Original Article

Methylenetetrahydrofolate Reductase C677T Gene Polymorphism in Osteosarcoma and Chondrosarcoma Patients

(osteosarcoma / chondrosarcoma / MTHFR / polymorphism)

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Abstract. This study was designed to investigate the association of *MTHFR* C677T polymorphism and the risk of two common musculoskeletal sarcomas, osteosarcoma and chondrosarcoma. *MTHFR* genotypes were determined in 56 patients (44 osteosarcoma, 12 chondrosarcoma) and 44 controls using the PCR-RFLP technique. In the gender subgroup analysis, wild-type A allele frequency was higher in male osteosarcoma patients than in male control subjects (P = 0.064). Mutant V allele and mutant VV genotype were similar in the control group compared to the sarcoma groups (P > 0.05). No correlation could be proved between patient tumour site, presence of metastasis, and local tumour relapse and *MTHFR* polymorphism. The *MTHFR* C677T polymorphism may not be important in an individual's susceptibility to osteosarcoma and chondrosarcoma in Turkey and may not be a useful marker for identifying patients at high risk of developing sarcomas.

Introduction

Methylenetetrahydrofolate reductase (MTHFR) plays a key role in the regulation of cellular methylation reaction by catalyzing the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate; the latter is the major form of folate in blood, and the primary methyl donor for the remethylation of homocysteine (Hcy) to methionine (Frosst et al.,1995). It has a crucial role in the metabolism of folate, which acts in DNA metabolism particularly in the synthesis of purine and thymidine nucleotides, DNA repair mechanisms, DNA methylation process, and cell division (James et al., 1994; Kim et al., 1996).

Frosst et al. (1995) described C → T substitution in the *MTHFR* gene at bp 677 that produces an alanine to valine amino acid substitution in position 222 in the catalytic domain of the MTHFR enzyme (A – alanine corresponds to 677C; V – valine corresponds to 677T) (Frosst et al.,1995). This polymorphism occurs in approximately 8 to 10 % of the population and reduces the activity of the MTHFR enzyme (75 % in the A/V heterozygous and 50 % in the V/V homozygous state, respectively) resulting in lower plasma folate levels (Jacques et al., 1996). The folate status shows a strong interactive effect with the C677T polymorphism. In folate-replete subjects, the VV genotype affords 50 % colorectal cancer risk reduction, whereas in subjects with low folate status, the VV genotype confers no protection or probably risk enhancement. This may be due to an effect on DNA synthesis resulting from increased availability of 5,10-methylenetetrahydrofolate (Chen et al., 1996; Ma et al., 1997).

MTHFR polymorphism has been reported to be related both to the carcinogenesis process and some complications of chemotherapy. Firstly, folate plays a critical role in regulation of gene expression, maintenance of genomic stability and DNA methylation, which are important steps in carcinogenesis (Lengauer et al., 1997; Kundu and Rao, 1999). A correlation between polymorphism in the *MTHFR* gene and increased risk of some cancer types has been shown in previous studies (Chen et al., 1996; Gershoni-Baruch et al., 2000). Several studies on the *MTHFR* C677T polymorphism and cancer risk indicate that the T allele protects against cancer in folate-replete subjects but increases the risk under conditions of impaired folate status. The protection might be related to abundant purines and pyrimidines available for DNA synthesis, leading to efficient DNA repair and essentially no uracil incorporation into DNA. The combination of low folate and

Received November 16, 2007. Accepted December 5, 2007.

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Abbreviations: Hcy – homocysteine, MTHFR – methylenetetrahydrofolate reductase

TT genotype impairs Hcy remethylation to methionine; this could thereby cause DNA hypomethylation, which is known to be involved in carcinogenesis (Ueland et al., 2001). Second, methotraxate (MTX) toxicity occurs in patients with a homozygous polymorphism (C677T) in the *MTHFR* gene (Toffoli *et al.*, 2003). Lower folate plasma levels increase the potency of MTX. Determination of genotype data and use of reduced doses of antifolates may help in reducing toxic complications of MTX treatment (Krajinovic et al., 2004; McLean et al., 2004).

This study aims to examine the prevalence of the *MTHFR* C677T mutation in osteosarcoma and chondrosarcoma patients, the two most common malignant bone tumours, and in a healthy control group, in order to investigate the associations between the *MTHFR* gene and carcinogenesis to support the relation of *MTHFR* mutation and cancer development.

Material and Methods

Patients

The study group consisted of 44 patients (22 female and 22 male) with a diagnosis of osteosarcoma (mean age: 23.84 ± 8.3 (range: 19–37)) and 12 patients (5 female and 7 male) with chondrosarcoma (mean age: 45 ± 11.7 (range: 22–59)). The diagnoses were established with histological examination in all cases. The control group consisted of 44 healthy individuals (mean age: 26.32 ± 5.15 (range 13–54); 36 female and 8 male) with a negative family history of neoplasia. This group primarily included the spouses of sarcoma patients and volunteers.

All the sarcoma patients and control subjects were Caucasians to ensure homogeneity of the ethnic background and to reduce genetic variability. They had an average socio-economical status, and were attending Istanbul University, Istanbul Faculty of Medicine, Department of Orthopaedics and Traumatology. The diagnosis of sarcoma was confirmed by clinical and laboratory examinations.

Six of the osteosarcoma and none of the chondrosarcoma patients had lung metastases. Informed consent was taken from all groups. The characteristics of the groups were summarized in Table 1. In this study, *MTHFR* polymorphism genotypes of C677T were investigated both in cancer patients and controls.

MTHFR Genotyping

DNA was extracted from the leukocyte pellets by sodium dodecyl sulphate lysis, ammonium acetate extraction, and ethanol precipitation (Miller et al., 1988). *MTHFR* C677T genotypes were determined following the PCR-RFLP method, according to a previously published protocol (Frosst et al., 1995).

Table 1. Characteristics of the study population

GROUP	Control (N = 44)	Osteosarcoma (N = 44)	Chondrosarcoma (N = 12)	Total patients (N = 56)
Gender (Male/ Female) (N)	8/36	22/22	7/5	29/27
Age (years) (mean ± SD)	26.32 ± 5.15	23.84 ± 8.3	45.00 ± 11.7	28.05 ± 12.28
Range of age (years)	13–54	19–37	22–59	19–59
***MTHFR* Genotypes**				
AA				
Total	25 (56.8 %)	24 (54.5 %)	7 (58.3 %)	31 (55.4 %)
Female	20 (55.6 %)	12 (54.5 %)	3 (60.0 %)	15 (55.6 %)
Male	5 (62.5 %)	12 (54.5 %)	4 (57.1 %)	16 (55.2 %)
VV				
Total	3 (6.8 %)	1 (2.3 %)	0 (0 %)	1 (1.8 %)
Female	1 (2.8 %)	1 (4.5 %)	0 (0 %)	1 (3.7 %)
Male	2 (25.0 %)	0 (0 %)	0 (0 %)	0 (0 %)
AV				
Total	16 (36.4 %)	19 (43.2 %)	5 (41.7 %)	24 (42.8 %)
Female	15 (41.7 %)	9 (40.9 %)	2 (40.0 %)	11 (40.7 %)
Male	1 (12.5 %)	10 (45.5 %)	3 (42.9 %)	13 (44.8 %)
***MTHFR* Alleles**				
A				
Total	66 (75 %)	67 (76 %)	19 (79 %)	86 (77 %)
Female	55(76.38)	33 (75.0 %)	8 (80.0 %)	41 (75.92 %)
Male	11 (68.75 %)	34 (77.27 %)	11 (78.57)	45(77.58 %)
V				
Total	22 (25 %)	21 (24 %)	5 (21 %)	26 (23 %)
Female	17 (23.61 %)	11 (25.0 %)	2 (20.0 %)	13 (24.04 %)
Male	5 (31.25 %)	10 (22.72 %)	3 (21.42 %)	13 (22.41 %)

N: number of individuals. Figures in parentheses are percentages

Statistical evaluation

Statistical analyses, using the SPSS version 10.0, included the χ^2 test for genotype and allele frequency comparison. A P value of less than 0.05 was regarded as being statistically significant. When cell frequencies were less than 5, frequency analyses were performed by Fisher's exact test. Student's *t*-test was used for the comparison of age between healthy and sarcoma patients.

Results

Distribution of *MTHFR* genotypes was similar in all study groups. Results are summarized in Table I. Of the total of 44 osteosarcoma patients, the distribution among genotypes was as follows: 24 AA (54.5 %), 19 (43.2 %) AV and one VV (2.3 %), which corresponds to allele frequencies of 67 for A and 21 for V (76.1 % of A and 23.8 % of V). Of the total of 12 chondrosarcoma patients, the distribution among genotypes was as follows: 7 AA (58.3 %), 5 AV (41.7 %), which corresponds to allele frequencies of 19 for A and 5 for V.

The wild-type allele (A allele) was found to be more frequent in the sarcoma patients (osteosarcoma and chondrosarcoma) compared to the healthy control patients, with no statistical significance ($\chi^2 = 1.625$, P = 0.202; OR: 0.94, 95% CI: 0.86–1.03). All chondrosarcoma patients had the wild-type (A) allele. In the gender subgroup analysis, A allele frequency was higher in male osteosarcoma patients than in male control subjects (Fisher's exact test, P = 0.064). However, mutant type allele (V allele) and mutant type genotype (VV genotype) occurrences were similar in the control group compared to the sarcoma groups (P > 0.05).

No association could be shown between *MTHFR* C677T polymorphism and lung metastasis. No correlation could be proved between local tumour relapse, the rate of metastasis, gender, and *MTHFR* genotypes in osteosarcoma patients (Table 2).

Analysis of association between the tumour site and *MTHFR* genotypes in the osteosarcoma patients revealed no significant correlation. The tumour was located in femur in 33.3 % of the cases (65.4 % (N = 17) AA and 34.6 % (N = 9) AV), in 31.8 % in tibia (28.6 % (N = 4) AA, 7.1 % (N = 1) VV and 64.3 % (N = 9) AV), in 4.5 % in humerus (100% (N = 2) AA), in 2.3 % in scapula (100 % (N = 1) AV) and in 2.3 % in clavicula (100 % (N = 1) AA). The tumour was localized in femur in 59.1 % of the cases (100 % (N = 3) AA), in 11.1 % in tibia (100 % (N = 1) AV), in 22.2 % in humerus (50 % (N = 1) AA and 50 % (N = 1) AV), in 11.1 % in sacroiliac region (100 % (N = 1) AV) and in 11.1 % in lumbopelvic region (100 % (N = 1) AV). No association was found between the tumour site and *MTHFR* genotypes in the chondrosarcoma patients; the tumour site was found to be independent of the *MTHFR* genotype.

Discussion

A common mutation in the *MTHFR* gene results from C to T transition at nucleotide 677, substituting alanine for valine at codon 222 (Frosst et al., 1995). This mutation is associated with increased thermolability and reduced enzyme activity, resulting in lower plasma folate levels (Jacques et al., 1996). Folate plays a role as a do-

Table 2. Comparison of the presence of local tumour relapse, metastasis, and gender and MTHFR C677T polymorphism in patients' groups

	Genotypes			**Alleles**	
	AA	**VV**	**AV**	**A**	**V**
Osteosarcoma (N = 44)					
Presence of local tumour relapse **(N = 4)**	2 (50.0 %)	0 (0 %)	2 (50.0 %)	4 (66.6 %)	2 (33.3 %)
Presence of metastasis **(N = 6)**	4 (66.7 %)	0 (0 %)	2 (33.3 %)	6 (75.0 %)	2 (25.0 %)
Female **(N = 22)**	12 (54.5 %)	1 (4.5 %)	9 (40.9 %)	21 (67.4 %)	10 (32.25 %)
Male **(N = 22)**	12 (54.5 %)	0 (0 %)	10 (45.5 %)	22 (68.75 %)	10 (31.25 %)
Chondrosarcoma (N = 12)					
Presence of local tumour relapse **(N = 2)**	1 (50.0 %)	0 (0 %)	1 (50.0 %)	2 (66.6 %)	1 (33.3 %)
Presence of metastasis	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)
Female **(N = 5)**	3 (60.0 %)	0 (0 %)	2 (40.0 %)	5 (71.42 %)	2 (28.57 %)
Male **(N = 7)**	4 (57.1 %)	0 (0 %)	3 (42.9 %)	7 (70.0 %)	3 (30.0 %)
Total patient group (N = 56)					
Presence of local tumour relapse **(N = 6)**	3 (50.0 %)	0 (0 %)	3 (50.0 %)	6 (66.6 %)	3 (33.3 %)
Presence of metastasis **(N = 6)**	4 (66.7 %)	0 (0 %)	2 (33.3 %)	6 (75.0 %)	2 (25.5 %)
Female **(N = 27)**	15 (55.6 %)	1 (3.7 %)	11 (40.7 %)	26 (68.42 %)	12 (31.57 %)
Male **(N = 29)**	16 (55.2 %)	0 (0 %)	13 (44.8 %)	29 (69.04 %)	13 (30.95 %)

N: number of individuals

nor of one carbon group in DNA methylation and nucleic acid synthesis. Folate deficiency causes not only defects in DNA methylation, but also genomic instability (Chen et al., 1998). Folate deficiency is also associated with chromosome breaks, because reduced DNA methylation decreases the synthesis of thymidine from uracil, leading to DNA strand breaks (Duthie and Hawdon, 1998). DNA methylation defects, loss of synthesis of purine and pyrimidine nucleotides and insufficient DNA repair mechanisms are some causes of DNA damage, which also are means of the carcinogenesis process (Goelz et al., 1985; Feinberg et al., 1988). Since MTHFR is indirectly related to these processes, *MTHFR* polymorphism or defects are hypothesized to be one of the risk factors for neoplastic diseases (Chiusolo et al., 2002; Zoodsma et al., 2005).

A correlation between *MTHFR* polymorphism and breast cancer or endometrial cancer has previously been demonstrated (Esteller et al., 1997; Campbell et al., 2002). Some studies focused on the combined effect of *MTHFR* polymorphism and dietary intake of folate on cancer formation. Beilby et al. observed that increased serum concentration of folate was associated with reduced risk of breast cancer, while this relation was stronger in cases with C677T genotype of the *MTHFR* gene (Beilby et al., 2004).

In the present study, the A allele frequency was higher in male osteosarcome patients compared to male control subjects in the gender subgroup analysis, but not statistically significant (P = 0.064). However, our results demonstrated that there is no association between *MTHFR* C677T polymorphism (the presence of V allele or VV genotype) and osteosarcoma or chondrosarcoma development.

Contrary to our study, an association between *MTHFR* genotype and colonic cancer was reported by some authors. Slattery et al. found that the presence of *MTHFR* valine/valine genotype and adequate folate intake resulted in 30–40% reduced risk for colon cancer, compared to wild-type *MTHFR* genotype and reduced folate intake (Slattery et al., 1999). Ma et al. found that the MTHFR valine/valine genotype had a 50% reduced colorectal carcinoma risk compared to alanine/valine and alanine/alanine genotypes (Ma *et al.,* 1997). In another study, it was shown that the valine/valine genotype was associated with a statistically significant decrease in the risk for colorectal carcinoma (Yin *et al.,* 2004). However, some researchers failed to show any association between the *MTHFR* genotype and colorectal carcinoma (Marugame *et al.,* 2000; Ulrich *et al.,* 2000).

In conclusion, our findings do not support the hypothesis that the V allele or VV genotype of *MTHFR* is a reducing factor for carcinoma**.** The present article is one of the preliminary studies about *MTHFR* gene polymorphism in osteosarcoma and chondrosarcoma patients. Studies performed on a larger number of subjects are needed to achieve a better understanding of this relationship.

Acknowledgement

The authors would like to thank Dr. Hakan Camlica for statistical analysis. Dr. Camlica is an Assistant Professor at the University of Istanbul, Institute of Oncology, Division of Cancer Epidemiology and Biostatistics, Istanbul, Turkey.

References

Beilby, J., Ingram, D., Hahnel, R., Rossi, E. (2004) Reduced breast cancer risk with increasing serum folate in a case-control study of the C677T genotype of the methylenetetrahydrofolate reductase gene. *Eur. J. Cancer* **40**, 1250-1254.

Campbell, I. G., Baxter, S. W., Eccles, D. M., Choong, D. Y. (2002) Methylenetetrahydrofolate reductase polymorphism and susceptibility to breast cancer. *Breast Cancer Res.* **4**, R14.

Chen, J., Giovannucci, E., Kelsey, K., Rimm, E. B., Stampfer, M. J., Colditz, G. A., Spiegelman, D., Willett, W. C., Hunter, D. J. (1996) A methylenetetrahydrofolate reductase polymorphism and the risk of colorectal cancer. *Cancer Res.* **56**, 4862-4864.

Chen, R. Z., Pettersson, U., Beard, C., Jackson-Grusby, L., Jaenisch, R. (1998) DNA hypomethylation leads to elevated mutation rates. *Nature* **395**, 89-93.

Chiusolo, P., Reddiconto, G., Casorelli, I., Laurenti, L., Sora, F., Mele, L., Annino, L., Leone, G., Sica, S. (2002) Preponderance of methylenetetrahydrofolate reductase C677T homozygosity among leukemia patients intolerant to methotrexate. *Ann. Oncol.* **13,** 1915-1918.

Duthie, S. J., Hawdon, A. (1998) DNA instability (strand breakage, uracil misincorporation and defective repair) is increased by folic acide depletion in human lymphocytes in vitro. *FASEB J.* **12**, 1491-1497.

Esteller, M., Garcia, A., Martinez-Palones, J. M., Xercavins, J., Reventos, J. (1997) Germ line polymorphisms in cytochrome-P450 1A1 (C4887 CYP1A1) and methylenetetrahydrofolate reductase (MTHFR) genes and endometrial cancer susceptibility. *Carcinogenesis* **18**, 2307-2311.

Feinberg, A. P., Gehrke, C. W., Kuo, K. C., Ehrlich, M. (1988) Reduced genomic 5-methylcytosine content in human colonic neoplasia. *Cancer Res.* **48**, 1159-1161.

Frosst, P., Blom, H. J., Milos, R., Goyette, P., Sheppard, C. A., Matthews, R. G., Boers, G. J., Den Heijer, M., Kluijtmans, L. A., Van Den Heuvel, L. P. (1995) A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat. Genet.* **10**, 111-113.

Gershoni-Baruch, R., Dagan, E., Israeli, D., Kasinetz, L., Kadouri, E., Friedman, E. (2000) Association of the C677T polymorphism in the MTHFR gene with breast and/or ovarian cancer risk in Jewish women. *Eur. Cancer* **36**, 2313-2316.

Goelz, S. E., Vogelstein, B., Hamilton, S. R., Feinberg, A. P. (1985) Hypomethylation of DNA from benign and malignant human colon neoplasms. *Science* **28**, 187-190.

Jacques, P. F., Bostom, A. G., Williams, R. R., Ellison, R. C., Eckfeldt, J. H., Rosenberg, I. H., Selhub, J., Rozen, R. (1996) Relation between folate status, a common mutation

in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. *Circulation* **93**, 7-9.

James, S. J., Basnakian, A. G., Miller, B. J. (1994) In vitro folate deficiency induces deoxynucleotide pool imbalance, apoptosis, and mutagenesis in Chinese hamster ovary cells. *Cancer Res.* **54**, 5075-5080.

Kim, Y. I., Pogribny, I. P., Salomon, R. N., Choi, S. W., Smith, D. E., James, S. J., Mason, J. B. (1996) Exon-specific DNA hypomethylation of the p53 gene of rat colon induced by dimethylhydrazine. Modulation by dietary folate. *Am. J. Pathol.* **149**, 1129-1137.

Krajinovic, M., Lemieux-Blanchard, E., Chiasson, S., Primeau, M., Costea, I., Moghrabi, A. (2004) Role of polymorphisms in MTHFR and MTHFD1 genes in the outcome of childhood acute lymphoblastic leukemia. *Pharmacogenomics J.* **4**, 66-72.

Kundu, T. K., Rao, M. R. (1999) CpG islands in chromatin organization and gene expression. *J. Biochem.* **125**, 217-222.

Lengauer, C., Kinzler, K. W., Vogelstein, B. (1997) DNA methylation and genetic instability in colorectal cancer cells. *Proc. Natl. Acad. Sci. USA* **94**, 2545-2550.

Ma, J., Stampfer, M. J., Giovannucci, E., Artigas, C., Hunter, D. J., Fuchs, C., Willett, W. C., Selhub, J., Hennekens, C. H., Rozen, R. (1997) Methylenetetrahydrofolate reductase polymorphism, dietary interactions, and risk of colorectal cancer. *Cancer Res.* **57**, 1098-1102.

Marugame, T., Tsuji, E., Inoue, H., Shinomiya, S., Kiyohara, C., Onuma, K., Hamada, H., Koga, H., Handa, K., Hayabuchi, H., Kono, S. (2000) Methylenetetrahydrofolate reductase polymorphism and risk of colorectal adenomas. *Cancer Lett.* **151,** 181-186.

Mclean, R. R., Karasik, D., Selhub, J., Tucker, K. L., Ordovas, J. M., Russo, G. T., Cupples, L. A., Jacques, P. F., Kiel, D. P. (2004) Association of a common polymorphism in the methylenetetrahydrofolate reductase (MTHFR) gene with bone phenotypes depends on plasma folate status. *J. Bone Miner. Res.* **19**, 410-418.

Miller, S. A., Dykes, D. D., Polesky, H. S. (1988) Simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* ***16***, 1215.

Slattery, M. L., Potter, J. D., Samowitz, W., Schaffer, D., Leppert, M. (1999) Methylenetetrahydrofolate reductase, diet, and risk of colon cancer. *Cancer Epidemiol. Biomarkers Prev.* **8**, 513-518.

Toffoli, G., Russo, A., Innocenti, F., Corona, G., Tumolo, S., Sartor, F., Mini, E., Boiocchi, M. (2003) Effect of methylenetetrahydrofolate reductase 677C-->T polymorphism on toxicity and homocysteine plasma level after chronic methotrexate treatment of ovarian cancer patients. *Int. J. Cancer* **103**, 294-299.

Ueland, P. M., Hustad, S., Schneede, J., Refsum, H., Stein, S. E. (2001) Biological and clinical implications of the MTHFR C677T polymorphism. *Trends Pharmacol. Sci.* ***22***, 195-201.

Ulrich, C. M., Kampman, E., Bigler, J., Schwartz, S. M., Chen, C., Bostick, R., Fosdick, L., Beresford, S. A. A., Yasui, Y., Potter, J. D. (2000) Lack of association between the C677T MTHFR polymorphism and colorectal hyperplastic polyps. *Cancer Epidemiol. Biomarkers Prev.* **9**, 427-433.

Yin, G., Kono, S., Toyomura, K., Hagiwara, T., Nagano, J., Mizoue, T., Mibu, R., Tanaka, M., Kakeji, Y., Maehara, Y., Okamura, T., Ikejiri, K., Futami, K., Yasunami, Y., Maekawa, T., Takenaka, K., Ichimiya, H., Imaizumi, N. (2004) Methylenetetrahydrofolate reductase C677T and A1298C polymorphisms and colorectal cancer: The Fukuoka Colorectal Cancer Study. *Cancer Sci.* **95**, 908-913.

Zoodsma, M., Nolte, I. M., Schipper, M., Oosterom, E., Van Der Steege, G., De Vries, E. G., Te Meerman, G. J., Van Der Zee, A. G. (2005) Methylenetetrahydrofolate reductase (MTHFR) and susceptibility for (pre)neoplastic cervical disease. *Hum. Genet.* **116**, 247-254.