

Prevalence of the Mutation C677 → T in the Methylene Tetrahydrofolate Reductase Gene Among Distinct Ethnic Groups in Brazil

Valder R. Arruda,^{1*} Lucia H. Siqueira,¹ Marilda S. Gonçalves,² Paula M. von Zuben,¹ Manoel C.P. Soares,³ Raimundo Menezes,³ Joyce M. Annichino-Bizzacchi,¹ and Fernando F. Costa¹

¹Hematology-Hemotherapy Center, Department of Clinical Medicine, State University of Campinas, Campinas-SP, Brazil

²Federal University of Bahia, Salvador-BA, Brazil

³Evandro Chagas Institute, Belém-PA, Brazil

Vascular disease is a serious public health problem in the industrialized world, and is a frequent cause of death among the adult population of Brazil. Mild hyperhomocysteinemia has been identified as a risk factor for arterial disease, venous thrombosis, and neural tube defects. Individuals homozygous for the thermolabile variant of methylenetetrahydrofolate reductase (MTHFR-T) are found in 5–15% of the general population and have significantly elevated plasma homocysteine levels which represent one of the genetic risk factors for vascular diseases. We have analyzed the prevalence of individuals homozygous for the MTHFR-T in 327 subjects representing the three distinct ethnic groups in Brazil. The prevalence of homozygotes for the mutated allele MTHFR-T was high among persons of Caucasian descent (10%) and considerably lower among Black (1.45%) and Indians persons populations (1.2%). These data suggest that screening for the MTHFR-T allele should help in identifying individuals with a high risk of vascular disease among populations with a heterogeneous background. *Am. J. Med. Genet.* 78:332–335, 1998.

© 1998 Wiley-Liss, Inc.

KEY WORDS: methylenetetrahydrofolate reductase gene; homocysteine; folic acid; arterial disease; venous thrombosis

INTRODUCTION

A World Health Organization (WHO) report [1990] has suggested that cardiovascular diseases, the principal cause of death in developed countries, are also emerging as a prominent public health problem in developing countries. Duncan et al. [1992], when analyzing mortality data among adults from southeastern Brazil, showed that cardiovascular and cerebrovascular diseases are the most common cause of death. The primary prevention of these diseases is an important measure, even in developing countries.

According to a WHO Scientific Group [1994], the new areas for research in cardiovascular disease risk factors include further knowledge of the role of hemostatic factors levels, nutritional aspects, and the control of high levels of plasma homocysteine. Mild hyperhomocysteinemia is an independent risk factor for peripheral and coronary arterial disease [McCully, 1969; Clarke et al., 1991; Boushey et al., 1991], and also for venous thrombosis [Falcon et al., 1994; den Heijer et al., 1996]. Abnormal homocysteine levels may result from interaction between genetic and nutritional factors, and offer a potentially interesting area for research on the prevention of these diseases [Motulsky, 1996; McCully, 1996; Verhoef et al., 1996].

Of inherited hyperhomocysteinemias, the thermolabile variant of the methylenetetrahydrofolate reductase (MTHFR-T) described by Kang et al. [1988] is the most prevalent in the general population [Kang et al., 1991; Engbersen et al., 1995]. The MTHFR gene has recently been cloned by Frost et al. [1995] and the molecular basis for the thermolability has been shown to be C → T substitution at nucleotide 677, which converts an alanine to residue valine. The reduced specific activity of the enzyme in persons homozygous for the valine allele is associated with elevated plasma homocysteine levels as well as lower levels of an activated form of folic acid. These data suggest that the mutation C677 → T in the MTHFR gene may represent an important genetic risk factor for vascular disease [Kluijtmans et al., 1996; Gallagher et al., 1996] and also for

*Correspondence to: Valder R. Arruda, M.D., Hematology-Hemotherapy Center, State University of Campinas UNICAMP, C.P. 6108, C.E.P. 13081-970, Campinas-SP, Brazil.

Received 21 February 1997; Accepted 3 June 1997

neural tube defects [van der Put et al., 1995; Ou et al., 1996]. The frequency of individuals homozygous for the mutation varies widely from 5% to 12% among Caucasians and it is also common among the Japanese and Middle Eastern populations [Motulsky, 1996]. The lowest incidence of the mutation (1.4%) has been described among African-Americans (1.4%), which agrees with previous data referring to the genetic resistance of this group to hyperhomocysteinemia and the lower incidence for both coronary arterial disease and neural tube defects in this population [Ubbink et al., 1995; McCully, 1996; Motulsky, 1996; Stevenson et al., 1997]. These data raise a question as to the importance of screening for this MTHFR mutation among distinct populations in order to identify with high risk of vascular disease.

The ethnic origin of the Brazilian population is highly heterogeneous and is composed of immigrants from Europe, Africa, Asia, as well as Indian groups, which results in a complex race admixture. We have previously described that in the Brazilian population, homozygotes for the mutated allele MTHFR-T have a fivefold greater risk of arterial disease and a threefold greater risk of venous thrombosis [Arruda et al., 1997]. In the present study, we have determined the prevalence of the mutation C677 → T among individuals from three distinct racial groups in Brazil.

METHODS

Diagnosis of Mutation C677 → T in the MTHFR Gene

Genomic DNA was obtained from peripheral blood samples from all groups using a standard method [Miller et al., 1988]. A fragment of the MTHFR gene was amplified by the polymerase chain reaction (PCR) [Saiki et al., 1988], in a mixture of 54 mM Tris-HCl, pH 8.8, 5.4 mM MgCl₂, 5.4 μM EDTA, 13.3 mM (NH₄)₂SO₄, 8% DMSO, 8 mM β-mercaptoethanol, 0.4 mg of BSA/ml, 0.8 mM of each nucleoside triphosphate, 400 ng of each of the primers described by Froost et al. [1995]: sense (5'-TGAAGGAGAAGGTGTCTGCGGGA-3') and antisense (5'-AGGACGGTGCGGTGAGAGTG-3'), genomic DNA and 2 U of Taq polymerase. The reaction involved 30 cycles of incubation at 94°C (1 minute), 55°C (1 minute) and 72°C (2 minutes) and yielded a fragment of 198 bp. Subsequently, 10–15 μl of this PCR product was digested with 2.5 U of Hinf I. After digestion of the mutated MTHFR gene 677T, two fragments of 175 and 23 bp were observed following electrophoresis in a 2% agarose gel. When the normal allele 677C was present, there was no cleavage site for Hinf I and the fragment remained 198 bp (Fig. 1).

Selection of Groups

The populations studied were from three distinct regions of Brazil and represented samples from the three major ethnic groups which make up this country's population. These ethnic groups were selected as part of a research program for the evaluation of the inherited risk factors for vascular disease carried out at the State University of Campinas [Arruda et al., 1996].

The first group comprised 83 Amazonian Indians (40

males and 43 females) from the Tupi tribe known as Parakanã. These subjects had a median age of 32.9 years (ages ranging from 14 to 70 years), and were from two different villages in the Oriental Amazonian. Their samples were collected by two of the authors (M.C.P.S. and R.M.) during a campaign to control viral hepatitis.

The second group consisted of 137 (67 males and 80 females) Brazilian Blacks ranging in age from 12 to 60 years (median age, 33 years). This group contained the students and staff of laboratories in the Federal University of Bahia, State of Bahia, northeastern Brazil, where the majority of the population is of African descent derived from the slave trade [Curtin, 1969]. The samples in this group were collected by M.S.G.

The third group comprised 107 individuals of Caucasian descent (55 males and 52 females), recruited by L.H.S. from the laboratory staff, students, and physicians of the State University of Campinas, State of São Paulo, southeastern Brazil. These subjects ranged in age from 19 to 62 years (median age, 33.8 years). This latter group contained no individuals with a known admixture of races in the last three generations. Their ancestors were usually from Italy, Spain, Portugal, Austria, and Germany.

Statistical Analysis

The statistical significance of differences between groups as calculated by the chi-square or by Fischer's exact tests. The 95% confidence intervals were calculated using Epi Info [Dean et al., 1994].

RESULTS

The distribution of the MTHFR genotypes is shown in Table I. The prevalence of homozygotes for the mutated allele MTHFR-T was higher among Caucasians (10%) than among Blacks (1.45%) or the local Indian population (1.2%). There was no significant difference in the frequency of homozygotes for the mutated allele between Blacks and Indians ($P = 0.68$). As shown in Table II, there was a significant difference in the distribution of the alleles of (677T) between Caucasian and Black subjects (37% vs. 20%; $\chi^2_{(1)} 17.99$; $P < 0.001$) as well as between Caucasians and Indians (37% vs. 11.4%; $\chi^2_{(1)} 32.64$; $P < 0.001$). Similarly, the distribution of this 677T was significantly higher among Blacks than among Indians ($\chi^2_{(1)} 5.50$ $P = 0.019$). No related gender differences were observed.

TABLE I. MTHFR Genotype Distribution Among Distinct Ethnic Groups from Brazil

Genotype ^a	Caucasians ($n = 107$)	Blacks ($n = 137$)	Indians ($n = 83$)
+/+	10.3% ($n = 11$)	1.45% ($n = 2$)	1.2% ($n = 1$)
+/-	54.2% ($n = 58$)	37.2% ($n = 51$)	20% ($n = 17$)
-/-	35.5% ($n = 38$)	61.3% ($n = 84$)	78.5% ($n = 65$)

^aThe genotypes are represented by homozygous for normal allele (-/-) or mutated allele (+/+), and heterozygous (+/-). The number of homozygous (+/+) differs significantly when the Caucasians were compared to the Black population ($\chi^2_{(1)} 9.27$; $P = 0.002$) or to the Indians ($\chi^2_{(1)} 6.51$; $P = 0.01$). No difference was found between Black and Indian populations ($P = 0.68$).

TABLE II. Distribution of the Mutated Alleles of MTHFR Among Distinct Ethnic Groups

	Caucasians	Blacks	Indians
Total alleles	$\eta = 214$	$\eta = 274$	$\eta = 166$
Allele 677T ^a	37.3% ($\eta = 80$)	20% ($\eta = 55$)	11.4% ($\eta = 19$)
95% C.I. ^b	30.9–44.2	15.5–25.4	7.2–17.5

^aThe frequency of the allele 677T differs among Caucasians when compared to the Black population ($\chi^2_{(1)} 17.9$; $P < 0.001$) or to Indians ($\chi^2_{(1)} 32.6$; $P < 0.001$), and also between Black and Indian populations ($\chi^2_{(1)} 5.50$; $P = 0.019$).

^b95% C.I. denotes 95% confidence intervals.

DISCUSSION

The MTHFR-T allele is one of the most common genetic factors underlying vascular disease and neural tube defects in the general population, particularly since the presence of this mutation may lead to hyperhomocysteinemia [Kang et al., 1988, 1991; Franken et al., 1994; Kluijtmans et al., 1996]. In this study, we examined the incidence of the C677 → T in the MTHFR gene among normal subjects from three distinct ethnic groups in Brazil in order to identify those in which genetic screening would be useful as an indicator of vascular disease. Among Brazilians in general, the incidence of homozygosity for the mutation based on a nonselected group of consecutive newborns was found to be 4%, which is similar to the 5% described among normal Dutch, Finnish, and Irish controls [Kluijtmans et al., 1995; Motulsky, 1996; Gallagher et al., 1996]. By determining the distribution of homozygotes for the MTHFR-T allele in each ethnic group, we have shown here that the highest prevalence was among those individuals of Caucasian descent (10%). Among Brazilian Blacks, the incidence was 1.45%, a level similar to that previously described for African-Americans (1.4%). The Brazilian Blacks in the northeastern region originated principally from Angola, Congo, Mozambique, Nigeria, and Ghana and therefore differ from the African-derived group previously studied [Curtin, 1969]. Together, these data suggest a uniform low incidence of

homozygote MTHFR-T among Black populations. It is interesting to note that the most common inherited defect for venous thrombosis, a mutation in factor V of blood coagulation, known as factor V Leiden, is also uncommon among the Black population [Rees et al., 1995; Arruda et al., 1996]. Homozygotes for the MTHFR-T allele were found in 1.2% of Amazonian Indians. The low frequency (11.4%) of the mutated allele (677T) observed in this group contrasts with the 20% and 37% found among Blacks and Caucasians, respectively. South American Indians are probably related to Oriental populations and are descendants of a migration which originated in northeastern Asia and entered America through the Bering Strait [Cavalli-Sforza et al., 1988]. The Parakanã Indians represent a population almost unaffected by civilized society with which they have only recently established regular contact. No European or African blood groups were found in members of this tribe born before 1974 [Black et al., 1980]. It is possible that, in this group, marriage between closely related partners contributed to the lower incidence of the MTHFR-T allele. As already described for the Black population of Brazil, factor V Leiden was not detected in any of the Amazonian Indians [Arruda et al., 1996]. It has recently been estimated that factor V Leiden arose 21,000 to 34,000 years ago, i.e., after the evolutionary divergence of African from non-African populations [Zivelin et al., 1997]. A similar process may also have occurred with the MTHFR gene.

We have shown that MTHFR-T is a common risk factor for vascular disease among Brazilians (Arruda et al., 1997) and, based on the results of the present study, this risk is especially high among those of Caucasian descent. These data suggest that an increase in the food intake of folic acid and vitamin B-6, in order to counteract hyperhomocysteinemia and to decrease vascular disease and neural tube defects [U.S. Public Health Service, 1992], should be encouraged as a preventive health care measure. This suggestion is supported by the data of Duncan et al. [1992] who pointed out the high incidence of death due to vascular disease among Brazilian adults.

REFERENCES

- Arruda VR, von Zuben PM, Soares MCP, Menezes R, Annichino-Bizzacchi JM, Costa FF (1996): Very low incidence of Arg506 → Gln mutation in the factor V gene among the Amazonian Indians and the Brazilian black population. *Thromb Haemost* 75:860–861.
- Arruda VR, von Zuben PM, Annichino-Bizzacchi JM, Costa FF (1997): The mutation Ala677 → Val in the methylenetetrahydrofolate reductase gene: A risk factor for premature arterial disease and venous thrombosis. *Thromb Haemost* 77:818–821.
- Black FL, Salzano FM, Layrisse Z, Franco MHL, Harris NS, Weimer TA (1980): Restriction and persistence of polymorphisms of HLA and other blood genetic traits in the Parakanã Indians of Brazil. *Am J Phys Anthr* 52:119–132.
- Boushey CJ, Beresford SAA, Omenn GS, Motulsky AG (1995): A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. *JAMA* 274:1049–1057.
- Cavalli-Sforza LL, Piazza A, Menozzi P, Montain J (1988): Reconstruction of human evolution: Bringing together genetic, archaeological and linguistic data. *Proc Natl Acad Sci USA* 85:6002–6006.
- Clarke R, Daly L, Robinson K (1991): Hyperhomocysteinemia: An independent risk factor for vascular disease. *N Engl J Med* 324:1149–1155.

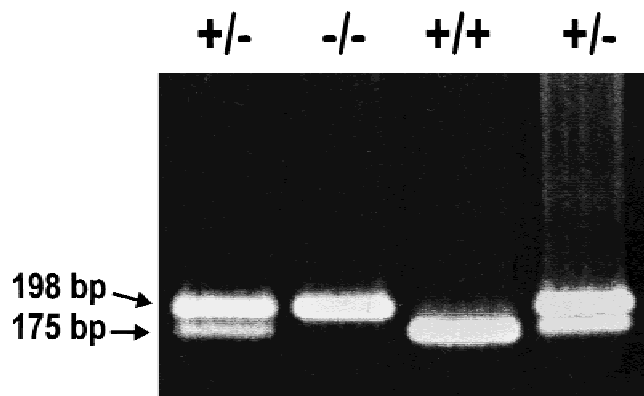


Fig. 1. Ethidium bromide-stained 2% agarose gel showing PCR products corresponding to a fragment of MTHFR gene after digestion with *Hinf*I. When the nucleotide 677 substitution C → T was present, it created a restriction site to *Hinf*I, resulting in fragments of 175 and 23bp, allele (+) or remained 198bp when the normal allele (-) was present. Heterozygous (+/-), homozygous for normal allele (-/-) or mutated allele (+/+) are shown in the figure.

- Curtin PD (1969): "The Slave Atlantic Trade: A Census." Milwaukee: University of Wisconsin Press.
- Dean AG, Dean JA, Coulombier D, Brendel KA, Smith DC, Burton AH, Dicker RC, Sullivan KM, Fagan RF, Arner TG (1994): Epi Info, version 6: A word processing, database, and statistics program for epidemiology on microcomputers. Atlanta: Center for Disease Control and Prevention.
- den Heijer M, Koster T, Blom HJ, Bos GMJ, Briët E, Reitsma PH, Vandenbroucke JP, Rosendaal FR (1996): Hyperhomocysteinemia as a risk factor for deep-vein thrombosis. *N Engl J Med* 334:759-762.
- Duncan BB, Schmidt MI, Polanczyk CA, Mengue SS (1992): High mortality rates in Brazilian adult populations—an international comparison. *Rev Ass Med Brasil* 38:138-144.
- Engbersen AMT, Franken DG, Boers GHJ, Stevens EMB, Trijbels FJM, Blom HJ (1995): Thermolabile 5,10-methylenetetrahydrofolate reductase as a cause of mild hyperhomocysteinemia. *Am J Hum Genet* 56:142-150.
- Falcon CR, Cattaneo M, Panzeri D, Martinelli I, Mannucci PM (1994): High prevalence of hyperhomocyst(e)inemia in patients with juvenile venous thrombosis. *Arterioscler Thromb* 14:1080-1083.
- Franken DG, Boers GHJ, Blom HJ, Trijbels JMF, Kloppernborg PWC (1994): Treatment of mild hyperhomocysteinemia in vascular disease patients. *Arterioscler Thromb* 14:465-470.
- Froost P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GHJ, den Heijer M, Kluijtmans LAJ, van den Heuvel LP, Rozen R (1995): A candidate genetic risk factor for vascular disease: A common mutation in methylenetetrahydrofolate reductase. *Nature Genet* 10:111-113.
- Gallagher PM, Meleady R, Shields DC, Tan KS, McMaster D, Rozen R, Evans A, Graham IM, Whitehead ASD (1996): Homocysteine and risk of premature coronary heart disease. *Circulation* 94:2154-2158.
- Kang SS, Wong PWK, Susmano A, Sora J, Norusis M, Ruggie N (1991): Thermolabile methylenetetrahydrofolate reductase: An inherited risk factor for coronary artery disease. *Am J Hum Genet* 48:536-545.
- Kluijtmans LAJ, van den Heuvel LPWJ, Boers GHJ, Frosst P, Stevens EMB, van Oost BA, den Heijer M, Trijbels FJM, Rozen RI (1996): Molecular genetic analysis in mild hyperhomocysteinemia; A common mutation in methylenetetrahydrofolate reductase gene is a genetic risk factor for cardiovascular disease. *Am J Human Genet* 58:35-41.
- McCully KS (1969): Vascular pathology of homocysteinemia. *Am J Pathol* 56:111-128.
- McCully KS (1996): Homocysteine and vascular disease. *Nature Medicine* 2:386-389.
- Millar SA, Dykes DD, Polesky HF (1988): A simple salting out procedure for extracting DNA from nucleated cell. *Nucleic Acids Res* 16:1215.
- Motulsky AG (1996): Nutritional ecogenetics: Homocysteine-related arteriosclerotic vascular disease, neural tube defects, and folic acid. *Am J Hum Genet* 58:17-20.
- Ou CY, Stevenson RE, Brown VK, Schwartz CE, Allen WP, Khoury MJ, Rozen R, Oakley GP Jr, Adams MJ Jr (1996): 5,10 Methylenetetrahydrofolate reductase genetic polymorphism as a risk factor for neural tube defects. *Am J Med Genet* 63:610-614.
- Rees DC, Cox M, Clegg JB (1995): World distribution of factor V Leiden. *Lancet* 346:1133-1134.
- Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA (1988): Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 239:487-491.
- Stevenson RE, Schwartz CE, Du Y-Z, Adams MJ Jr (1997): Differences in methylenetetrahydrofolate reductase genotype frequencies, between whites and blacks. *Am J Hum Genet* 60:229-230.
- Ubbink JB, Vermaak WJH, Delport R, van der Merwe A, Becker PJ, Potgieter H (1995): Effective homocysteine metabolism may protect South African blacks against coronary heart disease. *Am J Clin Nutr* 62:802-808.
- U.S. Public Health Service (1992): Recommendation for the use of folic acid to reduce the number of cases of spina bifida and other neural tube defects. *MMWR* 41:1-7.
- van der Put NMJ, Steegers-Theunissen RPM, Froost P, Trijbels FJM, Eskes TKAB, van den Heuvel LP, Mariman ECM, den Heyer M, Rozen R, Blom HJ (1995): Mutated methylenetetrahydrofolate reductase as a risk factor for spina bifida. *Lancet* 346:1070-1071.
- Verhoef P, Stampfer MJ, Buring JE, Gaziano JM, Allen RH, Stabler SP, Reynolds RD, Kok FJ, Hennekens CH, Willett WC (1996): Homocysteine metabolism and risk of myocardial infarction: Relation with vitamin B6, B12, and folate. *Am J Epidemiol* 143:845-859.
- WHO Technical Report Series No. 797 (1990): "Diet Nutrition, and the Prevention of Chronic Diseases." Report of a WHO Study Group.
- WHO Technical Report Series, No. 841 (1994): "Cardiovascular Disease Risk Factor: New Areas for Research." Report of a WHO Scientific Group.
- Zivelin A, Griffin JH, Xu X, Pabinger I, Samama M, Conard J, Brenner B, Eldor A, Seligsohn U (1997): A single genetic origin for a common caucasian risk factor for venous thrombosis. *Blood* 89:397-402.