



*Short Communication*

# Prevalence of thymidylate synthase gene 5'-untranslated region variants in an Argentinean sample

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**ABSTRACT.** Thymidylate synthase (TYMS) is a key enzyme in nucleotide synthesis and therefore, an important target of many chemotherapeutic agents. Expression of TYMS mRNA is thought to be modulated by a 28-bp tandem repeat polymorphism within its 5'-untranslated region, raising the question of this variant's utility in predicting the efficacy and toxicity of cancer treatment regimens. The aim of the present research was to describe the distribution of this TYMS polymorphism in the Argentinean population. A total of 199 randomly

selected DNA samples from healthy volunteers were analyzed using polymerase chain reaction and polyacrylamide gel electrophoresis. The 2R and 3R alleles were present in 47.74 and 52.26% of samples, respectively, with frequencies of 21.6 (43), 52.3 (104), and 26.1% (52) recorded for the 2R/2R, 2R/3R, and 3R/3R genotypes, respectively. No significant difference regarding gender was observed. Our prevalence data are similar to those reported for other Caucasian populations. This opens a discussion concerning the reference population valid for comparisons and the clinical importance of this genotyping test as an additional tool in personalized medicine.

**Key words:** Thymidylate synthase; TYMS; Pemetrexed; 5-Fluorouracil; Pharmacogenomics

## INTRODUCTION

Thymidylate synthase (TYMS) is a key enzyme in nucleotide synthesis. It catalyzes the conversion of deoxyuridine monophosphate to deoxythymidine monophosphate (dTMP) through a methylation process. This TYMS-dependent reaction is the only source of dTMP in cells. Given its essential role in DNA replication, TYMS is an important direct and indirect target of many chemotherapeutic agents, including 5-fluorouracil and pemetrexed (Ulrich et al., 2000).

The mRNA expression of the TYMS gene is thought to be affected by a 28-bp tandem repeat polymorphism within its 5'-untranslated region (UTR; Kaneda et al., 1987). The majority of human TYMS alleles harbor either a double (2R) or a triple repeat (3R), resulting in the genotypes 2R/2R, 2R/3R, and 3R/3R. Although there have been reports of four- and five-repeat variants in some populations, their global prevalence is very low (Marsh et al., 1999).

Individuals harboring the 3R/3R genotype may express TYMS at higher levels than those carrying the 2R/2R genotype (Horie et al., 1995). In addition, a C/G single nucleotide polymorphism (SNP) in the second repeat of the 3R allele has been described, the variants of which are named 3RC and 3RG. The 3RC variant may attenuate the increased transcription caused by the presence of three repeats.

Although the exact mechanism responsible is not well understood, it has been postulated that two upstream stimulating factor (USF) family E-box consensus sequences are found within the tandem repeats of the 3R allele. USF proteins bind to these regions, enhancing transcription, and the C/G SNP influences this interaction (Mandola et al., 2003).

The clinical impact of these genotypes may involve both the toxicity and efficacy of chemotherapy. Basic knowledge of the distribution of these polymorphic variants in our country and region is needed, as no data regarding TYMS genotype frequencies in the Argentinean population has been reported to date. Our aim was to describe the frequency of TYMS 5'-UTR variable number tandem repeat (VNTR) polymorphisms in a sample of this population.

## MATERIAL AND METHODS

### Study population

Two hundred and two random samples from healthy controls were retrieved from

the Hospital Italiano de Buenos Aires DNA Bank and analyzed. Informed consent from participants and approval from the appropriate institutional review boards were obtained.

### Sample collection and DNA extraction

Following obtainment of informed consent, 10 mL peripheral blood was collected from each subject in 5-mL tubes containing ethylenediaminetetraacetic acid. Whole blood samples were stored at 4°C until use. Genomic DNA was extracted and purified using a QIAmp DNA Blood Mini kit (QIAGEN, Hilden, Germany).

### Genotyping of TYMS 5'-UTR polymorphism

Genotyping of the TYMS 5'-UTR polymorphism (rs45445694 in the reference sequence GenBank accession No. NM\_001012716.2) was performed by polymerase chain reaction (PCR) and polyacrylamide gel electrophoresis. In each reaction, genomic DNA was amplified using the same primers specific to the TYMS 5'-UTR region (forward, 5'-AGGCGCGCGGAAGGGGTCCT-3'; and reverse, 5'-TCCGAGCCGGCCACAGGCAT-3'; IDT, Coralville, IA, USA), yielding amplicons of 113 and 141 bp for the 2R/2R and 3R/3R genotypes, respectively (Kawakami and Watanabe, 2003).

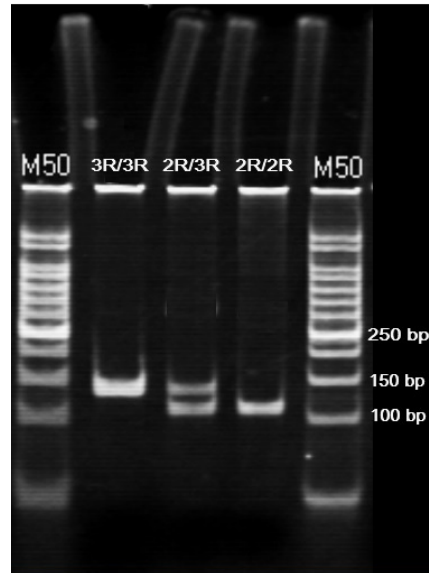
Each PCR comprised a 50- $\mu$ L mixture containing 1X PCR buffer (Invitrogen, Carlsbad, CA, USA), 1.5 mM magnesium chloride (Invitrogen), 200  $\mu$ M deoxynucleotides (Sigma, St. Louis, MO, USA), 500 nM each primer, 1.5 U Taq polymerase (Invitrogen), 1X betaine (QIAGEN), and 100 ng genomic DNA. Reactions were performed in an Applied Biosystems (Foster City, CA, USA) thermocycler. Cycling parameters for amplification of the TYMS 5'-UTR region were as follows: initial denaturation at 95°C for 15 min, then 30 cycles of 91°C for 1 min, 60°C for 1 min, and 72°C for 1 min, before a final extension step at 72°C for 10 min. Samples were subsequently held at 4°C until needed.

In order to characterize amplicons, DNA fragments of all expected sizes from homozygous samples were submitted to an external laboratory (Macrogen, Korea) for purification and sequencing. Sequence quality was analyzed manually using the program Chromas 1.56 (<http://www.techneysium.com.au/chromas.html>), and each sequence was compared for similarity with others deposited in the GenBank database using the Basic Local Alignment Search Tool on the National Center for Biotechnology Information website (<http://www.ncbi.nlm.nih.gov>).

Aliquots of PCR products were electrophoresed on an 8% polyacrylamide gel and stained with ethidium bromide. DNA bands were visualized with an ultraviolet transilluminator to carry out TYMS 5'-UTR genotyping (Figure 1).

### Statistical analysis

Allelic and genotypic frequencies are reported as absolute values and percentages of the study sample. Two hundred samples were used to estimate 2R/2R genotype prevalence, previously reported as 19% in the European population, with a precision of 5.4% (Marsh et al., 1999). The results obtained were further evaluated for stratification by gender and conformance with Hardy-Weinberg equilibrium. We used Stata v14.1 (StataCorp, College Station, TX, USA) for all analyses.



**Figure 1.** Genotyping of thymidylate synthase 5'-untranslated region variants by polymerase chain reaction and electrophoresis on an 8% polyacrylamide gel stained with ethidium bromide. A 141-bp band corresponding to the 3R/3R genotype, two bands of 141 and 113 bp signifying the 2R/3R heterozygous genotype, and a 113-bp band resulting from the 2R/2R genotype are shown. A 50-kb reference ladder is visible to the right and left of the sample lanes.

## RESULTS

We attempted to genotype all samples according to the method described above. Of the 202 subjects analyzed, only 199 were genotyped due to technical problems with the remaining samples. Regarding the demographic characteristics of our sample of healthy volunteers, 106 (53.3%) were men and 93 (46.7%) were women; the median age was 42 years, with a 25-75% interquartile range of 33-43 years.

In our study sample, 2R and 3R allele counts were 190 (47.74%) and 208 (52.26%), respectively, and no 4R variant was identified. Genotype counts were as follows: 2R/2R, 43 (21.6%); 2R/3R, 104 (52.3%); 3R/3R, 52 (26.1%).

Concerning stratification of the data by sex, the following distribution of genotypes was observed among women: 2R/2R, 21 (22.6%); 2R/3R, 49 (52.7%); 3R/3R, 23 (24.7%). That among men was as follows: 2R/2R, 22 (20.8%); 2R/3R, 55 (51.9%); 3R/3R, 29 (27.3%). The difference between the male and female groups was not significant based on a two-sided Fisher's exact test ( $P = 0.89$ ).

The distributions were also analyzed for Hardy-Weinberg equilibrium. The expected genotype frequencies were 2R/2R, 45.4; 2R/3R, 99.3; and 3R/3R, 54.4. A P-value of 0.5 was returned from a two-sided chi-square test with one degree of freedom, failing to reject the null hypothesis of a population in equilibrium.

## DISCUSSION

To our knowledge, this is the first report of the prevalence of TYMS 5'-UTR variants

in an Argentinean population sample. The functional effects of this polymorphism have been analyzed previously in many *in vitro* and *in vivo* contexts. Compared with the 3RG/3RG genotype, the 2R/2R, 2R/3RC, and 2R/3RG variants are associated with lower intratumoral TYMS mRNA levels (Morganti et al., 2005).

In terms of the possible clinical impact of this phenomenon, studies of germline carriers of these variants have shown that compared with high-expression genotypes (2R/3RG, 3RG/3RC, and 3RG/3RG), those resulting in lower TYMS expression (2R/2R, 2R/3RC, and 3RC/3RC) are associated with better 5-fluorouracil response in liver-only metastatic colorectal cancer (Graziano et al., 2008). Moreover, in comparison with the 2R/3R and 2R/2R variants, the 3R/3R genotype has been linked to greater overall response rate, overall survival, and a tendency for increased progression-free survival in wild-type epidermal growth factor receptor patients with non-small cell lung cancer (NSCLC) receiving pemetrexed (Arévalo et al., 2014).

Germline carriers of the TYMS 2R/2R genotype are at increased risk of severe diarrhea when treated with 5-fluorouracil, compared to those with the 2R/3R or 3R/3R genotype (Lecomte et al., 2004; Schwab et al., 2008). In contrast, grade 3-4 hematologic toxicities are more frequent among Japanese carriers of the 3R/3R genotype with NSCLC treated with pemetrexed in combination with carboplatin (Kanazawa et al., 2014).

Concerning tumorigenesis, a meta-analysis carried out by Wang et al. (2014) revealed that the 2R/3R and 2R/2R genotypes confer a lower risk of developing colorectal cancer among Caucasians. However, no association has been established between the TYMS 5'-UTR VNTR polymorphism and the incidence of gastric tumors, breast cancer, or leukemia (Nazki et al., 2012; Quintero-Ramos et al., 2014; Araújo et al., 2015).

Given its links to chemotherapy efficacy and toxicity and tumorigenesis, further pharmacogenetic research involving this particular TYMS polymorphism is warranted in the context of oncology. The present study constitutes the first study of TYMS 5'-UTR VNTR polymorphism prevalence in an Argentinean population sample. The allelic and genotypic frequencies recorded here were similar to those previously reported by Marsh et al. (1999), who found the 2R/2R, 2R/3R, and 3R/3R genotypes to be present in 19, 43, and 38% of a Caucasian population represented by 96 samples.

One limitation of the present study should be addressed. We did not genotype the C/G SNP in the second repeat of the 3R allele, yet this variation may be important in determining the efficacy and toxicity of certain drugs, as described above.

The comparability of our findings with those of other studies of the prevalence of this polymorphism may be due to the genetic backgrounds of the populations considered. The contemporary Argentinian population is thought to include components of European, Native American, and African genetic ancestry, but in relative proportions appear to depend on the genetic material considered. Argentines carry a large fraction of their European genetic heritage in their Y chromosomal (94.1%) and autosomal (78.5%) DNA, but their mitochondrial gene pool is mostly of Native American ancestry (53.7%). However, the African contribution is small in all three genetic systems (<4%; Corach et al., 2010). This large European element in the autosomal genome may in part account for the similarity between our results and previously reported frequencies based on Caucasian samples.

Our investigation provides additional data useful to future research concerning the TYMS 5'-UTR polymorphism in Argentina and other countries with similar genetic ancestry. This benefits studies of the efficacy and toxicity of the many chemotherapeutic agents targeting the TYMS enzyme, as well as tumorigenesis research, where a particular patient group may be

compared with healthy controls and/or the general population in relation to the prevalence of this polymorphism in a country and/or region.

### Conflicts of interest

The authors declare no conflict of interest.

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