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Accepted 4 November 2001.

ANALYSIS OF TWO MUTATIONS IN THE MTHFR GENE ASSOCIATED WITH MILD HYPERHOMOCYSTEIN-AEMIA — HETEROGENEOUS DISTRIBUTION IN THE SOUTH AFRICAN POPULATION

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Objective. The frequencies of mutations 677C→T and 1298A→C in the methylenetetrahydrofolate reductase (MTHFR) gene, previously shown to be associated with decreased enzyme activity that may lead to hyperhomocysteinaemia and consequently increased risk of cardiovascular disease (CVD), were determined in the South African population.

Methods. *HinfI* (677C→T) and *MboII* (1298A→C) restriction enzyme analyses were performed on amplified DNA samples of 76 white, 73 coloured and 60 black subjects.

Results. The mutant alleles of mutations 677C→T and 1298A→C were more common in the white (allele frequencies 0.36 and 0.37, respectively) than in the black population (0.04 and 0.09), while intermediate frequencies were detected in the coloured population (0.18 and 0.30). Homozygosity for mutation 677C→T was not detected in the black cohort, while this genotype was detected in 1 coloured (1.4%) and 8 white (10.5%) subjects. In the black population, 5% of the 60 subjects analysed were homozygous for mutation 1298A→C, compared with approximately 12% in both the white and coloured populations.

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Conclusions. Since hyperhomocysteinaemia is a risk factor for premature CVD, the heterogeneous distribution of the 677C→T and 1298A→C mutations across ethnic groups may partly explain ethnic differences in heart disease risk through decreased enzyme activity and hence increased homocysteine levels.

S Afr Med J 2002; 92: 464-467.

Cardiovascular disease (CVD) is a multifactorial condition caused by an interaction of genetic and environmental factors. In addition to well-known risk factors including low high-density lipoprotein (HDL) and raised low-density lipoprotein (LDL) cholesterol, triglycerides, fibrinogen and lipoprotein(a) (Lp(a)), elevated plasma homocysteine concentration has consistently been identified as a risk factor for the development of CVD.¹ In 1995 Frosst *et al.*² identified a C to T base change at nucleotide position 677 in the methylenetetrahydrofolate reductase (MTHFR) gene, which is responsible for increased thermolability of the enzyme, causing mild hyperhomocysteinaemia. Although various studies have demonstrated a positive association between CVD and this polymorphism, lack of association has also been reported, indicating that environmental interaction and the genetic background of the study population are important determinants of risk imposed by mutation 677C→T.^{3,5} More recently, a second common mutation, 1298A→C, resulting in decreased MTHFR activity, has been identified in the MTHFR gene.⁶ This sequence variant is not associated with elevated plasma homocysteine levels or a lower plasma folate concentration, although combined heterozygosity for both mutations results in increased thermolability of the enzyme and elevated homocysteine levels. Van der Put *et al.*⁶ indicated that mutation 1298A→C may be an additional risk factor for neural tube defects (NTDs), but its possible role in CVD has not yet been defined.

Since the public health relevance of MTHFR mutations causing hyperhomocysteinaemia would largely depend on the frequency of disease-related mutations within a population, we analysed the 677C→T and 1298A→C MTHFR mutations in the South African population. We attempted to determine whether the distribution of MTHFR mutations among ethnic groups might be associated, at least in part, with ethnic differences in risk of CVD. The pattern of CVD differs in various population groups in South Africa,^{7,8} and this phenomenon has been attributed largely to differences in lifestyle and diet.^{7,9} During recent years several studies have contributed to a growing awareness of the significant role of genetic factors predisposing an individual to different forms of heart disease, including the demonstration that three founder-related low-density lipoprotein receptor (LDLR) gene mutations are responsible for

the high prevalence (1/70) of familial hypercholesterolaemia (FH) in the South African Afrikaner population.¹⁰ These mutations shown to be responsible for the disease in approximately 90% of affected Afrikaners were absent in the black population, while detected in 10 - 20% of coloured FH patients.¹¹ The detection of multiple founder-type LDLR gene mutations originating from European populations provided direct genetic evidence that Caucasoid admixture contributes significantly to the apparently high prevalence (> 1/500) of FH in the South African coloured population. This finding has demonstrated the potential consequences of recent admixture in populations with different disease risks, a phenomenon that may also be of relevance to the present study of MTHFR mutations in the general South African population.

MATERIALS AND METHODS

Subjects

Blood samples of 209 individuals from three different ethnic groups in South Africa (Table I) were collected after obtaining informed consent: 60 Xhosa, Pedi and Zulu individuals (black population), 73 of mixed ancestry (coloured population) and 76 Caucasians (white population). These included healthy blood donors, farm workers and laboratory personnel. In this study 'white' or 'Afrikaner' refers to an individual of European descent, mainly Dutch, French, German and British origin; 'coloured' refers to an individual of mixed ancestry, including Khoisan, African Negro, Madagascar, Javanese and European origin; and 'black' refers to South Africans of central African descent.

Mutation detection

Genomic DNA was extracted from whole blood according to the method of Miller *et al.*,¹² and amplified by the polymerase chain reaction (PCR) using previously described oligonucleotides.^{2,6} Amplified products were digested with *HinfI* and *MboII* restriction enzymes for detection of mutations 677C→T and 1298A→C, respectively. *HinfI* digested products were electrophoresed on a 12% polyacrylamide gel and *MboII* digestions on a 20% polyacrylamide gel. Bands were visualised under ultraviolet light following ethidium bromide staining.

Statistical analysis

Chi-square values were calculated and their significance levels determined by two-way contingency tables. *P*-values of < 0.05 were regarded as statistically significant.

RESULTS AND DISCUSSION

The frequencies of the 677C→T and 1298A→C MTHFR gene mutations were determined in the diverse South African population in order to evaluate their potential in predicting



Table I. Comparison of genotype distribution and allele frequencies of two MTHFR gene mutations in three ethnic groups in South Africa

MTHFR mutations	Genotype/allele	White* (N = 76)		Coloured† (N = 73)		Black‡ (N = 60)	
		N	%	N	%	N	%
677C→T	CC	30	39	47	65	55	92
	CT	38	50	25	34	5	8
	TT	8	11	1	2	0	0
Allele	C	98	64	119	82	113	96
Frequency	T	54	36	27	18	5	4
1298A→C	AA	29	38	40	55	52	87
	AC	38	50	24	33	5	8
	CC	9	12	9	12	3	5
Allele	A	96	63	93	70	109	91
Frequency	C	56	37	39	30	11	9

Genotype distributions and allele frequencies differed significantly:

* White v. coloured: $P < 0.03$ (677 C→T mutation only).

† Coloured v. Black: $P < 0.004$ (both mutations).

‡ Black v. white: $P < 0.0001$ (both mutations).

CVD risk in different ethnic groups. The results obtained following *HinfI*² and *MboII*⁶ restriction enzyme analysis in the unselected white, black and coloured populations are shown in Table I. Statistically significant differences for the two mutations were observed among the different South African population groups, with regard to both genotype distribution and allele frequencies ($P < 0.01$). However, no significant difference could be detected between whites and coloureds for mutation 1298A→C. The mutant alleles of mutations 677C→T and 1298A→C were more common in the white (allele frequencies 0.36 and 0.37, respectively) than in the black population (0.04 and 0.09), while intermediate frequencies were detected in the coloured population (0.18 and 0.30).

Homozygosity for mutation 677C→T was not detected in black subjects, consistent with the findings of Ubbink *et al.*,¹³ who indicated that this genotype does not constitute a genetic risk factor for NTDs in South African blacks. These results are in accordance with the lower homocysteine levels reported previously in this population compared with Caucasians.^{14,15} Three of 60 black individuals analysed (5%) were homozygous for mutation 1298A→C. The frequency of the mutant allele of mutation 677C→T among whites and blacks was similar to those previously reported for the different ethnic groups.¹⁶ Mutation 1298A→C has not yet been studied extensively, but the frequency of the C-allele in South African Caucasians (0.37) was similar to that in control individuals (0.33) previously studied in the Netherlands.⁶

The heterogeneous distribution of two MTHFR gene mutations among different ethnic groups in South Africa may be one of several factors underlying the differences observed in the risk of heart disease. This may particularly be the case for the extensively studied 677C→T mutation, which can be considered an established risk factor for CVD.²³ The study

participants were recruited from the general South African population, where heart attack deaths in the coloured population are less common than in the white population but more prevalent than in the black population.¹⁷ The intermediate frequency of the two common MTHFR mutations observed in the coloured population is in accordance with previous findings indicating an increased manifestation of coronary heart disease (CHD) in this population,¹⁸ possibly as a consequence of Caucasoid admixture.¹¹

While various studies have demonstrated that mild hyperhomocysteinaemia is associated with CHD, no conclusive reports have yet been published on its possible role in the development of CHD in FH patients. In a pilot study (performed in the Western Cape), a significant association was found between homozygosity for mutation 677C→T and CHD in patients with the common Afrikaner founder mutation D206E ($P = 0.027$), suggesting that the 677-TT genotype is associated with a high CHD risk in these FH patients.¹⁹ This finding could, however, not be replicated in an extended group of Afrikaner FH heterozygotes from a different geographical region (Gauteng) in South Africa (M J Kotze, C L Scholtz, F J Raal — unpublished data). The contradictory findings in patients with similar genetic backgrounds may be suggestive of environmental differences such as vitamin intake, and/or may reflect differences in selection criteria used at different lipid clinics. The allele frequency of mutation 1298A→C was not significantly higher in the CHD-positive group compared with the CHD-negative group, or with combined genotypes for both mutations (M J Kotze, C L Scholtz and F J Raal — unpublished results). The 677C→T mutation occurred at a significantly lower frequency ($P < 0.05$) in 102 molecularly characterised Afrikaner FH index cases (above the age of 25 years) compared with control individuals drawn from the same population,



which raises the possibility that the MTHFR gene represents a modifier locus for FH.

It has also previously been noted that the effect of mutation 677C→T on homocysteine concentration may differ in separate studies as a result of a variable intake of folate, since this mutation leads to elevated plasma homocysteine concentrations only in individuals with a low folate status.²⁰ Biochemical analysis has indeed indicated higher folate levels in 20 FH heterozygotes (18.9 ± 19.4 nmol/l) (from the Afrikaner group where association between CHD and the MTHFR mutation could not be detected) compared with 20 controls (14.4 ± 6.2 nmol/l).²¹ The significantly lower plasma homocysteine concentrations detected in these FH patients may be related to a healthier lifestyle/diet in families known to be affected with FH. In light of recent reports on possible beneficial effects of vitamin supplementation in the prevention of CVD,²² dietary considerations should form an important aspect of future studies on the role of MTHFR mutations in CHD risk in FH.

Although it may be too laborious and expensive to include dietary information and perform all the relevant biochemical tests for such a study, conclusive results would probably only be obtained in the absence of possible confounding factors. A recent study performed by Tonstad *et al.*²³ in children with FH addressed this issue. These authors convincingly demonstrated a moderately elevated plasma homocysteine level associated with a parental history of CVD, and demonstrated that homozygosity for mutation 677C→T occurs more frequently in FH children with than in a group without a parental history of CVD.

The wide spectrum of phenotypic variability observed in FH patients sharing the same defective allele²⁴⁻²⁶ suggests that other important predictors of CHD risk in FH remain to be identified. A population-based approach would only reveal major additive factors, since different CHD risk factors could be present in different FH families. The key to unravelling the various factors likely to be involved in the development of CHD in FH heterozygotes are probably, as suggested previously,²⁴ to be found in families where the clinical expression of FH varies among relatives.

In summary, we have demonstrated a heterogeneous distribution of MTHFR genotypes among different ethnic groups, which may partly explain differences¹⁴ in the risk for heart disease in the genetically distinct populations of South Africa. Although it is possible that deleterious MTHFR genotypes will only emerge as a risk factor for CVD in populations with a low folate status, it appears appropriate to screen all patients with a history of premature atherosclerosis for established risk factors, including elevated homocysteine levels, which can be normalised. Assessment of genetic risk factors in families with a history of CVD, and timely implementation of appropriate measures before the onset of

disease, may represent an important strategy towards prevention of CVD predicted to become the leading cause of death in developing countries by the year 2020.

This study was supported by the Universities of Stellenbosch and Pretoria, the South African Medical Research Council and the Technology and Human Resources for Industry Programme. C L Scholtz received a student bursary from the Harry Crossley Foundation. R Hillermann is a recipient of a Stellenbosch University postdoctoral fellowship.

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Accepted 8 December 2001.