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

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Abstract

Tumorigenesis is a multiphasic process in which genetic alterations guide the progressive transformation in cancer cells. 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.\(^6\)

Timidylate 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...
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.\textsuperscript{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.\textsuperscript{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** | **RESTRICTION ENZYME** |
|---------------------------------------------------------------|
| **TYMS (2R/3R)** |
| rs34743033 | F-5': GTGGCTCCCTGCCTCCGCTCCC - 3' | 62° | Drg 1 |
| **TYMS (DEL/INS 6 bp)** |
| rs16430 | F-5': CACAGCATTATTCTGGGAAATATTT - 3' | 56° | — |
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Eur J Transl Myol 2020; 30 (3): 8970. doi: 10.4081/ejtm.2020.8970

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 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    |

**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    |
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. To gastric toxicity, the treatment 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-Fluoruracil, a chemotherapy agent that belongs to the family of antimitabolesites 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 thymidilate synthetase, which become 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,
EXIT5 - Escape from X-Inactivation Tumor Suppressor
SNPs - single nucleotide polymorphisms
TYMS - thymidylate synthase
VNTR - variable number tandem
5'-UTR – untranslated end
5'-DFUR - 5'-deoxy-5-fluorouridine
5'-DFCR - 5'-deoxy-5-fluorocytidine

**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.

**Acknowledgments**

The authors would like to thanks all the patients involved in the study and the healthcare professionals that cooperated with us.

**Funding** None

**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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References

1. Pradeepkiran JA, Sainath SB, Kumar KK, et al. CGMD: An Integrated Database of Cancer Genes and Markers. Sci Rep 2015;5:12035. doi: 10.1038/srep12035.

2. Ebadi MR, Aghdam MK, Lima ZS, Younesi L. Investigation into breast cancer and partial breast reconstruction: A review. Eur J Transl Myol 2019;29(2):8157. doi: 10.4081/ejtm.2019.8157

3. Bloomfield M, Duesberg P. Inherent variability of cancer-specific aneuploidy generates metastases. Mol Cytogenet. 2016;9:90. doi:10.1186/s13039-016-0297-x

4. Abbasi A, Heydari S. Studying the expression rate and methylation of Reprimo gene in the blood of patients suffering from gastric cancer. Eur J Transl Myol 2018;28(2):7423. doi: 10.4081/ejtm.2018.7423.

5. Dunford A, Weinstock DM, Savova V, et al. Tumor-suppressor genes that escape from X-inactivation contribute to cancer sex bias. Nat Genet 2017;49(1):10-16. doi: 10.1038/ng.3726

6. Galvan A, Ioanomidou JP, Dragani TA. Beyond genome-wide association studies: genetic heterogeneity and individual predisposition to cancer. Trends Genet 2010;26:132-41. doi: 10.1016/j.tig.2009.12.008

7. Ulrich CM, Bigler J, Velicer CM, et al. Searching expressed sequence tag databases: discovery and confirmation of a common polymorphism in the thymidylate synthase gene. Cancer Epidemiol Biomarkers Prev 2000;9:1381-5.

8. Guan X, Liu H, Ju J, et al. Genetic Variant rs16430 6bp > 0bp at the microRNA-Binding Site in TYMS and Risk of Sporadic Breast Cancer Risk in Non-Hispanic White Women Aged ≤55 Years. Mol Carcinog 2015;54:281-90. doi: 10.1002/mc.22097. Epub 2013 Oct 26.

9. Vilmos A, Hitre E, Köves I, et al. Heterozygote deficiency in thymidylate synthase enhancer region polymorphism genotype distribution in Hungarian colorectal cancer patients. Int J Cancer 2005;108: 852-6.

10. Wang X, Wang Y, Wang Y, et al. Association of thymidylate synthase gene 3’-untranslated region polymorphism with sensitivity of non-small cell lung cancer to pemetrexed treatment: TS gene polymorphism and pemetrexed sensitivity in NSCLC. Journal of biomedical science, 2013;20:5. Doi 10.1186/1423-0127-20-5

11. Mo A, Zhao Y, Shi Y, et al. Association between polymorphisms of thymidylate synthase gene 5’- and 3’-UTR and gastric cancer risk: meta-analysis. Biosci rep 2016;36, e00429. org/10.1042/BSR20160273

12. Fu Z, Jiao Y, Li Y, et al. TYMS presents a novel biomarker for diagnosis and prognosis in patients with pancreatic cancer. Medicine (Baltimore). 2019;98:e18487. doi: 10.1097/MD.0000000000018487.

13. Bai W, Wu Y, Zhang P, Xi Y. Correlations between expression levels of thymidylate synthase, thymidine phosphorylase and dihydropyrimidine dehydrogenase, and efficacy of 5-fluouracil-based chemotherapy for advanced colorectal cancer. Int J Clin Exp Pathol 2015;2015;8:12333–45.

14. Chu E, Callender MA, Farrell MP, Schmitz JC. Thymidylate synthase inhibitors as anticancer agents: from bench to bedside. Cancer Chemother Pharmacol 2003;52 Suppl 1:S80-9. doi: 10.1007/s00280-003-0625-9.

15. Pellicer M, García-González X, García MI, et al. Identification of new SNPs associated with severe toxicity to capecitabine. Pharmacol Res 2017;120:133-7. doi: 10.1016/j.phrs.2017.03.021

16. Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. Cancer 1981;47:207–14. doi: 10.1002/1097-0142 (19810147:1<207::aid-cncr2820470134>3.0.co;2-6.

Submissions: March 19, 2020
Revision received: May 13, 2020
Accepted for publication: May 13, 2020