The 677C→T mutation of the methylene-tetra-hydrofolate reductase gene in the pathogenesis of osteonecrosis of the femoral head

We investigated the presence of the 677C→T methylene-tetrahydrofolate reductase (MTHFR) mutation in 66 patients with osteonecrosis (ON) of the femoral head and 300 healthy controls. Homozygosity for the MTHFR mutation was present in 26.1% of patients with idiopathic ON and in 10% of controls (odds ratio: 3.2 [95% confidence interval: 1.2-8.7]). The homozygous state of the 677C→T MTHFR mutation appears to be associated with idiopathic osteonecrosis.

The etiology of non-traumatic osteonecrosis (ON) of the femoral head has not been elucidated. However, intravascular coagulation appears to constitute a pathogenetic mechanism through which various environmental and genetic risk factors lead to bone ischemia and death.[1] Protein C and S deficiency, elevated lipoprotein (a), and the factor V Leiden mutation represent genetic risk factors for hypercoagulability and ON.[2-4] The 677C→T mutation of the 5,10-methylene-tetrahydrofolate reductase (MTHFR) gene has been identified as a common cause of MTHFR enzyme deficiency and has been postulated as a genetic risk factor for thrombotic events.[5,6] The purpose of our study was to evaluate the potential association of this MTHFR mutation with non-traumatic ON of the femoral head.

The study population consisted of 66 consecutively identified Caucasian patients with non-traumatic ON of the femoral head and 300 healthy controls. There were 48 male and 18 female patients with a mean age of 31 years. In 43 patients the disease was considered secondary to various factors (administration of steroids in 34 patients, alcoholism in 7, and systemic lupus erythematosus in 2) and was considered idiopathic in the remaining 23 patients, since no risk factor was known to be present. The control group consisted of 300 consecutive Caucasian healthy blood donors (mean age 36 years) who presented at the University Hospital of Ioannina. The presence of the homozygous (677TT genotype) and heterozygous (677CT genotype) state of the 677C→T mutation in the MTHFR gene was investigated by restriction enzyme digestion of an amplified gene fragment using the primers and polymerase chain reaction conditions described by Frosst *et al.*[7] Odds ratios with 95% confidence intervals were calculated for the homozygous versus the other genotypes.

Allele frequencies at the 677 locus were found to be in Hardy-Weinberg equilibrium for both patients and controls. The prevalence of homozygosity for the 677C→T mutation was 18.2% (12 of 66) in the patient group and 10% (30 of 300) in the control group. The odds ratio was 2.0 (95% confidence interval: 1.0- 4.2), which was marginally not statistically significant with the numbers available. In the idiopathic ON subgroup, homozygosity for the mutation was present in 26.1% of patients (6 of 23). The odds ratio was 3.2 (95% confidence interval: 1.2-8.7) and this was statistically significant (Table 1).

The 677C→T mutation of the MTHFR gene leads to hyperhomocysteinemia, which is associated with an increased risk of thrombosis,[8] and the MTHFR mutation has been postulated as a genetic risk factor for thrombotic events.[5,6] Intravascular coagulation is considered a common pathogenetic mechanism for development of ON of the femoral head in adults. Studies have demonstrated a thrombotic potential in up to 66% of ON patients.[2-4] However, in a considerable proportion of ON patients no thrombotic potential was detected and this may be explained by the presence of coagulation disorders not yet identified and evaluated. The 677C→T MTHFR mutation may be one of these. Interestingly, Glueck *et al.* observed increased homocysteine levels in a significantly greater proportion of ON patients than controls.[9]

Our study demonstrated an increased prevalence of the homozygous 677C→T MTHFR mutation in patients with idiopathic femoral head ON. We analyzed the idiopathic ON subgroup separately, because differences in criteria used to select groups of patients may in part explain the various associations between the MTHFR mutation and vascular disease reported in the literature. Gemmati *et al.* pointed out that both the prevalence of the MTHFR mutation and the odds ratio increased when patients without associated risk factors were examined separately.[6] Similarly, in our study the association of the mutation with ON was clearly evident only in the idiopathic subgroup. We report on patients and controls of the same race and geographic area, which is important because of the variable prevalence of the MTHFR mutation in different populations.

Nevertheless, our study has limitations. Although an association between the MTHFR mutation and idiopathic ON was observed, the hypothesis that the MTHFR mutation may lead to the disease by promoting intravascular coagulation remains to be proven. The MTHFR mutation is not considered a definite risk factor for thrombosis,[10] and our study did not include evaluation of the hemostatic mechanism. Moreover, we did not measure homocysteine levels. In conclusion, homozygosity for the 677C→T MTHFR mutation appears to be associated with idiopathic femoral head ON in adults. Despite the relatively small sample size, our findings constitute the basis for further investigation of the MTHFR mutation to clarify its exact role in the complex pathophysiology of ON.

Table 1. Comparison of the osteonecrosis group and the idiopathic osteonecrosis subgroup with the controls for homozygosity for the 677CT mutation of the MTHFR gene. Odds ratios with 95% confidence intervals were calculated for the homozygous subjects (677TT genotype) versus subjects with the other genotypes (677CT and 677CC).

	Osteonecrosis patients n=66		*Controls n=300*		
Genotype	*N*	*%*	*N*	*%*	*Odds ratio (95% CI)*
Homozygous 677TT	12	18.2%	30	10.0%	2.0 (1.0-4.2)
Heterozygous 677CT	32	48.5%	153	51.0%	
Normal 677CC	22	3.3%	117	39.0%	
	idiopathic osteonecrosis patients n=23		*controls n=300*		
Genotype	*N*	*%*	*N*	*%*	*Odds ratio (95% CI)*
homozygous 677TT	6	26.1%	30	10.0%	3.2 (1.2-8.7)
heterozygous 677CT	10	43.5%	153	51.0%	
normal 677CC	7	30.4%	117	39.0%	

ON: osteonecrosis; CI: confidence interval.

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