

Correlation between polymorphism of TYMS gene and toxicity response to treatment with 5-fluoruracil and capecitabine

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Abstract

Tumorigenesis is a multiphasic process in which genetic alterations guide the progressive transformation in cancer cells¹. In order to evaluate the possible correlation between some gene variants and the risk of the toxicity development onset, two of the polymorphisms of the thymidylate synthase (TYMS), rs34743033 (2R/3R) and rs16430 (DEL/INS) were investigated. We enrolled in our study 47 patients from the Hospital of Sicily. Our preliminary findings suggest that there could be a linkage between the genotypes discussed and the development of the toxicity following the chemotherapy treatment. These results need to be confirmed by further studies, however this short paper offers some initial insight into the relationships between genetic background and the better outcome for patients.

Key Words: Cancer, genetics, thymidylate synthase, polymorphisms, toxicity.

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Cancer is a pathology characterized by dynamic alterations in the genome.¹ Tumorigenesis is a multiphasic process in which the succession of genetic alterations, capable of conferring a growth advantage, guide the progressive transformation of a normal cell into a tumor cell.² The unregulated growth of cancer cells derives from the sequential acquisition of somatic mutations and/or alterations in the expression of genes that control growth, differentiation and apoptosis; or that guarantee the integrity of the genome, i.e. those mechanisms that normally regulate cell proliferation and homeostasis.³ In recent times, numerous studies have attempted to identify predisposition factors of cancer. Genes have been identified whose reduced expression can act as biomarkers for the early diagnosis of tumors, such as Reprimo gene for gastric cancer,⁴ or reduced cancer incidence such as biallelic expression of EXITS genes in females explains a portion of the reduced cancer incidence compared to males across a variety of tumor types.⁵ The probability that an individual affected by these mutations will develop a tumor is variable and depends on various factors. These include the type of mutated allele, as well as the age and the action of other

genes called modifiers. The influence of other factors such as diet, lifestyle and environmental factors remains unclear. The data obtained to date, suggests that for any type of tumor, only one or a few loci can explain the genetic risk, regardless of the type of the genetic variant (i.e., single nucleotide polymorphisms, SNPs or copy-number variations, CNVs), or frequency (whether it is common, rare or very rare). The risk of developing a tumor would instead be associated with a polygenic inheritance in which hundreds or thousands of genetic variants would be involved. A theoretical model is thus governed by a complex architecture and a multiplicity of genetic loci in which mutations in the individual genes and genetic variants present in different loci would generate tumorigenic conditions in otherwise normal tissue. This predisposing risk would then be modulated differently in each individual from environmental factors leading to tumor development.⁶

Thymidylate Synthetase (TYMS) is an enzyme that is essential for DNA replication and repair and is also an important target for a variety of chemotherapy drugs, playing an important role not only in cancer therapy but also possibly in cancer prevention.⁷ Polymorphisms in the TYMS gene (rs16430) can therefore lead to altered

enzymatic function, which can influence susceptibility to cancer.⁷ Among these, a deletion/insertion of 6 base pairs has been observed at the untranslated end (3'-UTR). Therefore, there exists different genotypes related to insertion and deletion: (a) a homozygosity for deletion (Del/Del 6 bp), (b) a homozygosity for insertion (Ins/Ins 6bp) or (c) a condition of heterozygosity (Del/Ins 6 bp). The correlation between rs16430 and the susceptibility of many tumors including gastric cancer, colorectal cancer and breast cancer has also been highlighted in the literature, exemplifying the importance of this polymorphism in a discussion of potential cancer treatments. The risk of breast cancer has been associated with the homozygous deletion genotype, and has been more evident in older, postmenopausal women, and in those in whom menarche occurred at an older age. The homozygous insertion genotype, on the other hand, has been associated with a significantly decreased risk of breast cancer; suggestive of a protective effect given by the levels of sex hormones during the life of the subjects. The same data was subsequently confirmed in 2015 by Guan and collaborators in a case-control study carried out on non-Hispanic white women aged less than or equal to 55 years.⁸ The rs16430 polymorphism of *TYMS* also appears to influence the susceptibility of colorectal cancer, as confirmed by Vilmos and colleagues.⁹ In particular, the Ins 6/Del 6 heterozygotes are less susceptible to the onset of this type of tumor. Another variant is 28 bp variable number tandem (VNTR) polymorphism is found in 5' untranslated region (5'UTR) of *TYMS*,¹⁰ occurring with variable number generated two different alleles:¹¹ two tandem repeats (2R) or three tandem repeats (3R). While 3R represents the wild-type form, 2R represent the mutant form and the three possible genotype are: 2R/2R, 2R/3R and 3R/3R.¹²

5-Fluorouracil is a chemotherapy agent that has entered the practice clinic for more than 40 years as an elective drug for the treatment of colorectal, stomach, pancreas cancers and, in some specific cases, breast cancer.¹³ The drug interferes with the synthesis of thymidine nucleotides, fundamental for DNA synthesis, acting on thymidylate synthetase, which is the target of chemotherapy¹⁴. Capecitabine is a non-cytotoxic fluoropyrimidine carbamate, which acts as a precursor, administered orally, of 5-Fluorouracil; is a pro-drug enzymatically converted to 5-Fluorouracil and its pathway

of activation involves three enzymatic steps and two intermediate metabolites, the 5'-deoxy-5-fluorocytidine (5'-DFCR) and 5'-deoxy-5-fluorouridine (5'-DFUR) to form the 5-fluorouracil.¹⁵ After oral administration it comes absorbed quickly and extensively, it is transported in the bloodstream bound to albumin and eliminated via the kidneys. The chemotherapy toxicity is almost always the greatest limitation in choosing the appropriate therapeutic dosage that could ensure a better outcome for patients, in terms of improving both quality of life and survival.¹⁶

The aim of our study was to evaluate the possible correlation between the aforementioned gene variants and the degree of toxicity observed following chemotherapy.

Materials and Methods

Patients

We enrolled 47 patients from the Oncology Operative Unit of the Sant'Elia Hospital in Caltanissetta, Sicily. The group consisted of 24 women and 23 men aged between 39 and 85, 8 with breast cancer and 39 with gastric cancer. The aforementioned patients performed the chemotherapy treatment according to the therapeutic protocol appropriate to their pathology; particularly 17 patients in the adjuvant stage whilst the others 30 in the metastatic stage. The patients received adjuvant 5-FU-based chemotherapy mixed with a different concentration of capecitabine. Toxicity was recorded according to the criteria of the World Health Organization. Physical examination and a full blood count were performed before the chemotherapy treatment. All the patients who had received at least one course of chemotherapy were evaluated for toxicity. The same chemotherapy regimen was maintained until disease progression or severe toxicity.

DNA extraction and Genotyping

After signed informed consent from each patient, the blood sample was collected in sterile tubes containing the anticoagulant ethylenediamine tetraacetic acid (EDTA), and stored at -90°C until the analysis. Genomic DNA was isolated from an aliquot of peripheral blood using a PureLink blood kit (PureLink Genomic DNA, ThermoFisher Scientific). This was used as a template for polymerase chain reaction and subsequent enzymatic

Table 1. Information on genotyping methods for each polymorphism.

GENE POLYMORPHISM AND REFERENCE ID	NUCLEOTIDE SEQUENCE	ANNEALING TEMPERATURE	RESTRICION ENZYME
<i>TYMS</i> (2R/3R) rs34743033	F-5'- GTGGCTCCTGCGTTTCCCC - 3' R-5'- GCTCCGAGCCGCCACAGGCATGGCGCGG -3'	62°	Dra I
<i>TYMS</i> (DEL/INS 6 bp) rs16430	F-5'- CACAAGCTATTTTGGAAAATT - 3' R-5'- GACGAATGCAGAACACTTCT - 3'	56°	—

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Table 2. Crosstab: Hematological -toxicity*Independent variables

Independent Variable	Level	Toxicity (subjects number)	No- Toxicity (subjects number)	Total
Gender	Male	13	10	23
	Female	16	8	24
Total		29	18	47
Genotype 1	2R/2R	5	0	5
	2R/3R	14	10	24
	3R/3R	10	8	18
Total		29	18	47
Genotype 2	Ins/Ins	7	8	15
	Del/Ins	13	7	20
	Del/Del	9	3	12
Total		29	18	47
Tumor type	Gastric	23	16	39
	Breast	6	2	8
Total		29	18	47
Tumor stage	Adjuvant	9	8	17
	Metastatic	20	10	30
Total		29	18	47

digestion when request, to detect the genotype of *TYMS* gene variants (Table 1).

Statistical analysis

In order to evaluate the association between dependent variables (gastric and hematological toxicity) and independent variables (genotype 1, genotype 2, tumor

stage, tumor type, gender). The distribution frequencies of independent variable parameters, for all subjects were calculated. Dichotomic variables were expressed as number and percentage and analyzed with the Fisher's exact probability test. The chi-square test was possible perform only between the dependent variable

Table 3. Crosstab: Gastric-toxicity*Independent variables

Independent Variable	Level	Toxicity (subjects number)	No- Toxicity (subjects number)	Total
Gender	Male	2	21	23
	Female	6	18	24
Total		8	39	47
Genotype 1	2R/2R	0	5	5
	2R/3R	5	19	24
	3R/3R	3	15	18
Total		8	39	47
Genotype 2	Ins/Ins	1	14	15
	Del/Ins	4	16	20
	Del/Del	3	9	12
Total		8	39	47
Tumor type	Gastric	7	32	39
	Breast	1	7	8
Total		8	39	47
Tumor stage	Adjuvant	1	16	17
	Metastatic	7	23	30
Total		8	39	47

Table 4. Univariate analysis: statistical data of Fisher's test and chi-square test, ($p < 0.05$ is significant).

Independent variables	Hematological toxicity		Gastric toxicity	
	Fisher's Exact Test value	Exact Significant value (p-value)	Fisher's Exact Test value	Exact Significant value (p-value)
Gender	.512*	.556*	2.303	.245
Genotype 1	3.33	.225	.820	.862
Genotype 2	2.33	.325	1.86	.380
Tumor type	.758	.692	.149	1
Tumor stage	2.06	.334	2.45	.361

* The result refers to the Pearson chi-square test.

hematological toxicity and the independent variable gender. Results were expressed as p-value. Significance was defined as value of p (alpha error) < 0.05 . The analyzes were performed using IBM SPSS Statistics 23.0 (IBM SPSS Inc., Chicago, IL).

Results and Discussion

From the entire treatment sample 61.70% shown hematological toxicity, while only 17,02% shown gastric toxicity. The remaining patients didn't develop any toxicity. Descriptive analyses for each variable are shown in Table 2 and Table 3. The univariate analysis didn't show any statistically significant effect between hematological toxicity after treatment and independent variable: gender $p > .05$ chi-square .512, genotype 1 (2R/3R) $p > .05$, genotype 2 (Del/Ins) $p > .05$, tumor type (gastric or breast) $p > .05$, tumor stage $p > .05$. As to gastric toxicity, the univariate analysis didn't show any statistically significant effect between treatment and the independent variable: gender $p > .05$, genotype 1 (2R/3R) $p > .05$, genotype 2 (Del/Ins) $p > .05$, tumor type (gastric or breast) $p > .05$, tumor stage $p > .05$ (Table 4). The treatment of the different cancer with these two effective drugs often collides with their toxic. This factor represents the greatest limitation in choosing appropriate therapeutic dosage that could ensure a better outcome for the patients. During the past fifty years the use of 5-Fluorouracil, a chemotherapy agent that belongs to the family of antimetabolites and, specifically, it is an analogue of pyrimidines continued to improve. The drug interferes with the synthesis of thymidine nucleotides, fundamental for DNA synthesis, acting on thymidylate synthetase, which became the target of chemotherapy.¹⁴ Capecitabine is a non-cytotoxic fluoropyrimidine carbamate, which acts as a precursor to 5-Fluorouracil. In this present study, we investigated whether the analysis of two common polymorphisms of the TYMS gene in gastric and breast cancer patients who received 5-FU-based chemotherapy mixed with Capecitabine, could be used to predict the toxicity to the chemotherapy treatment. We investigated 47 patients who were treated at the Sant'Elia Hospital, Caltanissetta Sicily.

In genotypes distribution, the most frequent genotype for the TYMS gene polymorphism rs16430 was the heterozygous Del/Ins (42.5%) while the rarest one was

Del/Del genotype (25.5%). With regards to the polymorphism rs34743033, there was a high incidence of the 2R/3R genotype (51%) and a low presence of the 2R/2R genotype (10.6%). No statistically significant correlation between the genotypes and the development of the toxicity was detected but particular attention must be paid to genotype 1 where all the 5 subjects with 2R/2R genotype showed hematological toxicity, but not gastric toxicity development after treatment. These preliminary results need further investigations analyzing a wider variety of polymorphisms on control subjects and on a larger cohort of patients.

List of acronyms

CNVs - copy-number variations,
 EXITS - Escape from X-Inactivation Tumor Suppressor
 SNPs - single nucleotide polymorphisms
 TYMS - thymidylate synthase
 VNTR - variable number tandem
 3'-UTR - untranslated end
 5'-DFCR - 5'-deoxy-5-fluorocytidine
 5'-DFUR - 5'-deoxy-5-fluorouridine
 5'-UTR - untraslated end

Authors contributions

IDL, SV, PP made substantial contributions to conception and design. RRR and CV contributed to acquisition of data. AA performed statistical analysis and interpretation of data. CMDL and GS drafted the article with the help of GM. PP revised the article critically for important intellectual content. All authors approved the final version and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy and integrity of any part of the work are appropriately investigated and resolved.

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Conflict of Interest

The authors declare they have no financial, personal, or other conflicts of interest.

Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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