Single-Nucleotide Polymorphisms of Tight Junction Component Claudin-1 Associated with Leukoaraiosis

Binod Kumar Yadav, PhD,*† and Byoung-Soo Shin, MD, PhD*‡

Background: The blood–brain barrier (BBB) plays a major role in the development of leukoaraiosis (LA). The junctional complex of BBB consists of tight junction (TJ) and adherens junction (AJ). Claudin-1 is the integral component of TJ. The aim of this study was to evaluate whether genetic variations in claudin-1 gene are associated with the development of LA. *Methods:* LA has to be diagnosed based on images. A total of 228 LA cases and 203 controls were enrolled from the individuals who underwent brain magnetic resonance imaging with obtainable vascular risk factors. Genotyping of claudin-1 single-nucleotide polymorphisms (SNPs) (*rs17501010*, *rs893051*, and *rs9290927*) was performed by real-time polymerase chain reaction with LightSNiP reagents (coupled primer and probe) and FastStart DNAMaster HybProbe (Roche Diagnostic, GmBH, Mannheim, Germany) in LightCycler 2.0. *Results:* Among the 3 SNPs of claudin-1, a significant genetic difference was found only between control and LA (both LA-periventricular white matter [PVWM] and LA-subcortical deep white matter) with SNP *rs9290927*. However, their haplotypes G-G-T and G-C-A were significantly different between LA-PVWM and control, which increase the development of LA-PVWM with odds ratios of 1.45 and .57, respectively. *Conclusions:* This study demonstrated first evidence of genetic polymorphism of TJ component claudin-1 and their haplotypes associated with LA. **Key Words:** Leukoaraiosis—claudin-1—blood–brain barrier—genetic polymorphism.

Introduction

Leukoaraiosis (LA), one of the manifestations of cerebral small-vessel disease,[1] is defined as confluent white matter irregularities, demonstrating hypodensity on computed tomography, or hyperintensity on T2-weighted or fluid-attenuated inversion-recovery image on magnetic resonance imaging (MRI). LA can be focal, patchy, or diffuse area in periventricular white matter (PVWM) and/or subcortical deep white matter (DWM).[2] Several studies from neuroimaging, neuropathology, epidemiology, and experimental models concluded that disruption of the blood–brain barrier (BBB) plays a key role in LA and lacunar stroke.[3] BBB is composed of endothelial cells, capillary basement membrane, astrocyte end-feet ensheathing the vessels, and pericytes embedded within the basement membrane. Brain microvascular endothelial cells situated at the interface between the blood and the brain are the main component of BBB and perform major biological functions.[4] The junctional complexes in the BBB include adherens

From the *Research Institute of Clinical Medicine of Chonbuk National University-Biomedical Research Institute of Chonbuk National University Hospital, Jeonju, Chonbuk, Republic of Korea; †Department of Biochemistry, Maharajgunj Medical Campus, Institute of Medicine, Tribhuvan University, Kathmandu, Nepal; and ‡Department of Neurology, Chonbuk National University Medical School, Jeonju, Chonbuk, Republic of Korea.

Received March 13, 2015; accepted March 25, 2015.

This study was supported by fund of Biomedical Research Institute, Chonbuk National University Hospital, South Korea. The biospecimens and data used in this study were provided by the Biobank of Chonbuk National University Hospital, a member of the Korea Biobank Network, which is supported by the Ministry of Health, Welfare and Family Affairs. All samples derived from the Korea Biobank Network were obtained with informed consent under institutional review board–approved protocols.

All authors declare that there are no conflict of interests.

Address correspondence to Byoung-Soo Shin, MD, PhD, Department of Neurology, Chonbuk National University Medical School, 20 Geonji-ro, Jeonju, Chonbuk 561-712, Republic of Korea. E-mail: sbsoo@jbnu.ac.kr.

1052-3057/$ - see front matter

http://dx.doi.org/10.1016/j.jstrokecerebrovasdis.2015.03.038

junctions (AJs) and tight junctions (TJs). AJs are responsible for keeping the cells together, giving structural support to tissues in BBB. They are also required for the formation of tight junctions. The disruption of AJs results in barrier disruption.[5] Tight junctions (TJs), present between the cerebral endothelial cells, form a diffusion barrier, which selectively excludes most blood-borne substances from entering the brain. TJs are composed of TJ proteins occludin, claudins, junctional adhesion molecules, zonula occludens (ZO1, ZO2, ZO3), cingulin, AF6, and 7H6, which are responsible for controlling the permeation of polar solutes between the endothelial cells and from the blood plasma to the brain extracellular fluid.[6,7]

Claudins are 22-kDa phosphoprotein having 4 transmembrane domains.[8] There are around 20 isoforms of claudin known (claudin1-20).[9] Claudins are one of the major components of TJs, which are placed entirely at TJ strands. Claudins form dimers and bind homotypically to other claudin molecules in adjacent brain microvascular endothelial cells to form primary seal of the TJ.[8] Both claudin-1 and claudin-5, together with occludin, are present in endothelial TJs forming the BBB[10,11] and are associated with maintenance of normal BBB function.[12] Several studies have demonstrated the loss of clauidn-1 from cerebral vessel under pathologic conditions such as colorectal carcinoma,[13] breast cancer,[14] and stroke.[15] All these findings showed that claudin-1 is one of the important structural and functional units of BBB and it seems, therefore, a good candidate gene for LA. Different studies have already demonstrated the association of claudins genetic polymorphism with colon carcinoma,[16] inflammatory bowel disease,[17] contact sensitization,[18] autoimmune encephalitis,[19] and schizophrenia.[20] Till date, none of the studies has evaluated the genetic roles of clauidn-1 in LA, and we hypothesized that alternation in clauidn-1 gene may be one of the causes of BBB abnormality, a well-known cause of LA. To prove this hypothesis, we designed this case–control association study in patients with LA.

Materials and Methods

Study Subjects

LA has to be diagnosed based on images. After getting the ethical committee approval from Chonbuk National University Hospital, a total of 228 LA cases (LA-PVWM, 183; LA-DWM, 156) and 203 controls were enrolled from the individuals visiting the neurology clinic, who underwent brain MRI with obtainable vascular risk factors. The selection of patient and the clinical check-up procedures were the same as our previous study.[21] LA was defined as a bilateral and symmetrical area in the periventricular and centrum semiovale white matter lesion with hyperintensities on T2-weighted and fluid-attenuated inversion-recovery images. Extension and severity of white matter changes were rated according to the scale of Fazekas et al.[22] Fazekas scale graded LA as follows: 0 (absent or no lesion), 1 ("cap" or pencil-thin lining), 2 (smooth "halo"), and 3 (irregular periventricular signal extending into the DWM) of PVWM and 0 (absent or no lesion), 1 (punctate foci), 2 (beginning confluence), and 3 (large confluent areas) of DWM. As punctuate and "cap" lesions have no clinical significance in neurological disease, the subjects were categorized into control (grades 0 and 1) and case (grades 2 and 3). The history of hypertension, diabetes mellitus, and vascular risk factors was recorded.

Blood Collection

In fasting state, a total of 10 ml of venous blood was collected from each participant and divided into 2 vials:

Table 1. *Demographic and clinical characteristics in control and patients with LA*

Characteristics	Control (n = 203)	LA-PVWM (n = 183)	*P*	LA-DWM (n = 156)	*P*
Age	61.66 ± 13.01	74.46 ± 7.76	<.0001	73.44 + 8.87	<.0001
Gender (male), n (%)	127 (62.6)	91 (49.7)	.014	81 (51.9)	.012
Hypertension, n (%)	112 (55.4)	134 (73.2)	<.0001	113 (72.4)	.016
Diabetes (%)	58 (28.7)	65 (35.5)	.157	52 (33.3)	.855
Cholesterol (mg %)	166.13 ± 43.51	169.21 ± 41.13	.47	168.97 + 41.29	.617
Triglyceride (mg %)	147.12 ± 130.59	131.62 ± 104.08	.20	126.46 + 98.13	.81
HDL-Cholesterol (mg %)	40.40 ± 10.87	41.26 ± 12.02	.45	41.36 + 12.37	.39
LDL-Cholesterol (mg %)	98.43 ± 34.89	97.92 ± 31.91	.88	98.36 + 31.74	.973
ApoA1 (mg %)	1.16 ± .24	1.19 ± .26	.43	1.20 + .27	.212
ApoB (mg %)	.96 ± .29	.93 ± .25	.30	.92 + .26	.237
Lp(a) (mg %)	30.42 ± 26.09	28.23 ± 26.13	.46	26.21.86	.14
Glucose (mg %)	144.81 ± 65.91	141.79 ± 81.76	.59	140.52 + 85.08	.523
HbA1C (%)	6.36 ± 3.53	6.21 ± 1.38	.69	6.19 + 1.40	.568
tHCy (μmol/L)	13.26 ± 6.30	14.34 ± 5.78	.08	14.48 + 5.67	.057

Abbreviations: Apo A1, Apolipoprotein A1; ApoB, Apolipoprotein B; DWM, deep white matter lesion; HDL, high-density lipoprotein; LA, leukoaraiosis; LDL, low-density lipoprotein; Lp(a), lipoprotein a; PVWM, periventricular white matter lesion; tHCy, total plasma homocysteine.