Neuroscience Letters 629 (2016) 33–37

Contents lists available at ScienceDirect

Neuroscience Letters

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

Research article

DNA methylation in the *NCAPH2/LMF2* promoter region is associated with hippocampal atrophy in Alzheimer's disease and amnesic mild cognitive impairment patients

Shunichiro Shinagawa[a,*], Nobuyuki Kobayashi[b], Tomoyuki Nagata[a,c], Akira Kusaka[a], Hisashi Yamada[c], Kazuhiro Kondo[b], Kazuhiko Nakayama[a]

[a] Department of Psychiatry, The Jikei University School of Medicine, Tokyo, Japan
[b] Department of Virology, The Jikei University School of Medicine, Tokyo, Japan
[c] Division of Molecular Genetics, The Jikei University School of Medicine, Tokyo, Japan

HIGHLIGHTS

- Mechanism *NCAPH2/LMF2* methylation affects the pathogenesis of AD/aMCI is uncertain.
- We investigated relationships between *NCAPH2/LMF2* methylation and other factors.
- Only hippocampal atrophy is correlated with *NCAPH2/LMF2* methylation levels.

ARTICLE INFO

Article history:
Received 25 May 2016
Received in revised form 21 June 2016
Accepted 25 June 2016
Available online 26 June 2016

Keywords:
DNA methylation
Hippocampal atrophy
Alzheimer's disease: mild cognitive impairment patients

ABSTRACT

Several studies have noted an effect of DNA methylation on the pathogenesis of Alzheimer's disease (AD). We have already reported that DNA methylation levels in the *NCAPH2/LMF2* promoter region can be a useful biomarker for the diagnosis of AD and amnesic mild cognitive impairment (aMCI). However, there is still uncertainty about the mechanism by which *NCAPH2/LMF2* methylation affects the pathogenesis of AD and aMCI. In this study, we investigated relationships between *NCAPH2/LMF2* methylation and other factors. AD (n = 30) and aMCI (n = 28) subjects were included in this study. *NCAPH2/LMF2* methylation levels were measured by pyrosequencing. Correlations between methylation levels and other factors including age at onset, sex, duration of disease, education, mini-mental state examination (MMSE) and frontal assessment battery (FAB) scores, *APOE* genotype, degree of hippocampal atrophy, and total brain atrophy were measured. Degrees of hippocampal atrophy and total brain atrophy were measured by VSRAD (Voxel-Based Specific Regional Analysis System for Alzheimer's Disease). Regression analysis revealed that only hippocampal atrophy according to VSRAD is a significant dependent variable correlated with *NCAPH2/LMF2* methylation levels. Our results suggest that DNA methylation in the *NCAPH2/LMF2* promoter region is associated with hippocampal atrophy through apoptosis.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder in which patients typically show memory deficit, decline in activities of daily living, and behavioural and neuropsychiatric problems [1]. The disease slowly progresses through a prodromal phase to the stage of amnesic mild cognitive impairment (aMCI) when symptoms are manifested.

In the last few years, several studies have reported that DNA methylation is associated with specific genes in AD patients [2–5]. Recently, we reported that the DNA methylation in the *NCAPH2/LMF2* promoter region, examined by pyrosequencing, was significantly different in AD and aMCI subjects, compared to normal subjects [6]. We concluded that *NCAPH2/LMF2* methylation levels can potentially be a convenient and useful biomarker for diagnosis of AD and aMCI. However, the mechanism by which *NCAPH2/LMF2* methylation affects the pathogenesis of AD and aMCI remains uncertain.

One commonly used approach to uncover the AD pathologic process is to study associations with well-established disease

* Corresponding author.
E-mail address: shinagawa@jikei.ac.jp (S. Shinagawa).

http://dx.doi.org/10.1016/j.neulet.2016.06.055
0304-3940/

characteristics. According to the amyloid cascade theory, amyloid beta (Aβ) accumulation is the earliest event in the pathophysiological process of AD, which is associated with synaptic dysfunction, neurodegeneration, and eventual neuronal loss [7]. Age, *APOE* – the major genetic risk factor for AD – as well as other specific host factors, such as cognitive reserve including education, may influence the pace of progression toward the clinical manifestations of AD [1]. Neurodegeneration and neuronal loss are represented by cortical atrophy, which usually begins in the entorhinal cortex and hippocampus area before spreading into other parts of the temporal and parietal cortex and finally to most association cortex, based in part on magnetic resonance imaging (MRI) studies [8,9]. Thus, in patients with early AD or aMCI, cortical atrophy of the entorhinal cortex and hippocampus area is a characteristic finding of brain MRI and this serves as a valuable biological marker.

NCAPH2 is the gene that codes for Condensin-2 complex subunit H2, which is associated with mitosis and meiosis. This means that *NCAPH2/LMF2* methylation can affect the apotosis of neural cells [10]. In order to investigate a possible mechanism by which *NCAPH2/LMF2* methylation affects the pathogenesis of AD and aMCI, we hypothesized that *NCAPH2/LMF2* methylation levels are associated with neuronal loss, especially as represented by hippocampal atrophy and other cortical atrophy- the most well established structural AD biomarkers to date.

Recently, a statistical imaging analysis system, which has been given the name voxel-based specific regional analysis system for Alzheimer's disease (VSRAD) and uses Statistical Parametric Mapping (SPM) to quantify the volume of medial temporal structures including the entire region of the entorhinal cortex and hippocampus using an unbiased automated method has been developed [11,12]. With VSRAD, we can easily measure the atrophy in the hippocampal area, and the extent of the region with significant atrophy in the whole brain.

In the present study, we aimed to determine a possible mechanism by which *NCAPH2/LMF2* methylation affects the pathogenesis of AD and aMCI, and investigated relationships between *NCAPH2/LMF2* methylation levels and other background factors, including hippocampal atrophy and total brain atrophy, using VSRAD.

2. Materials and methods

2.1. Ethics statement

The study was approved by the Ethics Committee of the Jikei University School of Medicine, and written informed consent was obtained from all subjects. For participants whose capacity to consent was compromised, caregivers who were the spouse or a relative within the second degree consented on their behalf. For patients with aMCI or AD who were able to sign the consent form, if there was any possibility of them forgetting that they had consented to participation, written informed consent was obtained from both patients and caregivers.

2.2. Subjects

From among consecutive memory clinic outpatients visiting the Jikei University Hospital (Tokyo) or the Jikei University Kashiwa Hospital (Kashiwa City, Chiba Prefecture), 30 patients with AD and 28 patients with aMCI were enrolled (same sample as used in our previous study) [6]. AD was diagnosed based on the US National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria and aMCI by the criteria defined by Peterson [13,14]. aMCI included both amnestic MCI-single domain- and MCI-multiple domain-type subjects. The neuropsychiatric symptoms for AD were assessed based on information from a structured interview with each patient's caregiver by geriatric psychiatrists using the behavioral pathology in Alzheimer's disease (Behave-AD) scale [15]. Two neuropsychological tests, mini-mental state examination (MMSE) and frontal assessment battery (FAB), were administered to AD and aMCI patients by a clinical psychologist [16,17]. MMSE is widely used for assessment of cognitive function and FAB is an effective tool for assessment of executive function. Global disease severity was measured by The Clinical Dementia Rating Scale Sum of Boxes (CDR-SOB) score [18]. Genomic DNA was extracted from peripheral blood leucocytes using a standard method [19].

2.3. APOE *genotyping*

APOE genotypes (rs429358 and rs7412) were determined by allelic discrimination on an Applied Biosystems 7300 real-time PCR System (Applied Biosystems). The amplifications were performed in duplicate in a total volume of 25 μl containing 12.5 μl of 2× TaqMan Universal PCR Master Mix II, No AmpErase UNG, 0.625 μl of 40× Primer and TaqMan Probe dye mix (assay ID C____3084793_20 and C ____904973_10) (Applied Biosystems), 1.0 μl of the genomic DNA, and 10.875 μl of PCR-grade water. The thermal profile was 50 °C for 2 min and 95 °C for 10 min, followed by 40 cycles of 92 °C for 15 s and 60 °C for 1 min.

2.4. *Pyrosequencing*

We chose a pyrosequencing method, in order to figure the methylation level of specific *NCAPH2/LMF2* promoter region. Pyrosequencing was conducted according to the standard protocol. Briefly, 500 ng of each genomic DNA sample was bisulfite-converted using EpiTect Plus DNA Bisulfite Kit (Qiagen, Inc.) and the bisulfited DNA was sequenced using PyroMark Q24 System (Qiagen, Inc.). The details of the pyrosequencing procedure are given in our previous paper [6]. Four CpGs were included in the pyrosequencing area, and the first one was analyzed as it is considered to have the most representative methylation levels.

2.5. *Brain MRI imaging*

All the subjects' brain MRI examinations were performed using a 1.5-T MRI system (EXCELART, Toshiba, Japan). Three-dimensional volumetric acquisition of a T1-weighted gradient echo sequence at 22.4/5.5/1 (TR/TE/excitation) produced a gapless series of continuous, thin sagittal sections with the following parameters: flip angle, 35°; matrix, 256 × 256; field of view, 22 × 22 cm; section thickness, 2.00 mm.

2.6. *Voxel-based MRI analysis*

Voxel-based morphometry (VBM) analysis using SPM8 plus DARTEL has been developed for the automated diagnosis of very early Alzheimer's disease (AD), and given the name voxel-based specific regional analysis system for Alzheimer's disease (VSRAD) [11,12]. First, brain MRIs were spatially normalized with only a 12-parameter affine transformation to the SPM template so as to correct for differences in brain size. These linearly transformed images were nonlinearly transformed and then modulated to the customized template for DARTEL, followed by smoothing using an 8-mm FWHM kernel. This procedure revealed a significant decline in gray matter (GM) in the bilateral entorhinal cortex and parahippocampal gyrus. The region of interest (ROI) was fixed within the bilateral entorhinal cortex and the parahippocampal gyrus, and the severity of the gray matter atrophy in