NEUROSCIENCE
RESEARCH ARTICLE
B. Zhu et al. / Neuroscience 369 (2018) 261–268

The Choline Acetyltransferase (*CHAT*) Gene is Associated with Parahippocampal and Hippocampal Structure and Short-term Memory Span

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Abstract—The *CHAT* gene encodes choline acetyltransferase, which is an enzyme responsible for the biosynthesis of the neurotransmitter acetylcholine in the brain. This study collected structural MRI, genetic, and behavioral data from 324 healthy Chinese adults, and examined the associations between *CHAT* genetic variants, parahippocampal and hippocampal structure, and short-term memory span. After controlling for intracranial volume, sex, and age, *CHAT* SNP rs12246528 had the strongest association with parahippocampal structure, with the A allele being linked to smaller volume, surface area, and thickness. SNP rs1917814 had the strongest association with hippocampal volume, with the T allele being linked to larger hippocampal volume. After controlling for sex and age, *CHAT* rs3729496 had the strongest association with memory span, with the T allele being associated with a greater memory span. Finally, the left parahippocampal gyrus surface area was positively associated with memory span. This study provides the first evidence for the involvement of the *CHAT* gene in parahippocampal and hippocampal structures and memory span in healthy Chinese adults.

Key words: CHAT, parahippocampal gyrus, hippocampus, memory span, gene.

INTRODUCTION

The *CHAT* gene encodes a key enzyme for the synthesis of the neurotransmitter acetylcholine. Pharmacological studies of humans, monkeys, and rats have suggested that cholinergic receptors in the parahippocampus play a significant role in memory (Hasselmo, 2006). Studies of clinical samples (i.e., patients with Alzheimer's disease [AD], mild cognitive impairment [MCI], depression, and nicotine dependence) have provided indirect evidence linking *CHAT* to cognitive functions and parahippocampal and hippocampal structure.

First, *CHAT* has been associated with AD and MCI in a number of studies (Mubumbila et al., 2002; Kim et al., 2004; Cook et al., 2005; Ahn Jo et al., 2006; Ozturk et al., 2006; Tang et al., 2008; Grunblatt et al., 2009; Scacchi et al., 2009; Grunblatt et al., 2011; Lee et al., 2012; Xu et al., 2013). C*HAT* genetic polymorphisms were also associated with responsiveness to drug treatment (i.e., acetylcholinesterase inhibitors) in AD (Harold et al., 2006; Lee et al., 2015; Yoon et al., 2015). At the same time, AD patients have been found to show reduced parahippocampal (Echávarri et al., 2011) or hippocampal volume (Hanggi et al., 2011b), as well as impaired memory span (Ewers et al., 2012). For example, using 800 Alzheimer's Disease Neuroimaging Initiative (ADNI) participants, a recent study suggested that AD patients had a shorter digit span than healthy controls (Gibbons et al., 2012). Taken together, these results suggest that *CHAT* may be associated with hippocampal and parahippocampal structure and related cognitive functions. It should be noted, however, that a few studies did not find significant associations between SNPs in the *CHAT* gene and AD (Harold et al., 2003; Schwarz et al., 2003), which may be due to their selection of different SNPs of the *CHAT* gene or their usage of different samples (e.g., early-onset rather than late-onset AD).

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https://doi.org/10.1016/j.neuroscience.2017.11.021
0306-4522/

Second, *CHAT* genetic variants have been associated with other disorders that show hippocampal or parahippocampal abnormalities. For example, *CHAT* has been linked to depression in elderly subjects (Grunblatt et al., 2009, 2011), and late-life depression subjects have smaller parahippocampal volume than healthy controls (Andreescu et al., 2008). In addition, a smaller hippocampal volume was associated with higher severity of depression (Brown et al., 2014). *CHAT* is also associated with nicotine dependence and smoking cessation (Ray et al., 2010; Wei et al., 2010), and smaller parahippocampal gyrus volume was found in smokers than nonsmokers (Gallinat et al., 2006; Hanlon et al., 2014). In summary, the *CHAT* gene has been associated with several neuropsychological disorders that are accompanied by abnormal parahippocampal or hippocampal structure.

Finally, in a non-clinical sample of elderlies, *CHAT* genetic variants were modestly associated with cognitive function (Mengel-From et al., 2011). Specifically, the A allele of *CHAT* rs3810950, a risk allele for AD, was associated with lower composite scores on cognitive tests including forward and backward digit span.

In the present study, we explored whether *CHAT* genetic variations were associated with parahippocampal gyrus volume, surface area, and thickness; hippocampal volume; and memory span in a sample of healthy young Chinese adults. In addition, we also explored if memory span was correlated with parahippocampal or hippocampal structural indices.

EXPERIMENTAL PROCEDURES

Participants

Three hundred and twenty four healthy Han Chinese college students were recruited from a university in Beijing (age = 20.41 ± 0.88, ranging from 18 to 22 years old; 59% female). All of them had valid brain imaging, genotype, and behavioral data. Subjects with poor quality MRI scans were excluded. Based on self-report, they had normal vision and no history of mental or physical diseases. They were all unrelated to one another. Written consent was obtained from each participant. The study was approved by the Institutional Review Board of Beijing Normal University, China.

Brain imaging data collection and analysis

Brain imaging measurements were performed on a 3 T Siemens Magnetom Trio whole-body MRI scanner equipped with a 12-channel head coil at Beijing Normal University Brain Imaging Center. T1-weighted structural brain imaging data were acquired with the three-dimensional magnetization-prepared rapid gradient-echo (MPRAGE) pulse sequence (TE = 3.75 ms, TR = 2,53 0 ms, flip angle = 7°; FOV = 256 mm × 256 mm, voxel size = 1 × 1×1.33 mm^3, slice thickness = 1.33 mm, number of slices = 128). As shown in Fig. 1, FreeSurfer segmentation software (http://surfer.nmr.mgh.harvard.edu, version 6.0) was used to extract the volume of the hippocampus; the volume, surface area, and thickness of parahippocampal gyrus; and the volume of intracranial volume (ICV) (Fischl et al., 2002; Iglesias et al., 2015). The accuracy and reliability of the parcellation using FreeSurfer was evidenced by previous studies (Fischl, 2012; Bailey et al., 2013; Iglesias et al., 2015).

Genotyping

We extracted DNA from blood samples. Genotyping was performed using the standard Affymetrix genotyping protocol (Affymetrix, Inc.). As described in Table 1, 20 SNPs within the *CHAT* gene on chromosome 10 were selected (minor allele frequency (MAF) > 0.10, Hardy–Weinberg equilibrium (HWE) $p > 0.05$, and genotype call rate > 0.95), which covered most of the linkage disequilibrium (LD) blocks in the *CHAT* gene. The allele frequencies in our sample were very similar to those of the Chinese sample in the HapMap dataset.

Memory assessment

To assess memory span, all subjects were tested with the digit span task. It is a subtest of Wechsler Adult Intelligence Scale-Revised (Chinese version), which has well-established reliability and validity (Gong, 1992). It includes forward and backward digit span subtests. For the forward digit span subtest, the examiner read a list of digits at the rate of one per second, and the subject repeated the digits aloud verbatim. For the backward digit span subtest, the subject repeated the digits aloud in the reverse order. The length of the digit sequences gradually increased, starting with a sequence of three numbers to a sequence of 12 numbers in the forward subtest, and starting with a sequence of two numbers to a sequence of 10 numbers in the backward subtest. The length of the digit sequences was increased with an increment of one digit until the subject fails two consecutive trials of the same length. The span was established as the length of the longest list recalled correctly. The forward and backward digit spans were combined to form a total score, with a maximum score of 22. The total score was used as an index for memory span in the current study.

Statistical analysis

PLINK v1.07 was used for quantitative trait genetic association analysis (Purcell et al., 2007), including allelic association tests between individual SNPs and the phenotypes (i.e., the volumes, surface areas, and thickness of left and right parahippocampal gyrus; the volumes of left and right hippocampus; and memory span). The max(T) permutation approach in PLINK (10,000 permutation) was used for multiple testing correction for individual SNPs. In order to detect the associations between each SNP and the phenotypes, additive genetic linear regression models (i.e., additive effects of allele dosage) were used. MANCOVA tests were also performed with covariates in SPSS 16.0 to test genotype group differences in phenotypes. In addition, we explored correlations between memory span and parahippocampal/hippocampal structural indices in the total sample.