Psychiatry Research 226 (2015) 399–402

Contents lists available at ScienceDirect

Psychiatry Research

journal homepage: www.elsevier.com/locate/psychres

Brief report

Allelic variation at *5-HTTLPR* is associated with brain morphology in a Chinese population

Jiewei Liu [a,h,1], Yin Mo [b,1], Tian Ge [c,d,1], Yi Wang [e], Xiong-jian Luo [f], Jianfeng Feng [c,d], Ming Li [g,*], Bing Su [a,**]

[a] *State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan, China*
[b] *Imaging Center, The First Affiliated Hospital of Kunming Medical College, Kunming, Yunnan, China*
[c] *Centre for Computational Systems Biology, School of Mathematical Sciences, Fudan University, Shanghai, China*
[d] *Department of Computer Science, University of Warwick, Coventry, UK*
[e] *State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing, China*
[f] *Key Laboratory of Animal Models and Human Disease Mechanisms of the Chinese Academy of Sciences and Yunnan Province, Kunming Institute of Zoology, Kunming, Yunnan, China*
[g] *Lieber Institute for Brain Development, Johns Hopkins University, Baltimore, MD, USA*
[h] *Kunming College of Life Science, University of Chinese Academy of Sciences, Beijing, China*

ARTICLE INFO

Article history:
Received 25 June 2014
Received in revised form
19 January 2015
Accepted 22 January 2015
Available online 30 January 2015

Keywords:
5-HTTLPR
VBM
Han Chinese

ABSTRACT

Previous studies have reported significant associations of *5-HTTLPR* with brain structures mainly in Europeans, but the situations in other ethnic groups remain largely unknown. Here we examined the association of *5-HTTLPR* with regional gray matter volume in Han Chinese, and observed significant association in the postcentral gyrus and precuneus cortex.

1. Introduction

Since its first description in 1993 (Ramamoorthy et al., 1993), the human serotonin transporter gene (*SLC6A4*, also known as *5-HTT*), which encodes the serotonin transporter protein, has been implicated as a candidate gene in a variety of neuropsychiatric disorders. *SLC6A4* has potential connection with mental disorders, given the importance of serotonergic functions on mood-related temperamental phenotypes (e.g., emotional processing), as well as the wide-spread clinical use of the selective serotonin reuptake inhibitors in the treatment of affective and anxiety disorders (Nemeroff and Owens, 2002; Wong and Licinio, 2001). In particular, the short allele (*s*) of an insertion/deletion polymorphism (*5-HTTLPR*) in the promoter region of *SLC6A4* has been shown to be associated with an increased risk of major depression, especially following exposure to stressful life events (Caspi et al., 2003, 2010; Eley et al., 2004). This variation has also been shown to influence transcriptional activity and subsequent availability of *5-HTT* both in vitro and in vivo (Lesch et al., 1996; Murphy et al., 2004), prompting further studies into the genetic mechanism of *5-HTTLPR* underlying the susceptibility to mental disorders.

In vivo neuroimaging studies have detected altered volumes of hippocampus and other brain regions (e.g., anterior cingulum, prefrontal cortex, etc.) in mental disorders (Bell-McGinty et al., 2002; Campbell et al., 2004), suggesting that brain image derived morphological features may serve as potential biomarkers for mental illnesses. Several previous imaging genetic studies have reported decreased regional gray matter (GM) volumes in individuals carrying *s* allele of *5-HTTLPR* among European populations (Canli et al., 2005; Frodl et al., 2008; Kobiella et al., 2011; Little et al., 2014; Pezawas et al., 2005; Selvaraj et al., 2010), although controversial results have also been reported (Scherk et al., 2009). In addition, the interactions between *5-HTTLPR* and other factors (e.g., stress and sex) also showed effects on brain structures (Cerasa et al., 2014; Everaerd et al., 2012; Frodl et al., 2010). Considering the function of this polymorphism in serotonergic alterations, *5-HTTLPR* may play a pivotal role in neurodevelopment and the onset of mental disorders.

Though provocative, it is known that genetic factors underlying a specific mental disorder often vary across different ethnic groups

---

[*] Corresponding author.
[**] Corresponding author. Tel.: +86 871 6512 0212; fax: +86 871 6519 3137.
E-mail addresses: limingkiz@gmail.com, liming@mail.kiz.ac.cn (M. Li), sub@mail.kiz.ac.cn (B. Su).
[1] These authors contributed equally to this work

http://dx.doi.org/10.1016/j.psychres.2015.01.022
0165-1781/

(Li et al., 2014a). While associations have been reported among Europeans, the genetic susceptibility of *5-HTTLPR* to mental disorders and brain volumetric changes in other ethnic groups remains largely unknown. In this study, we investigate the relationship between *5-HTTLPR* and regional GM volume in a healthy Han Chinese population.

2. Methods

2.1. Sample description

A total of 278 unrelated healthy Han Chinese (174 females, 104 males, mean age 36.21 ± 12.58 years) were recruited from hospital staff, students, and company employees with no history of alcohol dependence, mental disorder, drug abuse, or brain injury to examine the potential effects of *5-HTTLPR* on brain GM volume. The screening of healthy controls was ascertained by face-to-face interviews where subjects were asked if they had suffered from an episode of depression, mania, or psychotic experiences or if they had received treatment for any psychiatric disorder. All participants were also required to answer the questions of self-rating depression scales, and no subject with potential depressive symptoms was found. Written informed consent was obtained from each subject prior to this study. This imaging sample has been used previously and shown to be effective for the detection of genetic effects on the features extracted from magnetic resonance imaging (MRI) scans with negligible population stratification (Li et al., 2012, 2013, 2014b; Li and Su, 2013). All research protocols were approved by the internal review board of Kunming Institute of Zoology, Chinese Academy of Sciences.

2.2. MRI acquisition and image preprocessing

Structural MRI data were acquired using a Philips MRI scanner (Achieva Release 3.2.1.0) operating at 3T. High-resolution whole-brain T1-weighted images were acquired sagittally with an inversion-recovery prepared 3-D spoiled gradient echo (SPGR) pulse sequence (Repetition Time=7.38 ms, Echo Time=3.42 ms, flip angle=8°, voxel dimensions=$1.04 \times 1.04 \times 1.80\ \text{mm}^3$, slice thickness=1.2 mm).

All preprocessing were performed using SPM8 (Wellcome Trust Centre for Neuroimaging, London, UK. http://www.fil.ion.ucl.ac.uk/spm/software/spm8/). Specifically, high resolution T1 images were first segmented into gray matter, white matter and cerebrospinal fluid in the native space using SPM's new segmentation. The gray matter images were then iteratively aligned to an increasingly crisp average template by DARTEL (Ashburner, 2007). Finally, all images were normalized to the standard Montreal Neurological Institute (MNI) template, modulated to account for volume changes in the warping, and resampled to $2 \times 2 \times 2\ \text{mm}^3$. Modulated gray matter images were smoothed with an 8 mm Gaussian kernel. A gray matter analysis mask was constructed by thresholding the averaged gray matter image that maximized the sample Pearson correlation between the binarized mask and the original average image (Ridgway et al., 2009).

2.3. Genotyping method

We genotyped *5-HTTLPR* in all subjects, using the methods described in the previous study (Nakamura et al., 2000). Specifically, venous blood was collected from all participants, and genomic DNA was extracted from the blood sample using the standard phenol–chloroform method. DNA samples were randomly distributed in the DNA sample plates. To generate the S- and L- fragments, polymerase chain reaction (PCR) was carried out using the reported system (Nakamura et al., 2000). The PCR products were purified, and sequenced using the BigDye Terminator Cycle Sequencing kit v3.1 (Applied Biosystems, USA) on an ABI 3130XL Genetic Analyzer (Applied Biosystems, USA). Among the 278 subjects, *5-HTTLPR* genotypes were successfully determined in 275 individuals.

2.4. Statistical analysis

The association between *5-HTTLPR* and the brain structure was analyzed using a linear regression model at each voxel, adjusting for age, gender and the total intracranial volume (the total volume of gray matter, white matter and cerebrospinal fluid). Voxel-wise *p*-Values were then corrected over the entire brain to control family wise error (FWE) rate. In particular, a non-parametric permutation method was used to account for the correlation structure in brain imaging data and accurately estimate the significance of an observed peak (Ge et al., 2012). For each permutation, the regressor encoding the polymorphism was randomly permuted and the maximal voxel-wise statistic over the brain was saved. The *p*-Value for an observed statistic *T* was then computed as the proportion of the permutation distribution as or more extreme than *T*. A total of 10,000 permutations were performed.

Table 1
The genotypic frequencies of *5-HTTLPR* in European and Han Chinese sample (n=275). As a comparison, we also show the genotypic frequencies of *5-HTTLPR* in a European sample (n=74) from a previously published study (Nakamura et al., 2000). The *5-HTTLPR* polymorphism includes an embedded A/G substitution (rs25531). Considering the genotype of both *5-HTTLPR* and rs25531, a detailed genotypic combination was also indicated, where the 14-B and 14-D alleles are short-G (S_G, combination), the 16-D allele is long-G (L_G), and the other alleles carry A-allele of rs25531 (S_A or L_A).

Genotype	European (n=74)	Chinese (n=275)	Broad genotype	
14-A/14-A	15 (20.3)	146 (52.9)	SA/SA	*s/s*
14-A/14-B	0 (0.0)	1 (0.4)	SA/SG	
14-A/16-A	30 (40.5)	23 (8.3)	SA/LA	*l/s*
14-A/16-B	0 (0.0)	5 (1.8)	SA/LA	
14-A/16-C	0 (0.0)	24 (8.7)	SA/LA	
14-A/16-D	3 (4.1)	48 (17.4)	SA/LG	
14-A/16-E	1 (1.4)	0 (0.0)	SA/LA	
14-A/16-F	1 (1.4)	0 (0.0)	SA/LA	
14-D/16-A	1 (1.4)	0 (0.0)	SG/LA	
16-A/16-A	19 (25.7)	2 (0.7)	LA/LA	*l/l*
16-A/16-D	4 (5.4)	1 (0.4)	LA/LG	
16-C/16-D	0 (0.0)	10 (3.6)	LA/LG	
16-D/16-D	0 (0.0)	3 (1.1)	LG/LG	
14-A/20	0 (0.0)	11 (4.0)	Other	
16-C/20	0 (0.0)	1 (0.4)		

3. Results

We identified 147 *s/s* homozygotes and 116 individuals with *l/l* or *l/s* in our sample (Table 1). The remaining subjects (n=13) carry a rare allele (with a total of 20 repetitive elements), which is different from either *s* (with a total of 14 repetitive elements) or *l* (with a total of 16 repetitive elements) (Table 1). Due to the unique genotype and small sample size, these individuals were excluded, leaving 263 subjects for subsequent analyses (147 *s/s* and 116 *l/l*+*l/s*). No deviation from Hardy–Weinberg equilibrium was found for the genotypes of *5-HTTLPR* (p=0.85).

A whole-brain VBM analysis of these subjects revealed significantly reduced GM volume in the postcentral gyrus and precuneus cortex (peak statistic t=4.62, uncorrected $p=3.05 \times 10^{-6}$, FWE-corrected p=0.039, MNI coordinates [−6 −42 60], Fig. 1) among *s/s* individuals, compared to *l/l*+*l/s* individuals. A secondary analysis showed that the results were similar if the 16 *l/l* carriers were removed (peak statistic t=4.66, uncorrected $p=2.61 \times 10^{-6}$, FWE-corrected p=0.036), and the location of the most associated voxel remained unchanged (MNI coordinates [−6 −42 60]). Therefore, it appears that the effects of *5-HTTLPR* were mainly driven by the difference between *s/s* and *l/s* carriers.

4. Discussion

Previous imaging studies in European populations reported decreased regional GM volume among *s* allele carriers (*s/s*+*l/s*) relative to *l/l* individuals (Pezawas et al., 2005; Selvaraj et al., 2010). Here, we only compared *s/s* homozygotes with *l/s*+*l/l* carriers, due to the heterogeneous genotypic distributions of *5-HTTLPR* between Han Chinese and European populations, and the small number of *l/l* individuals in our sample. As a comparison, Table 1 also shows the genotypic distribution of *5-HTTLPR* in a sample of European ancestry (Nakamura et al., 2000). The frequency of *s* allele is 44.6% in Europeans while is as high as 73.7% in Han Chinese, which indicates that *l/l* homozygotes may be rather rare in the entire Han population.

Our findings support earlier observations that *5-HTTLPR* is associated with regional brain structures. However, we note that our statistical analysis was based on the *a priori* assumption that *s* allele carriers have a smaller regional GM volume than *l* allele