Available online at www.sciencedirect.com
ScienceDirect
Clinical Biochemistry 42 (2009) 1493 – 1499
CLINICAL BIOCHEMISTRY

Haplotype-based case–control study between human apurinic/apyrimidinic endonuclease 1/redox effector factor-1 gene and cerebral infarction

Takahiro Naganuma [a,b], Tomohiro Nakayama [a,c,*], Naoyuki Sato [a], Zhenyan Fu [a], Mai Yamaguchi [a], Masayoshi Soma [d], Noriko Aoi [a], Ron Usami [b], Nobutaka Doba [e], Shigeaki Hinohara [e]

[a] *Division of Molecular Diagnostics, Department of Advanced Medical Science, Nihon University School of Medicine, Tokyo, Japan*
[b] *Department of Biological Applied Chemistry, Toyo University Graduate School of Engineering, Saitama, Japan*
[c] *Division of Laboratory Medicine, Department of Pathology and Microbiology, Nihon University School of Medicine, Ooyaguchi-kamimachi, 30-1 Itabashi-ku, Tokyo 173-8610, Japan*
[d] *Division of General Medicine, Department of Medicine, Nihon University School of Medicine, Tokyo, Japan*
[e] *Life Planning Center, Tokyo, Japan*

Received 28 February 2009; received in revised form 4 July 2009; accepted 14 July 2009
Available online 23 July 2009

Abstract

Objectives: The aim of this study was to investigate the relationship between cerebral infarction (CI) and the human apurinic/apyrimidinic endonuclease 1/redox effector factor-1 (APE1/REF-1) gene using single-nucleotide polymorphisms (SNPs) and a haplotype-based case–control study.

Design and methods: We selected 5 SNPs in the human APE1/REF1 gene (rs1760944, rs3136814, rs17111967, rs3136817 and rs1130409), and performed case–control studies in 177 CI patients and 309 control subjects.

Results: rs17111967 was found to have no heterogeneity in Japanese. The overall distribution of the haplotype-based case–control study constructed by rs1760944, rs3136814 and rs1130409 showed a significant difference. The frequency of the G-C-T haplotype was significantly higher in the CI group than in the control group (2.5% vs. 0.0%, $p > 0.001$).

Conclusions: Based on the results of the haplotype-based case–control-study, the G-C-T haplotype may be a genetic marker of CI, and the APE1/REF-1 gene may be a CI susceptibility gene.

Keywords: Cerebral infarction; Apurinic/apyrimidinic endonuclease 1/redox effector factor-1; Single-nucleotide polymorphism; Haplotype; Case–control study

Introduction

Cerebral infarction (CI) is the most common type of stroke, and often causes long-term disability [1]. In Japan, CI has recently become a major cause of death in the elderly population. CI is considered to be a multifactorial disease that results from interaction between genetic and environmental factors [2].

Apurinic/apyrimidinic endonuclease 1/redox effector factor-1 (APE1/REF-1) is a protein with multifunctional roles impacting a wide variety of important cellular functions [3]. APE1/REF-1 has 2 major functions: it acts as an apurinic/apyrimidinic endonuclease during the second step of the DNA base excision repair pathway, which is responsible for the repair of cellular oxidative DNA damage [4]; and it is also known as redox effector factor-1 (REF-1), which is important for the activation of transcription factors, such as activator protein 1 (AP1), p53 and nuclear factor kappaB (NF-kB) [5,6]. The human APE1/REF-1 gene is located on chromosome 14q11.2–q12, which consists of 5 exons and 4 introns spanning 2.64 kilobase pairs [7]. Human APE1/REF-1 is encoded by a gene is composed of 318 amino acids [8]. The apurinic/apyrimidinic endonuclease activity of APE1/REF-1 resides in the N-terminus, while the redox activity resides in the C-terminus [8,9].

Oxidative stress, or excess generation of reactive oxygen species (ROS), results in cellular damage and is thought to be a factor in various diseases, such as cancer, leukemia, CI,

* Corresponding author. Division of Laboratory Medicine, Department of Pathology and Microbiology, Nihon University School of Medicine, Ooyaguchi-kamimachi, 30-1 Itabashi-ku, Tokyo 173-8610, Japan. Fax: +81 3 5375 8076.
E-mail address: tnakayam@med.nihon-u.ac.jp (T. Nakayama).

0009-9120/$ - see front matter © 2009 The Canadian Society of Clinical Chemists. Published by Elsevier Inc. All rights reserved.
doi:10.1016/j.clinbiochem.2009.07.016

myocardial infarction (MI) and hypertension [10,11]. DNA is one of the biological molecules damaged by oxidative stress, and it has been reported that oxidative DNA damage is elevated in hypertension and cardiovascular diseases [12,13].

There have been no studies examining the association between the human APE1/REF-1 gene and cerebral infarction. However, it has been reported that mice heterozygous for the APE1/REF-1 allele (APE1/REF-$1^{+/-}$) were significantly more hypertensive than wild-type mice (APE1/REF-$1^{+/+}$) [9,14]. Therefore, hypertension is closely related to CI, and the APE1/REF-1 gene is thought to be a mediator in CI. Jeon et al. [9] showed that APE1/REF-1 regulated H-ras expression through its reducing function. Moreover, they placed H-ras upstream of phosphoinositide-3 kinase (PI3-K) and Akt kinase in the calcium sensitization of endothelial NO synthase by APE1/Ref1. The reducing function of APE1-REF1 may be associated with cerebral infarction.

The aim of this study was to investigate the relationship between CI and the human APE1/REF-1 gene using single-nucleotide polymorphisms (SNPs) and a haplotype-based case–control study.

Methods

Subjects

Participants in whom CI was diagnosed were recruited at Nihon University Itabashi Hospital in Tokyo, Japan, as well as neighboring hospitals, between 1993 and 2003. We selected 177 patients with CI diagnosed by neurologic examination and the findings of computed tomography (CT), magnetic resonance imaging (MRI) or both, and those who had neurologic deficit ratings greater than grade 3 on the modified Rankin Scale. Patients with hemorrhage stroke diagnosed by CT, MRI or both were excluded from this study group.

A total of 309 Japanese subjects were enrolled as control subjects. Control subjects were members of the New Elder Citizen Movement in Japan who lived in Tokyo, or in the suburbs of Tokyo, and who had vascular risk factors such as hypertension, hypercholesterolemia or diabetes mellitus, but no history of CI. They were confirmed as being grade 0 on the modified Rankin Scale. In this study group, participants with cancer or autoimmune diseases, including antiphospholipid antibody syndrome, were excluded. Patients with cerebral embolism caused by atrial fibrillation, diagnosed by anamnesis and findings of electrocardiography, echocardiography, CT or MRI were excluded. However patients in whom cerebral thrombosis was diagnosed were included. No participants had a history of peripheral arterial occlusive disease. Informed consent was obtained from each subject in accordance with the protocol approved by the Human Studies Committee of Nihon University [15,16].

Biochemical analysis

Blood samples were obtained from subjects in the morning, after resting in the sitting position for at least 30 min without eating. In the clinical laboratory department of our university hospital, these blood samples were used to measure plasma concentrations of total cholesterol and serum concentrations of creatinine [17].

Genotyping

As the detailed data for SNPs in the APE1/REF-1 gene on the Hap Map website were not clear, information on allelic

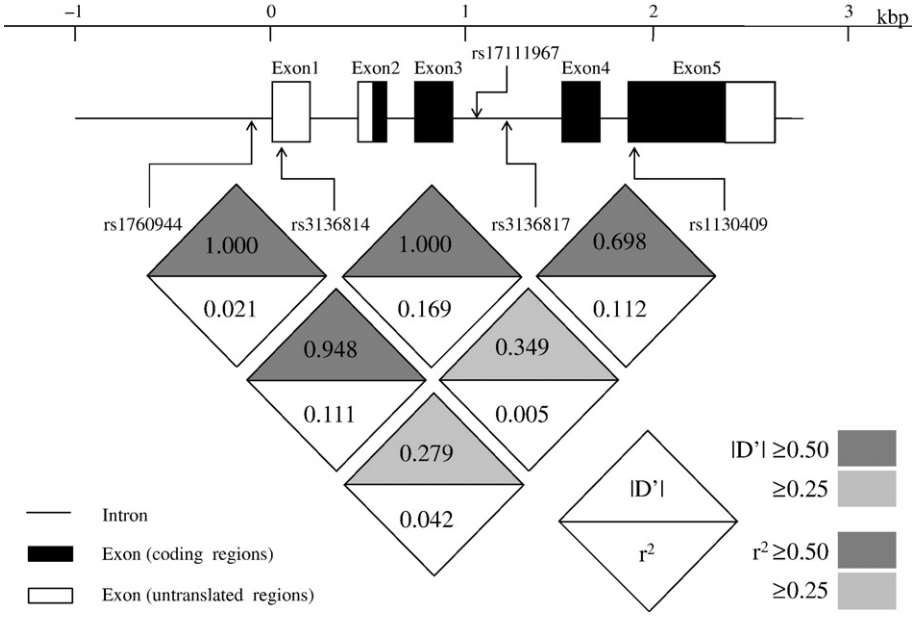

Fig. 1. Organization of the gene encoding human apurinic/apyrimidinic endonuclease-1/redox effector factor-1 (APE1/REF-1), locations of single-nucleotide polymorphisms (SNPs) used in present association study, and pair-wise LD in the APE1/REF-1 gene, as evaluated by $|D'|$ and r^2. Closed boxes indicate exons, and lines represent introns. Pair-wise LD among the 4 marker pairs studied in the human APE1/REF-1 gene were computed, upper triangles indicate $|D'|$ and lower triangles represent r^2. Pairs in LD ($|D'|$ or $r^2 \geq 0.5$) are shown as deep gray-shaded values, and pairs in LD ($|D'|$ or $r^2 \geq 0.25$) are shown as light gray-shaded values.