The more impaired the amygdala–PFC pathway was in participants, the higher the anxiety or depression scores they exhibited within the normal range. The findings that functional coupling between the amygdala and PFC was negatively correlated with anxiety but not depression scores, whereas the opposite pattern was found with structural coupling, may indicate that the functional coupling measured involved more pathways than just the UF. Alternatively, the link with anxiety scores may simply reflect changes within the amygdala and PFC rather than in the UF, whereas depression scores may be more associated with altered integrity of the UF. In any event, it is clear that functional and structural measures of amygdala-PFC pathway function may prove to be a particularly useful endophenotype linking genes and the risk of developing a mood disorder.

However, in the sub-group who did exhibit significant levels of depression and anxiety, indicative of potential pathology, there was no impact of 5-HTTLPR on functional and structural coupling in the amygdala-PFC pathway. This might simply have been due to the smaller sample size, but may also indicate that gene effects on coupling in this pathway are more linked with the potential risk of pathology rather than with severity of clinically relevant symptoms. Indeed, our results showing a greater proportion of L-alleles in the sub-group actually suffering from significant depression provide some support for this possibility.

The L-allele of 5-HTTLPR is increasingly being associated with psychopathy in Caucasian populations[52]. In general, it has been argued that in these populations the L-allele conveys a greater efficacy in transporting serotonin, resulting in reduced serotonin levels. This would be consistent with increased risk-taking and psychopathic behavior, although it is not fully established[52]. It does however raise an interesting question about whether, in Asian populations, the S rather than the L form of the allele is associated with risk-taking and psychopathy, and reduced serotonin levels. This clearly requires further investigation, as does the fascinating question as to why these functional allelic differences may have evolved in Caucasian and Asian populations.

In summary, we provide a behavioral and functional and structural connectivity analysis establishing that, in complete contrast to Caucasians, in Chinese Han participants it is the L- rather than the S-allele of 5-HTTLPR that is associated with depression and anxiety and impaired coupling between the amygdala and PFC. This raises serious concerns about taking into account ethnic background when attempting to establish links between specific gene polymorphisms and emotional disorders. It also raises concerns about the potential efficacy of selective serotonin-reuptake inhibitor treatments for mood disorders in Asian populations, since nearly all available biochemical evidence has been obtained from human cells of Caucasian ancestry and indicates lower transcription activity with the S-allele. Clearly, new biochemical evidence based on Asian populations would help substantiate our current findings and their potential therapeutic implications.

ACKNOWLEDGEMENTS

This work was partly supported by the National Key Basic Research and Development Program (973) (2011CB707800), the Strategic Priority Research Program of the Chinese Academy of

Sciences (XDB02030300), the Natural Science Foundation of China (91132301 and 81000582), and the Beijing Nova Program (2010B06).

Received date: 2012-12-12; Accepted date: 2012-12-19

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