Polymorphisms of methylenetetrahydrofolate reductase and the risk of prostate cancer: a nested case–control study
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It has been proposed that folate and polymorphisms of the enzyme methylenetetrahydrofolate reductase (MTHFR), which regulates influx of folate from DNA synthesis and repair to methylation reactions, are involved in the aetiology of cancer. To relate the MTHFR 677C→T and 1298A→C polymorphisms to the risk of prostate cancer, taking into consideration prospective plasma levels of folate, vitamin B₁₂ and homocysteine. The design was a case–control study of 223 prostate cancer cases and 435 matched controls nested within the population-based Northern Sweden Health and Disease Cohort. Neither the MTHFR 677C→T nor the MTHFR 1298A→C polymorphism was statistically significantly associated with the risk of prostate cancer in univariate analysis by conditional logistic regression. After adjustment for MTHFR 1298A→C, plasma folate, vitamin B₁₂, homocysteine, body mass index and smoking, the odds ratios were, for the 677 CT genotype, 1.52 [95% confidence interval (CI) 1.02–2.26], and for TT, 0.91 (95% CI 0.41–2.04). Our previously reported observation of a possible increase in the risk of prostate cancer at high plasma folate levels was attributable in this study to subjects having the MTHFR 677C→T polymorphism. We found that the MTHFR 677C→T polymorphism is not likely to have a major role in the development of prostate cancer, although it may possibly increase the risk in combination with high plasma folate levels. Further investigation in larger studies is warranted. European Journal of Cancer Prevention 15:46–50 @ 2006 Lippincott Williams & Wilkins.

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Introduction
It has been proposed that folate, a B vitamin found primarily in vegetables, has a protective effect in the development of cancer. Suggested mechanisms have focused on the key role of folate as the donor of one-carbon groups for nucleotide synthesis and methylation reactions. These two pathways of folate metabolism are separated by an irreversible reaction regulated by the enzyme methylenetetrahydrofolate reductase (MTHFR). A C→T substitution at base 677 of the MTHFR gene results in a thermolabile enzyme with a reduction in activity of up to 35% in heterozygotes and 70% in TT homozygotes (Frosst et al., 1995). A 1298A→C polymorphism has also been found to decrease enzyme activity, although to a lesser extent (Weisberg et al., 1998). The MTHFR polymorphisms can thus provide insight into the relative importance of different aspects of folate metabolism in the aetiology of cancer (Fig. 1).

The substrate of the MTHFR reaction, 5,10-methylene-tetrahydrofolate, promotes nucleotide synthesis. Of particularly importance is the synthesis of thymidylate from uracil, which minimizes the misincorporation of uracil in DNA and, consequently, double-strand breaks, and ensures adequate mismatch repair. The product of the MTHFR reaction, 5-methyltetrahydrofolate, provides single-carbon groups for the methylation of the amino acid homocysteine to methionine, a reaction for which vitamin B₁₂ is a co-enzyme. Methionine is the precursor of S-adenosylmethionine, the universal one-carbon donor for methylation reactions. Hypomethylation of global DNA, associated with genome instability, is a common trait in tumours (Jones and Baylin, 2002). It might be speculated therefore that MTHFR polymorphisms, and the consequent decrease in enzyme function, either protect against the development of cancer by providing more folate for DNA synthesis and repair, or increase the risk by reducing the availability of methyl groups (Friso and Choi, 2005).

In the prostate, however, gene-specific hypermethylation appears to have a particularly important role in tumorigenesis (Nelson et al., 2003). Methylation of CpG island sequences in the promoter region of genes silences their expression, which, in the case of tumour suppressor and other DNA protective genes such as the caretaker
glutathione-S-transferase π (GSTP1), may contribute to the development of prostate tumours (Nelson et al., 2003). Most preliminary evidence from epidemiological studies has tended to support an increased risk of prostate cancer associated with the MTHFR polymorphisms (Kimura et al., 2000; Heijmans et al., 2003; Cicek et al., 2004; Singal et al., 2004), but the data remain inconclusive.

The aim of the present study was to relate the MTHFR 677C→T and 1298A→C polymorphisms to the risk of prostate cancer, taking into consideration possible interactions with prospective plasma levels of folate, vitamin B₁₂ and homocysteine.

Materials and methods

The Northern Sweden Health and Disease Cohort

This was a case-control study nested within the population-based Northern Sweden Health and Disease Cohort (NSHDC), which for men includes the Västerbotten Intervention Project (VIP) (Hallmans et al., 2003) and the Northern Sweden WHO Monitoring of Trends and Cardiovascular Disease study (MONICA) (Stegmayr et al., 2003). VIP is an ongoing, community-based intervention study founded in 1985, with the aim of reducing cardiovascular disease and cancer through lifestyle changes. Each year, all residents of the county of Västerbotten who are 40, 50 or 60 years of age are invited to participate. The MONICA study includes a randomly selected, population-based sample of 2000 healthy subjects recruited from the counties of Västerbotten and Norrbotten every 4 years since 1986. In both projects, subjects undergo a health examination at which height and body weight are measured (to the nearest 0.2 kg and cm, respectively) and a 20 ml blood sample is collected. For the large majority of participants, blood collection takes place in the morning after a fast of at least 8 h, and all blood samples are stored at −80°C, either directly or after, at most, 1 week at −20°C. Both VIP and MONICA also include an extensive self-administered questionnaire addressing demographic, medical and lifestyle characteristics. As of July 2001, the time of case identification for the present study, a total of 37776 men had been recruited to NSHDC, 2708 of whom were from the MONICA study.

Study subjects

All incident cases of prostate cancer were identified using national personal identification numbers to link the Northern Sweden Health and Disease Cohort with the regional cancer registry. This resulted in identification of 299 histologically confirmed cases of prostate cancer and 617 controls for whom analysis of polymorphisms and/or plasma factors involved in folate metabolism was possible. The mean follow-up time between recruitment and the diagnosis of prostate cancer was 4.9 years (SD = 2.8). For each case, two controls were randomly selected from among men matching the index case for age (±2 years) and date of blood sampling (±2 months), and who were alive and free of cancer at the time of diagnosis of the index case. Tumour characteristics were extracted from medical records by a research nurse, verified by the treating physician, and reported to the Prostate Cancer Registry at the Oncological Center, Umeå University Hospital, which includes more than 98% of all prostate cancer cases diagnosed in northern Sweden. No formal screening programme has ever been in operation in the catchment area of the cohort, and in the present study approximately 12% of cases were asymptomatic at diagnosis and identified through health check-ups. This is in accordance with the background population (Stattin et al., 2003) and suggests little exposure for opportunistic prostate-specific antigen (PSA) screening for early detection of prostate cancer.

Laboratory and statistical analyses

Genotypes for MTHFR 677C→T and 1298A→C were generated using the TaqMan allelic discrimination method. TaqMan assays and reagents were from Applied Biosystems (Foster City, California, USA). Polymerase chain reactions (PCRs) were performed on the GeneAmp PCR system 9700, and PCR programs were according to the manufacturer (ABI). PCR products were analysed at the ABI PRISM 7900HT Sequence Detection System. The plasma analyses have been described previously (Hultdin et al., 2005). In brief, concentrations of folate and vitamin B₁₂ in heparin plasma were analysed by Quantaphase II radioassay (BioRad Diagnostic Group, Hercules, California, USA), and total plasma homocysteine was measured by a fluorescence polarization immunoassay on an IMx unit (Abbott Laboratories, Abbott Park, Illinois, USA). All coefficients of variation for the plasma analyses were under 7.5%. Genotyping analyses were performed at the Center for Genome Research, plasma analyses at Clinical Chemistry, both of the Department of Medical Biosciences, Umeå University, Sweden.
Risk estimates for prostate cancer were obtained by conditional logistic regression analysis, with $P_{\text{trend}}$ determined by assigning ordinal values to genotypes (homozygous wild-type = 1, heterozygous = 2, homozygous for the mutation = 3), and midpoint values to quartiles of plasma variables, and including them as continuous variables in analyses. To evaluate the effect of the $MTHFR$ polymorphisms on the prostate cancer risk estimates for plasma folate, vitamin B$_{12}$ and homocysteine reported in Hultdin et al. (2005), we calculated odds ratios (ORs) in genotype subgroups by non-conditional logistic regression because case-control tripllets could not be conserved). SPSS version 11.5 (SPSS Inc., Chicago, Illinois, USA) was used for all statistical testing. A $P$ value of 0.05 was considered significant, and all analyses were two-sided.

**Ethics approval**

This study was approved by the Research Ethics Committee of Umeå University Hospital, and all subjects provided informed consent at recruitment for the use of their blood samples in future research.

**Results**

For both $MTHFR$ polymorphisms, the full study group, as well as cases and controls separately, were in Hardy–Weinberg equilibrium. Genotype frequencies and baseline characteristics for the study group are presented in Table 1. The $MTHFR$ 677C→T polymorphism was statistically significantly associated with lower plasma folate and vitamin B$_{12}$ levels and higher homocysteine levels, while the $MTHFR$ 1298A→C polymorphism was not related to any of the plasma variables (Table 2).

Neither the $MTHFR$ 677C→T nor 1298A→C polymorphism, nor any haplotype of the two, was statistically significantly associated with the risk of prostate cancer in univariate analysis, although the OR for the 677 CT vs. CC genotype became statistically significant after adjustment for potential confounding factors in analyses. To evaluate the effect of the $MTHFR$ polymorphisms on the prostate cancer risk estimates for plasma folate, vitamin B$_{12}$ and homocysteine reported in Hultdin et al. (2005), we calculated odds ratios (ORs) in genotype subgroups by non-conditional logistic regression because case-control tripllets could not be conserved). SPSS version 11.5 (SPSS Inc., Chicago, Illinois, USA) was used for all statistical testing. A $P$ value of 0.05 was considered significant, and all analyses were two-sided.

None of the ORs for the $MTHFR$ polymorphisms differed materially by disease state (advanced disease defined as locally advanced tumour (T3 or T4), and/or lymph node metastasis (N1), and/or metastasis on bone scan (M1), and/or serum PSA over 50 ng/ml) (data not shown).

High plasma folate levels were associated with a statistically significant increased risk of prostate cancer in the combined $MTHFR$ 677 CT and TT subgroup (OR 2.30 (95% CI 1.07–4.93) for highest vs. lowest quartile, $P_{\text{trend}} = 0.06$), but not in the CC subgroup (Table 4). No interaction with genotype was observed for plasma vitamin B$_{12}$ or homocysteine (data not shown).

**Discussion**

In this population-based study, neither the $MTHFR$ 677C→T nor the 1298A→C polymorphism showed a dose-dependent association with the risk of prostate cancer, although heterozygosity for $MTHFR$ 677C→T was associated with a statistically significant moderate risk increase after adjustment for potential confounding factors.
The *MTHFR* polymorphisms mimic a lifelong reduced exposure to folate, and thus, much like a randomized clinical trial, allow study of folate metabolism in cancer development with less risk of bias than epidemiological studies. This is the principle of Mendelian randomization (Davey Smith and Ebrahim, 2004). Although, to date, our studies. This is the principle of Mendelian randomization development with less risk of bias than epidemiological clinical trial, allow study of folate metabolism in cancer exposure to folate, and thus, much like a randomized the TT genotype. This may be due to the low number of TT subjects, or it may suggest a chance finding for the CT group.

In our previous article (Hultdin et al., 2005), in which plasma vitamin B<sub>12</sub> was found to be a strong risk factor for prostate cancer, we hypothesized that an increase in the available pool of methyl donors might increase susceptibility to hypermethylation and consequent silencing of genes such as *GSTP1* and tumour suppressors in the prostate, a phenomenon believed to have an important role in prostate tumorigenesis (Nelson et al., 2003). However, the increased risk of prostate cancer observed in the present study for the combination of high plasma folate and the *MTHFR* 677C→T polymorphism might also suggest an increase in the availability of folate for DNA synthesis, and thus cell proliferation, in an undiagnosed prostate tumour. Given the high prevalence of prostate cancer in the general population (Thompson et al., 2003), the possibility of a role for folate metabolism in tumour growth may be important. Furthermore, prostate epithelial cells express high levels of prostate-specific membrane antigen (PSMA), a folate hydrolase that is a marker and therapeutic target in prostate cancer (Ghosh and Heston, 2004).

In a recent family-based study, the *MTHFR* 677 T allele increased risk of less advanced prostate cancer but demonstrated a protective effect against more advanced disease (Cicak et al., 2004). In line with the latter observation is that polyamines, important regulators of prostate cell proliferation and differentiation whose production is dependent on methylation by S-adenosylmethionine,
are currently being investigated as targets for prostate cancer chemotherapy (Schipper et al., 2003). However, Kimura et al., (2000) reported a weak positive association between MTHFR 677C→T and tumour grade, Singal et al., (2004) observed a possible protective effect in earlier, less advanced prostate cancer, and risk estimates in the present study did not differ by disease state.

In colorectal cancer, the role of the MTHFR 677C→T polymorphism is believed to depend largely on folate status, being protective in combination with high dietary intake/biological levels of folate (Sharp and Little, 2004). A protective effect has also been observed in acute lymphatic leukaemia (Robien and Ulrich, 2003), while for cancers of the oesophagus and stomach, preliminary reports have suggested a possible increased risk (Shen et al., 2001; Song et al., 2001; Miao et al., 2002).

For breast cancer, results have been mixed (Campbell et al., 2002; Sharp et al., 2002; Langsenlehner et al., 2003; Semenza et al., 2003). Thus, it appears that the role of MTHFR activity in the development of cancer, and thereby the relative importance of the DNA synthesis and repair versus methylation pathways of folate metabolism, may vary by cancer site and folate status.

In conclusion, our results suggest that the MTHFR 677C→T polymorphism is not likely to have a major role in the development of prostate cancer, although it may moderately increase risk in combination with high plasma folate levels. Further investigation, based on larger study groups, is warranted.

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References


