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Plasma Homocysteine, Folate, and Vitamin B_{12} and the Risk of Hip Fracture: The Hordaland Homocysteine Study

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ABSTRACT: Homocysteine and related factors were evaluated as risk factors for subsequent hip fractures among 4766 elderly men and women. High levels of homocysteine and low levels of folate predicted fracture, whereas vitamin B_{12} and genotypes were not related to fracture risk. High homocysteine may be a modifiable risk factor for hip fracture.

Introduction: Elevated plasma total homocysteine (tHcy) and deficiencies of folate and vitamin B_{12} are associated with risk of osteoporosis and fracture. We examined whether plasma levels of tHcy, folate, and vitamin B_{12} and the methylenetetrahydrofolate reductase (MTHFR) 677C→T and 1298C→T polymorphisms predicted hip fracture.
Materials and Methods: This was a population-based prospective study of 2639 women and 2127 men who were 65–67 yr at enrollment in 1992–1993. Information on hip fracture was obtained from computerized records of discharge diagnoses from all hospitalizations in the region in the period between enrollment and November 30, 2005. Cox proportional hazard regression was used to estimate fracture risk according to levels of plasma tHcy, folate, and vitamin B_{12} and for different genotypes.
Results: Over a median follow-up period of 12.6 yr, hip fracture was recorded in 184 (7.0%) women and 90 (4.2%) men. The adjusted hazard ratio (95% CI) for fracture in subjects with high (≥15 μM) compared with low levels (<9.0 μM) of tHcy was 2.42 (1.43–4.09) among women and 1.37 (0.63–2.98) among men. Dose-response analyses indicated a positive association between plasma tHcy and risk of fracture in both sexes and a negative association between plasma folate and risk of fracture among women only. Plasma vitamin B_{12} level or MTHFR genotype was not significantly related to risk of fracture after adjustments for confounding factors. The association between tHcy and risk of hip fracture was only slightly weakened by adjustments for plasma levels of vitamin B_{12} and folate.
Conclusions: tHcy seems to be a predictor for hip fracture among elderly men and women. Folate was a predictor among women only, whereas vitamin B_{12} and MTHFR genotype did not predict hip fracture. Our data corroborate the hypothesis that homocysteine may play a role in the pathogenesis of osteoporotic fractures.
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Key words: homocysteine, folate, vitamin B_{12}, polymorphisms, hip fracture

INTRODUCTION

Bone health and risk of fracture have recently been related to components of one-carbon metabolism, including plasma levels of total homocysteine (tHcy), folate, and vitamin B_{12}, and the 677C→T polymorphism in the methylenetetrahydrofolate reductase (*MTHFR*) gene. These studies were motivated by observations in patients with homocystinuria, an inborn error of metabolism characterized by very high plasma levels of homocysteine and, among several clinical manifestations, premature osteoporosis and fractures.[1] Some[2–4] but not all[5,6] studies have shown an inverse relationship between levels of tHcy and BMD of the hip. Two large, recent studies showed that moderately elevated plasma level of tHcy was a risk factor for osteoporotic fractures in elderly men and women.[5,7]

Folate and vitamin B_{12} are the major nutritional determinants of tHcy levels,[8] but these B vitamins may have

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effects on bone tissue independent of tHcy. In some studies, vitamin B_{12} deficiency seems to be associated with an increased risk of osteoporosis[3,9] and hip fractures,[10] possibly because of suppression of osteoblast activity.[11] In other studies, however, subtle degrees of vitamin B_{12} deficiency have not been associated with BMD.[4,6] In a study of elderly Italians, low serum folate was a predictor of fracture, whereas tHcy had no independent effect once serum folate was taken into account.[12] Notably, in a Japanese study, combined treatment with folate and vitamin B_{12} effectively reduced the risk of hip fracture after stroke.[13]

A common polymorphism exists for the gene that encodes MTHFR. Individuals homozygous for a C-to-T transition at base pair 677 are prone to elevated tHcy under conditions of impaired folate status,[14] and this genotype is a strong determinant of plasma tHcy in the general population.[15] The TT genotype has been associated with low BMD in some studies,[16,17] but not in others,[4,18] whereas some find this association only under conditions of low folate[19] or low riboflavin.[20] Results of studies of this polymorphism and fracture risk are conflicting.[16,18,21] We could not identify any publications on the relation between the MTHFR1298A→C polymorphism and risk of fracture. Recent data have shown that the 1298A→C polymorphism has effect on one-carbon metabolism that cannot be accounted for by disequilibrium with 677C→T.[22] Thus, we found it relevant to explore the association between 1298A→C and hip fracture.

The aim of this study was to examine whether baseline plasma levels of tHcy, folate, and vitamin B_{12}, and the MTHFR677C→T and MTHFR1298A→C polymorphisms were associated with subsequent hip fracture in a large population-based cohort.

MATERIALS AND METHODS

Study population

The baseline examination of the Hordaland Homocysteine Study was conducted in 1992–1993 as a collaboration between the University of Bergen, local health services, and the National Health Screening Service.[23] Men and women 41–67 yr of age were invited according to two main age groups (41–42 and 65–67 yr). Because hip fractures in the younger age group were too few to be examined separately, these analyses were restricted to the 4766 participants (attendance rate, 73.2%) who were 65–67 yr at baseline. This cohort was followed for 13 yr, and the period of follow-up for each participant was calculated as the time from inclusion in the study to the first hip fracture, death, or end of study, whichever occurred first. The Regional Committee for Medical Research Ethics, the Data Inspectorate, and the Directorate for Health and Social Affairs approved the study protocol.

Laboratory measures

Nonfasting EDTA blood samples were collected, chilled, and centrifuged within 1–3 h. Plasma fraction and packed blood cells were separated and stored at –20°C until analysis. Plasma tHcy was determined with a fully automated high-performance liquid chromatography (HPLC) assay.[24,25] Our analyses included 20 participants who had a tHcy concentration ≥40 μM. These persons were made aware of their high concentrations and offered a clinical consultation and treatment with folic acid or vitamin B_{12}.[14] Exclusion of these individuals did not materially change the reported results. Plasma folate and vitamin B_{12} was measured by microbiological assays.[26,27] Before baseline folate and vitamin B_{12} measurements were performed, the plasma samples had been stored at –20°C up to 10 yr. Plasma vitamin B_{12} is stable, but plasma folate declines during frozen storage.[28] However, we have previously reported that, although the folate levels declined ~20% between 1995 and 2000, the strength of associations with tHcy and other factors remain unaltered.[29] DNA was extracted from packed blood cells, and the MTHFR 677C→T and 1298A→C polymorphisms were determined by real-time PCR.[30]

Assessment of fractures

Information on fracture was obtained from computerized records containing discharge diagnoses for all hospitalizations occurring between the baseline examination and November 30, 2005 at the six hospitals serving Hordaland County. Hip fracture was defined as the first fracture of the proximal femur that occurred during the observation period. Only hip fractures confirmed by a concurrent code of an adequate surgical procedure were included. The discharge diagnoses used to classify a hip fracture were according to the International Classification of Diseases, Ninth Revision (ICD-9):820–820.9 and Tenth Revision (ICD-10): S720-S722. Surgical treatments for each hospital stay are coded according to the Norwegian Classification of Surgical Procedures (version 2, 1989 and 3, 1995) and later the NOMESCO Classification of Surgical Procedures (version 1, 1999-version 3, 2004). All hospital discharges with an identified hip fracture diagnosis were searched for adequate surgical treatment. Missing surgical code resulted in exclusion of 16 hip fractures. Information on time of death was obtained from the Norwegian National Population Register.

Collection of additional data

Height and weight were measured in light clothing, and body mass index (BMI, kg/m^2) was calculated. Self-administered questionnaires provided information on education, physical activity, consumption of coffee, smoking habits, and use of vitamin and estrogen supplements. Information on diabetes mellitus, epilepsy, arthritis, and renal and cerebrovascular diseases was also self-reported. Based on this information, smokers were categorized into five groups: never smokers, former smokers, light smokers (1–9 cigarettes/d), moderate smokers (10–19 cigarettes/d), or heavy smokers (≥20 cigarettes/d). Participants marked the category that best fitted their average degree of physical activity in leisure time for the last year: sedentary or no activity, walking, cycling or other type of moderate physical activity for at least 4 h/wk, or exercise with physical exertion for at least 4 h/wk. Coffee consumption was recorded as cups consumed per day. Vitamin D supplementation was measured as use of vitamin D containing tablets or cod liver

oil for >5 d/wk year around. Education was dichotomized at the level of high school completion. Estrogen use was categorized as current or no use. Further details on data collection have been previously published.[31]

1997–1999 data collection

During 1997–1999 the 4766 subjects who were 65–67 yr at baseline were invited to a second examination, where 3340 (70.1%) participated. This examination included essentially the same variables. In addition, serum creatinine level was measured by a standard alkaline picrate method. Cognitive function was assessed by, among other tests, the Kendrick Object Learning Test (KOLT),[32] and dietary and supplemental intake of calcium and vitamin D were measured by a quantitative food frequency questionnaire.[33] BMD of the hip was obtained by a stationary DXA (EXPERT-XL; Lunar, Madison, WI, USA).[4]

Statistical analyses

Kaplan-Meier estimation and the Cox proportional hazards model were used to estimate the associations between levels of plasma tHcy, folate, and vitamin B_{12} and subsequent hip fracture. Plasma tHcy was divided into four categories based on clinical cut-offs used previously[4,34–36] (tHcy, <9.0 [reference], 9.0–11.9, 12.0–14.9, and ≥15 μM). To explore if there was a further increase in fracture risk at higher levels of tHcy, we subdivided the highest category of plasma tHcy into 15.0–19.9 and ≥20.0 μM.[35] The cut-off values for plasma vitamin B_{12} and folate were chosen to obtain similar proportions of subjects in the four categories as chosen for tHcy (~10% of the total group in the most extreme categories for each variable). A linear representation of the indicator variables was used to test for trend. We also tested for differences among the exposure groups without any assumption about the shape of the dose-response (*p* for homogeneity). We tested the proportional hazards assumption in an extended Cox model with a time-dependent covariate and detected no statistically significant deviations.

Analyses were adjusted for BMI, smoking status, consumption of coffee, level of physical activity, regular use of vitamin D supplements, and educational level, and for women, use of estrogen. To assess the independence of each factor and to compare the strength of tHcy, folate, and vitamin B_{12} on fracture risk, we included all three variables in the same model. Cox regression analysis was also performed to explore the possibility of synergistic effects of tHcy, folate, and vitamin B_{12}, by analyzing the effect of one factor in the presence of plasma levels above and below sex-specific medians of one of the other factors, using product terms. Measurements from the second data collection (1997–1999) were included as covariates in additional analyses to adjust for potential confounders that were not obtained at the baseline data collection in 1992–1993. We also performed the analyses with exclusion of subjects with diabetes mellitus, stroke, epilepsy, renal disease, and arthritis, because these diseases are known to increase tHcy levels and risk of fracture. Graphic representations of dose-response were obtained by fitting a Cox model with a *p*-spline function (pspline function of S-PLUS),[37] which allows for a flexible (nonlinear) effect estimation.[38] On the *y* axis, the model generates a relative reference scale, where 1 is set to represent the fracture risk (hazard) at the mean tHcy or folate concentration. These dose-response curves were adjusted for the same covariates as listed above.

Cox regression was also used to estimate the associations between MTHFR677C→T polymorphism and fracture adjusted for age and sex. To examine the possible effect modification of plasma folate level on this association, plasma folate was dichotomized at 4 nM. This value is close to the median folate level for the TT group and was chosen to assure sufficient number of individuals at both high and low levels of folate in this group. We compared hip fracture risk among genotypes separately for each folate level and fracture risk between folate groups within each genotype. Mean tHcy levels for the MTHFR/folate group were calculated. In addition to S-PLUS, statistical analyses were performed with the use of SPSS for Windows, version 13.0.

RESULTS

Demographics and blood indices

During a median follow-up period of 12.6 yr (range, 0.02–13.0 yr), hip fracture was recorded among 184 women (7.0%) and 90 men (4.2%). Baseline characteristics according to fracture status are presented in Table 1. The proportion of current smokers was significantly higher among subjects who later suffered a hip fracture than among those who did not. Compared with the group without fractures, those with fractures had higher plasma tHcy and lower BMI, women had lower plasma folate, and men had lower plasma vitamin B_{12} and vitamin D intake (Table 1). There were no significant differences in mean KOLT score, serum creatinine, or intake of calcium between the fracture group and the no-fracture group (data not shown).

Plasma levels of tHcy and risk of hip fracture

There was a strong relation between plasma tHcy level and cumulative incidence of hip fracture throughout the follow-up period among women (Fig. 1; Table 2). Among men, the dose-response relationship was J-shaped. When subjects having tHcy concentrations of 9.0–11.9, 12.0–14.9, and ≥15.0 μM were compared with those having a tHcy concentration <9.0 μM, adjusted HRs were 0.86, 1.59, and 2.42 for women and 0.42, 0.73, and 1.37 for men. However, significant tests for trend indicated a positive association between plasma tHcy and risk of fracture in both sexes. Adjustments for level of folate and vitamin B_{12} did not materially change this association (Table 2). There was a further increase in fracture risk when we subdivided the highest category of plasma tHcy into 15.0–19.9 and ≥20.0 μM. The adjusted HRs (95% CI) for those having a tHcy concentration ≥20.0 μM compared with those having a tHcy concentration <9.0 μM were 3.08 (1.47–6.46) and 1.70 (0.62–4.66) for women and men, respectively. Exclusion of subjects with diabetes mellitus, stroke, epilepsy, arthritis, and renal disease did not materially alter the results. The association between tHcy and fracture was essentially the

TABLE 1. CHARACTERISTICS OF THE STUDY POPULATION AT BASELINE IN RELATION TO LATER HIP FRACTURE

	Women					*Men*				
	*Total N**	*Without fracture*		*With fracture*		*Total N**	*Without fracture*		*With fracture*	
	No fracture/ hip fracture	*N*	*(%)*	*N*	*(%)*	*No fracture/ hip fracture*	*N*	*(%)*	*N*	*(%)*
Coffee consumption ≥5 cups/d	2455/184	370	(15.1)	43	(23.4)	2036/90	546	(26.8)	29	(32.2)
Current smokers	2455/184	506	(20.6)	58	(31.5)	2037/90	578	(28.4)	38	(42.2)
Education ≤12 yr	2236/163	2015	(90.1)	152	(93.3)	1896/80	1454	(76.7)	69	(86.3)
No regular physical activity	2454/184	537	(21.9)	52	(28.3)	2036/90	315	(15.5)	18	(20.0)
Use of vitamin D supplementation	2131/154	914	(42.9)	65	(42.2)	1767/75	678	(38.4)	19	(25.3)
Use of estrogen supplementation	2407/182	220	(9.1)	16	(8.8)					
		Mean	*(SD)*	*Mean*	*(SD)*		*Mean*	*(SD)*	*Mean*	*(SD)*
BMI (kg/m^2)	2449/184	26.0	(4.3)	24.8	(4.1)	2034/90	25.7	(3.2)	25.0	(3.1)
tHcy (μM)	2455/184	11.5	(4.1)	12.7	(5.0)	2037/90	13.0	(5.8)	14.5	(6.0)
Folate (nM)	2453/184	6.0	(3.5)	5.3	(3.1)	2037/90	5.2	(2.7)	5.3	(2.9)
Vitamin B_{12} (pM)	2450/184	379	(285)	485	(943)	2037/90	360	(279)	343	(201)

* The sample number varies between the reported variables according to different numbers of missing data.

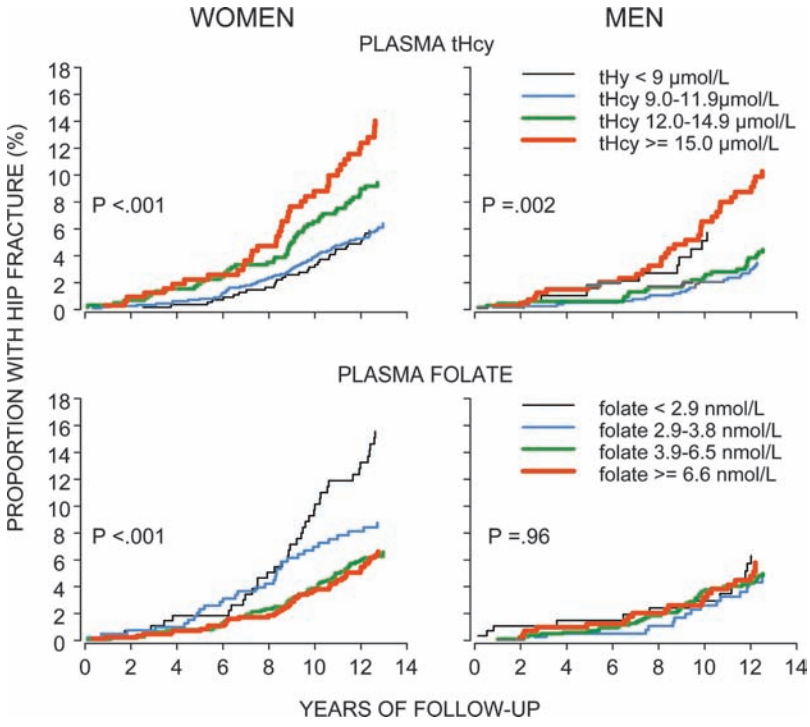

FIG. 1. Unadjusted survival curves (Kaplan-Meier) for cumulative incidence of hip fracture by levels of plasma tHcy and folate among women (left) and men (right). Each plot gives the *p* value for log-rank test for trend across categories.

same in the subgroup that attended the second examination as in the total cohort. Further adjustment for calcium and vitamin D intake, KOLT score, or plasma creatinine measured in 1997–1999 did not change the observed relationship between tHcy and fracture in this subgroup. Notably, the association between tHcy and risk of hip fracture weakened, but remained significant, after adjustment for femoral neck or total hip BMD measured in 1997–1999 (data not shown).

On the logit scale, the increase in fracture risk was close to linear within the tHcy range of 7–20 μM for both men and women; however, CIs were wider among men than among women (Fig. 2).

Plasma folate and vitamin B_{12} and risk for hip fracture

Women within the lowest folate category have an increased risk of hip fracture (Fig. 1; Table 2). The adjusted HR (95% CI) for fracture for low (<2.9 nM) versus high (≥6.6 nM) levels of folate was 2.40 (1.50–3.84) among women and not significant among men. The association among women was somewhat weakened, but still statistically significant, after adjustments for tHcy and vitamin B_{12}. The negative relation between plasma folate levels and risk of fracture among women was close to linear within the folate range of 2–10 nM (Fig. 2). There was no relationship

TABLE 2. HAZARD RATIOS FOR HIP FRACTURE ACCORDING TO DIFFERENT PLASMA LEVELS OF TOTAL HOMOCYSTEINE, FOLATE, AND VITAMIN B_{12}

		Women						*Men*					
	*Range**	*At risk*	*Events (%)*	*HR*[†]	*(95% CI)*	*HR*[‡]	*(95% CI)*	*At risk*	*Events (%)*	*HR*[†]	*(95% CI)*	*HR*[‡]	*(95% CI)*
		2639	184 (7.0)					2127	90 (4.2)				
tHcy	<9.0	569	31 (5.4)	1.00		1.00		196	10 (5.1)	1.00		1.00	
	9.0–11.9	1144	65 (5.7)	0.86	(0.54–1.38)	0.85	(0.52–1.38)	832	24 (2.9)	0.42	(0.18–0.95)	0.44	(0.19–1.01)
	12.0–14.9	600	50 (8.3)	1.59	(0.98–2.59)	1.53	(0.91–2.57)	674	24 (3.6)	0.73	(0.34–1.60)	0.81	(0.36–1.86)
	≥15.0	326	38 (11.7)	2.42	(1.43–4.09)	2.16	(1.20–3.89)	425	32 (7.5)	1.37	(0.63–2.98)	1.52	(0.64–3.58)
p for trend				<0.001		0.001				0.009		0.009	
p for homogeneity				<0.001		0.002				0.002		0.003	
Folate	<2.9	279	37 (13.3)	2.40	(1.50–3.84)	1.90	(1.13–3.17)	275	13 (4.7)	1.00	(0.48–2.12)	0.65	(0.29–1.48)
	2.9–3.8	398	32 (8.0)	1.15	(0.68–1.94)	1.00	(0.58–1.73)	413	15 (3.6)	0.80	(0.39–1.62)	0.60	(0.28–1.29)
	3.9–6.5	1120	65 (5.8)	1.02	(0.68–1.54)	0.98	(0.64–1.50)	1010	42 (4.2)	0.81	(0.45–1.46)	0.69	(0.37–1.29)
	≥6.6	840	50 (6.0)	1.00		1.00		429	20 (4.7)	1.00		1.00	
p for trend				<0.001		0.03				0.97		0.29	
p for homogeneity				<0.001		0.03				0.84		0.57	
Vitamin B_{12}	<230	300	28 (9.3)	1.14	(0.68–1.90)	0.78	(0.46–1.34)	310	18 (5.8)	1.63	(0.74–3.58)	1.46	(0.65–3.28)
	230–279	420	27 (6.4)	0.82	(0.49–1.37)	0.68	(0.40–1.15)	342	18 (5.3)	1.79	(0.84–3.81)	1.64	(0.76–3.53)
	280–414	1165	75 (6.4)	0.82	(0.56–1.20)	0.79	(0.53–1.16)	1000	40 (4.0)	1.30	(0.67–2.52)	1.34	(0.69–2.61)
	≥415	749	54 (7.2)	1.00		1.00		475	14 (2.9)	1.00		1.00	
p for trend				0.85		0.24				0.14		0.30	
p for homogeneity				0.49		0.47				0.43		0.65	

* tHcy in μM, folate in nM, and vitamin B_{12} in pM.

† Hazard ratio for hip fracture adjusted for age, body mass index, smoking status, consumption of coffee, level of physical activity, regular use of vitamin D supplements, and educational level, and for women, use of estrogen.

‡ Hazard ratio for hip fracture adjusted for the above covariates and in addition tHcy analyses are adjusted for levels of plasma folate and vitamin B_{12}, folate analyses are adjusted for levels of tHcy and plasma vitamin B_{12}, and vitamin B_{12} analyses are adjusted for levels of tHcy and plasma folate.

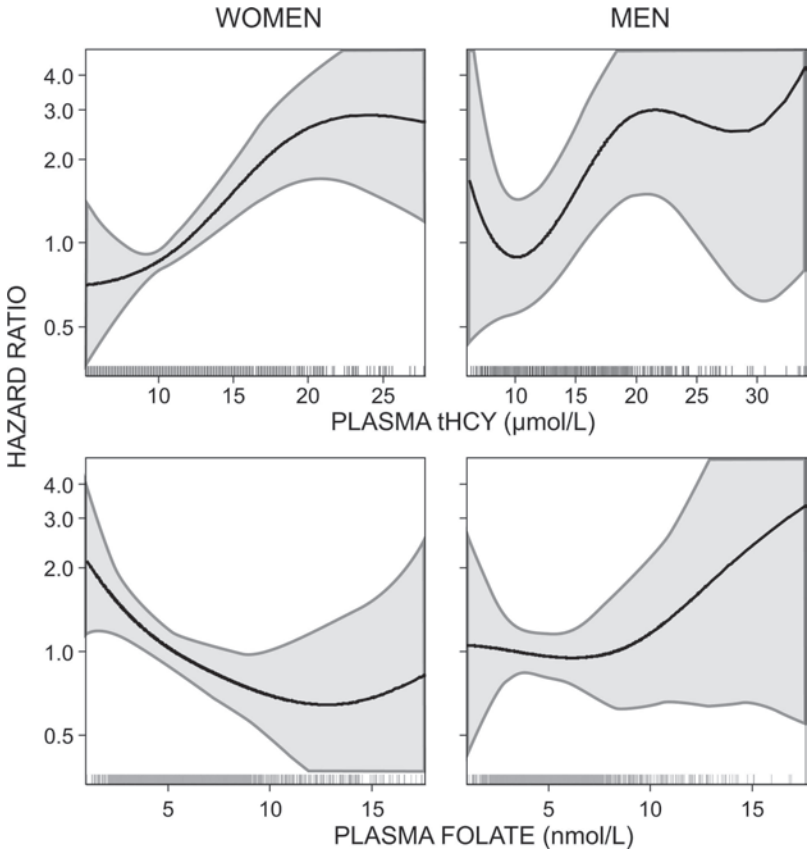

FIG. 2. Dose-response curves for the relation between plasma tHcy and plasma folate and hazard ratio for hip fracture among women (left) and men (right). Analyses are adjusted for body mass index, smoking status, consumption of coffee, level of physical activity, intake of vitamin D supplements, and educational level, and for women, use of postmenopausal estrogen therapy. Shaded area represents 95% CIs. Ranges from 1 to 99 percentiles of exposure variables are included. The rug plot at the bottom of each graph depicts distribution of observations.

TABLE 3. HAZARD RATIOS FOR HIP FRACTURE ACCORDING TO MTHFR 677C→T GENOTYPE AND FOLATE LEVEL

	At risk	*Events (%)*	*tHcy* (SD)*	*HR†*	*(95% CI)*	*p for trend*
All folate levels						
CC	2367	122 (5.2)	11.8 (3.6)	1.00		
CT	2003	130 (6.5)	12.2 (4.8)	1.25	(0.98–1.60)	0.22
TT	393	22 (5.6)	15.2 (10.0)	1.08	(0.69–1.70)	
Folate <4 nM						
CC	659	41 (6.2)	13.3 (3.7)	1.00		
CT	667	49 (7.3)	13.8 (4.7)	1.18	(0.78–1.78)	0.30
TT	168	14 (8.3)	18.9 (13.4)	1.34	(0.73–2.46)	
Folate ≥4 nM						
CC	1708	81 (4.7)	11.2 (3.3)	1.00		
CT	1336	81 (6.1)	11.3 (4.7)	1.26	(0.93–1.72)	0.70
TT	224	8 (3.6)	12.4 (4.6)	0.73	(0.35–1.50)	

* Mean total homocysteine (μM).
† Hazard ratio for hip fracture adjusted for age and sex.

between level of vitamin B_{12} and fracture risk (Table 2), and no threshold effect was observed in the dose-response curves (data not shown).

MTHFR genotypes and risk for hip fracture

The prevalences of MTHFR677C→T genotypes (CC: 49.7%, CT: 42.1% ,and TT: 8.3%) and 1298A→C genotypes (AA: 44.1%, AC: 45.1%, and CC: 10.8%) were in Hardy-Weinberg equilibrium ($p > 0.2$). The 677C→T polymorphism was not associated with risk of fracture, neither in the total population nor in the subgroup with folate levels <4 nM (Table 3). Although there were no significant differences or trend between genotypes, in those with low folate, there was a suggestion of an increased risk in the TT group compared with CC. Within each genotype, there was a slightly increased risk of fracture among individuals with low folate levels compared with those with levels >4 nM (Fig. 3). The increased risk associated with low plasma folate level was larger among subjects with the TT genotype compared with the other genotypes. Among TT individuals,

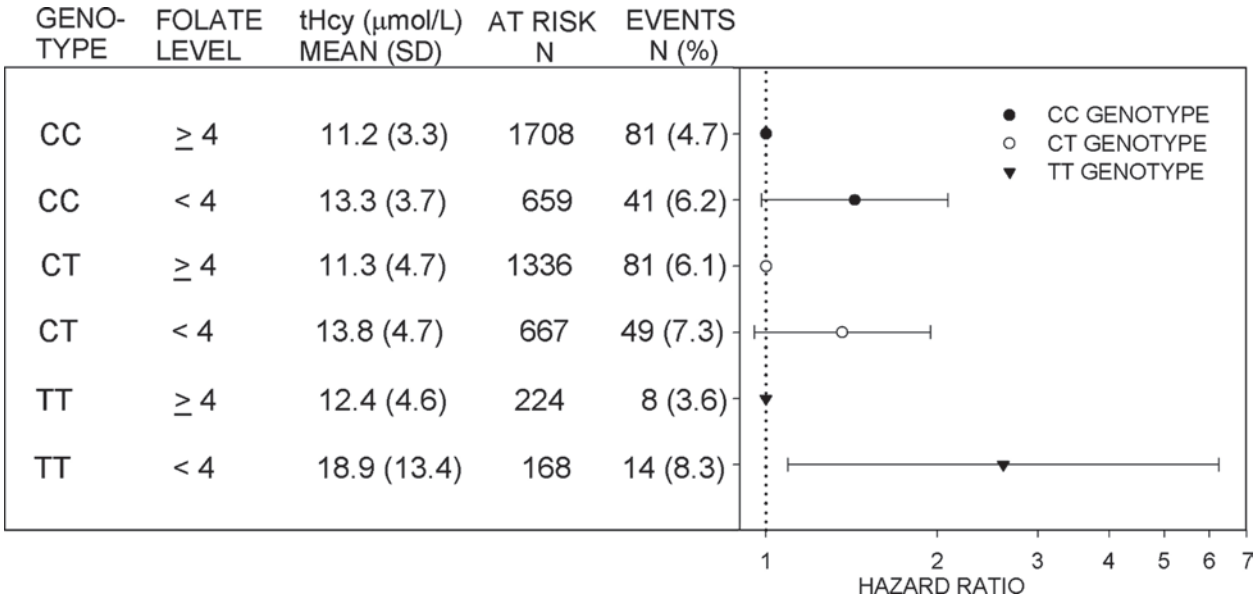

FIG. 3. Age- and sex-adjusted hazard ratio for hip fracture according to the MTHFR677C→T polymorphism and baseline folate level (≥ 4 and <4 nM). Comparisons are made within each genotype. Brackets indicate 95% CIs.

there was a large (almost 3-fold) and statistically significant increase in the risk of fracture for those with low folate. However, interaction by folate level was not statistically significant. The difference in mean tHcy between folate groups was greater in TT individuals compared with the other two genotypes. There was no association between the MTHFR1298A→C polymorphism and risk of hip fracture (data not shown).

DISCUSSION

Results of this prospective, population-based study of 4766 elderly men and women suggest that women with high tHcy or low folate have an increased risk of hip fracture. Dose-response analyses indicated a positive association between high plasma tHcy and risk of fracture also among men. Vitamin B_{12} was not associated with fracture risk in either men or women. Also, although MTHFR genotype was not associated with fracture, TT individuals with low folate had twice the fracture risk of TTs with high folate.

Strengths of our study include a large, population-based cohort with subjects in a relevant age group, a prospective design, and a relatively large number of hip fractures. The study included measures of established risk factors for osteoporosis and hyperhomocystinemia, such as smoking, low physical activity, consumption of coffee, and low estrogen exposure.[8,39]

Because our study is observational, it cannot resolve the question of whether homocysteine plays a causal role in hip fracture. Plasma samples for determination of folate and vitamin B_{12} levels had been stored for up to 10 years before analyses, and this may have led to weakening of associations.[28] Because the validity of hospital discharge diagnoses have been questioned,[40] we included only the first hip fracture diagnosis, validated by a concurrent code of an adequate surgical procedure. This should reduce the overestimation of fractures related to rehospitalizations, transferals, or miscoding. We may have missed a few hip fractures that occurred outside Hordaland County, but this should not invalidate our findings.

Our results agree with findings from the Framingham cohort[7] and the LASA/Rotterdam study,[5] both showing tHcy as a strong predictor of hip fractures among older persons. Neither of these studies measured folate or vitamin B_{12}. Although the dose-response relationship between tHcy and fracture was strongest for women in our study, we observed a similar pattern among men. The discrepancy between men and women may be caused by the lower number of endpoints among men, but biological differences cannot be ruled out. In agreement with our results among women, low levels of folate were associated with increased risk of fracture in a smaller cohort of elderly Italians.[12] Low levels of vitamin B_{12} increased fracture risk among elderly Dutch women but not among men.[10] We found no association between vitamin B_{12} levels and fracture risk in adjusted analyses. Reasons for these discrepancies may relate to differences in age at inclusion, different definitions of vitamin B_{12} deficiency, and folate fortification in the area of subject recruitment. There is no B vitamin fortification of food in Norway.

The MTHFR677TT genotype is associated with elevated tHcy concentrations.[8] The MTHFR677C→T polymorphism has been associated with increased risk of fracture[16] and decreased BMD[16,17] in some but not all[2,4,18] studies. The lack of influence by the 677C→T polymorphism on the risk of hip fracture in this study could be caused by insufficient statistical power. TT individuals have mean plasma tHcy concentrations 2.7 μM higher than those with the CC genotype.[15] If the risk conferred by the TT genotype is caused by its effect on tHcy alone, the expected relative risk

corresponds to a HR (95% CI) per 3-μM increase in tHcy of 1.07 (1.02–1.13) for men and 1.17 (1.10–1.25) for women. Detection of a relative risk in the range of 1.10–1.15 with a power of 80% and a significance level of 5% will require 7800–16,300 cases and an equal number of controls.[41] The greater increase in fracture risk associated with low plasma folate among TT individuals than among subjects with other genotypes in our study suggests that the 677C→T transition is associated with increased folate responsiveness, and this supports the role of tHcy or folate for fracture risk.

Currently, it is unclear how homocysteine may affect the risk of fracture. Studies of patients with homocystinuria have revealed disturbed cross-linking of collagen.[42] There is also experimental[43,44] and epidemiological[10,45] support for the hypothesis that homocysteine increases bone resorption. Our group[4] and others[2,3] have reported an inverse association between tHcy levels and BMD. The associations between tHcy and fracture risk in the Rotterdam/LASA study, however, seemed independent of BMD.[5] Our study showed that the association between tHcy and hip fracture remained significant after adjustment for BMD (measured in 1998). Furthermore, a Japanese randomized controlled trial found that combined treatment with folate and vitamin B_{12} reduced the risk of hip fracture after stroke,[13] without any associated changes in BMD. These data suggest that the effect of homocysteine on bone may to some extent be independent of BMD.

High levels of tHcy often reflects low dietary intake of folate, cobalamin, riboflavin, or vitamin B_6.[8] Such a diet may also imply low dietary intake of nutrients important to bone health, such as vitamin D and calcium.[46] We found that the observed relationship between tHcy and fracture was independent of intake of vitamin D and calcium among participants that attended the second examination (1997–1999). However, residual confounding from dietary factors cannot be ruled out.

Vitamin B_{12} may influence bone tissue independently of tHcy, possibly because of suppression of osteoblast activity.[11] The relationship between tHcy and risk of fracture observed in our study could not be explained by low levels of folate or vitamin B_{12}, indicating an independent effect of homocysteine on bone. Elevated levels of tHcy may be a risk factor for impaired cognitive function[32] and Alzheimer disease[47] in the elderly, conditions that increase the risk of falling. Whereas >90% of hip fractures are associated with falls, tHcy does not seem to be related to recurrent falling,[5,10] and cognitive function measured by mini mental status could not explain the relationship between tHcy and fracture in previous studies.[5,7] Similarly, we found that the association between tHcy and fracture was unaffected by cognitive function (measured by KOLT in 1997–1999) among those who participated in the second examination.

Reduced glomerular filtration rate increases the level of tHcy,[8] and renal failure is associated with increased risk of fracture.[48] There is, however, no evidence that the age-related decline in glomerular filtration rate is independently associated with BMD or fracture; in fact, only patients with end-stage renal disease have increased risk of fracture.[48] Excluding participants with self-reported renal disease did not change our results, and adding creatinine level (measured in 1997–1999) to the model strengthened the relationship between plasma tHcy levels and fractures in this group. Thus, renal impairment is an unlikely explanation for the observed association between the level of tHcy and hip fracture.

Low estrogen levels may be a common determinant for osteoporosis and hyperhomocystinemia among women.[49,50] Even though we adjusted for use of estrogen supplements, we cannot rule out the possible confounding effect of endogenous estrogen levels in our study.

If moderately elevated levels of tHcy are causally related to risk of osteoporotic fractures, the public health implications are large because plasma tHcy is easily lowered by B vitamin supplementation.[51] Randomized trials will, hopefully, clarify the role of homocysteine in osteoporosis and the relevance of B vitamin supplementation for prevention.

Results of this prospective, observational study indicate that there is a strong association between plasma tHcy levels and the risk of hip fracture, particularly among women. Low plasma folate was also associated with increased fracture risk in women. These observations, combined with increasing experimental evidence for an effect of homocysteine on bone metabolism, further corroborate the hypothesis that homocysteine plays a role in the pathogenesis of osteoporotic fractures.

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