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The synergistic effect of bone mineral density and methylenetetrahydrofolate reductase (MTHFR) polymorphism (C677T) on fractures

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Abstract A functional polymorphism in methylenetetrahydrofolate reductase (MTHFR) has been identified at codon 677 (C677T). The T-allele variant (valine type) has lower enzyme activity than the wild type (C-allele or alanine type), resulting in a slightly elevated homocysteine level, which has been recently recognized as a risk factor for fracture. However, whether subjects bearing the T allele have higher susceptibility to fractures is still controversial. We have investigated the effects of MTHFR polymorphism on fracture susceptibility in Japanese postmenopausal women. A total of 502 postmenopausal ambulatory Japanese women were followed up for 5.1 ± 3.4 (mean ± SD) years, and a total of 155 patients with incident fractures (121 patients with vertebral fractures and 34 cases with fractures at other sites) were recorded. When compared with the patients without any fractures, the patients with incident fractures were older, had more prevalent fractures, had higher urinary levels of bone turnover markers as well as plasma homocysteine level, but were shorter in body height and had lower bone mineral density. The prevalence of the TT genotype of MTHFR was significantly higher in the patients with incident fractures compared to the other genotypes. The subjects with the TT genotype had a higher incidence rate of fracture and higher plasma level of homocysteine than the subjects bearing the non-TT genotype. This relationship was observed in both osteoporotic and nonosteoporotic groups. The hazard ratio for TT genotype without osteoporosis, non-TT genotype with osteoporosis, and TT genotype with osteoporosis was 1.49 (0.91–2.45), 3.64 (2.50–5.29), and 7.21 (4.34–11.97), respectively, compared to the non-TT genotype without osteoporosis. A higher hazard ratio for the TT genotype with osteoporosis was still apparent after adjustment for age, body size, and number of prevalent vertebral fractures. These results indicate that the TT genotype of MTHFR may be a risk factor for future fracture in addition to the traditional risk factors.

Key words osteoporosis · fracture · methylenetetrahydrofolate reductase (MTHFR) · gene polymorphism · homocysteine

Introduction

Osteoporosis, which is characterized by deterioration in bone strength [1], is a national burden in an aging society because of the high susceptibility of osteoporotic patients to bone fractures, which could jeopardize the patient's quality of life [2–4] or increase mortality [5,6]. Because bone fracture prevention is the primary aim of osteoporosis treatment, an assessment of bone strength is absolutely required to determine fracture risk in an individual patient. According to the definition of osteoporosis proposed by the National Institutes of Health [1], bone strength is determined by the bone mass (bone mineral density) and bone quality. Among these two components, bone mineral density has been known to be the major determinant of future fracture risk. In fact, the measurement of bone
mineral density (BMD) has been utilized to diagnose osteoporosis [7,8]. In contrast, bone quality assessment has not been applied to clinical practice, except for bone turnover marker measurement, which has been employed to assess future fracture risks [9–12]. Methylenetetrahydrofolate reductase (MTHFR) is an important enzyme in the methionine cycle, involved in the removal of circulating homocysteine, and lies within a linkage region for the regulation of BMD in chromosome 1p36 [13]. A functional polymorphism has been identified in exon 4 of the gene that results in an alanine to valine amino acid change at codon 677 (C677T). The T-allele variant (valine type) has been reported to have a lower enzyme activity than the wild type (C-allele or alanine type) because of its heat-labile form, and to be associated with a moderately elevated homocysteine level, which has been recently recognized as a significant risk factor for fracture [14–16]. It was confirmed that this risk for fractures is independent of age and BMD, because it is the inhibition of collagen cross-links that disturbs bone calcification [17,18]. Meanwhile, clinical evidence regarding the role of MTHFR polymorphism on BMD [19–23] or fracture susceptibility is still controversial [14,24–27]. The aim of the present study was to clarify the role of MTHFR polymorphism on fracture susceptibility in relationship to BMD and serum homocysteine levels.

Materials and methods

Subject selection

The study design was a prospective observational study that started in 1993 and continued to 2006 [12]. The baseline examination was conducted with informed consent from 561 unrelated ambulatory postmenopausal volunteers living in Nagano Prefecture, Japan. Exclusion criteria were endocrine disorders such as hyperthyroidism, hyperparathyroidism, adrenal disease, diabetes mellitus with insulin treatment, renal disease, and a history of extensive gastrointestinal surgery and use of medications known to affect bone metabolism. Patients who had sustained fractures from major traumas were also excluded from the analysis. The period of follow-up for each participant was calculated as the time from inclusion in the study to the first fracture, death, missing, or to the end of 2006, whichever occurred first. All the subjects in the present study were followed up for more than 1 year (mean observational period and SD, 5.1 ± 3.4 years), except for those whose first fracture event was observed within 1 year. The termination of the observation was at the end of 2006; i.e., the longest observation time was 13 years.

Bone mineral density (BMD) measurements

Axial BMD (lumbar spine BMD, LBMD) was measured by dual-energy X-ray absorptiometry (DXA) using a Lunar DPX-L or DPX–IQ (Lunar Corporation, Madison, WI, USA). The interassay variance of LBMD in our laboratory was 0.5% ± 0.5% [coefficient of variation (CV) ± SD] [28]. To guard against machine drift, a quality assurance test was carried out at every measurement.

Definition of prevalent and incident vertebral fractures

Prevalent and incident vertebral fractures were diagnosed by a semiquantitative visual method [29] using lateral thoracolumbar spine radiographs in accordance with the method reported previously [30]. To detect incident vertebral fractures, spine radiographs were repeatedly taken at 1-year intervals, and additional X-rays were taken when the subjects complained of symptoms suspicious of new clinical vertebral fractures. Both new clinical and morphometric fractures were counted as incident vertebral fractures. Incident long bone fractures were identified from medical records or confirmed on X-ray films. Incident clinical fractures in vertebrae or other parts of the bone structure were easily recognized when they had occurred, whereas morphometric vertebral fractures were sometimes difficult to detect clinically. As the exact time of the event could not be determined for some of the patients with morphometric incident vertebral fractures, the time of obtaining the spinal radiograph showing the fractures was taken as the time of the fracture.

Diagnosis of osteoporosis

Diagnosis of osteoporosis was made in accordance with the osteoporosis diagnostic criteria (2000 version) proposed by the Japanese Society for Bone and Mineral Research [8]. Briefly, osteoporosis is diagnosed as the presence of fragility fractures in any bone lesion in a person with BMD less than 80% (−1.63 SD) of a young adult mean (YAM). Osteoporosis is also diagnosed when the LBMD is less than 70% (−2.45 SD) of a YAM even if the person is without prevalent fragility fracture.

Biochemical indices

Nonfasting serum, plasma, and urine samples were collected as baseline data at the time of enrollment. Routine biochemical examination, including serum and urinary levels of calcium and creatinine, serum levels of total protein, alkaline phosphatase (Al-P) activity, blood urea nitrogen (BUN), inorganic phosphate, total cholesterol, triglycerides, and blood sugar, were analyzed immediately. Plasma levels of total homocysteine were measured by a high performance liquid chromatography (HPLC) system [31]. Vitamin B₁₂ and folate were measured at Mitsubishi Kagaku BCL Laboratory (Tokyo, Japan) using chemiluminescence assay kits for vitamin B₁₂ and folate (Bayel Medical, Tokyo, Japan).