Genetic variants in the enhancer region of the thymidylate synthase gene in the Chilean population

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Aims
Thymidylate synthase (TYMS) is an important target enzyme for the fluoropyrimidines. The *TYMS* gene enhancer region possesses tandemly repeated (*TSER*) sequences that are polymorphic in humans and different among ethnic groups. The aims of this study were to estimate the frequencies of the *TSER* variants in two hospital samples located in the northern (HSJ) and eastern (CLC) parts of Santiago, Chile, and compare them with the frequencies in other populations of different ethnic origin.

Methods
Genotyping of *TSER* variants in 368 Chilean subjects (HSJ = 178 and CLC = 190) by polymerase chain reaction; products of amplification were electrophoresed, obtaining fragments of 250 bp for allele *TSER*3* and 220 bp for allele *TSER*2.*

Results
The two hospital samples had different degrees of Amerindian admixture (HSJ 34.5%; CLC 15.9%), which was not reflected in the observed frequencies of the CLC *TSER*3*: 56.8% and HSJ *TSER*3*: 53.4%.

Conclusions
Our results are unexpected, considering that genetic markers in the Chilean population generally show allele frequencies between those observed in European Caucasians and Amerindians and that the percentage of Amerindian admixture in CLC is lower than in HSJ. Both hospitals should have had greater frequencies of *TSER*3* than were found and the frequency should have been greater in HSJ than in CLC; the only logical explanation of our results is that the frequency of this allele in aboriginal Chilean people is much lower than the 80% estimated for Mongoloid populations.

Thymidylate synthase (TYMS) catalyses the intracellular conversion of deoxyuridine-5′-monophosphate (dUMP) to 2′ - deoxythymidine - 5′ - monophosphate (dTMP), which is essential for DNA replication. TYMS is an important target for cancer chemotherapy drugs such as 5-fluorouracil and raltitrexed. These drugs prevent DNA synthesis by forming stable complexes with TYMS and its folate cofactor, thus blocking the conversion of dUMP to dTMP [1]. Therefore, the *in vivo* regulation of TYMS is important both in normal tissue biology and in cancer therapeutics [2]. The human *TYMS* promoter has recently been characterized, identifying several important mechanisms for gene regulation [2, 3]. Tandem repeat sequences were identified near the initiation site. Analysis of the 5′-untranslated region of the *TYMS* gene identified a tandem repeat sequence that is a *cis*-acting enhancer element. This *TYMS* enhancer region (*TSER*) was shown to be polymorphic, containing

either two or three 28-bp tandem repeats. *In vitro* expression studies suggest that the presence of a triple repeat (*TSER*3*) results in a 2.6-fold increase in mRNA expression compared with a double repeat (*TSER*2*) [4]. *In vivo* studies in human gastrointestinal tumours have shown a significant increase in TYMS protein levels and functional activity in patients with *TSER*3* compared with individuals with *TSER*2* [5].

There are few population studies of the *TSER* locus in the literature. The frequencies reported for allele *TSER*3* in Japanese [6], Chinese [7], South-west Asians [8], British Caucasians [8], American Caucasians [9, 10], African-Americans [9], Ghanaians [9], Kenyans [9], Northern Irish [11], Italians [12] and Australians [13] were 0.81, 0.82, 0.62, 0.60, 0.54, 0.54, 0.52, 0.56, 0.49, 0.52, 0.50 and 0.56, respectively. The highest population frequencies of *TSER*3* appear in the Mongoloids and the lowest in the Negroids. Negroid and Mongoloid populations also have alleles with *TSER*4, TSER*5* and *TSER*9* repetitions, with frequencies between 0.18% and 3.76% [7, 9].

In Chile, allele frequencies for genetic markers are known to vary according to socioeconomic stratum. For example, the *ABO*A* allele of the ABO blood group and the *RHD*d* allele of the Rh system are more frequent in the higher socioeconomic stratum, which has more genes of European origin, while native Amerindian alleles are more prevalent in the lower socioeconomic stratum [14]. This is explained by the origin of the Chilean population, which is a mixture of Spanish and Chilean aborigines. Since the period of colonization (1541), aborigines and mestizos were considered to belong to a lower social stratum than the Spanish, for whom the government, property and occupations with the highest prestige and power were reserved. Hard labour was given first to aborigines and then to mestizos. These factors led to a cline of mixture between the two extreme classes (Aborigines and Spanish) [14].

The objective of this study was to estimate the allelic frequencies of *TSER* in two urban subpopulations of Santiago, Chile, according to socioeconomic stratum and to compare these frequencies with those of other populations. There are no studies for the *TSER* locus in Chilean populations. Our results provide an important starting point for pharmacogenomic analysis in cancer chemotherapy.

Materials and methods

EDTA-blood samples were obtained from healthy individuals from the San José Hospital (SJH), which belongs to the National Health Service and serves mainly low socioeconomic classes, and from Clínica Las Condes (CLC), a private hospital serving principally high socioeconomic classes. The SJH and CLC are located in the northern and eastern parts of Santiago, respectively. The purpose of the study was explained to all individuals and each participant signed an informed consent. The study was approved by the Ethics Committee of the Medicine Faculty of the University of Chile. To determine the *TSER* variants, DNA was isolated by routine methods and amplified by polymerase chain reaction (PCR) [15]. Two oligonucleotide primers were synthesized specifically to amplify the polymorphic region of *TSER* (sense primer 5′-GTGGCTCCTGCGT TTCCCCC-3′ and antisense primer 5′-GCTCCGAGC CGG CCACAGGCATGGCGCGG-3′) [6]. Amplification reactions were performed in a 25-µl volume containing 0.2 µg genomic DNA, 0.2 mM $dNTP_s$, 0.5 µM of each primer, 1.0 units of Taq polymerase (New England BioLabs, Ipswich, MA, USA) and 2.5 µl of 10 × Taq PCR buffer. Cycling conditions were: one cycle at 94 °C for 5 min with hot start; 30 cycles of 40 s at 94 °C, 1 min at 62 °C and 40 s at 72 °C; and one elongation cycle for 5 min at 72 °C. Reaction products were analysed on 4% agarose gels; the 28-bp difference in allele size (250 bp for *TSER*3* and 220 bp for the *TSER*2*) discriminated between alleles [6]. Allele frequencies were calculated by allele counting. No technical problems were encountered in these processes, and less than 2% of the 374 samples could not be genotyped. The fact that the genotypic frequencies observed did not differ from Hardy–Weinberg equilibrium is indirect evidence against technical errors.

The χ^2 test was used to compare genotype distributions and allele frequencies between the studied groups and to test for Hardy–Weinberg equilibrium in the genotype distributions [16, 17]. For both samples the percentage of aboriginal admixture was estimated (Bernstein) [18], using ABO and Rh (anti-D serum) systems as genetic markers, and published estimates of their frequencies in the parental populations: $ABO*O = 0.65$, $RHD*d = 0.41$ for the Spanish population [19], and $ABO*O = 0.98$, $RHD*d = 0.0$ [20, 21] for Chilean aborigines. The nominal level of significance for all analyses was $P < 0.05$ [17].

Results

Table 1 presents the phenotype and allele frequencies for the variants of *TSER, ABO* and *Rh* loci and the Amerindian component in the two hospital samples, based on the ABO and Rh systems. The results reveal that the degree of Amerindian admixture in SJH was higher than in CLC, as expected. The *TSER*3* allele was more frequent in both groups. The frequencies of the

Table 1
Genotype or phenotype distribution, allele frequencies for *TSER*, *ABO* and Rh loci, and Amerindian component in the San José Hospital (SJH) and Clínica Las Condes (CLC) samples

TSER Genotype	HSJ No	*Freq.* ± CI (95%)	CLC No	*Freq.* ± CI (95%)
*TSER*2/TSER*2*	40	22.47 ± 6.13	38	22.00 ± 5.69
*TSER*2/TSER*3*	86	48.31 ± 7.34	88	46.32 ± 7.09
*TSER*3/TSER*3*	52	29.21 ± 6.68	64	33.68 ± 6.72
Total	178		190	
Allele				
*TSER*3*		53.37 ± 5.18		56.84 ± 4.98
ABO				
Phenotype				
AB	2	1.40 ± 1.93	2	1,31 ± 1.80
A	50	34.97 ± 7.82	68	44.44 ± 7.87
B	11	7.69 ± 4.37	14	9.15 ± 4.57
O	80	55.94 ± 8.14	69	45.10 ± 7.88
Total	143		153	
Allele				
*ABO*A*		20.26 ± 4.66		26.49 ± 4.94
*ABO*B*		4.66 ± 2.44		5.40 ± 2.53
*ABO*O*		75.08 ± 5.01		68.10 ± 5.22
Rh				
Phenotype				
DD+Dd	130	94.89 ± 3.69	137	90.73 ± 4.63
dd	7	5.11 ± 3.69	14	9.27 ± 4.63
Total	137		151	
Allele				
*RHD*d*		22.60 ± 4.95		30.45 ± 5.19
Amerindian component		34.51 ± 16.30		15.87 ± 17.60

genotypes and alleles were not significantly different between the samples ($P > 0.05$ in all cases). None of the genotype frequencies of *TSER* or *ABO* deviated significantly from those predicted by Hardy–Weinberg equilibrium.

Table 2 compares the allele frequencies of *TSER*3* in SJH and CLC samples with those of previous studies in other populations. The frequency of allele *TSER*3* was lower in SJH, but not significantly different ($53 \pm 2.6\%$) from that in CLC ($57 \pm 2.5\%$); and the overall Chilean sample frequency ($55.2 \pm 1.8\%$) was similar to that of American Caucasians [9, 10] and African groups [9], and significantly lower than Chinese ($81 \pm 1.1\%$) [7] and Japanese ($81 \pm 6.1\%$) [6]. The *TSER*3* frequency observed in CLC was significantly higher ($P \leq 0.05$; *Z*-test) than for Italians [12] and Kenyans [9]; however, its frequency in SJH was significantly lower ($P \leq 0.05$; *Z*-test) than that of South-west Asia [8]. Curiously, the frequency reported by Marsh *et al.* [9] in American Caucasians was between the CLC and SJH (CLC 57%; USA 54%; SJH 53%). Although *TSER*4, TSER*5* and *TSER*9* repetitions were not found in HSJ and CLC samples, it is possible that these alleles exit at frequencies too low to be observed in a study of this scale.

Discussion

Considering that genetic markers in the Chilean population have generally shown allele frequencies between those observed in European Caucasians and Amerindians (of Mongoloid origin) [14, 22, 23] and that the percentage of Amerindian admixture in CLC is lower than in SJH, our results for the *TSER* locus are surprising; we expected to find a greater frequency of allele *TSER*3* in HSJ than in CLC. In order to estimate expected frequencies, we must suppose that: (i) the Spanish population which mixed with the indigenous group had a *TSER*3* allele frequency similar to that found in the current Spanish population (57%) [24]; (ii) the indigenous Chilean population had a *TSER*3* allele frequency similar to that found in current Mongoloid groups (81%) [7]; and (iii) the estimates of admixture are close to the real values. Using Bernstein's formula [18] and 34.51% and 15.87% as the estimate of degree of Amerindian admixture for SJH and CLC, respectively (Table 1), the expected frequencies would be 65.3% for

Table 2
Frequencies of the *TSER *3* allele in different populations

Population	Alleles, *N*	*TSER*3* (% ± SE)	Reference
American Caucasian	208	(54.0 ± 3.4)	[9]
American Caucasian	2280	(54.1 ± 1.0)	[10]
British Caucasian	192	(60.0 ± 3.5)	[8]
Italian	272	(50.0 ± 3.0)	[12]
Spanish	ND	(57.0 ± ND)	[24]
Hungarian	200	(53.5 ± 3.5)	[25]
North Irish	784	(52.2 ± 1.8)	[10]
Australian	182	(56.0 ± 3.7)	[13]
South-west Asian	190	(62.0 ± 3.5)	[8]
Chinese	192	(82.5 ± 2.7)	[8]
Chinese	1336	(81.0 ± 1.1)	[7]
Japanese	42	(81.0 ± 6.1)	[6]
African-American	184	(52.0 ± 3.7)	[9]
Kenyan	196	(49.0 ± 3.6)	[9]
Ghanaian	496	(56.0 ± 2.2)	[9]
Chilean			
HSJ	356	(53.0 ± 2.6)	Present study
CLC	380	(57.0 ± 2.5)	Present study
Total	736	(55.2 ± 1.8)	Present study

ND, No data.

HSJ and 60.8% for CLC. The frequency of the allele *TSER*3* in the Chilean population appears to be incompatible with its ethnic origin. By contrast, all previous population studies of genes in the Chilean population have produced results consistent with the hypothesis of ethnic mixture [14, 22, 23]. Which factors may have been important in producing the current observed frequencies? At present we have no explanation for our results, but we suspect that the frequency of *TSER*3* in aboriginal Chilean populations, when estimated, will prove to be much lower than the 80% found in Mongoloid populations [7], which might have occurred by genetic drift during the early colonization of South America.

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