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Original Article

Gene polymorphism frequency of c677t (rs1801133) MTHFR in colombian population

Frecuencia del polimorfismo c677t (rs1801133) del gen MTHFR en individuos colombianos

Consuelo Romero-Sánchez[1,4,5] ,Alberto Gómez-Gutierrez[1,2] ,Piedad Elena Gómez[1], Maria Consuelo Casas-Gomez[1], Ignacio Briceño[1,2,3]

[1] Instituto de Referencia Andino. Bogotá, Colombia
[2] Instituto de Genética Humana, Facultad de Medicina, Pontificia Universidad Javeriana. Bogotá, Colombia
[3] Facultad de Medicina, Universidad de La Sabana. Bogotá, Colombia
[4] Servicio de Reumatología e Inmunología, Hospital Militar. Bogotá, Colombia
[5] Instituto UIBO - Unidad de Investigación Básico Oral, Universidad El Bosque. Bogotá, Colombia.

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Abstract

Introduction: Abnormal levels of the enzyme methylenetetrahydrofolate-reductase (MTHFR) are associated with both cardiovascular and cerebrovascular risk and higher concentrations of homocysteine. They are also related to birth defects, pregnancy complications, cancer and toxicity to Methotrexate (MTX). Polymorphisms of MTHFR affect the activity of the enzyme. Genetic associations have been related to treatment efficacy.

Objective: To establish the frequency of the polymorphism C> T at nucleotide 677 of the MTHFR gene in a group of Colombian individuals

Methods: Data of pharmacogenetic microarrays which include MTX sensibility associated polymorphisms were collected retrospectively (Pathway Genomics®). The frequency of the polymorphism C> T MTHFR rs1801133 marker polymorphism was analyzed.

Results: Microarrays from 68 men and 84 women were analyzed. Comparisons of genotype C/C vs. C/T and T/T were statistically significant (p= 0. 001 and p= 0,026 respectively) as well as the comparison between C/T and T / T (p= 0. 0001).

Conclusions: Results for C/C and C/T genotypes in Colombia are similar to other groups of healthy subjects previously studied. Subjects of our population might be at risk of developing diseases associated with MTHFR polymorphisms and might present toxicity and adverse effects if treated with MTX, which suggests the need to evaluate therapeutic alternatives based on individual pharmacogenetic studies.

Resumen

Introducción: Las alteraciones de la enzima metilen-tetrahidrofolato reductasa (MTHFR) se asocian con riesgo cardiovascular y cerebrovascular y con presencia de concentraciones altas de homocisteína. Se relacionan también con defectos congénitos, complicaciones en embarazo, cáncer y toxicidad del Metotrexato (MTX). Los polimorfismos del gen MTHFR afectan la actividad de la enzima. Se han descrito asociaciones genéticas con la eficacia del tratamiento con MTX.

Objetivo: Establecer la frecuencia del polimorfismo C>T en el nucleótido 677 del gen MTHFR en un grupo de individuos Colombianos.

Métodos: Estudio descriptivo de corte transversal. Se recolectaron retrospectivamente resultados de microarreglos farmacogenéticos que incluyen polimorfismos asociados con la sensibilidad al MTX (PathwayGenomics®). Se analizó la frecuencia del polimorfismo C>T del polimorfismo rs1801133 del gen MTHFR.

Resultados: Se analizaron microarreglos de 68 hombres y 84 mujeres. Las comparaciones del genotipo C/C frente a C/T y a T/T fueron estadísticamente significativas (p= 0.001 y p= 0.026 respectivamente) tanto como la comparación entre C/T y T/T (p= 0.0001).

Conclusiones: Los genotipos C/C y C/T en Colombia son tan variables como en otros grupos sanos en otras poblaciones. Los sujetos de nuestra población podrían tener riesgo para el desarrollo de enfermedades asociadas al polimorfismo del genMTHFR y con genotipos de riesgo de presentar toxicidad y efectos adversos del MTX, lo cual sugiere la necesidad de evaluar alternativas terapéuticas con estudios farmacogenéticos.

Corresponding author:
Consuelo Romero-Sánchez. Unidad de Investigación Básica Oral, UIBO.
E-mail: romeromaria@unbosque.edu.co

Introduction

Sequencing of the human genome and understanding the potential of the study of single nucleotide polymorphisms (SNPs: Single Nucleotide Polymorphisms) have generated a considerable amount of data that reveal the basis of genetic association of hundreds of diseases and toxicity or efficacy of various drugs. The second area of study is called "pharmacogenetics". The technology applied to reveal these associations is available and costs are reduced progressively against the benefit of optimization and clinical application of their findings.

The response of each individual to the same treatment can be partly inherited; creating differences in the efficacy in subjects due to polymorphism of genes encoding enzymes involved in the metabolism of certain drugs, in their transporters and/or specific treatment targets[1]. A gene is described as "polymorphic" when allelic variants are present, the activity of the protein can vary when compared to the original allele. Pharmacogenetics may additionally explain individual differences in response to treatment and may have predictive value for patient response to different drugs[2,3].

A classic example of pharmacogenetics is shown in rheumatoid arthritis (RA), inflammatory disease that affects between 0.5% and 1% of the population. One of the most widely used therapies in this disease and more effectively from the clinical standpoint, is methotrexate (MTX). Several reports describe it´s use since 1951, and clinical trials show its effectiveness but also demonstrate its toxicity, including increased nodes, pneumonitis, neurologic involvement, gastrointestinal complications such as nausea, vomiting and diarrhea, increased transaminases, hematologic abnormalities, skin rash, stomatitis and alopecia[4-6].

methylenetetrahydrofolate reductase (MTHFR) gene genetic variants related to the efficacy of MTX were studied. The C677T polymorphism enzyme activity was reduced by 35% in carriers and has a prevalence of 40% in the population. MTX activity is reduced 50-70% in the homozygous TT at position 677 with a prevalence of 8-10% in the population[7].

Additionally, MTX inhibits dihydrofolate reductase (DHFR) and folate dependent enzymes such as timidilatosintasa (TS) and MTHFR. It was shown that their polymorphisms affect enzyme activity, resulting in elevated homocysteine levels and toxicity[8].

Besides the study of MTHFR enzyme has acquired much interest after the fundamental discovery of Kang *et al.*[7], who reported that a variant of the thermo labile enzyme was associated with increased cardiovascular risk and a higher concentration of total plasma homocysteine. Today the concentration of homocysteine in plasma is considered a risk factor for coronary heart disease, cerebrovascular disease, and vascular occlusion[9]. It has also been linked to the occurrence of neural tube defects and other birth defects, complications associated with pregnancy[10,11] and cancer[12].

Genetic analysis can be requested independently on the *MTHFR* gene or in the context of molecular genetic profiles as part of preventive medicine. These profiles evaluate pharmacogenetic associations with drugs including MTX. The polymorphism analysis can be performed as well by direct sequencing of the entire gene or a fragment thereof, by analysis of restriction enzyme polymorphisms (RFLP) or by microarray technique. The spectrum of the frequency of the gene *MTHFR* polymorphism C677T in a group of healthy Colombian individuals was determined in order to evaluate the genetic background in relation to disease susceptibility and pharmacogenetic applications.

Materials and Methods

A retrospective cross-sectional study in 152 healthy Colombian individuals over 18 yrs was conducted, using molecular genetic profile. DNA samples were collected from saliva sample in the area of preventive medicine of "Instituto de Referencia Andino", including all applications from medical orders and / or direct individuals received between the years 2012-2014. All individuals signed the informed consent. Data from subjects were demographic data was not complete were excluded.

The pharmacogenetic polymorphism panel from Pathway Genomics® in Medication DNA Insight™genetic test evaluates 15 metabolism associations for drug metabolism including MTX in addition to: Abacavir® hypersensitivity, aminoglycoside-induced ototoxicity, response to beta blockers, carbamazepine hypersensitivity, clopidogrel metabolism, estrogen supplementation, alpha interferon, metoprolol metabolism, metabolism and hypersensitivity to phenytoin, proton-pump inhibitor, simvastatin induced myopathy, metabolism of voriconazole and warfarin.

DNA microarrays were used to measure expression levels of probes, corresponding to allele mutations "C" and "T" in the *MTHFR* gene. Each microarray position contains 10-12 picomoles of a specific DNA sequence under high stringency. Probe-target hybridization is detected and quantified by fluorophores attached to the probe.

The frequency of the C677T polymorphism, consisting of a C>T substitution in the MTHFR gene marker rs1801133 was analyzed.

Each participant was identified by a code to be included into a database, where the descriptive analysis for continuous variables such as age determination was performed with measures of central tendency and normality test of Shapiro Wilk; the description of categorical variables (origin, sex, genotype and allele) was performed by frequency analysis. Given the variable nature of the study association levels, they were evaluated by the statistical test Chi^2 with a confidence level of 95% and significance. In addition, Hardy Weinberg equilibrium was tested. The results were transferred to the statistical program SPSS® for Windows V20. They were not stratified by ethnic group or socioeconomic levels.

Ethical considerations

Study was approved by the Ethics Committee of Fundacion Instituto de Reumatología Fernando Chalem.

Results

The study included 152 individuals, 68 men (44.7%) and 84 women (55.3%) aged 40 ±13 and 41 ±13 yrs, respectively. The origins of the samples were as follows: Medellin (15 individuals: 9.9%), Cali (10

individuals: 6.6%), Bogotá (119 individuals: 78.3%), Barranquilla and Cartagena (6 subjects: 3.9%), Pasto (2 Individuals: 1.3%).

The most frequently observed genotype was the heterozygous C/T (80 individuals, 52.6%), followed by the homozygous C/C with 52 individuals (34.2%). The homozygous genotype T/T had the lowest frequency in the population studied (20 individuals) who corresponded to 13.2%. Comparisons of genotype C/C vs. C/T and T/T were statistically significant (p= 0.001 and p= 0.026 respectively) and similarly for comparison of C/T and T/T (p= 0.0001) (Fig. 1).

No statistically significant associations between genotype and allele with sex, p= 0.799 and p= 0.615 respectively were observed by the statistical test Chi² (Table 1). The population is in Hardy-Weinberg equilibrium (p= 0.211).In assessing the allele frequency in the population, the C allele was found in 86.3% (n= 306) of individuals and the T allele in 13.7% (n= 42).

Discussion

There are functional polymorphisms in genes encoding enzymes involved in metabolism that may produce susceptibility to diseases; for that reason, MTHFR gene polymorphisms are among the most studied[13-15].

In the current study the population was in Hardy-Weinberg equilibrium for alleles of MTHFR gene and therefore there is no evidence of selection for any of them. Whereas it has been found that this polymorphism affects MTHFR enzyme activity, it is important to assess its frequency and its effect on the population.

MTHFR enzyme is a critical protein in the metabolism of 5-methyl tetrahydrofolate reductase, which is the donor in several biochemical reactions involved in the methylation of homocysteine. A dozen polymorphisms of MTHFR gene have been described, but two main variants from the functional point

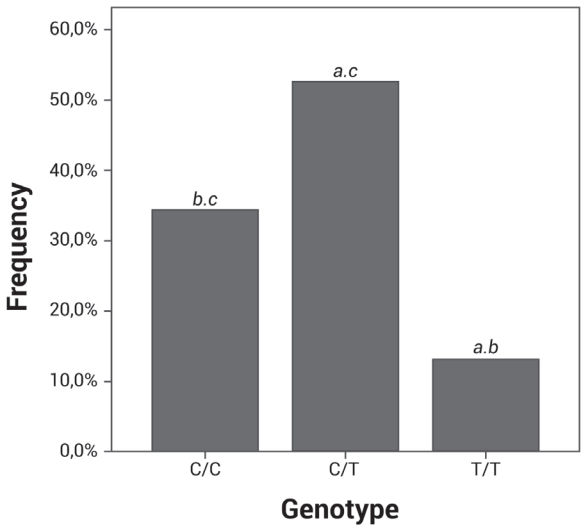

Figure 1. Comparison of the three genotypes in the total sample: a significant statistical differences for C/C, b significant statistical differences for C/T, c significant statistical differences for T/T. All the analysis were obtained by Chi square p< 0.05.

Table 1. Genotype and allele frequency.

rs1801133 MTHFR		Male (n= 68)	Female (n= 84)	*p*
Genotype Frecuency	CC*	25 (36.8)	27 (32.1)	0.799
	CT	35 (51.5)	45 (53.6)	
	TT	8 (11.8)	12 (14.3)	
Allele frequency	C	114 (87.7)	150 (85.2)	0.615
	T	16 (12.3)	26 (14.8)	

*n (%)

of view are the most studied, A1298C and C677T. The organic effect of these polymorphisms has been determined, generating predictive results for patient response to MTX [7]. The results of the meta-analysis evaluating the C677T polymorphism, homozygous or heterozygous, show great variability between populations[16-19].

Most genotype frequency observed in the population corresponded to the heterozygous C / T, followed by C/C and the lowest frequency was for T/T genotype. The C677T polymorphism produces a change of alanine for valine at codon 222, corresponding to a thermolabile MTHFR enzyme variant generating a decrease of activity and increased plasma levels of homocysteíne[20] . The homozygous genotype T/T has 30% of the original activity with a prevalence of 8 to 10% in the general population. Heterozygotes have about 60% activity with a prevalence of 40% in the population[20].

In a study in Mexico, where the frequency of polymorphisms of MTHFR gene in women with and without cervical cancer was evaluated, the results showed a genotype C/C in 22.4% of the 89 control women with an average age of 44 yrs, lower than 32.1% found in the present study. Moreover, genotype C/T was present in 55.1% of the study population, similar to that found in the analyzed group of Colombian women (53.6%). Finally, the T/T genotype was found in 22.5% of the population, a figure significantly higher than in the present study, considering that this is the homozygous variant that is associated with an increased risk of cancer. Allele frequencies of both C and T were 0.5 in the Mexican study[21], which contrasts with the population here analyzed where the predominant was the C allele.

In Colombia there has been some previous studies[22-25] in which the allele and genotype frequencies of this polymorphism were analyzed by comparing cases and controls, always with the aim to determine their association with different pathologies.

Bermudez *et al*., studied 102 volunteers, of both sexes, from various parts of Colombia residing in Bogota, aged between 18 and 50 years, 53 women and 49 men[22]. This population was not stratified by origin, ethnicity or socioeconomic status. In contrast, the T/T genotype showed a frequency of 27.4%, higher than the value found in the present study. In women the frequency was 13.2%, a 14.3% increase. In another evaluation of Colombian individuals, healthy subjects were included, and different genotype frequencies to this report were found: C/C allele in 22.5% and T/T 27.4%, with minor allele frequencies for C (47.5%) and T (52.4%), predominantly the allele associated with toxicity for MTX[23]. These reports in healthy Colombian individuals include smaller numbers. They were analyzed with RFLP methodology unlike this study where microarrays were used.

In a study of a population sample of 206 students in Bogota Colombia, overall frequencies were calculated using data from healthy controls reported in other studies, finding similar frequencies of genotypes and alleles detected in the group of subjects analyzed in our study[26]. In this sample there was no Hardy-Weinberg equilibrium, while the overall Colombian data of the present study was found in equilibrium.

When comparing the frequency of TT homozygosity in other populations worldwide, this varies from 0% to over 30%[26]. However, the results found in our study are similar to those reported by González *et al*., in a greater number of healthy Colombian individuals[19]. These differences were also found in China[27,28] and United States[29], where analyzes were reported in subjects from the same geographic population, in which very different results were obtained. In the United States, in a study involving different ethnic groups a lower frequency of allele T was found in the African Americans and a higher frequency in the Hispanic population[29], in contrast to our results where the T allele was found in 13.7% of the population tested. Worldwide frequencies range from 1% in the population of south east India30 to 43% in China[27,31].

In summary, a great variability was observed in the frequencies of C and T alleles worldwide. In Colombia, some studies show a predominance of allele C and others of allele T. Differences between populations suggest that both allele frequencies in certain circumstances could produce selective advantages and disadvantages. Some reports suggest that the T allele can produce resistance to infections such as malaria and cytomegalovirus, and may also protect against hypertension[32,33]. Similarly, the C allele protects against birth defects and cancer, and is associated with higher fertility and later onset of neurological diseases[34]. These findings allow postulating that the frequencies of C and T alleles may be conditioned locally in different populations by environmental selective pressures in different regions of Colombia and the world.

The results of this study are limited to a Colombian population who applied to study their genetic profile in the cities of Bogota, Medellin, Barranquilla and Cali, which does not allow giving a comprehensive understanding of the Colombian population. The findings in different studies have shown a variable behavior, even when people have been analyzed in the same geographical area. Possibly the differences found in the different studies may result from different methodological approaches used and the ethnic heterogeneity of the subjects[24,25].

Some subjects of our population with MTHFR gene polymorphism genotypes associated with toxicity and adverse defects to MTX could be at high risk, suggesting the need to evaluate therapeutic alternatives with pharmacogenetic studies for MTX genotype T/T. From the clinical perspective, the classification of patients for polymorphisms of MTHFR gene before administering medication would offer alternative treatments without subjecting patients to harmful effects, optimizing therapy and reducing costs for health systems.

Conclusion

Results for the genotype C/C and C/T in Colombia are as variable as in other groups of healthy individuals studied in other populations, and this variability may be subject to selective pressures of the environment in different regions of Colombia.

Conflict of interests: The authors declare no conflict of interest.

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