Research Article

Impact of MTHFR Polymorphisms on Clinical Outcomes of Breast Cancer Patients Treated by 5-FU Based Chemotherapy

Sedrati K[1], Abadi N[1,2], Sifi K[1,2], Chirouf A[3], Filali T[3]
[1]Biologic and Molecular Genetics Laboratory, Constantine 1 university, Algeria.
[2]Biochemical Laboratory, CHU, Constantine, Algeria.
[3]Anticancer Center and Chemotherapy Unit, CHU, Constantine, Algeria.
***Corresponding author's E-mail:**

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ABSTRACT

Two common single-nucleotide polymorphisms (SNPs) of Methylenetetrahydrofolate reductase (MTHFR) gene, C677T (rs1801133) and A1298C (rs1801131), reduce enzyme activity. These SNPs seems to influence the effectiveness of treatment with fluoropyrimidine in breast cancer chemotherapy, but further studies have yielded contradictory results. We tested whether these two polymorphisms are determinants of clinical outcome. 117 breast cancer patients receiving 5-FU based chemotherapy was genotyped for the tow variants in MTHFR gene using PCR-RFLP, relationships between genetic variants and tumor response were assessed. The response rate (48.7 % responders, 51.3 % non-responders) was related to 5-FU pharmacogenetic. MTHFR genotypes for C677T were associated to response (p=0.01, $\chi 2$ = 8.18). However, no association was observed for the A1298C polymorphism (p=0.79, $\chi 2$ = 0.46). Our data provide that C677T MTHFR polymorphism affect the clinical outcome for fluoropyrimidine chemotherapy. The MTHFR polymorphism may be a useful pharmacogenetic determinant for predict of benefit from 5-FU based chemotherapy.

Keywords: Breast cancer, MTHFR polymorphisms, chemotherapy, 5-FU, response.

INTRODUCTION

Breast cancer (BC) is the commonest cause of cancer death among women worldwide[1], treatment for BC has progressed significantly over the last years with the use of active chemotherapeutics agents including fluoropyrimidines.

Mapping of the variety of epigenetic and genomic alterations in tumor genomes and correlating these finding with tumor characteristics, prognosis and response to therapy are the first steps towards generating personalized therapy[2]. Treatment decisions for BC patients are currently based on a small number of crude predictive markers of outcome[3]. However, the outcome of anticancer therapy varies greatly from patient to patient, and it is becoming clear that the individual genetic profile plays a dominant role[4]. Some molecular markers for the prediction of response to therapy have been verified, among them MTHFR gene.

Methylenetetrahydrofolate reductase (MTHFR) plays a critical role in the regulation of intracellular folate concentrations. This enzyme catalyzes the irreversible conversion of 5,10-methylenetetrahydrofolate (5,10-MTHF) to methyltetrahydrofolate (5-MTHF). The substrate 5,10-MTHF is required for DNA synthesis and for maintaining the balance of the nucleotide pool, whereas 5-MTHF is required for methylation reactions, including the methylation of homocysteine to methionine and the maintenance of DNA methylation patterns. 5,10-MTHF is the methyl donor for the nonreversible methylation, catalyzed by thymidylate synthase, of deoxyuridine-5 –monophosphate (dUMP) to deoxythymidine-5 -monophosphate (dTMP), a precursor for DNA synthesis. 5,10-MTHF is also involved in de novo purine biosynthesis[5].

Although, in general terms, 5-FU has several potential cytotoxic mechanisms, it is considered to be a folate antimetabolite. Two metabolites of 5-FU, 5-fluoro-2 -deoxyuridine-5-triphosphate (5-FdUTP) and 5-fluorouridine-5 –triphosphate (5-FUTP), can be incorporated into DNA and RNA, respectively, resulting in DNA instability and interfering with RNA processing and function[6]. 5-FU can also form a ternary complex involving 5-fluoro-2 -deoxyuridine-5 - monophosphate (5FdUMP; the active metabolite of 5-FU), thymidylate synthase which catalyze the conversion of dUMP to dTMP, and 5,10-MTHF. The formation of this complex inhibits thymidylate synthase activity. The lack of intracellular dTMP leads to decreased DNA synthesis, dUMP misincorporation into DNA, and DNA strand breaks followed by cell apoptosis[7].

Two common nonsynonymous single nucleotide polymorphisms (SNPs) have been noted in the MTHFR gene, which functionally alter the protein product[8]. C677T, found in Exon 4, (rs1801133, Ala222Val) results in a reduced-activity thermolabile variant, which has decreased stability and specificity of action[9] and is associated with decreased MTHFR activity and increased homocysteine levels[10]. A1298C (rs1801131, Glu429Ala), found in Exon 7, also reduces MTHFR activity, though seemingly less severely than C677T[11]. Both of the C677T

and A1298C SNPs in the MTHFR gene are associated with a 50% to 60% decrease in catalytic activity.

Various experimental[12-14] and clinical studies[15,16] have been carried out to test the association between genetic variants of MTHFR and 5-FU treatment effect. However, inconsistent results have been obtained. To investigate the possible association of polymorphisms in MTHFR gene with clinical response to 5-FU based chemotherapy in breast cancer patients, the present study was carried out.

MATERIALS AND METHODS

Study Population

We performed a prospective cohort study of 117 patients newly diagnosed with locally advanced breast cancer, who were consecutively treated in the Anti-Cancer Center CHU Constantine Algeria, between September 2011 and June 2013. All the cases for the current study were histologically confirmed. Everyone was asked to sign an informed consent document.

The patients received combination chemotherapy every three week, consists of cyclophosphamide (C), epirubicine (E) or doxorubicine (A) and 5-fluorouracil (F) (=CEF and CAF treatment protocol).

Tumor response was assessed using Response Evaluation Criteria in Solid Tumors (RECIST) criteria[17]. Subjects presenting greater than or equal to 30% reduction in tumor diameter upon clinical measurements were considered responders (R), whereas those showing a less than 30% reduction in tumor diameter, or presenting disease stability or progression were classified as non-responders (NR)

Genotyping of MTHFR variants

Both polymorphic sites C677T and A1298C were screened by Polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP), Peripheral blood samples were obtained from patient and collected into EDTA tubes, DNA was extracted by a salting-out method[18].

The C677T polymorphism was determined as follows: 50 ng/µL DNA was amplified in a total reaction volume of 50 µL containing 20 mM Tris- HCl, 25 mM MgCl2, 2 mM of each deoxynucleotide triphosphate, 100 µM of each primer, and 2 U AmpliTaq DNA polymerase, we used the forward primer 5'- TGA AGG AGA AGG TGT CTG CGG GA-3' and the reverse primer 5'- AGG ACG GTG CGG TGA GAG TG- 3'. PCR was carried out in an Eppendorf Gradient Thermocycler, The PCR reaction mixture was pretreated at 95°C for 5 minutes followed by 30 cycles of 95°C for 30 s, 65°C for 30 s, and 72°C for 1 minute. The final extension was at 72°C for 10 minutes. The primers generated an amplified fragment of 198 bp. The PCR product was digested with 4U of HinfI (Fermentas) at 37°C over-night and electrophoresed on a 3% agarose gel and stained with ethidium bromide. The wild genotype (CC) is represented by a single band of 198 pb , the homozygous mutant (TT) is represented by two bands of 175 and 23 bp and the heterozygous (CT) genotype is characterized by three bands 198, 175, 23 bp.

To consider the A1298C MTHFR polymorphism, we have used the flowed forward and the reverse primer sequence: 5' –GGA GTG TGC CCT GAC CTC T – 3' and 5' – GTG AGT GAT GCT GGA GTG G – 3' respectively. The PCR mixture consisted of the following reagents: 10 mM Tris-HCl, 2 mM MgCl2, 10 pmol of each primer, 0.4 mM of the deoxynucleoside triphosphates, 1.5U of Taq DNA polymerase, and 100 ng of genomic DNA template, for a total of 25 µL per sample. The PCR conditions consisted of an initial denaturation step for 5 min at 95°C, followed by 30 cycles altering of denaturation at 95°C for 15 s, annealing at 60°C for 15 s and extension at 72°C for 25 s. The terminal extension was performed at 72°C for 8 min. The amplification was followed by digestion of the amplified product (236 bp) with the MboII restriction enzyme as described in the manufacturer's instructions (Thermo Scientific; FastDigest). After digestion, all the fragments were resolved on NuSieve 3:1 Agarose gels. The homozygous normal allele (AA) produced 2 fragments of 106 and 130 bp, the heterozygous (AC) produced 4 fragments of 130, 106, 72 and 58 bp, whereas the homozygous mutant (CC) produced 3 fragments of 106, 72 and 58 bp.

Statistical analysis

For each polymorphism, genotype frequencies were calculated, and result were regrouped as "wildtype" versus "any variant". The Hardy–Weinberg equilibrium was assessed for each polymorphism using Chi-squared test. The Statistical Package for Social Sciences (SPSS) software version 16.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. The association of polymorphisms of MTHFR with response to chemotherapy in breast cancer patients was calculated by odd ratio (OR) with a corresponding 95% confidence interval (CI). The relative risk [hazard ratio (HR)] and 95% CI were calculated with the Cox regression model. The P-value less than 0.05 was considered statistically significant.

RESULTS

Table 1: Genotypes distribution among the study population

Variant	genotype	n. (%)
C677T (rs1801133)	CC	49 (41.88)
	CT	53 (45.29)
	TT	15 (12.83)
A1298C (rs1801131)	AA	69 (58.97)
	AC	36 (30.77)
	CC	12 (10.26)

There were 117 patients treated and evaluated for assessment of response outcome, among them 57

(48.7%) were considered to be responders and 60 (51.3%) non responders according to RECIST criteria.

MTHFR genotypes were determined for the entire patients group for the C677T and A1298C polymorphisms. The corresponding genotypes are given in the table 1. Both of the tow polymorphisms agree with the Hardy-Weinberg equilibrium.

We analyzed the effect of the MTHFR C677T and A1298C polymorphisms on the clinical outcome. Table 2 shows the prevalence of the study genetic polymorphisms among patients, stratified by their clinical response to 5FU based chemotherapy (non-responders vs. responders). Statistical analysis revealed that the C677T polymorphism was linked to clinical response (χ^2 tests: CC vs. CT vs. TT, p=0.01, $\boldsymbol{\chi^2}$ = 8.18) on the other hand, there was no difference between non-responders and responders for A1298C polymorphism (χ2 tests: AA vs. AC vs. CC, p=0.79, χ2 = 0.46)

Table 2: Distribution of MTHFR polymorphisms on objective tumor response.

Variant	genotype	Responders N=57 n. (%)	Non responders N=60 n. (%)	p value
C677T (rs1801133)	CC	31 (54.39)	18 (30)	—
	CT	22 (38.59)	31 (51.67)	**0.02**
	TT	4 (7.02)	11 (18.33)	**0.01**
A1298C (rs1801131)	AA	36 (63.15)	33 (55)	—
	AC	16 (28.08)	20 (33.33)	0.83
	CC	5 (8.77)	7 (11.67)	0.50

DISCUSSION

The main result of this study is that the C677T polymorphism of MTHFR gene is associated with significant higher rates of response to 5-FU based chemotherapy. Several clinical studies have investigated the potential predictive role of these genetic variants in toxicity and efficacy of 5-FU, but contradictory data has been published[19]. Some of these studies, considering both tumor material and normal tissue, have reported that C677T genetic variant, but not A1298C, is significantly associated with increased tumor response rate to 5-FU-based therapy[20]. However, the polymorphic A1298C does not reduce the enzyme activity of MTHFR to the same degree as the C677T[21]. Etienne evaluated the MTHFR polymorphisms in patients with metastatic colorectal cancer who were treated with 5-FU and leucovorin, their response rate was significantly associated with the C677T genotype, but not connected with the A1298C genotype[22]. In the study by Jakobsen the response rate was 66% for TT compared with 33% and 21% for 677CC and 677CT, respectively. No correlation was observed in the case of A1298C polymorphism[23].

However, the 1298CC polymorphic genotype in an advanced colorectal cancer population study has been correlated with an increased risk of developing severe adverse events after 5-FU based chemotherapy[24]. In recent years, several pharmacogenetic analyses have been performed to examine the association between C677T and A1298C and the outcome of patients treated with fluoropyrimidine-based chemotherapy but they do not confirm the role of MTHFR polymorphisms as pharmacogenetic determinants of 5-FU therapy outcome in patients with breast cancer[25]. Large clinical trials are necessary to confirm the effect of the MTHFR polymorphisms on treatment response in breast cancer patients receiving 5-FU based chemotherapy.

The combination of MTHFR C677T and A1298C genetic variants with the activity or polymorphisms of thymidylate synthase, the key target enzyme of 5-FU, appeared to have a better predictive power on 5-FU-based chemotherapy response compared to the approach that takes into consideration the C677T polymorphism alone[26]. When multiple markers are potentially involved, association of an outcome with combination of different polymorphisms rather than a single polymorphism has a greater chance of success[27,28].

From a theoretical point of view, there is no reason to think that susceptibility to 5-FU treatment is a monogenic trait since several proteins are involved in the pharmacokinetics and pharmacodynamics of 5-FU (and indeed all drugs) [29,30]. An approach, which combines several of the genes involved in 5-FU metabolism and mechanisms, will yield a better, more logical model for explaining individual variations in 5-FU efficacy.

In conclusion, our data indicates that the MTHFR C677T polymorphism may be an important pharmacogenetic determinant of fluoropyrimidines based chemotherapy in breast cancer. These finding needs to be validated in a large cohort and with other candidate genes. Such research could help to establish prognostic or predictive models for providing rational and effective tailored chemotherapy.

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