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Research paper

# Methylenetetrahydrofolate reductase C677T polymorphism is associated with increased risk of coronary artery disease in young South African Indians

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ABSTRACT

Methylenetetrahydrofolate reductase (MTHFR) reduces 5′,10′-methylenetetrahydrofolate to 5′-methyltetrahydrofolate, and is involved in remethylation of homocysteine to methionine, two important reactions involved in folate metabolism and methylation pathways. The common MTHFR C677T single nucleotide polymorphism (SNP) (rs1801133) has been associated with raised levels of homocysteine, a well known risk factor for coronary artery disease (CAD). CAD is a major cause of mortality worldwide. The age of onset of this chronic disorder is on the decline, particularly in the Indian population. Indians in South Africa (SA) have a higher prevalence of premature CAD compared to Black South Africans. The MTHFR C677T SNP has not been investigated in the SA Indian population. The present study therefore investigated the MTHFR C677T SNP in young SA Indian males with CAD compared to young Indian and Black male controls. A total of 290 subjects were recruited into this study which included 106 CAD patients (diagnosed on angiography, mean age 37.5, range 24–45 years), 100 Indian male controls (mean age 37.5, range 28–45 years), and 84 Black male controls (mean age 36.4, range 25–45). Polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) was used to genotype CAD patients and healthy controls. Data for clinical markers were obtained from pathology reports. There was a significant association between the 677 MTHFR variant (T) allele and CAD patients compared to the healthy Indian controls ($p = 0.0353$, OR = 2.105 95% CI 1.077–4.114). Indian controls presented with a higher frequency of the variant allele compared to Black controls (7% vs. 2% respectively, $p = 0.0515$ OR = 3.086 95% CI 0.9958–9.564). The MTHFR C677T SNP did not influence levels of total cholesterol, LDL, HDL, triglycerides, fasting glucose, fasting insulin, HbA1c or hsCRP. The higher frequency of the MTHFR 677 variant allele in South African Indians may be a contributing factor to the higher risk profile for the development of premature CAD in Indians.



## 1. Introduction

Methylenetetrahydrofolate reductase (MTHFR) is an enzyme that catalyses the reduction of 5′,10′-methylenetetrahydrofolate to 5′-methyltetrahydrofolate; a major form of folate in plasma, and a carbon donor for the remethylation of homocysteine to methionine (Kluijtmans and Whitehead, 2001; Vasisht et al., 2002). The enzyme is therefore responsible for reducing levels of homocysteine in the plasma.

The 2.2 kb long MTHFR gene is located on the short arm of chromosome 1 at 1p36.3. A common SNP at position 677 of the MTHFR gene (rs1801133) in which a cytosine is converted to thymine, results in the substitution of an alanine residue to valine in the enzyme (Anderson et al., 1997). The encoded protein has reduced activity at 37 °C and higher, and thus the C677T SNP is termed "thermolabile" (Vasisht et al., 2002). The MTHFR 677 TT genotype has been linked to increased plasma homocysteine levels (Anderson et al., 1997; Clarke et al., 2012; Mehlig et al., 2013; Nienaber-Rousseau et al., 2013).

Elevated total plasma homocysteine (tHcy) is an established risk factor for CAD that appears to occur independently of other conventional risk factors (Doshi et al., 2002). There are several proposed mechanisms to explain the association between homocysteine and CAD, including

*Abbreviations:* MTHFR, methylenetetrahydrofolate reductase; C, cytosine; T, thymine; SNP, single nucleotide polymorphism; CAD, coronary artery disease; AMI, acute myocardial infarction; SA, South African; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; OR, odds ratio; CI, confidence interval; ECG, electrocardiogram; LDL, low-density lipoprotein; HDL, high-density lipoprotein; HbA1c, glycated haemoglobin; hsCRP, high-sensitivity C-reactive protein; BMI, body mass index; tHcy, total plasma homocysteine; DNA, deoxyribonucleic acid; bp, base pair; HWE, Hardy–Weinberg equilibrium; ns, non-significant; ACE-Is, angiotensin-converting-enzyme inhibitors.

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endothelial cell dysfunction, enhanced platelet aggregation, production of free radicals, and stimulation of oxidation of low-density lipoprotein (LDL) (Stanger et al., 2002; Coffey et al., 2003).

Dietary intake and status of folate, and to a lesser extent the status of vitamins B2, B6, and B12, are classical determinants of plasma homocysteine (Muskiet, 2005). Oral folate supplements can effectively lower plasma homocysteine levels (Clarke, 1998). The metabolism of homocysteine and folate are interrelated, and increasing folate intake enhances remethylation of homocysteine. A daily dose of 0.4–0.5 mg of folate is reported to reduce tHcy by 25% (Doshi et al., 2002). This has led to the suggestion that folate supplementation may reduce the risk of CAD and other vascular-related disease by reducing tHcy.

Coronary artery disease is a multifactorial disease that depends on the interaction between environmental risk factors and several predisposing genes (Andreassi et al., 2003). On a genetic basis, SNPs in key genes may play a vital role in an individual's susceptibility and response to the disease.

Although the relationship between the MTHFR C677T variant and risk of CAD is not yet fully understood, the SNP may be important in disease mechanisms underlying CAD due to its influence on tHcy levels. The role of the MTHFR C677T SNP in CAD has been investigated across a range of ethnic groups (Gudnason et al., 1998; Friso et al., 2002; Sun et al., 2005). Indian populations worldwide have the highest prevalence of early-onset CAD compared to other ethnic groups (Rajeshwari et al., 2005). Early-onset or premature CAD is defined as cardiac events occurring before the age of 55 in men and 65 in women, and before the age of 40 in its severe form (Rissam et al., 2001).

People of Indian ancestry in particular develop heart disease when they are younger than 40, a phenomenon unheard of in other populations (Sastry et al., 2011). The pattern with regard to early-onset CAD in the Indian population is similar in various parts of the world. In India, 25–40% of patients suffering from acute myocardial infarction (AMI) were below the age of 45 (Girija, 1997). In Great Britain, AMI occurred in Indians younger than 40 years old — and the incidence was 10 times higher than the local Caucasian population (Rajadurai et al., 1992). Studies in Singapore reported that mortality due to CAD below 30 years of age occurs 10 times more in the Indian population compared to the local Chinese (Hughes et al., 1990). In the Middle East, 80% of patients who experienced AMI were Indians below the age of 40, yet Indians account for approximately 10% of the population in this region (Rissam et al., n.a.; Rissam et al., 2001). Angiographically, Indians have a 15 times higher rate of CAD than Chinese and 10 times higher rate than Malays below 40 years of age (Rissam et al., 2001).

The occurrence of CAD is on the rise in developing countries such as South Africa. Most South African Indians are descendants of indentured labourers who were brought to KwaZulu-Natal from India between 1860 and 1911 (Seedat, 2005). Indians in India and South Africa are predisposed to the early onset of CAD, one to two decades earlier than other population groups, indicating a genetic link (Ranjith et al., 2005; Sharma and Ganguly, 2005). In 1966, Wainwright reported a high incidence of early-onset CAD in South African Indians (Wainwright, 1966). In 2006, reports still showed that the highest death rates for early-onset CAD in South Africa occur in Indians, followed by mixed race, White, and Black (Norman et al., 2006). This suggests that regardless of the number of generations of Indian immigrants who have lived in South Africa, there remains a differing pattern of early-onset CAD mortality compared to the natives of the country, despite them sharing the same environment and to some extent similar diets and cultural habits (Lal, 2004). There is currently no literature available on South African Indians with regard to the MTHFR C677T SNP. We set out to assess this SNP in a cohort of young South African Indians with CAD compared to Indian and Black male controls.

**Table 1**
Frequency of MTHFR C677T genotypes and alleles in SA Indian CAD patients and controls (Indian and Black).

| | South African Indians | | South African Blacks |
|---|---|---|---|
| | CAD patients (n = 106) | Controls (n = 100) | Total n = 84 |
| *Genotypes* | | | |
| CC n (%) | 79 (74.5) | 86 (86) | 80 (95) |
| CT n (%) | 25 (23.5) | 14 (14) | 4 (5) |
| TT n (%) | 2 (2) | 0 (0) | 0 (0) |
| *Alleles* | | | |
| C n (%) | 183 (86) | 186 (93) | 164 (98) |
| T n (%) | 29 (14) | 14 (7) | 4 (2) |
| HWE p value | | 0.753 | 0.975 |
| p-Value | 0.0353[a] | 0.0515[b] | |

Fisher's exact test for heterogeneity between [a] healthy SA Indian and coronary artery disease (CAD) patients and [b] healthy SA Black and healthy SA Indian.
HWE: Hardy–Weinberg equilibrium.

## 2. Methods

### 2.1. Patient recruitment and sample collection

A total of 106 young SA Indian male CAD patients (mean age 37.5, range 24–45 years), 100 Indian male controls (mean age 37.5, range 28–45 years), and 84 Black male controls (mean age 36.4, range 25–45) were enrolled following institutional ethical approval (BE067/14). A full pathology report of clinical markers was assessed by routine laboratory testing at Global Clinical and Viral Laboratory (Amanzimtoti, South Africa) — a South African National Accreditation System (SANAS) certified laboratory. The following parameters were tested: Haematology (Roche Sysmex 1800XT), Chemistry (Beckman Coulter DXC600), Endocrinology and hsCRP (Siemens Centaur XP), Serology (BD Biosciences FACS Calibur) as per international standards to obtain levels of total cholesterol, HDL, LDL, triglycerides, fasting glucose, 2 h glucose, fasting insulin, glycosylated haemoglobin, sodium, potassium, bicarbonate, chloride, urea, creatinine, glomerular filtration rate, cluster of differentiation (CD) 4 count, CD8 count, CD45 count and CD3 count. The physical measurements of weight, height, abdominal circumference, waist circumference, and patient history were conducted by the clinician.

The inclusion criteria for young CAD patients were: Indian ancestry and unrelated, adults aged <45 years, and stable CAD confirmed on angiography. Exclusion criteria for patients were an acute coronary syndrome/revascularisation procedure in the preceding three months, chronic renal or liver disease, malignancy and known active inflammatory or infectious disease. Indian and Black male controls who did not have heart disease were recruited. Inclusion criteria for controls were Indian/African ancestry, unrelated to one another, adult males below the age of 45 years and no known or suspected atherosclerotic vascular disease. For control subjects, a blood sample was drawn and in addition an exercise electrocardiogram (ECG) was recorded in order to exclude heart disease. The exclusion criteria for controls were as for cases, and included the following: symptoms or clinical evidence of atherosclerotic vascular disease i.e. CAD, stroke/carotid disease or peripheral vascular disease, evidence of ischaemia/infarction on 12 lead resting ECG or evidence of ischaemia on treadmill exercise stress testing done to predict maximum heart rate. A positive family history was recorded if any first degree relative (father, mother, brother or sister) had CAD (Table 2).

### 2.2. DNA extraction

Genomic DNA was extracted from the whole blood sample of each patient and control according to the method described by Sambrook et al. 2001 (Sambrook and Russell, 2001). Cells were transferred to 600 μl lysis buffer (0.5% sodium dodecyl sulphate (SDS), 150 mM