BIOMARKERS, VOL. 9, NO. 3 (MAY–JUNE 2004), 291–297

Taylor & Francis
healthsciences

Evaluation of high sensitive C-reactive protein and 5-10 methylenetetrahydrofolate reductase genotype in Japanese young adults

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Received 19 July 2004, revised form accepted 6 October 2004

Primary objective: To carry out a preliminary evaluation of subclinical inflammation and its genetic background in young adults.

Research design: Fifty-five healthy Japanese young adults aged 19–27 years (37 males and 18 females, mean age: 22.3 years), and 58 healthy Japanese adults aged 40 to 60 years (21 males and 37 females, mean age: 51.5 years) were included in this study.

Methods and procedures: We measured plasma high-sensitive C-reactive protein (hs-CRP) levels and screened for the C677T polymorphism of the 5-10 methylenetetrahydrofolate reductase gene (*MTHFR*), which is considered a genetic risk factor for atherosclerosis, by *HinfI* digestion.

Main outcomes and results: Hs-CRP levels of the young adult group were significantly lower than the levels of the middle aged group (0.014 ± 0.030 mg/dl vs. 0.031 ± 0.040 mg/dl, $p = 0.005$). The levels were significantly higher in males than in females (0.028 ± 0.019 mg/dl vs. 0.013 ± 0.010 mg/dl, $p = 0.008$) among young adults. Furthermore, we evaluated the relationship of the C677T genotype and hs-CRP values, but found no association between them.

Conclusions: Although the sample size is limited, our preliminary study demonstrated the profiles of hs-CRP in Japanese young adults. Further investigation will be needed to establish the guidelines for customized school health education using sensitive laboratory and genetic markers.

Keywords: young adult, high sensitive CRP (hs-CRP), 5-10 methylenetetrahydrofolate reductase (MTHFR), School Health Counseling.

Introduction

C-reactive protein (CRP) levels predict coronary events in patients with cardiovascular disease (CVD) as well as in apparently healthy subjects (Liuzzo *et al.* 1994, Ridker *et al.* 1997). Recently, assays of high-sensitive CRP (hs-CRP) have been developed that allow variation in normal hs-CRP levels to be studied within a person and between subjects (Macy *et al.* 1997). In adults, elevated levels of hs-CRP are associated with smoking, increasing age, obesity, triglycerides levels, and various markers of cardiovascular diseases (Haverkate *et al.* 1997, Reynolds and Vance 1987). Several prospective studies have shown that hs-CRP

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Biomarkers ISSN 1354-750X print/ISSN 1366-5804 online © 2004 Taylor & Francis Ltd
http://www.tandf.co.uk/journals
DOI: 10.1080/13547500400017846

concentration is the most sensitive predictor of cardiovascular events in adults (Mendall *et al.* 1996, Ridker 1998).

Accumulated evidence suggests that the risk of arteriosclerosis begins in childhood continuing through adulthood when several cardiovascular risks appear. Obesity in children and young adults is associated with the development of hypertension, dyslipidemia, and hyperinsulinemia during adulthood (Srinivasan *et al.* 1996, Misra 2000). Although screening of hs-CRP in children and adolescents has been performed (Cook 2000, Visser *et al.* 2001, Ford *et al.* 2001, Barbeau *et al.* 2002, Jarvisalo *et al.* 2002, Vikram *et al.* 2003), less attention has been given to young adults. However, with regards to the relationship between the beginning age of smoking and vascular damage, the importance of hs-CRP screening in this generation should be emphasized.

Hyperhomocysteinemia has been identified as a risk factor for cerebrovascular, peripheral vascular, and coronary heart diseases (De Bree *et al.* 2002). Elevated levels of homocysteine can result from genetic, nutritional, and lifestyle factors (Engbersen *et al.* 1995, Refsum *et al.* 1998), and ongoing debate exists about the relative contribution of each one. The cDNAs of cystathionine β-synthase (CBS), methyleneterahydrofolate reductase (MTHFR), methionine synthase (MTR), and methionine synthase reductase (MTRR) have been cloned and analyzed for functional polymorphisms that affect homocysteine/folate metabolism. The most extensively studied variant is a C677T (Ala222Val) transition in MTHFR leading to a mildly dysfunctional "thermolabile" enzyme (De Bree *et al.* 2002, Frosst *et al.* 1995). Although several studies on linkage between hs-CRP and C677T have been conducted in the general population (De Bree *et al.* 2002), data on young adults are scanty. For young adults, the most important risk for vascular damage is considered to be smoking. However, genotype may also contribute to small vascular damage even in the early stages.

For the appropriate health education at an early stage to prevent the future atherosclerosis, it is important to establish the sensitive makers to detect the micro vascular changes even in adolescents and young adults. Furthermore, it is also important to know the genetic background on the risk of atherosclerosis, in order to evaluate its effects to the vascular changes among them. In this point of view, we evaluated the hs-CRP levels in healthy Japanese young adults and their MTHFR genotype to establish recommendations for "tailor-made school health education" for them.

Materials and methods

Before the study, ethical approval was obtained from the special committee of the Nagasaki University (project registration no. 04011900500). After obtaining informed consent, blood samples were obtained from 55 healthy students at Nagasaki University of Medical School aged 19–27 years (37 males and 18 females, mean age: 22.3 years), and from 58 healthy adults aged 40–60 years (21 males and 37 females, mean age: 51.5 years), living in "O" town of Nagasaki Prefecture. The mean body mass index (BMI) of the young adults were 21.8 for males and 19.9 for females, respectively. For the middle-aged adults, the BMIs for males and females were 22.8 and 23.6, respectively.

Plasma hs-CRP concentrations were measured using N-Latex CRP II (Dade Behring, Tokyo, Japan). The lowest detectable limit was 0.001 mg/dl.

Genomic DNA was extracted from blood cells using a QIAamp DNA blood mini kit (Qiagen, Tokyo, Japan). The *MTHFR* gene was amplified by polymerase chain reaction (PCR) using a set of primers that we previously prepared (Takamura *et al.* 2004). The C to T substitution at nucleotide 677 in the

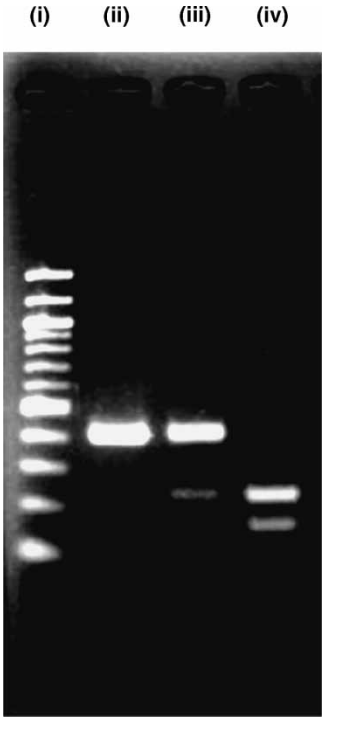

Figure 1. Genetic screening to detect C677T genotype by HinfI digestion. i) size marker, ii) wild type (−/−), iii) heterozygous (+/−) and iv) homozygous (+/+) of C677T.

MTHFR gene introduces a restriction site for the *Hinf*I enzyme. This enzyme was used to distinguish the alleles by cleaving the mutant fragment into 226 and 165 base pair fragments on 2% agarose gel (figure 1).

Results of hs-CRP concentrations are expressed as mean ± standard deviation (SD). Comparisons between young adults and middle-aged adults and between males and females were evaluated using the *t*-test.

Results and discussion

Plasma levels of hs-CRP in young adults ranged from 0.002 to 0.138 mg/dl. As shown in figure 2, the level of hs-CRP in young adults was significantly lower than that of the middle aged adults (0.021 ± 0.030 mg/dl vs. 0.041 ± 0.040 mg/dl, $p = 0.005$). In young adults, the median level of hs-CRP was significantly higher in males than in females (0.029 ± 0.019 mg/dl vs. 0.013 ± 0.010 mg/dl, $p = 0.008$) (figure 3). Furthermore, we evaluated the relationship of the C677T genotype and the level of hs-CRP; however, no relationship between them was observed (wild type ($n = 20$) and heterozygous ($n = 26$) vs. homozygous ($n = 9$): 0.020 ± 0.036 mg/dl vs. 0.015 ± 0.009 mg/dl, $p = 0.231$).

In this study, we demonstrated that the level of hs-CRP is lower in young adults than in middle-aged adults. Also we showed that the level of hs-CRP is higher in males than in females in young adults. In our group, obesity (BMI ≥ 25) was not observed, and only six of the 53 young adults were smokers (four males and two females) in young adults. Furthermore, the BMIs of the middle-aged adults were

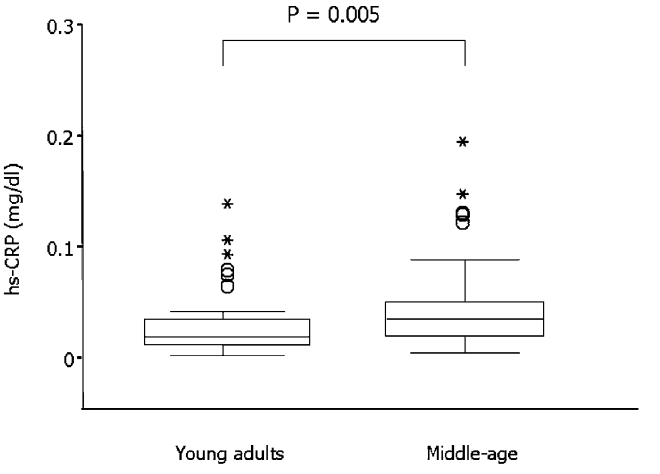

Figure 2. The comparison of plasma hs-CRP between young adults and middle age.

24.3 ± 2.7 for males and 22.9 ± 3.2 for females, and five females and one male were obese. In these observations, we believe that both groups (young adults and middle age) are appropriate choices as a "healthy" population for the evaluation of subclinical inflammation markers.

Although several studies have reported the measurements of hs-CRP in healthy children or adolescents (Cook *et al.* 2000, Visser *et al.* 2001, Ford *et al.* 2001, Barbeau *et al.* 2002, Jarvisalo *et al.* 2002), little data on healthy young adults is available. Vikram *et al.* screened healthy adolescents and young adults aged 14–25 years in urban North India for hs-CRP levels, and found that the median level of hs-CRP was higher in males (Vikram *et al.* 2003). Although our sample size was small, the current study also showed a difference in hs-CRP levels between males and females in Japanese young adults. Besides adipose tissue, which is an important source of proinflammatory cytokines, interleukin-6 (IL-6) and estrogen should be

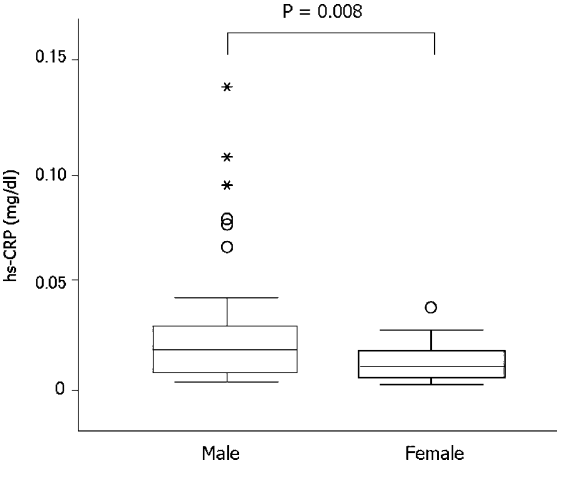

Figure 3. The comparison of plasma hs-CRP between males and females in young adults.

considered key factors for this difference. Several studies have shown the link between menopause and an increased incidence of cardiovascular disease. Interestingly, recent studies have shown that oral postmenopausal hormone treatment is associated with increased hs-CRP levels (Cushman *et al.* 1999; Pradhan *et al.* 2002); however, increased hs-CRP concentrations in these studies were not accompanied by an elevation in other acute phase reactants, such as IL-6, E-selectin, and fibrinogen. These findings suggest that the effects of postmenopausal hormones on hs-CRP do not represent a generalized proinflammatory effect mediated through upstream cytokines such as IL-6 but arise from a secondary mechanism (Miller *et al.* 2003). Furthermore, the finding that hs-CRP levels do not elevate by transdermal estradiol suggests that this secondary mechanism might be a first pass effect of hs-CRP production in the liver after oral estrogen absorption, an effect that is avoided by transdermal delivery (Decensi *et al.* 2002, Vongpatanasin *et al.* 2003). From these observations, an intrinsic estrogen, which differs from extrinsic estrogen, might play a role in the reduction of circulating hs-CRP levels in females. Further research will be needed to clarify this point.

In this study, we could not identify a relationship between hs-CRP levels and the C677T polymorphism of *MTHFR* in young adults. Although our small sample size could explain the lack of association, it may suggest that association between hs-CRP levels and the C677T polymorphism occurs at a later age, according to aging (Demuth *et al.* 1998). Since it has been reported that mild homocysteinemia caused by a homozygous C677T genotype is a significant risk factor for cerebrovascular, peripheral vascular and coronary heart diseases (De Bree *et al.* 2002), careful follow-up of this generation will be important to understanding the significance of the genotype in the development of arteriosclerosis.

Our study demonstrated the profiles of hs-CRP levels and *MTHFR* genotype in Japanese young adults. In this study, the sample size was relatively small. Further studies are needed to evaluate the hs-CRP levels in healthy young adults and their MTHFR genotype, in order to establish recommendations for "tailor-made school health education" for them. Further investigation will be needed to establish the guideline of "tailor made" school health education, based on the development of sensitive laboratory and genetic markers.

Acknowledgements

We are indebted to the study participants and staff of Nagasaki University and "O" town in Nagasaki Prefecture.

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