Gene Polymorphisms MTHFR C677T and MTR A2756G as Predictive Factors in Adjuvant Chemotherapy for Stage III Colorectal Cancer

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Abstract. Background: The aim of this study was to explore the effect in stage III colorectal cancer of functional gene polymorphisms methylenetetrahydrofolate reductase (MTHFR C677T) and methionine synthase (A2756G), in the folate metabolism on outcome and risk of toxicity for adjuvant chemotherapy. A secondary aim was to investigate any possible interdependency between the two genes. Patients and Methods: one hundred and fifty randomly chosen patients with stage III colorectal cancer, treated with adjuvant chemotherapy, were genotyped by real-time PCR. Patient and treatment data were retrieved and assessed for demography, pathology, chemotherapy tolerability and survival after adjuvant therapy. The polymorphisms were studied separately and in combination to discover possible interactions. Results: Patients with MTHFR 677 CC genotype carried lower risks of suffering from nausea (p=0.027), parasthesia (p=0.0042) and need for dose reduction (p=0.025). The CC genotype was also associated with better survival (p<0.034). There was interdependency with MTR A2756G. Patients with MTR AG/GG in combination with MTHFR CT/TT genotypes carried the highest risk of side-effects. Conclusion: Functional polymorphisms of MTHFR C677T and MTR A2756G can affect outcome and risk of toxicity during adjuvant chemotherapy in stage III colorectal cancer. Their possible interdependence brings attention to the function of folate metabolism overall regarding its association with 5-fluorouracil related toxicity. Our results could explain some of the difficulties of obtaining reproducible and uniform results when using single polymorphisms as predictive markers.
but also by genetic variations in the form of functional polymorphisms. One of the most studied enzymes is TS, for which several gene polymorphisms have been described (7, 8). MTHFR is less studied but two functional polymorphisms, A1298G and C677T, have currently been identified (9, 10) whereas only one, A2756G, has been described for MTR.

Chemotherapy is not a risk-free treatment. Adverse events are common which can affect not only the patients health and well-being but also the chance of treatment completion and even the outcome (11). Great effort is directed at finding predictive factors that can aid the oncologist in providing the optimal treatment for the individual patient (12). Predictive factors could suggest what regimes to use in order to optimise the clinical efficacy and reduce the risk of serious side-effects.

The aim of the present study was to explore the effect of gene polymorphisms MTHFR C677T and MTR A2756G on outcome and risk of toxicity for patients with stage III colorectal cancer when treated with adjuvant 5-FU-based chemotherapy. A secondary aim was to explore a possible interdependence between the two genes.

Patients and Methods

Patients. The study was conducted at a high-volume university hospital, where blood samples are routinely collected from patients operated on for colorectal cancer. A total of 649 patients were operated on for stage III colorectal cancer in the period 1999-2006. Subsequently, 429 of these patients (66.1%) received postoperative adjuvant chemotherapy and 150 of them, randomly chosen, were genotyped by Single Nucleotide Polymorphism (SNP) analysis of genomic DNA as described below. All included patients were treated according to the Nordic FLV-protocol based on 500 milligram per m² of 5-FU in combination with 60 milligrams per m² of leucovorin. The chemotherapy was given as a weekly intravenous bolus injection for 6 months. Sixteen patients, proportionally distributed among the groups, also received oxaliplatin at 85 milligrams per m² as an i.v. infusion every other week. Patient and treatment data were retrieved and assessed for demography, pathology and chemotherapy tolerability, including any side-effects, along with data from systematic follow-up. Survival was calculated from the start of adjuvant treatment until death. The polymorphisms were analysed separately as well as in combination to assess a possible interaction. As the prevalence of patients having MTHFR 667 TT and MTR 2756 GG was low, each of these groups were pooled with the corresponding heterozygote. The study was approved by the local Ethics Committee and all patients gave their written informed consent.

Genotyping of the MTHFR and MTR genes. Genomic DNA was extracted from fresh-frozen blood using QIAamp DNA Mini Kit according to the manufacturer’s instructions (Qiagen, Hilden, Germany). Genotype analyses of the genes MTHFR (rs1801133, assay no. C_1202883_20) and MTR (rs18050087, assay no. C_12005959_10) were run on an ABI PRISM® 7900HT sequence detection system (Applied Biosystems) using real-time PCR and TaqMan chemistry. The SNP assays (Applied Biosystems) and the TaqMan PCR master mix (Applied Biosystems, Foster City, CA,
USA) were aliquoted into a 384-well plate using a liquid-handling Biomek FX robot (Beckman Coulter Inc., San Diego, CA, USA). Reactions were characterized by comparing the threshold cycle (CT) values as described by the manufacturer. Laboratory staff members employed in genotyping were blinded to clinical outcome. All genotype analyses were performed by the Genomics Core Facility in Gothenburg, Sweden.

Statistics. JMP 8.0 /SAS software (SAS institute, Cary, NC, USA) was used for statistical analysis. The data was analysed by distribution statistics and t-tests for parametric data. For the non-parametric data, contingency tables with the chi-square test were used. The Kaplan-Meier method and Wilcoxon test was used for survival assessment. The confidence level was set at 95%.

Results

Patient characteristics and genotype prevalence. The patient characteristics are summarized in Table I. The median age of the patients was 66 years. They had all been radically operated on, with no evidence of remaining tumour tissue, or distant metastasis. All patients had stage III disease, with a median number of 15 assessed lymph nodes. All patients received chemotherapy as describe above. There was no association between any polymorphism and demographic factors or pathological data, such as local tumour stage or differentiation grade. The MTHFR C677T and MTR A2756G genotype distributions were in Hardy Weinberg equilibrium. The overall genotype distribution was for MTR 2756, AA 95 (63.3%), AG 43 (28.7%), and GG 12 (8.0%); and for MTHFR 677, CC 71 (48.6%), CT 64 (43.8%) and TT 11 (7.6%). There was no association between MTHFR and MTR genotype prevalence. Genotype data for MTHFR was inconclusive for four patients. Median follow-up was 70 months.

Side effects, survival and interdependency of genotype. Patients with MTHFR CC genotype had a lower risk of suffering nausea (p=0.027) and parasthesia (p=0.0042) and more often needed a dose reduction (p=0.025) than patients with the CT/TT genotype (Table II). The parasheets side-effect was to a large extent also associated with the oxaliplatin combination therapy. Patients with the MTHFR CC genotype also had a better survival after adjuvant chemotherapy (Figure 2, p=0.034). The findings were only statistically valid for the patients who also had the MTR AA genotype. The risk of suffering side-effects was dependent upon the combination of genotypes, where patients with a MTHFR 667 CC and MTR 2756 AA genotype carried the lowest risk of 5-FU-related toxicity (Table III). There was no statistical association between MTR 2756 genotype by itself and survival or the risk of side-effects. A sub-group analysis, excluding patients who due to high age or co-morbidity started with a lower dose, showed that patients with the genotype MTR AG/GG were more likely to need a reduction of dose (58.6% vs. 76.6%, p=0.038) and pre-emptive treatment termination than those with the AA genotype (relative risk 2.1, p<0.03).

Discussion

Chemotherapy is used in treatment of colorectal cancer in several ways related to the indication (3). The treatment itself carries risks of side-effects, which, in milder cases can affect the patients’ well-being and quality of life. However, the treatment can also lead to life-threatening conditions such as severe infections and toxicity of the bone marrow.

Adjuvant treatment is used for patients who has confirmed lymph node metastasis, but are without radiological evidence of distant metastasis and thus having stage III disease. The risk assessment can be difficult and differs from the palliative situation. There are some prognostic measures, such as lymph node ratios, which can assess the risk at group level, but the risk for the individual patient is unknown (13). Thus, it can be hard to ascertain the benefit of the treatment for the individual patient. Development of predictive factors is important in order to increase treatment accuracy and minimize patient toxicity. An individualized chemotherapy regimen, by the use of predictive factors, should have several important benefits, such as less patient suffering and a better chance of treatment completion by reducing the risk of side-effects.

Patients with the MTHFR 677 CC genotype had a significantly lower risk of chemotherapy related toxicity and a better survival than those with CT/TT genotypes. These findings concur with those that have been previously reported, including from our own group (14, 15). The main target of 5-FU is folate metabolism, by inhibiting TS (4). The associated enzyme MTHFR converts 5,10MTHF to 5THF. The product provides a methyl group to convert homocysteine to methionine which is supported by MTR. Thus, a less active enzyme system, with MTHFR 667 CC being more effective than MTHFR 667 CT/TT, could theoretically result in increased 5,10MTHF-levels. This could lead to a higher efficacy of 5-FU in binding and inhibiting TS and could thus increase the toxicity. Moreover, it could also lead to a lower level of substrate for the conversion of homocysteine to methionine by MTR. This could affect the
availability of methyl groups needed in DNA gene regulation through the methylation process and might even have a prognostic impact.

Previous findings on TS and MTHFR gene polymorphisms as predictive factors looked promising as described in several publications (7, 8, 16). However, the data have been hard to validate and have even occasionally been contradicted (17, 18). Thus, no real clinical utilization has yet been found, as noted by Tejpar et al. (19). There are many difficulties in the search for predictive factors, including the increasing molecular heterogeneity of the colorectal cancer itself (20, 21). The absence of validated conclusive findings could also suggest that the molecular heterogeneity makes the use of single markers more difficult.

The MTR A2756G polymorphism has been suggested to affect the risk of developing cancer but has otherwise, to our knowledge, not been clinically studied in cancer disease (22, 23). In the present paper, there was a link between MTR genotype and the risk for a need for dose reduction due to toxicity. The lack of findings for toxicity in association with MTR itself suggested a possible functional interdependency.

Thus, the following exploration of a connection between the different genotypes of MTHFR and MTR gave findings that challenge and modify previous results. A patient who has an optimal enzymatic activity of MTHFR, which genotype 677 CC provides, in combination with MTR 2756 AA has the least risk of side-effects. The risk of side-effects increases for patients who instead have the MTR AG or GG genotypes. The pattern is similar but more pronounced for patients with MTHFR 667 CT and TT genotypes. The risk of nausea could serve as an example, where a patient with the MTHFR 667 CT or TT and MTR 2756 AG or GG genotype combination had 4.5 times higher risk (p<0.014) than patients with the MTHFR CC and MTR AA genotype combination. The functional interdependency did also, in this material, affect the survival, where again the difference by MTHFR function only is seen for the patients with MTR 2756 AA genotype.

Distinction can be made between prognostic and predictive factors where the latter relates to the prognosis for a specific treatment rather than being an overall indicator of survival. The MTHFR C677T polymorphism could then be...
suggested as a predictive factor for 5-FU treatment in colorectal cancer. However, that for MTHFR would be valid only for 95 out of 150 patients and the findings in the present study suggest that the outcome can be altered by MTR A2756G, which then should be added as a potential factor. Thus, it could be difficult to advocate the use of a single marker for prediction considering these findings. It is also highly plausible that the same connection and interdependence could exist with other enzymes such as TS. It is not unreasonable that the findings could, at least partly, explain some of the difficulties in reaching coherent and conclusive results with single predictive markers for use in tailoring and individualising chemotherapy. Identifying and testing markers and combinatory risk scores for the overall function of the metabolism could lead to further progress.

The study was performed at a single centre treating patients from a specific geographic region. Thus, the treatments and assessments were carried out coherently and along the same guidelines for all patients and also allowed for a good follow-up of the patients. The cancer staging was adequately performed, with adequate node assessment data, thus minimizing the risk of stage migration effects (24). The dietary folate content has been suggested to possibly influence the effect of the polymorphisms. The actual folate levels in blood or colorectal mucosa was not measured in this material, which is a possible weakness of the study. There is always an inter-individual variation in dietary intake and it has been suggested that low levels could affect the genotype-related risks. However, since the present patient cohort consists of an unselected material from a region without special dietary limitations, it is reasonably safe to assume that the dietary difference at a group level is not significant. There could also be geographical variations in genotype prevalence between different regions of the world, which could affect the findings from other research groups. The results here could support a hypothesis of MTHFR C677T being a predictive factor for 5-FU treatment in stage III colorectal cancer. However, the MTR A2756G gene polymorphism can affect and modify the risk of toxicity and the interdependency should not be neglected. It would be of interest to confirm these findings in further studies. A study with actual measurement of tissue levels of the folate metabolites would also be of interest and could bring new information on the complex function of folate metabolism as a whole.

**Conclusion**

The functional polymorphisms MTHFR C677T and MTR A2756G, influencing the activity of enzymes involved in folate metabolism, can affect the risk of toxicity during adjuvant 5-FU-based chemotherapy and outcome in stage III colorectal cancer. Variations in genetic combinations and enzyme interdependence are related to outcome and could explain some of the difficulties of obtaining uniform results when using single enzyme polymorphisms as predictive markers. More studies are needed to assess the genetic differences and the possible alterations that could be made in choice of treatment regimes.

**Conflict of Interest**

The Authors declare no conflict of interest.

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