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Cystathionine β-synthase 844Ins68 polymorphism is not associated with the levels of homocysteine and cysteine in an Indian population

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Abstract

Cystathionine β-synthase (CBS) is a key enzyme that plays a critical role in homocysteine metabolism and intracellular redox balance. We have analysed the association of the *CBS* 844Ins68 polymorphism alone and in combination with methylenetetrahydrofolate reductase (*MTHFR* C677T) and choline dehydrogenase (*CHDH* A119C) polymorphisms (the two polymorphisms recently shown to be associated with levels of homocysteine) with homocysteine, cysteine, folate and vitamin B_{12} in 817 individuals (397 patients with coronary artery disease and 420 controls). The *CBS* 844Ins68 polymorphism alone or in combination with *MTHFR* C677T and *CHDH* A119C polymorphisms was not significantly associated with any of the biochemical variables studied.

Keywords: *CBS 844Ins68; CHDH A119C; homocysteine; Indian population; MTHFR C677T*

Introduction

Homocysteine, a thiol amino acid, is a key intermediate in the methionine metabolism pathway that is ubiquitously present in all cell types. Methionine from a dietary source is converted to *S*-adenosyl methionine, which is then converted to *S*-adenosyl homocysteine via transmethylation reaction catalysed by various methyltransferases. *S*-Adenosyl homocysteine is then hydrolysed to homocysteine, which is then either remethylated back to methionine by methionine synthase or condenses with serine in the transsulfuration pathway to form cystathionine. The formation of cystathionine is catalysed by the enzyme cystathionine β-synthase (CBS). Cystathionine is then converted to cysteine and subsequently to the intracellular redox buffer glutathione. It is generally believed that homocysteine is equally metabolized via remethylation and transsulfuration pathways. Metabolism of homocysteine via the transsulfuration pathway is crucial as this helps in maintaining intracellular redox balance. Thus, defects in the enzymes involved in the transsulfuration pathway would lead to accumulation of homocysteine resulting in intracellular redox imbalance and could potentially lead to disease conditions.

An elevated level of homocysteine has been associated with various diseases and/or clinical conditions (Mills et al. 1995, McCaddon et al. 1998) and has been implicated as an independent risk factor for cardiovascular diseases (Robinson et al. 1995). Homocysteine levels are elevated due to deficiencies of micronutrients such as folate, vitamin B_{12}, vitamin B_6 (Mann et al. 1999, McKinley et al. 2001, Kumar et al. 2009b) or polymorphisms in the genes involved in methionine metabolism (Kumar et al. 2005, 2009a, Fredrisken et al. 2007). Among the single nucleotide polymorphisms (SNPs) that modulate homocysteine levels, the SNP in the methylenetetrahydrofolate reductase gene (*MTHFR* C677T) has been widely studied and individuals with the TT genotype have been reported to have significantly higher homocysteine levels in several

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populations (Fredrisken et al. 2007, Kumar et al. 2009a). Recently, we have shown that in addition to the *MTHFR* C677T polymorphism, the choline dehydrogenase gene (*CHDH* A119C) polymorphism also modulates the levels of homocysteine (Kumar et al. 2009a). Several mutations and polymorphisms in the *CBS* gene have been reported to influence the levels of homocysteine (Kraus et al. 1999). The G307S mutation in the *CBS* gene has been found to be the leading cause of hyperhomocysteinemia in Ireland (Gallagher et al. 1995). Sebastio et al. (1995) for the first time reported a variation in the *CBS* gene (*CBS* 844Ins68) where a 68-bp long insertion of DNA was found in exon 8. In a large study performed by Fredrisken et al. (2007), this polymorphism was found to be associated with altered levels of homocysteine. Studies have shown that mRNA formed from alleles carrying the insertion is transcribed poorly (Sperandeo et al. 1995). These authors also speculated that combined homozygosity for the *MTHFR* C677T polymorphism and heterozygosity for the *CBS* 844ins68 polymorphism might further hamper homocysteine metabolism (Sperandeo et al. 1995). Thus, in the present study carried out in an Indian population, we evaluated the effect of this insertion alone or in combination with *MTHFR* C677T and *CHDH* A119C on the levels of homocysteine, cysteine, vitamin B_{12} and folate and also assessed if this insertion is associated with coronary artery disease (CAD).

Materials and methods

A total of 817 individuals (397 patients with angiographically proven CAD and 420 treadmill test negative controls) were recruited from All India Institute of Medical Sciences, New Delhi, India. These individuals mainly belonged to the Northern part of India (Indo-European ethnicity). Written consent was obtained from all the participants and the study was conducted in accordance with the principles of the Helsinki Declaration, with the approval of the ethics committee.

Fasting blood samples were collected from the participants in tubes with and without anticoagulant, and serum/plasma was separated from the blood. DNA was isolated from leukocytes using the modified salting-out method as described previously (Miller et al. 1988). The region of interest in the *CBS* gene was amplified with forward (5′-AGTGTGAGGGTGAGTTACAG-3′) and reverse (5′-GTTGTTAACGGCGGTATTGG-3′) primers by polymerase chain reaction (PCR) using 30 ng of genomic DNA, 1.5 mM $MgCl_2$, 0.2 pmol μl^{-1} of each primer, 0.1 mM of each dNTP, 0.05 U μl^{-1} of Taq DNA polymerase (Bangalore Genie, India) and the buffer recommended by the supplier. The polymorphism was checked using denaturing high-performance liquid chromatography (dHPLC) (Transgenomic WAVE system Model D7000IF; Transgenomic USA, Omaha, NE, USA) equipped with an autosampler. Briefly, 10 µl of the PCR product was injected into the reverse-phase DNASepR column (Transgenomic USA). PCR products were eluted at 50°C by a linear acetonitrile gradient in 0.1 mM TEAA buffer, pH 7, at a constant flow rate of 0.9 ml min^{-1} using the double-stranded multiple fragment program (WAVEMAKER Ver4.1 software). The samples with insertions were identified based on their peak profile in dHPLC which was distinct from samples without insertions (Figure 1). Statistical analysis was performed using the Statistical Package for Social Sciences, Windows 10.0 (SPSS Inc., Chicago, Il, USA). Distribution of categorical variables

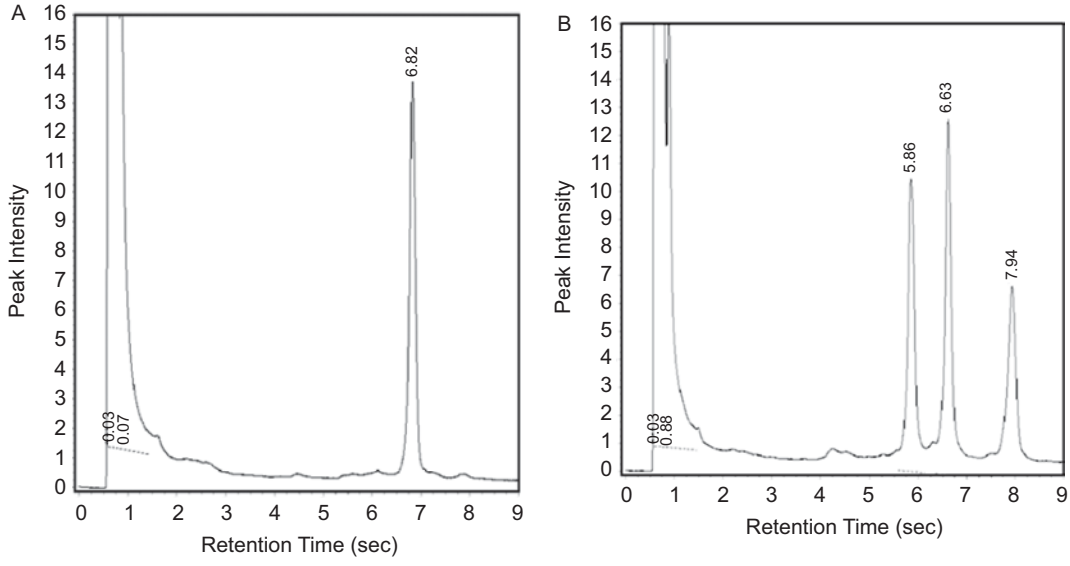

Figure 1. Representative denaturing high-performance liquid chromatography peaks for major homozygous (A) and heterozygous insertion (B) for the *CBS* 844Ins68 polymorphism.

was analysed employing the χ^2 test. All the continuous variables were analysed for the association with genotypes using the Kruskal–Wallis test or the Mann–Whitney test depending on the number of categories. Regression analysis was performed to capture the association of the studied polymorphisms after controlling for other confounding factors. A *p*-value <0.05 was considered significant.

Results and discussion

Several polymorphisms have been reported in the gene encoding the CBS enzyme, that play an important role in homocysteine metabolism. We have focused on the *CBS* 844Ins68 polymorphism present in exon 8 because of its wider prevalence and association with hyperhomocysteinemia in different populations. Demographic details of the participants included in the study are shown in the Table 1. Genotypic and allelic frequencies of the studied polymorphism are shown in the Table 2. Genotypic frequency was found to conform to the Hardy–Weinberg equilibrium (p >0.1). No significant difference in the genotypic or allelic frequency was observed between patients and controls (Table 2). Additionally, we did not observe any individuals who were homozygous for the 68-bp insertion in the *CBS* gene in our study population. The frequency of the *CBS* 844Ins68 polymorphism observed in the present study was comparable to that found in US, Iranian and Turkish populations (Ge et al. 2002, Akar & Koç 2009, Senemar et al. 2009). A study from the Eastern part of India also reported a similar frequency (Dutta et al. 2005). The maximum frequency for this polymorphism has been reported in a Spanish population (Pepe et al. 1999). Interestingly, this polymorphism was found to be completely absent in the Chinese and Japanese populations (Pepe et al. 1999).

As the insertion in the *CBS* gene results in decreased transcription, it may be presumed that the presence of the 68-bp insertion could potentially modulate the levels of the metabolites in the methionine cycle including homocysteine and cysteine. We thus checked the effect of this 68-bp insertion on the levels of homocysteine, cysteine, folate and vitamin B_{12} (Table 3). We did not observe any association of the *CBS* 844Ins68 polymorphism with any of these biochemical variables either in CAD patients or controls. Various confounding factors play an important role in controlling the levels of different blood biochemical variables (Mann et al. 1999, McKinley et al. 2001, Kumar et al. 2005, 2009a, b, Fredrisken et al. 2007). Even after controlling for various confounding factors (age, sex, smoking, diet, milk consumption, diabetes mellitus status, hypertension, body mass index (BMI), folate, vitamin B_{12}, etc.), we did not observe any association of the polymorphism with the levels of homocysteine, cysteine, vitamin B_{12} or folate. The factors that were found to be significantly associated with the levels of homocysteine after controlling for other confounding variables were disease status, levels of vitamin B_{12}, diet, diabetes status, sex, age and BMI. We analysed using different models and checking for the association of the *CBS* 844Ins68 polymorphism with the levels of homocysteine, cysteine, folate and vitamin B_{12} in all individuals as well as in the cases and controls separately but did not see any significant association. No significant interaction of the *CBS* 844Ins68 polymorphism with *MTHFR* genotypes or disease status was observed. Our observation agrees with other reports from Irish and Turkish populations (Gallagher et al. 1995, Akar & Koç 2009, Aléssio et al.

Table 1. General/clinical characteristic of the study population.

Characteristics	Controls (n=420)	Patients (n=397)	p-Value
Age (years), median (IQR)	48 (40–55)	54 (46–61)	0.09[a]
Sex (male), *n* (%)	322 (77)	350 (90)	<0.001[b]
Diet (vegetarian), *n* (%)	184 (45)	200 (52)	0.05[b]
Smoker, *n* (%)	133 (32)	109 (28)	0.28[b]
Diabetes mellitus, *n* (%)	70 (17)	76 (19)	0.36[b]
BMI (kg m^{-2}), median (IQR)	25 (22–28)	25 (22–27)	0.82[a]

[a]Mann–Whitney test; [b]χ^2 test followed by post test.
IQR, interquartile range; BMI, body mass index.

Table 2. Distribution of genotype and allele frequencies of the *CBS* 844Ins68 polymorphism.

	Genotypes		Allele frequency		
Individuals	WW, *n* (%)	WI, *n* (%)	W	I	p-Value[a]
Patients (n=397)	364 (92)	33 (8)	0.96	0.04	0.24[b]
Controls (n=420)	374 (89)	46 (11)	0.95	0.05	0.92[c]
Total (n=817)	738 (90)	79 (10)	0.95	0.05	

WW, homozygous major; WI, heterozygous insertion; W, major allele; I, insertion allele. Homozygous insertion was absent. [a]χ^2 test for comparison of genotype ([b]) and allele ([c]) frequency between patients and controls.

2008). However, a population-based study by Fredriksen et al. with a large number of individuals found a significant association of the *CBS* 844Ins68 polymorphism with homocysteine levels (Fredrisken et al. 2007). This is the first report from India where we have checked the association of the *CBS* 844Ins68 polymorphism with different biochemical parameters.

Recently, we showed that two polymorphisms, *MTHFR* C677T (rs1801133) and *CHDH* A119C (rs9001), modulate the levels of homocysteine (Kumar et al. 2009). We checked if the *CBS* 844Ins68 polymorphism had any effect on homocysteine levels in the presence of these two polymorphisms. We failed to observe any significant association of the *CBS* 844Ins68 polymorphism with any of biochemical variables tested in combination with the two polymorphisms (Table 4). This is in contrast to reports on Turkish and Brazilian populations where a significant association of the *CBS* 844Ins68 polymorphism was found with homocysteine levels in the presence of the *MTHFR* C677T polymorphism (Akar & Koç 2009, Aléssio et al. 2008). The difference in the results might be due to the different genetic backgrounds of our population from the other populations (Kumar et al. 2005, 2007, 2009a, Pemberton et al. 2008). It should also be noted that the frequency of the *MTHFR* TT genotype (risk genotype) is comparatively low in the Indian population compared with Caucasians (Kumar et al. 2005, 2009a). Additionally, in a recent study by our group, a high percentage of individuals was found to be deficient in vitamin B_{12}, a cofactor for enzyme methionine synthase that plays an

Table 3. Association of the *CBS* 844Ins68 polymorphism with various biochemical variables.

Parameter	WW	WI	*p*-Value[a]
Total individuals	*n* = 738	*n* = 79	
Homocysteine (μmol l^{-1})	15.1 (10.5–20.8)	15.0 (9.7–20.0)	0.82
Cysteine (μmol l^{-1})	207 (179–238)	212 (183–244)	0.24
Folate (nmol l^{-1})	8.8 (6.1–14.0)	8.6 (6.1–14.9)	0.78
Vitamin B_{12} (pmol l^{-1})	147 (112–198)	152 (114–197)	0.86
Controls	*n* = 374	*n* = 46	
Homocysteine (μmol l^{-1})	15.4 (10.5–21.2)	16.6 (12.6–20.4)	0.28
Cysteine (μmol l^{-1})	200 (177–232)	211 (183–247)	0.10
Folate (nmol l^{-1})	9.0 (6.4–14.1)	8.1 (6.2–14.0)	0.92
Vitamin B_{12} (pmol l^{-1})	155 (120–213)	155 (116–191)	0.43
Patients	*n* = 364	*n* = 33	
Homocysteine (μmol l^{-1})	15.0 (10.6–20.1)	13.2 (8.6–18.7)	0.08
Cysteine (μmol l^{-1})	212 (180–250)	219 (181–253)	0.85
Folate (nmol l^{-1})	8.6 (6.0–14.0)	8.7 (6.0–16.2)	0.65
Vitamin B_{12} (pmol l^{-1})	141 (106–183)	146 (109–215)	0.40

Values are median (interquartile range). WW, homozygous major; WI, heterozygous. [a]Mann–Whitney test.

Table 4. Comparison of biochemical parameters with the *CBS* 844Ins68 polymorphism in the presence of the *MTHFR* C677T and *CHDH* A119C polymorphisms.

Genotype	Hcy (μmol l^{-1})	Cys (μmol l^{-1})	Folate (nmol l^{-1})	Vit B_{12} (pmol l^{-1})
MTHFR 677CC				
CBS 68Ins WW (*n* = 501)	14.9 (10.4–20.3)	207 (177–241)	8.8 (6.1–14.2)	146 (114–191)
CBS 68Ins WI (*n* = 59)	14.0 (9.4–19.2)	213 (184–255)	8.1 (6.0–15.5)	157 (115–202)
p-Value[a]	0.56	0.18	0.73	0.52
MTHFR 677CT+TT				
CBS 68Ins WW (*n* = 230)	15.5 (11.1–22.1)	204 (183–233)	8.8 (6.2–13.6)	148 (108–212)
CBS 68Ins WI (*n* = 20)	15.8 (11.9–28.9)	207 (180–229)	10.2 (8.1–14.1)	129 (111–179)
p-Value[a]	0.45	0.92	0.23	0.56
CHDH 119AA				
CBS 68Ins WW (*n* = 512)	15.9 (11.0–21.5)	205 (178–241)	8.8 (6.2–14.7)	148 (111–200)
CBS 68Ins WI (*n* = 62)	14.9 (10.4–19.7)	211 (183–242)	8.8 (6.1–15.0)	146 (111–188)
p-Value[a]	0.55	0.33	0.77	0.87
CHDH 119AC+CC				
CBS 68Ins WW (*n* = 222)	13.7 (10.2–19.0)	208 (180–237)	8.8 (6.1–13.8)	145 (113–193)
CBS 68Ins WI (*n* = 16)	15.5 (7.0–20.1)	227 (167–285)	7.5 (6.1–15.0)	159 (117–216)
p-Value[a]	0.99	0.36	0.89	0.72

Values are median (interquartile range).
Hcy, homocysteine; Cys, Cysteine; Vit B_{12}, vitamin B_{12}; WW, major homozygous; WI, heterozygous insertion. [a]Mann–Whitney test.

important role in the homocysteine metabolism (Kumar et al. 2009b). In the background of environmental risk factors, the genetic changes tend to have synergistic effects thereby augmenting the risk for complex disorders. Furthermore, several instances of differences between Indian and other world populations are being increasingly revealed in complex disorders (Kumar et al. 2005, 2007, 2009a, Pemberton et al. 2008). Recently, a study from the Northern part of India analysed total homocysteine and cysteine levels along with *MTHFR* polymorphisms in cancer patients. They found no evidence of an association for the *MTHFR* polymorphism with levels of homocysteine. Furthermore, no evidence for a gene–virus interaction was found for the human papillomavirus infection (Kohaar et al. 2009). In conclusion, the polymorphism *CBS* 844Ins68 is not associated with the levels of homocysteine, cysteine, folate and vitamin B_{12} in an Indian population.

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Declaration of interest

All the authors declare no conflict of interest. The work has not been published or under consideration for publication anywhere else.

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