Polymorphisms of Thymidylate Synthase Enhancer Region (TSER) and Upstream Stimulatory Factor 1 (USF1 306G > A) Genes are Associated with Plasma Homocysteine Level and Susceptibility to Ischemic Stroke in a Korean Population

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ABSTRACT

The thymidylate synthase enhancer region (TSER) gene promoter has a variable tandem repeat polymorphism containing an upstream stimulatory factor (USF) family E-box binding site that affects expression of the enzyme. Also, the tandem repeat polymorphism of the TS promoter is known to affect plasma homocysteine (phcy) level. Upstream stimulatory factor 1 (USF1) is a transcription factor controlling expression of several genes involved in lipid and glucose homeostasis. We therefore investigated the influence of the TSER and the USF1 gene polymorphisms on susceptibility to ischemic stroke and phcy levels in a Korean population. The TSER and USF1 306G > A genotypes in 234 ischemic stroke patients and 203 age and sex-matched controls subjects were identified by PCR. Plasma hcy levels were measured by fluorescent polarizing immunoassay. Frequencies of USF1 306G > A genotypes and TSER variants were not significantly different between the controls and patients with ischemic stroke. The stroke patients, however, had significantly higher phcy levels with the TSER 2R3R+2R2R genotype than the control group. Also, phcy levels differed according to combined TSER and USF1 306G > A genotypes. The combined TSER 2R3R+2R2R and USF1 306GA+AA genotypes and the phcy concentration of the patient group were also higher than those of the control group (p = 0.027). In addition, the phcy concentration of the combined TSER 2R3R+2R2R and USF1 306AA genotype was higher than that of the combined TSER 3R3R and USF1 306GG genotype (p = 0.004) in the patient group. Our results suggest that TSER and USF1 306G > A polymorphisms may influence total homocysteine levels, leading to an increased risk of ischemic stroke.

Key words: thymidylate synthase enhancer region (TSER), upstream stimulatory factor (USF), ischemic stroke, homocysteine, polymorphism.

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INTRODUCTION

Thymidylate synthase (TS) is involved in the conversion of dUMP to dTMP in the folate metabolic pathway, which is an important process for DNA synthesis and repair. TS is an important target for a variety of chemotherapeutic agents such as 5-fluorouracil. Furthermore, altered TS gene expression is regarded as a risk factor for carcinogenesis (Danenberg 1977). The TS mRNA 5’-untranslated region (UTR), thymidylate synthase enhancer region (TSER), contains a tandem repeat element that influences the efficiency of TS gene expression. The 3R allele of the TSER gene is more actively expressed than the 2R allele in vitro and in vivo (Horie et al., 1995; Kawakami et al., 1999). Furthermore, the tandem repeats influence plasma folate and homocysteine levels. In healthy Singaporeans, the 3R3R genotype of TSER enhances the enzyme activity and the increased TS activity raises the plasma homocysteine level (Trinh et al., 2002). On the other hand, the 2R3R TS tandem repeat polymorphism has more influence on homocysteine concentration in healthy European adults (Brown et al., 2004).

Upstream stimulatory factor 1 (USF1) is a member of the basic helix-loop-helix leucine zipper family of transcription factors (Gregor et al., 1990) and regulates the expression of several genes involved in lipid metabolism and fatty acid synthase by binding to palindromic E-box motifs in their promoter regions. Thus, USF1 underpins whole-body energy homeostasis and maintenance of blood levels of cholesterol, triglycerides and glucose (Iynedjian et al., 1998; Casado et al., 1999; Qian et al., 1999; Ribeiro et al., 2003; Salero et al., 2003). The tandem repeat region of the TS promoter is an upstream stimulatory factor (USF) binding E-box site that is important for transcription or translation of TS.

The upstream stimulatory factor 1 (USF1) gene is located on chromosome1q22-23q. While the association between USF1 306G > A gene polymorphisms and metabolic abnormalities, such as diabetes (Ng et al., 2005; Gibson et al., 2005), hyperlipidemia (Shoulders and Naoumova 2004: Pajukanta et al., 2004), and cardiovascular disease (Choquette et al., 2007) has been investigated, such associations remain debatable.

The conventional risk factors for ischemic stroke include hyperhomocysteinemia, hypertension, diabetes mellitus, smoking and inflammation. We hypothesized that the USF1 gene polymorphism is associated with the risk of stroke and is involved in homocysteine regulation together with TSER tandem repeat variations. In the present study, we investigated whether the combined genotypes of TSER and USF1 are associated with an increased risk for ischemic stroke and influence the homocysteine level in a Korean population.

MATERIALS AND METHODS

Subjects and DNA isolation

The study population was composed of 234 patients with ischemic stroke and 203 age and sex-matched control subjects. The patients were enrolled from November 2000 to May 2002 at Bundang CHA Hospital, College of Medicine, Pochon CHA University. Ischemic stroke was diagnosed when neurological deficits were accompanied by corresponding abnormal magnetic resonance imaging (MRI) findings of the brain, as interpreted by two independent experienced neurologists. Ischemic stroke was excluded when these researchers did not agree, and patients with cerebral hemorrhage were also excluded (Choi et al., 2003). For the control subjects, we selected healthy gender- and age-matched individuals from those attending wellness examinations at Bundang CHA Hospital during the same period; these subjects were without a history of cerebrovascular disease or myocardial infarction. Some members of the ischemic stroke and control groups were diagnosed with hypertension or diabetes mellitus when the diagnostic criteria were fulfilled at the time of enrollment. Relevant information on past medical history and smoking habits were obtained from all subjects. Informed consent was obtained from all study participants after receiving a full explanation of the study.

DNA was extracted from leukocytes using a G-DEX™ II Genomic DNA Extraction Kit (iNtRON BIOTECHNOLOGY, Seongnam, South Korea) according to the manufacturer’s protocol.