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Chapter 7

The Prevalence of the C677T Polymorphism of the Methylenetetrahydrofolate Reductase (rs 1801133) and Classic Risk Factors in Costa Rican Women with Thrombotic Events

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Abstract

Thrombotic diseases develop as the result of multiple interactions between non-genetic and genetic risk factors. The known atherogenic risk factors played an important role as predictors in the development of the thrombotic diseases in the present female case-control study. The most important non-genetic risk factors found among the female patients are: age, hypercholesterolemia, DM, use of oral contraception, obesity, and fibrinogen levels. Moreover, the stratified effect of some of these factors according to the age of the patients is relevant for clinical risk assessment. Associations between the risk of venous thrombosis (VT) and genetic polymorphisms have been established. Some of these polymorphisms are highly prevalent in Caucasians, but there is a significant geographic variation in their prevalence among different populations. The MTHFR C677T (rs1801133), are analyses in females with stroke, venous thrombosis and myocardial infarction in a case-control study. The C677TMTHFR mutation were detected in 149 patients and 113 controls (P=0,001). In the present study, the results showed that hypercholesterolemia, diabetes mellitus, and elevated levels of fibrinogen were present in the younger group as risks factors. Family history of thrombotic event (TE) was statistically significantly associated among the older female group. Median values of total fibrinogen were significantly higher in the cases compared with the control group. The small numbers of carriers of these risk factors require a note of caution on any conclusion. The present data suggest that MTHFR C677T genotype may be an important factor in the life of female patients at risk of developing TE or cardiovascular disease, p=0,001 between cases and controls groups. Folic acid supplementation of foods is necessary (and mandatory in Costa Rica) before and during women's reproductive phase. However, our data presented suggest that a case for efforts to ensure a good intake of this vitamin in the postmenopausal phase. Especially if the high prevalence of the MTHFR 677T homozygosity and of classic risk factors in the Costa Rican population are taken into account. Further studies with more patients are necessary for final conclusions for these interactions to be drawn.

Introduction

The general definition of thrombotic event (TE) refers to a clot large enough to cause occlusion of flow through a blood vessel, either arterial or venous. The clinical manifestations of this event depend on the site of occlusion. Several pathophysiologic mechanisms have been described for this condition, such as intrinsic blood vessel involvement (most frequent), later participation of the humoral components of the primary coagulation pathways, and less frequent involvement of platelet activation.

The most common type of TE is acute coronary syndrome a component of coronary atherosclerotic disease causing ischemic heart disease, which is the leading cause of death in Costa Rica (Araya & Guzman, 2004) and, consequently, a significant proportion of the TE diagnoses in hospitals. Therefore, any effort to elucidate and identify factors that may intervene in primary and secondary prevention of this condition is clearly justified.

Thrombophilia is a disorder hemostasis where a tendency for the thrombosis in veins or arteries due to abnormalities in the composition, flow and vascular wall of the blood. This term is more related with venous thrombosis (VT): deep venous thrombosis (DVT) and pulmonary embolism (PE). VT is a serious disease with an annual age-dependent incidence of 1-3 individuals per 1000 per year (Naess et al., 2007), with a thirty da case-fatality rate 6.4% after first event and this rate is twice as high in PE than for DVT 4.6%, (Naess et al., 2007). VT is a complex and multifactorial disease both acquired and genetic is involved in the development of the disease. Acquired factors as surgery, immobilization, trauma, oral contraceptive or hormone replacement therapy use, pregnancy, malignancy and advance age (Koenderman and Reitsma, 2011)

A thrombophilia state in an individual is determined by conditions or individual risk factors. However, clinical studies have identified specific factors with statistically significant effects that are common among patients with recurrent thrombotic events. For example, for cardiovascular disease (CVD), well-known independent risk factors include advanced age, male gender and having relatives with a history of the CVD at an early age, smoking, hyperhomocysteinemia, diabetes mellitus, hypertension and / or dyslipidemia (Balleisen L, et al., 1985; De Stephano V,2000, Reiner A. et al., 2001) These risk factors are associated with the different clinical manifestations and some can be modified or attenuated, while others cannot. In recent decades, we have witnessed the description of "new" candidate vascular risk factors, many of which are based on measuring the levels of

various substances in biological fluids or on polymorphisms and gene mutations, such as the MTHFR C677T (rs1801133) polymorphism.

The MTHFR C677T (Rs1801133) Polymorphism and Hyperhomocysteinemia

Methylenetetrahydrofolate reductase (MTHFR) is an enzyme encoded by a gene located on chromosome 1p36.3. This gene has a size of 2.2 kilobases (kb) and consists of 11 exons (Goyette et al., 1998). In humans, the product of this gene is a protein of 77 kilodaltons (kDa) that catalyzes the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the major circulating form of folate (Botto and Yang, 2000). This form of folate participates in the transfer of one carbon atom during the synthesis of nucleotides, the synthesis of S-adenosylmethionine and methylation of DNA, proteins, neurotransmitters and phospholipids (Botto and Yang, 2000). Furthermore, it also acts as a donor of methyl groups on primary methylation of homocysteine to methionine, catalyzed by the enzyme methionine synthase (MS) (Botto and Yang, 2000).

The MTHFR enzyme activity helps maintain reserves of 5-methyltetrahydrofolate and methionine and negatively regulates circulating plasma homocysteine concentration (Botto and Yang, 2000). Homocysteine levels can increase due to environmental factors such as smoking, low folate and vitamin B12 intake and related genetic polymorphisms in genes encoding enzymes or transport proteins (Strandhagen E, et al.,2004).

Excess homocysteine can be harmful to developing tissues (Spotila et al., 2003) and has been associated with CVD (Kang et al., 1991), stroke (Bostom et al. 1999), birth defects (Botto and Yang, 2000) and postmenopausal depression (Slopien et al., 2008) among others.

Defects in the MTHFR enzyme have also been linked to VT (Arruda VR et al., 1977; Cataneo et al., 1997), Alzheimer's disease (Anello G et al., 2004), some types of cancer (Curtin K, et al., 2004), pregnancy complications (Vollset et al., 2000) and neural tube defects (Holt FA et al., 1998). The latter condition has been one of the most widely studied consequences of this deficiency and is one of the most common birth defects worldwide (Botto and Yang, 2000).

Variants for the MTHFR Gene

Different single nucleotide polymorphisms (SNPs) in the sequence of the MTHFR gene alter the affinity of the enzyme for its substrate, 5,10-methylenetetrahydrofolate (Botto and Yang, 2000; Wu et al., 2009). The most frequent polymorphisms are MTHFR SNPs causing the change of one amino acid for another in the protein chain. These are the change of adenine (A) for cytosine (C) at position 1298 of the gene (A1298C), and of cytosine (C) for thymine (T) at position 677 of the gene (C677T). Both SNPs cause a reduction of MTHFR enzyme activity of 50-60% of the normal levels when in individual homozygous form (Kluijtmans LAJ et al., 1996; Vizcaino G et al., 2001; Rallidis LS et al., 2008). Furthermore, it appears that the presence of both SNPs in heterozygous form also reduces enzyme activity in similar percentages. This activity can also be reduced in situations of drastically folate-deficient diet.

Among the SNPs, the variant most often associated with the disorders of folate metabolism is the C677T. This polymorphism, whose consequence is the substitution of an alanine for a valine residue, reduces the activity of the enzyme and increases its thermolability (Froost et al., 1995). The C677T polymorphism can be detected in amniotic fluid and blood (Botto and Yang, 2000) through polymerase chain reaction (PCR) and restriction fragment analysis, since the SNP creates a site for the restriction enzyme HinfI (Frosst et al., 1995; Botto and Yang, 2000).

Interference with the function of the MTHFR enzyme and its co-substrate in the remethylation of homocysteine, methionine, so that the action of the enzyme decreases for any reason (polymorphisms, cofactors or lack of reaction), results in an increase of homocysteine levels (Frosst et al., 1995; Kluijtmans LAJ et al., 1997; Holmes MV et al., 2011). The 677TT homozygote exhibits approximately 30% of the in vitro activity of wild-type MTHFR 677CC, whereas the heterozygous genotype 677CT has about 65% of the enzyme activity of the wild type (Frosst et al., 1995). Thus, homozygosity for the 677T allele is associated with high homocysteine levels, predominantly in individuals with low folate plasma levels (Vizcaino G et al., 2001).

The MTHFR 677T allele is present in 23,7-37% of the Caucasian population in Europe, 30,5-47,5% of the Hispanic population, 8,3-14,6% of the African-American population, and, in a homozygous state, in 11% of the Australian population (Franco RF et al., 1998, Herrmann FH et al., 1999).

This polymorphism is autosomal with incomplete dominance (Frosst et al., 1995; Botto and Yang, 2000). Many factors interact with the function of

the variant enzyme. The homozygous 677TT genotype can cause a significantly increased risk of coronary heart disease in the absence of group B vitamins and / or folate, as these are co-factors in the metabolic reaction (Radillis LS et al., 2008; Holmes et al., 2011). It has also been associated with all variants of TE, both arterial and venous, and other diseases such as dementia, certain types of tumors, and migraine (Boston AG, et al., 1999; De Stephano et al., 2000; Anello G et al., 2004; Curtin K et al., 2004). It can be stated that this variant allele is a disease-associated polymorphism in humans.

In populations in which there is a high frequency of folate deficiency, the determination of the MTHFR C677T SNP will identify a population group prone to developing a major and readily preventable cardiovascular risk factor such as hyperhomocysteinaemia.

Hyperhomocysteinemia is the most common clinical feature associated with the presence of this MTHFR polymorphism. Homocysteine is an amino acid resulting from the demethylation of methionine acquired in the diet by protein intake. Since 1976, homocysteine is known to damage the vascular matrix by oxidative mechanisms, reducing endothelial antithrombotic action and promoting the proliferation of vascular smooth muscle, being therefore favorable for atherosclerosis (Morita H et al., 1997; Lloyd M and Taylor Jr, 2003; Rallidis LS et al., 2008).

Hyperhomocysteinemia is an independent risk factor for CVD, coronary thrombosis and death (Lloyd M & Taylor Jr, 2003; Rallidis LS et al., 2008). This factor that has been identified as contributing not only to developing atherosclerosis but also to producing a pro-coagulant state in venous blood (Arruda VR, et al., 1997; Marosi K, et al., 2012)

Among the main causes of hyperhomocysteinemia are genetic factors such as polymorphisms in enzymes involved in its metabolism, most importantly the MTHFR C677T SNP because of its frequency, in combination with vitamin B12, vitamin B6 and folic acid deficiencies, because of their aforementioned role as cofactors in these metabolic pathways (Botto and Yang, 2000; Holmes MV et al., 2011). Indeed, the main factors affecting homocysteine concentrations are low folate intake and the MTHFR C677T SNP (Botto and Yang, 2000; Vizcaino G et al., 2001).

Studies are contradictory as to whether the sole presence of the risk allele without hyperhomocysteinemia is sufficient to increase the risk of thrombosis (Lloyd M & Taylor Jr, 2003; Rallidis LS et al., 2008). However, the situation seems to be clearer in the presence of other risk factors and DM, since the presence of the 677T allele in homozygous or heterozygous form increased

CVD risk without their necessarily being any hyperhomocysteinaemia or folate deficiency.

Given that in Costa Rica there is a high prevalence of the MTHFR 677T allele in homozygous form (29%) (Hermann FH et al., 2001; Salazar-Sanchez L., 2011), and that 35% of these people have risk homocysteine blood levels (i.e., 10 to 15 µmol/L) and 4% have hyperhomocysteinemia (levels greater than 15 µmol / L) (Monge-Rojas et al., 2005). This is important to assess the relationship between this polymorphism and TE in this country.

A protective factor for this condition in Costa Rica is the enrichment of rice, a common food in Costa Rica, with folic acid. This measure, established by an executive decree by the Presidency and the Ministry of Health in 2006 (decree number 30 031-S) (14), is likely to attenuate the effects of the polymorphism, as has been observed in other populations where, after such measures, homocysteine levels in the population, decrease (Tsai et al., 2008).

It is then important to consider this factor, since, according to Kaul (cited by Rallidis, LS et al., 2008), prescribing vitamin supplements can reverse hyperhomocysteinemia and could thus reduce cardiovascular risk. However, the latter has not been proven.

Since 1997, the Costa Rican Social Security Fund (CCSS) is able to analyze the MTHFR C677T SNP for its patients through the Center for Research in Hematology and Related Disorders (CIHATA), but the role of this polymorphism in the context of thrombotic events remains unknown for this country.

By assessing the individual's genotype in combination with other risk factors, the patients may be amenable to treatment and individualized approaches. Thus, the objective of this research was to study the prevalence and to evaluate the effects of known risk factors and of this polymorphism on vascular risk among Costa Rican women with thrombotic events.

Materials and Methods

Subjects

The C677T MTHFR (rs1801133) polymorphism and thrombosis risk factors were studied in 149 female patients with primary episodes of TE, including pulmonary embolism (PE), deep venous thrombosis (DVT), myocardial infarction (MI) or stroke. The patients were admitted to Social Security Hospitals in Costa Rica.

TE was diagnosed by ultrasonography and/or venography. The diagnosis of MI was based on ischemic chest symptoms, typical electrocardiographic (ECG) changes, and elevation of serum creatine kinase and its MB isoenzyme to more than twice the upper reference value limit.

Coronary angiography was performed in the patients. The severity of coronary atherosclerosis was determined by the number of coronary arteries with stenosis of more than 50 percent of the luminal diameter. Patients were included irrespective of concomitant risk factors for thrombosis such diabetes, hypercholesterolemia and smoking.

A complete clinical summary, with emphasis on personal and family history for MI, TE and stroke, and cardiovascular risk factors was obtained from all subjects by a specially trained staff. A subject was considered to have diabetes or hypercholesterolemia when he or she was receiving specific medications to treat these conditions and/ or when there was an established diagnosis of each disease. Information concerning with smoking habits, family history of CVD, use of oral contraceptives and body mass index (BMI) was also obtained for all subjects. Women with BMI >26 kg/m^2 were considered to be obese. Only subjects with history of regular cigarette consumption were included as smokers.

Healthy subjects (hospital and university staff, students and blood donors), without a history of TE and matched to the patients (age and sex) were selected for the control group. All data concerning demographic characteristics and the presence of major risk factors for TE o CVD were collected by the physicians.

Local ethics committees of participating institutions approved this study. All participants gave informed consent according to a protocol approved by the University of Costa Rica Human Subjects Committee and participating hospitals.

DNA Extraction and Polymorphism Determination

Blood samples were collected into tubes containing 1.7mM EDTA-K3. DNA was isolated from peripheral blood according to standard protocols (Miller et al., 1988). For some analyses blood samples soaked onto filter paper cards from the probands were used for polymerase chain reaction (PCR) as described previously (Herrmann et al., 1999).

Amplification was carried out on 50-µL volume samples in a Perkin Elmer-Cetus thermal cycler. Genotyping of C677T MTHFR, were performed

by PCR amplification of each of the target alleles from genomic DNA followed by restriction digestion with each of corresponding enzyme HinfI (Frosst et al.,1995; Herrmann et al., 1999).

Data Analysis

Statistical analysis was performed by the Statistical Package for the Social Sciences (SPSS) Version 19. To compare the basic characteristics, odds ratio (ORs) with 95% confidence intervals (CIs) was calculated and test for dichotomized variables. The means of continuous variables was evaluated with ANOVA or Kruskal Wallis test. The observed numbers of the polymorphism genotypes were compared with those expected for a population in Hardy-Weinberg equilibrium using the Chi^2 test. To assess the association between genotypes and MI, VT or stroke, ORs with 95% CIs was calculated. Adjustment was made for the confounding effects of dichotomized and continuous variables. The presence of interactions between traditional and genetic factors was estimated by stratified analysis. Multiple logistic regression analysis was also used in order to examine the relationships between variables. Statistical significance was taken as $P<0,05$.

Results

Traditional Risk Factors

The demographic characteristics and prevalence of traditional risk factors for thrombosis among patients and control subjects are present in the total group.

The mean ages for cases and controls were 45,0 (± 18,5) years and 54,0 (± 21,8) years, respectively. Of note, 36,6% of the cases were younger than 50 years; these subjects were considered for following analyses as the younger group. Also, 75,4% of the cases were older than 50 years; these subjects were considered as the older age group.

Table 1. Distribution of the MTHFR C677T genotypes among cases and controls

MTHFR 677C>T	Cases n (%)	Controls n (%)	p
677 CC	35 (23,5)	46 (40,7)	0,004
677CT	71 (47,7)	39 (34,5)	0,04
677 TT	43 (28,9)	28 (24,8)	0,55
Total	149 (100)	113 (100)	

p is significant <0,05

Of the traditional risk factors examined, the presence of obesity was significantly different (p=0,006) between the total cases (25,2 ± 4,9) and the control group (23,7 ± 4,1), also followed in decreasing order by use of oral contraceptives (p=0,003), family history of TE (p=0,002) and levels of fibrinogen (p=0,005) in the total group.

In addition, the prevalence of the *MTHFR* polymorphism was a significantly different between the groups (p=0,001) and in Table 1, is present the different value between the genotypes in cases and controls (677T:Wt; 677CT:Hz and 677TT:Ho).

Interaction of Combined Traditional TE Risk Factors in Groups of Cases and Controls by Age

A stratified analysis was performed to explore the interaction of between the age groups (age 50 or over and age under 50) and the risk factors. The results showed that hypercholesterolemia, diabetes mellitus, and elevated levels of fibrinogen were present in the younger group as risks factors. Family history of TE was statistically significantly associated among the older female group.

Median values of total fibrinogen were significantly higher in the cases compared with the control group. The main results of these analyses are given in Table 2.

Table 2. Demographic characteristics and prevalence of risk factors for thrombotic disease stratified by age groups among cases and controls

Variable/ Risk Factor	**Cases n=149**	**Controls n=113**	**Odds Ratio (95% CI)**	**p**
Age				
<50 years	36,6 ± 8,2	34,7 ± 10,5	N.A	0,3
≥50 years	75,4 ± 11,4	71,4 ± 12,8		0,14
BMI (kg/m^2)				
<50 years	24,5 ± 3,8	22,1 ± 2,7	N.A	0,000
≥50 years	27,6 ± 7,1	25 ± 4,7		0,066
Smoking, %				
<50 years	116	52		
Yes	16 13,8%	0 0%	N.A.	0,005
No	100 86,2%	52 100%		
≥50 years	33	61		
Yes	03 9,1%	10 16,4%	0,51 (0,13 – 2,1)	0,33
No	30 90,9%	51 83,6%		
Fibrinogen (mg/dL)				
<50 years	307,6 ± 114,6	257,8 ± 74,8	N.A	0.002
≥50 years	388,2 ± 167	297 ± 78,5		0,17
Diabetes mellitus, %				
<50 years	19	52		
Yes	09 47,4%	0 0%		0,04
No	10 52,6%	52 100%	N.A	
≥50 years	33	61		0,33
Yes	03 9,1%	10 16,4%	0,51 (0,13 – 2)	
No	30 90,9%	51 83,6%		
Family history				
<50 years	116	52		
Yes	40 34,5%	12 23,1%	1,7 (0,8 – 3,7)	0,14
No	76 65,5%	40 76,9%		
≥50 years	33	61		
Yes	11 33,3%	7 11,5%	3,8 (1,3 – 11,2)	0,01
No	22 66,7%	54 88,5%		

Table 2. (Continued)

Variable/ Risk Factor	Cases n=149	Controls n=113	Odds Ratio (95% CI)	p
Hypercholester-olemia				
<50 years	116	52		
Yes	33 28,4%	04 7,7%	4,8 (1,5 – 14,3)	0,003
No	83 71,6%	48 92,3%		
≥50 years	23	32	N.A.	0,13
Yes	01 4,3%	0 0%		
No	22 95,7%	32 100%		
Oral contraceptives*				
<50 years	114	52		
Yes	19 16,7%	03 5,8%	3,3 (0,9 – 11,6)	0,05
No	95 83,3%	49 94,2%		0,23
≥50 years	8	61		
Yes	1 12,5%	2 3,3%	4,2 (0,3 – 52,6)	
No	7 87,5%	59 96,7%		

Age, BMI and fibrinogen are expressed as mean ± standard deviation. On the remaining rows, the values denote numbers of cases or controls followed by percentage of the total group. Obesity: BMI ≥ 26 kg/m^2. Odds Ratio, 95%CI denotes confidence interval. **p** is significant <0,05.

Prevalence of the C677T MTHFR Genetic Polymorphism

The genotype distribution was in Hardy-Weinberg equilibrium in both groups. The results of the prevalence of the C677T MTHFR polymorphism are given in Table 3. Briefly, a significant difference in the prevalence was found between the overall age group (Table 1). However, in the stratified analysis it was as risk factor in the ≥50 years; old group.

A stratified analysis was performed to explore the interaction between each of the MTHFR C677T genotypes and the traditional risk factors. In the model of the logistic regression and stratified analysis according to age, the importance of the co-occurrences of some of the genotypes and the major risk factors (obesity, p=0,004; hypercholesterolemia p=0,003, use of oral contraceptives, p=0,05; and fibrinogen levels, p=0,002) were detected in the <50 years old group and family history (p=0,01) in the older group >50 years old.

Table 3. Prevalence of the *MTHFR* C677T polymorphism between the case and control groups and stratified by age.

Variable/ Risk Factor	Cases n=149	Controls n=113	Odds Ratio (95% CI)	p
MTHFR	n=116	n=52		
<50 years	Ho 35 30,2% Hz 53 45,7% Wt 28 24,1%	Ho 16 30,8% Hz 19 36,5% Wt 17 32,7%	N.A.	0,43
≥50 years	n=33 Ho 8 24,2% Hz 18 54,5% Wt 7 21,2%	n=61 Ho 12 19,7 Hz 20 32,8 Wt 29 47,5		0,037

The values denote numbers of cases or controls followed by percentage of the total group.

Odds Ratio, 95%CI denotes confidence interval. **p** is significant <0,05.

Conclusion

Thrombotic diseases develop as the result of multiple interactions between non-genetic and genetic risk factors. The known risk factors played an important role as predictors in the development of the thrombotic diseases in the present female case-control study. The most important non-genetic risk factors found among the female patients are: age, hypercholesterolemia, diabetes mellitus, use of oral contraception, obesity, and fibrinogen levels. Moreover, the stratified effect of some of these factors according to the age of the patients is relevant for clinical risk assessment.

In a large multicenter case-control study of thrombosis no association between MTHFR 677T homozigosity and arterial disease was found (pooled OR 1,1, 95%CI 0,9-1,4) (Brattström et al., 1998). The reasons given for the lack of association included nutritional or genetic differences between the populations of this study and those of others (Abbate et al., 1998; Lane and Grant, 2000). This is interesting, in the context of the high frequency of homozygosity of the variant (T) allele in the present Costa Rican female study as compared with Caucasian populations (Herrmann FH et al., 1999; Herrmann FH et al., 2000) and its significantly higher prevalence among the cases versus controls in the overall cohort. Future research in genetics for VT

and other multicausal diseases will be based on the new technologies for study the genetic architecture and the specific pathways that are important in the development of the personalized prediction and individual management.

In the present study, an increased risk for thrombosis was found in the older age group with MTHFR 677C>T polymorphism and the major risk factors (hypercholesterolemia p=0,003, use of oral contraceptives, p=0,05; and fibrinogen levels, p=0,002) were detected in the <50 years old group and family history (p=0,01) and obesity (p=0,004) in the older group >50 years old. The small numbers of carriers of these risk factors require a note of caution on any conclusion. Further studies with more patients are necessary for final conclusions for these interactions to be made. However, it is clear that the high prevalence of MTHFR T allele may participate as a risk factor for thrombosis in the older female group.

There has been ongoing discussion on the possibility that MTHFR genotypes could affect the methylation capacity of the estrogen receptor (Slopien et al., 2008). Evidence on the interaction between estrogen status, homocysteine concentration and MTHFR polymorphism and their effect on hormone replacement therapy has been reported (Ottaviano YL et al., 1994). Variations between the concentration of homocysteine among women of different ages and lifestyles that are consistent with effects attributable to estrogen status have been shown (Curtin K, et al., 2004). It seems therefore important to understand if the MTHFR 677TT genotype plays a role as risk factor for thrombosis either in pre or in postmenopausal women (Slopien et al., 2008).

Taken together, the present data suggest that MTHFR C677T genotype may be an important factor in the life of female patients at risk of developing TE or CVD. Folic acid supplementation of foods is necessary (and mandatory in Costa Rica) before and during women's reproductive phase. However, the data presented here suggests that a case for efforts to ensure a good intake of this vitamin in the postmenopausal phase can be made, especially if the high prevalence of the MTHFR 677T homozygosity and of classic risk factors in the Costa Rican population are taken into account. Larger additional studies among women with this genetic and environmental background looking at the combined effect of the MTHFR 677C>T polymorphism and traditional risk factors for thrombosis must be performed for final conclusions to be drawn.

References

Abbate R, Sardi I, Pepe G, Marcucci R, Brunelli T, Prisco D, Fatini C, Capanni M, Simonetti I, Gensini GF. The high prevalence of thermolabile 5-10 methylenetetrahydrofolate reductase (MTHFR) in Italians is not associated to an increased risk for coronary artery disease. *Thromb Haemost* 1998; 79:727-730.

Anello G, Guéant-Rodríguez RM, Bosco P, Guéant JL, Romano A, Namour B, Spada R, Caraci F, Pourié G, Daval JL, Ferri R. Homocysteine and methylenetetrahydrofolate reductase polymorphism in Alzheimer's disease. *Neuroreport*. 2004 Apr 9;15(5):859-861.

Araya M, Guzmán S. Evolución de la Mortalidad por Enfermedad Isquémico del Corazón e Infarto Agudo del Miocardio en Costa Rica, 1970-2001. *Rev. Panam Sal. Public*. 2004:16:295-301.

Arruda VR, von Zuben PM, Chiaparini LC, Annichino Bizzacchi JM, Costa FF. The mutation Ala677>Val in the methylene tetrahydrofolate reductase: a risk factor for arterial and venous thrombosis. *Thromb Haemost* 1997; 77: 818-821.

Brattström L, Wilcken DE, Ohrvik J, Brudin L. Common methylenetetrahydrofolate reductase gene mutation leads to hyperhomocysteinemia but not to vascular disease. *Circulation*. 1998; 98:2520–2526

Botto L & Yang Q. 5,10-Methylenetetrahydrofolate Reductase Gene Variants and Congenital Anomalies: A HuGE Review. Am J Epidemiol 2000; 151:862-77.

Bostom AG, Rosenberg IH, Silbershatz H, Jacques PF, Selhub J, D'Agostino RB, Wilson PW, Wolf PA Nonfasting plasma total homocysteine levels and stroke incidence in elderly persons: the Framingham Study. *Ann Intern Med*. 1999 Sep 7;131(5):352-355

Cataneo M, Tsai MY, Bucciarelli P, Taioli E, Zighetti ML, Bignell M, Mannucci PM. A common mutation in the methylenetetrahydrofolate reductase gene (C677T) increases the risk for deep-venous thrombosis in patients with mutant factor V (factor V:Q506*). Arterioscler Thromb Vasc Biol* 1997; 17: 1662-1666.

Curtin K, Bigler J, Slattery ML, Caan B, Potter JD, Ulrich CM. MTHFR C677T and A1298C polymorphisms: diet, estrogen, and risk of colon cancer. *Cancer Epidemiol Biomarkers Prev.* 2004 Feb;13(2):285-292.

Reglamento para el enriquecimiento del arroz. (Decreto Nª 30031-S) (02/01/02). *Publicado en La Gaceta Nº* 1 del 02 de enero del 2002.

De Stephano V, Casorelli I, Rossi E, Zappacosta B, Leone G. Interaction between hyperhomocysteinemia and inherited thrombophilic factors in venous thromboembolism. *Semin Thromb Haemost* 2000; 26: 305-311.

Franco RF, Araujo AG, Guerreiro JF, Elion J, Zago MA. Analysis of the 677 C→T mutation of the methylenetetrahydrofolate reductase gene in different ethnic groups. *Thromb Haemost* 1998; 79: 119-121.

Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJ, den Heijer M, Kluijmans LAJ, van den Heuvel LP, Rozen R. A candidate genetic risk factor for vascular diseases: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet* 1995; 10: 111-113.

Goyette P, Pai A, Milos R, Frosst P, Tran P, Chen Z, Chan M, Rozen R.Gene structure of human and mouse methylenetetrahydrofolate reductase (MTHFR) *Mamm Genome*. 1998 Aug; 9(8):652-656

Herrmann FH, Schröder W, Altman R, Jimenez Bonilla R, Perez-Requejo JL, Singh JR. Zur Prävalenz des G20210A-Prothrombin-Polymorphismus, der C677T-Mutation des MTHFR-Gens und der Faktor V-Leiden Mutation in Nordostdeutschland, Argentinien, Venezuela, Costa Rica und Indien. In: I. Scharrer/W. Schramm (Hrsg.) 28. *Hämophilie-Symposium Hamburg 1997*. Springer-Verlag Berlin Heidelberg 1999: 305-309.

Herrmann FH, Salazar-Sanchez L, Schröder W, Grimm R, Schuster G, Jimenez-Arce G, Chavez M, Singh JR. Prevalence of molecular risk factors FV Leiden, FVHR2, FII 20210G>A and MTHFR 677C>T in different populations and ethnic groups of Germany, Costa Rica and India. *Intl J Hum Genet* 2001; 1: 33-39.

Holmes MV, Newcombe P, Hubacek JA, Sofat R, Ricketts SL, Cooper J, Breteler MM, Bautista LE, Sharma P, Whittaker JC, Smeeth L, Fowkes FG, Algra A, Shmeleva V, Szolnoki Z, Roest M, Linnebank M, Zacho J, Nalls MA, Singleton AB, Ferrucci L, Hardy J, Worrall BB, Rich SS, Matarin M, Norman PE, Flicker L, Almeida OP, van Bockxmeer FM, Shimokata H, Khaw KT, Wareham NJ, Bobak M, Sterne JA, Smith GD, Talmud PJ, van Duijn C, Humphries SE, Price JF, Ebrahim S, Lawlor DA, Hankey GJ, Meschia JF, Sandhu MS, Hingorani AD, Casas JP. Effect Modification by population dietary folate on the association between MTHFR genotype, homocysteine, and stroke risk: a methanalysis of genetic studies and randomised trials. *Lancet* 2011; 378: 584-594.

Hol FA, van der Put NM, Geurds MP, Heil SG, Trijbels FJ, Hamel BC, Mariman EC, Blom HJ. Molecular genetic analysis of the gene encoding the trifunctional enzyme MTHFD (methylenetetrahydrofolate-dehydrogenase, methenyltetrahydrofolate-cyclohydrolase, formyltetra-

hydrofolate synthetase) in patients with neural tube defects. *Clin Genet*. 1998 Feb;53(2):119-122

Hunter KA, Garlik PJ, Broom I, Andersons SE, McNurlan MA. Effects of smoking and abstention from smoking on fibrinogen synthesis in human. *Clinical Science* 2001; 100: 459-465.

Kain K, Catto A, Kohler HP, Grant PJ. Haemostatic risk factors in healthy white and south asian populations in U.K. *Thromb Haemost* 1999; 82 (suppl.) 185.

Kang SS, Wong PW, Susmano A, Sora J, Norusis M, Ruggie N. Thermolabile methylenetetrahydrofolic acid reductase: an inherited risk factor for coronary artery disease. *Am J Hum Genet* 1991; 48: 536-545.

Kluijtmans LAJ, van den Heuvel LPWJ, Boers GHJ, Frosst P, Stevens EMB, van Oost BA, den Heijer M, Trijbels FJM, Rozen R, Blom HJ. Molecular genetic analysis in mild hyperhomocysteinemia: A common mutation in the methylenetetrahydrofolate reductase gene is a genetic risk factor for cardiovascular disease. *Am J Hum Genet* 1996; 58: 35-41.

Kluijtmans LAJ, Kastelein JJP, Lindemans J, Boers GHJ, Heil SG, Bruschke AVG, Jukema JW, van den Heuvel LPWJ, Trijbels JMF, Boerma GJM, Verheugt FWA, Willems F, Blom HJ. Thermolabile methylenetetra-hydrofolate in coronary artery disease. *Circulation* 1997; 96: 2573-2577.

Koenderman J and Reitsma PH. Geographic and Ethnic differences in the prevalence of thrombophilia. In *Thrombophilia* pp: 1-22 .InTech Publishers 2011: Rijeka, Croatia

Lane DA, Grand PJ. Role of hemostatic gene polymorphisms in venous and arterial thrombotic disease. *Blood* 2000; 95: 1517-1532.

Lloyd M, Taylor Jr. Elevated plasma homocysteine as risk factor for peripheral arterial disease-What is the evidence*? Seminars in vascular surgery* 2003; 16:215-222.

Marosi K, Agota A, Végh V, Joó JG, Langmár Z, Kriszbacher I, Nagy ZB. The role of homocysteine and methylenetetrahydrofolate reductase, methionine synthase, methionine synthase reductase polymorphisms in the development of cardiovascular diseases and hypertension. *Orv Hetil*. 2012 Mar 25;153(12):445-453.

Miller M, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988; 16: 121.

Monge-Rojas R, Barrantes M, Holst I, Nunez-Rivas H, Alfaro T, Rodriguez S, Cunningham L, Cambronero P, Salazar L, Herrmann FH. Biochemical

indicators of nutritional status and dietary intake in Costa Rican Cabecar Indian adolescents. *Food Nutr Bull*. 2005; Mar; 26(1):3-16.

Morita H, Taguchi H, Kurihara H, Kitaoka M, Kaneda H, Kurihara Y, Maemura K, Shindo T, Minamino T, Ohno M,Yamaoki K, Ogasawara K, Aizawa T, Suzuki S, Yazaki Y. Genetic polymorphism of 5,10-methylenetetrahydrofolate reductase (MTHFR) as a risk factor for coronary artery disease. *Circulation* 1997; 95: 2032-2036.

Naess I, A.Christianen, SC, Romundstad P, Cannegieter S, Rsendaal FR, Hammerstrom J. Incidence and mortality of venous thrombosis: a population-based study. *J.Thromb.Haemostast*. 2007; 5(4):629-699

Ottaviano YL, Issa JP, Parl FF, Smith HS, Baylin SB, Davidson NE. Methylation of the estrogen receptor gene CpG island marks loss of estrogen receptor expression in human breast cancer cells. *Cancer Res*. 1994 May 15;54(10):2552-2555

Rallidis LS, Gialeraki A, Komporozos C, Vavoulis P, Pavlakis G, Travlou A, Lekakis I, Kremastinos DT. Role of methylenetetrahydrofolate reductase 677 T polymorphism in the development of premature myocardial infarction. *Atherosclerosis* 2008; 200: 115-120.

Reiner A, Siscovick D, Rosendaal FR. Hemostatic risk factors and arterial thrombotic disease. Thromb Haemost 2001; 85: 584-595.

Salazar-Sanchez L. Geographic and Ethnic differences in the prevalence of thrombophilia. In *Thrombophilia* pp: 39-58.InTech Publishers 2011: Rijeka, Croatia

Słopien R, Jasniewicz K, Meczekalski B, Warenik-Szymankiewicz A, Lianeri M, Jagodziński PP.Maturitas. Polymorphic variants of genes encoding MTHFR, MTR, and MTHFD1 and the risk of depression in postmenopausal women in Poland. *Maturitas*. 2008 Nov 20;61(3):252-255.

Spotila LD, Jacques PF, Berger PB, Ballman KV, Ellison RC, Rozen R. Age dependence of the influence of methylenetetrahydrofolate reductase genotype on plasma homocysteine level. *Am J Epidemiol*. 2003 Nov 1;158(9):871-877.

Strandhagen E, Zetterberg H, Aires N, Palmér M, Rymo L, Blennow K, Landaas S, Thelle DS. The methylenetetrahydrofolate reductase C677T polymorphism is a major determinant of coffee-induced increase of plasma homocysteine: a randomized placebo controlled study. *Int J Mol Med*. 2004 Jun;13(6):811-815.

Tsai M, Loria M, Cao J, Kim Y, Siscovick D, Schreiner J, Hanson QN. Clinical utility of genotyping the 677C >T variant of methylenetetrafolate

reductase in humans is decreased in the Post-Folic acid fortification ERA 1, 2. *The Journal of Nutrition* 2008; 139:33-37

Vizcaino G, Diez-Ewald M, Herrmann FH, Schuster G, Perez-Resquejo JL. Relationships between homocysteine, folate and vitamin B12 levels with the methylenetetrahydrofolate reductase polymorphism in Indians from Western Venezuela. *Thromb Haemost* 2001; 85: 186-187.

Vollset SE, Refsum H, Irgens LM, Emblem BM, Tverdal A, Gjessing HK, Monsen AL, Ueland PM. Plasma total homocysteine, pregnancy complications, and adverse pregnancy outcomes: the Hordaland Homocysteine study. *Am J Clin Nutr.* 2000 Apr;71(4):962-968