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ORIGINAL ARTICLE

Genetic polymorphisms predisposing to hyperhomocysteinemia in cardiac transplant patients

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Summary

Genetic determinants for high homocysteine (Hcy) levels are now well known. We studied several single nucleotide polymorphisms (SNP) in Hcy-regulating genes [methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C; methionine synthase (MS) A2756G; methionine synthase reductase (MTRR) A66G] in relation to total plasma Hcy levels, transplant coronary artery disease and thromboembolic episodes in 84 heart transplant patients, and we compared the incidence of these polymorphisms with those in a healthy adult controls. At least one copy of the G allele of the MTRR A66G SNP was found in a significantly greater proportion of cardiac transplant (CTX) recipients compared with controls (94.0% vs. 79.9% respectively). None of the SNP analyzed were correlated with total Hcy plasma levels or the presence of transplant coronary artery disease. However, MS A2756G was significantly associated with cobalamin levels (AA genotype: 290 ± 122 pmol/l; AG: 381 ± 151 pmol/l and GG: 415 ± 100 pmol/l), as was MTRR A66G (AA: 478 ± 219 pmol/l, AG: 306 ± 124 pmol/l and GG: 306 ± 123 pmol/l). MTRR A66G was also correlated with serum folate. No association was found with thromboembolic events. In conclusion, there was a significant difference in the frequency of the G allele genotype of the MTRR A66G in CTX patients versus controls. Differences in cobalamin and folate levels with the MTRR A66G and MS A2756G polymorphisms were noted. Thus, SNP in Hcy-regulating genes may be important determinants of vitamin metabolism in CTX, raising the question of increased vitamin requirements to minimize increased plasma Hcy in this high-risk group.

Introduction

The success of heart transplantation is based on acceptance of the graft by the recipient's immune system. This depends on many factors, including differences between donor and recipient major histocompatibility complex genes. In the past few years, donor and recipient single nucleotide polymorphisms (SNP) have been also shown to correlate with graft outcomes [1].

Hyperhomocysteinemia is common after heart transplantation [2–4]. The consequences of this metabolic abnormality are not completely understood, but epidemiological data suggest an association between hyperhomocysteinemia and transplant coronary artery disease (TxCAD) [5,6]. Moreover, a recent study in rats showed that increased homocysteine (Hcy) levels might have detrimental effects on graft arteries. There are multiple reasons for the increased total plasma homocysteine (tHcy)

levels seen in cardiac transplant (CTX) recipients, and, as in healthy subjects, nutritional and genetics factors are important [4].

Methionine is a methyl donor for several important biochemical reactions, resulting in the formation of Hcy [7]. Homocysteine can be metabolized in two different ways: it can be degraded through the transsulfuration pathway, or it can be remethylated to regenerate methionine (Fig. 1). This later reaction is catalyzed by the enzyme complex, methionine synthase (MS), using cobalamin as the primary cofactor. The methyl group is donated by methyl tetrahydrofolate, yielding methionine and tetrahydrofolate. This last moiety is regenerated by acceptance of a methylene group, derived from serine and hydride transfer, and catalyzed by the methylenetetrahydrofolate reductase (MTHFR) enzyme. Although MS uses cobalamin as a cofactor, it must be present in the Co(III) form to be active. However, the metal ion is easily reduced [Co(III) → Co(II)] and must be reoxidized by MS reductase (MTRR) pathway to maintain catalytic activity.

Several functional SNP in the genes encoding the enzymes governing Hcy metabolism have been identified [8]. Some are known to modify the activity of the enzyme, and have been associated with abnormal Hcy metabolism. There are multiple studies linking MTHFR C677T to atherogenic and thrombogenic diseases, particularly in the setting of folate deficiency. Higher Hcy levels in CTX recipients homozygous for the T allele have been found [9,10] specifically in association with low folate levels. There is no apparent association between MTHFR C677T and TxCAD [9].

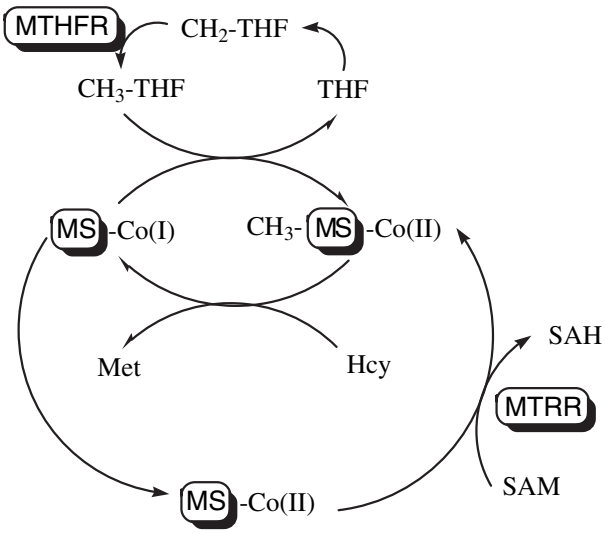

Figure 1 Homocysteine (Hcy) is metabolized to methionine (Met) by methionine synthase (MS) using as cofactor cobalamin and accepting a methyl group from 5′-methyl-tetrahydrofolate (CH_3-THF), yielding tetrahydrofolate (THF). This moiety is generated by methylenetetrahydrofolate reductase (MTHFR). Cobalamin is spontaneously oxidized (CoII) and needs to be regenerated to its active form (CoIII) by the enzyme methionine synthase reductase (MTRR) converting s-adenosylmethinine (SAM) to s-adenosil-homocysteine (SAH).

Hyperhomocysteinemia is known to alter the antithrombotic properties of the endothelium. Subjects with very high Hcy concentrations die at young age because of thromboembolic complications (TC). Hyperhomocysteinemia is prevalent after heart transplantation. A prothrombotic state, secondary to hyperhomocysteinemia, may be one reason for the increased incidence of thromboembolic episodes in CTX [11]. Moreover, the antithrombotic properties of graft vessels were shown to be abnormal. The long-term development of TxCAD is related to the deposition of fibrin in distal vessels early after transplantation [12]. It is likely that a combination of factors is responsible for the prothrombotic state in CTX, but hyperhomocysteinemia may well play a role, particularly in the face of relative insufficiency of vitamin cofactors required for Hcy remethylation.

The purpose of this study was to perform genotype analysis of common mutations in several enzymes involved in the metabolism of Hcy in our cohort of CTX recipients. We compared genotype frequencies in CTX recipients with those in a large health adult control population. We then analyzed circulating concentrations of tHcy, folate and cobalamin in relation to the different SNP. Finally, we conducted a correlation analysis of each of these genetic mutations with the presence of TxCAD.

Methods

Patients

Cardiac transplant recipients ($n = 84$) were consecutively enrolled from the heart transplant clinic at the Toronto General Hospital. Informed consent was required to participate in this study. The protocol was approved by the Hospital Ethics Committee. For the purpose of genetic analysis, controls were obtained from a large series of healthy young adults (mean age 41 ± 18 years; mean tHcy 6.5 ± 3.0 μmol/l) from the same urban Toronto population recruited through the Family Medicine Unit at the Toronto University Health Network [13].

Blood collection and sample preparation

Patients were fasting for at least 8 h. Peripheral blood was collected in the morning from an antecubital vein and placed in EDTA tubes. For Hcy analysis, blood was immediately placed in ice and centrifuged within 30 min after venipuncture. All plasma samples were frozen at −70 °C within 6 h until analysis. Whole anticoagulated blood samples DNA was extracted from peripheral blood

cells as described previously [13] then coded and analyzed in a blinded manner.

Biochemical assays

Total plasma Hcy and methionine levels were assayed using high-performance liquid chromatography with electrochemical detection and pulsed integrated amperometry [14]. Within-run and between-run coefficients of variation for tHcy assayed by this method are 3.1% and 3.8% respectively. Folate and cobalamin levels were measured with a radioimmunoassay (Quantaphase II; Bio-Rad Laboratories, Toronto, ON, Canada) as described previously [3]. Biochemical analyses were carried out in 72 patients in 1997. In 1998, folate food fortification was implemented nationwide in Canada. This had a substantial effect on Hcy levels in the general and CTX population, as we have reported [15,16]. Therefore, our analysis is restricted to those data collected prior to the onset of the national fortification program.

Genotyping methods

MTHFR C677T

MTHFR C677T genotypes were read from a single multiplex assay designed to report prothrombin 20210A and factor V Leiden polymorphisms as well [13]. The specific oligonucleotide primers for MTHFR C667T (Table 1) were designed on the basis of published data [17]. PCR was carried out using the GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, CA, USA). In each 20 μl reaction, 50 ng genomic DNA was mixed with 1x PCR buffer 2.5 mM $MgCl_2$, 0.2 mM each of dNTPs, 0.5U HotStarTaq™ (Qiagen, Mississauga, ON, Canada) and three pairs of primers (0.15 μmol/l for tMTHFR, 0.75 μmol/l for FV, and 1 μmol/l for PTB). The PCR reaction consisted of an initial 15 min of HotStarTaq™ activation at 94 °C, followed by 30 cycles (20 s each) of denaturation at 95 °C, annealing for 20 s at 57 °C, extension for 40 s (with an increment of 1 s for each subsequent cycle) at 72 °C, and a final extension step of 5 min at 72 °C. The PCR product was incubated with *Hin*dIII and *Taq*I endonucleases for 2 h at 37 °C followed by 2 h at 60 °C. Following digestion, 8 μl of the product was mixed with 2 μl of 5x loading dye [40% (w/v) sucrose; 0.01% bromphenol blue; 3.75x TAE buffer] and subjected to submerged electrophoresis on Clearose™ BG gels (Elchrom Scientific, Cham, Switzerland). The resolved amplicons were visualized with ethidium bromide under UV light. A blank control and known genotypes were run with each assay.

Table 1. Primers used in genetic analysis.

MTHFR 677-forward	5′-TGAAGGAGAAGGTGTCTGCGGGA-3′
MTHFR 677-reverse	5′-AGGACGGTGCGGTGAGAGTG-3′
MTHFR 1298C	5′-GGAGGAGCTGACCAGTGATGC-3′
MTHFR 1298A	5′-CAAGGAGGAGCTGCTGAAGATGTGGGGCC AGGAGCTGACCAGTGTAGA-3′
MTHFR 1298 IR	5′-GACCCAGCCTGTCTTTGCCT-3′
MTRR 66-forward	5′-GCAAAGGCCATCGCAGAAGACAT-3′
MTRR 66-reverse	5′-GTGAAGATCTGCAGAAAATCCA-3′
MS 2756-forward	5′-CATGGAAGAATATGAAGATATTAGAC-3′
MS 2756-reverse	5′-GAACTAGAAGACAGAAATTCTCTA-3′

MTHFR, methylene tetrahydrofolate reductase; MTRR, methionine synthase reductase; MS, methionine synthase.

MTHFR A1298C

A mutagenically separated PCR (MS-PCR) was designed to identify the MTHFR A1298C locus (Table 1). PCR was carried out using the GeneAmp® PCR System 9700 (Applied Biosystems). In each PCR reaction, 50 ng genomic DNA was used in 20 μl reaction mixture containing 1x PCR buffer, 1.5 mmol/l $MgCl_2$, 0.2 mmol/l dNTPs and 0.5 U HotStarTaq™ (Qiagen) and 4.0 μmol/l of each three primers. The cycling reaction consisted of an initial 15 min of HotStarTaq™ activation at 94 °C, followed by 30 cycles (30 s each) of denaturation at 95 °C, 20 s annealing at 57 °C, and 20 s of extension (with an increment of 1 s for each subsequent cycle) at 72 °C, and a final extension step of 5 min at 72 °C. Electrophoresis and visualization were performed as described above.

MTRR A66G

The A66G polymorphism in the MTRR gene was analyzed by restricted digestion with *Nde*I (New England BioLabs, Pickering, ON, Canada) as described by Wilson *et al.* [18]. A mismatched base was introduced in the forward primer to create a *Nde*I site in the normal sequence. The PCR was carried out in 20 μl mixture of 1x PCR buffer, 0.2 mM each of dNTPs, 0.25 U HotStarTaq™ (Qiagen), 50 ng of genomic DNA and 0.5 μM each of primers (Table 1). The reaction comprised an initial DNA denaturation and HotStarTaq™ activation at 95 °C for 15 min, 35 cycles of 94 °C for 20 s, 56 °C for 20 s and 72 °C for 20 s with an increment of 1 s after each cycle and a final extension at 72 °C for 5 min. Digestion of the 66 bp product was then carried out at 37 °C overnight and the resulting fragments were analyzed by 6% NuSieve 3:1 agarose gel electrophoresis (Biowhittaker Molecular Applications, Rockland, ME, USA). The normal (A) allele was cut giving fragments of 44 bp and 22 bp while the mutated (G) allele remained uncut.

MS A2756G

The A2756G polymorphic site in the MS gene was amplified using previously published primers (Table 1) [19].

The 20 μl reaction mixture contained 1x reaction buffer, 2 mM $MgCl_2$, 0.2 mM each of dNTPs, 0.5 μM each of the primers, 0.5 U HotStarTaq™ (Qiagen) and 50 ng genomic DNA. HotStarTaq™ was activated at 95 °C for 15 min, then the reaction went through 30 cycles of 94 °C for 20 s, 58 °C for 20 s and 72 °C for 20 s with an increment of 1 s after each cycle. There was a final extension at 72 °C for 5 min. The 189 bp product was then digested with *Hae*III (New England BioLabs) at 37 °C overnight and separated by electrophoresis using precast Clearose® BG gel. The mutant (G) allele was cut giving fragments of 159 bp and 30 bp while the normal (A) allele remained uncut.

TxCAD diagnosis

Before 1997, our center routinely performed yearly angiographic evaluation of the coronary tree for TxCAD. Since then, this practice changed to screening dobutamine stress echocardiogram yearly, and angiograms on an as needed basis. Of the transplant cohort, 48 patients had two or more angiograms available for comparison regarding TxCAD development. Two independent investigators reviewed these angiograms for TxCAD in a blinded manner, and stratified subjects into the presence or absence of TxCAD development in comparison with the baseline angiogram (year 1). The presence of TxCAD was considered positive if there was an obstructive lesion of 25% or more of the lumen, as previous cited criteria [5].

Thromboembolic complications

Retrospective analysis of patient charts was performed to identify the incidence of ischemic stroke, transient ischemic attack, deep vein thrombosis, pulmonary embolism and arterial embolism. Each event was recorded for analysis.

Statistical analysis

Data was analyzed with the SPSS 11.0 software package (SPSS Inc., Chicago, IL, USA). Student's *t*-test and ANOVA were used for analysis of the different SNP and blood assays. Quantitative data is expressed as mean ± SD, unless otherwise indicated. Chi-squared and Fisher's exact tests were used for the analysis of categorical variables. The significance level was set to 0.05.

Results

Data for 84 patients was available for analysis. The mean age was 53 ± 10 years, and 75 (89.2%) were males. All transplants were performed between 1985 and 1997; 83% of the patients were White people, 8% were south Asians, 5% African–Americans and 4% were from the Pacific Rim. The reason for cardiac transplantation was ischemic cardiomyopathy in 67.9%, idiopathic dilated cardiomyopathy in 19.0% and other cardiomyopathies in 13.1% of subjects. There was no statistical association between pretransplant diagnosis and any of the SNP (data not shown).

Analysis of different mutations involved in metabolism of Hcy, showed a significant difference in the MTRR A66G genotype (Table 2). In CTX recipients 28 (33.3%) subjects were homozygous for the GG genotype, while 51 (60.7%) subjects were heterozygous and five (5.9%) subjects were homozygous for the AA genotype. In the control population, the numbers were 56 (27.3%), 108 (52.6%) and 41 (20%) subjects, respectively ($P = 0.0075$). The G allele at position 66 of MTRR was present in 63.6% (107/168) of CTR compared with 53.6% (220/410) of controls ($P = 0.03$). The rest of the SNP were not significantly different between controls and CTX subjects.

Biochemical data were available for analysis in 72 subjects. There was no genotype-specific difference in tHcy or methionine plasma levels for any of the SNP. However, cobalamin levels varied significantly on the basis of MS A2756G genotypes. The mean level was 290 ± 122 pmol/l for the AA genotype ($n = 53$), 381 ± 151 pmol/l for the AG genotype ($n = 16$) and 415 ± 100 pmol/l for the GG genotype ($n = 3$, $P = 0.022$; Fig. 2) Comparison by allele showed that subjects with a G allele (AG or GG) had a significantly higher plasma cobalamin level (AG + GG, $n = 19$: 386 ± 142 pmol/l vs. AA, $n = 53$: 291 ± 122 pmol/l, $P = 0.006$). There were two (2.8%) patients with cobalamin deficiency (cobalamin <118 pmol/l) and

Table 2. Comparison between cardiac transplant patients and controls of the incidence of the analyzed mutations.

Enzyme/mutation	CTR [*n* (%)]	Controls [*n* (%)]	*P*-value
MTHFR 677			
CC	39 (46.4)	198 (48.6)	0.47
CT	33 (39.2)	169 (41.5)	
TT	12 (14.2)	40 (9.8)	
MTHFR 1298			
AA	38 (45.2)	193 (49.7)	0.26
AC	33 (39.2)	158 (40.7)	
CC	13 (15.4)	37 (9.5)	
MTRR 66			
AA	5 (5.9)	41 (20.0)	0.0075
AG	51 (60.7)	108 (52.6)	
GG	28 (33.3)	56 (27.3)	
MS 2756			
AA	60 (71.4)	106 (58.5)	0.12
AG	20 (23.8)	64 (35.3)	
GG	4 (4.7)	11 (6.0)	

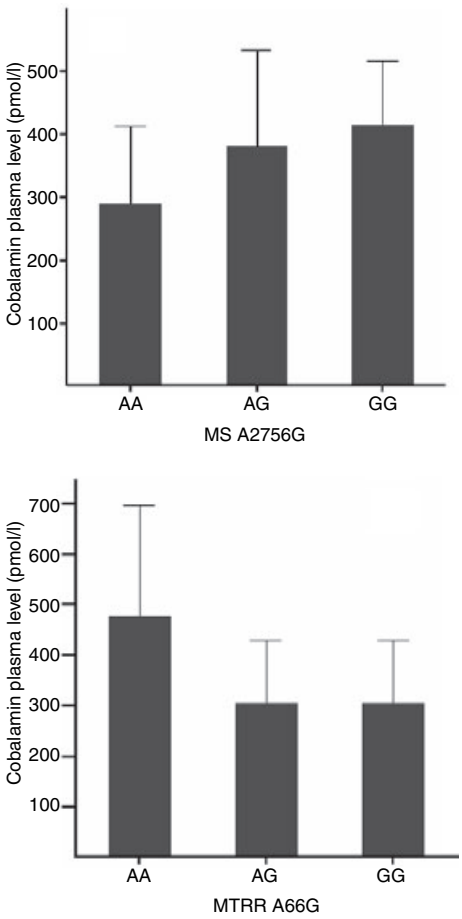

Figure 2 Cobalamin plasma level was significantly different for MS A2756G (290 ± 122 pmol/l for the AA genotype, 381 ± 151 pmol/l for the AG genotype, and 415 ± 100 pmol/l for the GG genotype; $P = 0.022$) (2A) and for MTRR A66G (477 ± 219 pmol/l in the AA group, 306 ± 124 pmol/l in the AG group, and 306 ± 123 pmol/l in the GG group; $P = 0.042$) (2B).

three (4.2%) patients with indeterminate cobalamin levels (cobalamin <148 pmol/l). All five patients with low cobalamin status were AA genotype.

MTRR A66G status was also significantly associated with cobalamin and folate levels. Cobalamin concentrations were 478 ± 219 pmol/l in the AA group ($n = 4$), 306 ± 124 pmol/l in the AG group ($n = 44$) and 306 ± 123 pmol/l in the GG group ($n = 24$, $P = 0.042$; Fig. 2). Two patients with cobalamin deficiency were GG genotype, three patients were in the indeterminate range and were GG in one case and GA genotype in two. Folate concentrations were 18.0 ± 18.2 μmol/l in AA group, 7.9 ± 7.3 μmol/l in AG group and 7.3 ± 3.0 μmol/l in the GG group ($P = 0.023$).

Data regarding the presence of TC was available in 81 subjects. Mean time of follow up after transplant was 8.7 ± 3.1 years, and mean time from transplant to the TC was 4.1 ± 3.5 years. Overall, 22 (26.2%) subjects had at least one thromboembolic complication. A total of 34 thromboembolic events were recorded, of which 15 (44.1%) were stroke/transient ischemic attack, 13 (38.2%) deep vein thrombosis, five (14.7%) pulmonary embolism and one (2.9%) arterial occlusion. Mean tHcy was 23.9 ± 5.8 μmol/l in patients with TC and 26.4 ± 7.6 μmol/l in patients without TC ($P = 0.2$). Folic acid, cobalamin, cyclosporin level and creatinine were not significantly different between groups (data not shown). However, patients with TC were older (56.7 ± 10.8 vs. 51.6 ± 9.4 years, $P = 0.056$). The occurrence of TC was not associated with any of the Hcy-related SNP (data not shown).

Table 3. Incidence of transplant coronary artery disease by genotype.

Enzyme/mutation	TxCAD [*n* (%)]	No TxCAD [*n* (%)]	*P*-value
MTHFR 677			
CC	9 (18.8)	9 (18.8)	0.56
CT	13 (27.1)	9 (18.8)	
TT	3 (6.3)	5 (10.4)	
MTHFR 1298			
AA	12 (25)	11 (22.9)	0.37
AC	9 (18.8)	11 (22.9)	
CC	4 (8.3)	1 (2.1)	
MS 2756			
AA	18 (37.5)	17 (35.4)	0.87
AG	5 (10.4)	5 (10.4)	
GG	2 (4.2)	2 (4.2)	
MTRR 66			
AA	1 (2.1)	2 (4.2)	0.67
AG	14 (29.2)	14 (29.2)	
GG	10 (20.8)	7 (14.6)	

As reported previously [6], 25 of 48 patients (52.1%) developed TxCAD after a mean time of 5.3 ± 2.8 years from the transplant. All patients presented Hcy levels above our upper normal limit of 15 μmol/l. The mean Hcy level in patients with TxCAD was 25.0 ± 5.9 μmol/l compared with 21.9 ± 3.4 μmol/l in patients with no TxCAD ($P = 0.029$). Hcy was an independent risk factor for TxCAD in this cohort. However, the analysis of several SNP related to Hcy metabolism did not reveal any association with TxCAD (Table 3).

Discussion

This paper describes a significantly increased incidence in the G variant allele of the MTRR A66G polymorphism in our CTX cohort, compared with a healthy population. However, heterozygosity or homozygosity in the G allele was not associated with tHcy concentrations. Secondly, of several frequent SNP affecting Hcy metabolism, none was associated with tHcy in our cohort. Thirdly, both MS

A2756G and MTRR A66G loci were associated with plasma cobalamin levels. The MTRR A66G SNP was found to be associated with folate concentrations as well. Fourthly, neither tHcy nor related SNP were associated with TC in our cohort. Finally, none of the SNP were associated with the development of TxCAD after a mean follow-up interval of 5 years.

Very high Hcy levels are known to cause vascular thrombosis, but only a decade ago it was recognized that mild increases in tHcy in the general population are also associated with increased incidence of atherosclerosis and thromboembolism [20]. In the last few years, this association has been extended to TxCAD [2,5,6]. Although there is a lack of information about the mechanism(s) by which tHcy is associated with TxCAD development, there is a possible connection between hyperhomocysteinemic atherosclerotic models and the role of oxidative stress in the pathogenesis of TxCAD [2].

There are a few well-described genetic polymorphisms associated with increased plasma Hcy levels, especially when folate or cobalamin levels are low [21]. Moreover, there is a correlation between these mutations and disease, including neural tube defects [22], thromboembolism [23] and atherosclerosis [21,24]. We compared the incidence of common SNP in genes involved in Hcy metabolism in our cohort of CTX patients versus healthy controls. We found a significant increase in the frequency of the MTRR variant G allele compared with controls. One plausible hypothesis is that subjects were preselected, based on their previous cardiomyopathy history. However, we did not find an association between MTRR A66G genotype and the pretransplant cardiomyopathy diagnosis. It could also be argued that our control population was not represented, but the genotype distribution of our control population is in accordance to that of Rady *et al.* [25] and similar to White people distributions reported elsewhere. Although our CTX cohort was mostly White people, it was not stratified to exclude individuals of non-White background. However, the genotype distribution of MTRR A66G genotypes in our CTX group differs significantly from all the ethnic groups reported by Rady *et al.* [25].

Of note, tHcy levels were not different in those patients with homozygosity or heterozygosity for the G allele, although altered cobalamin and folate levels were associated with this allele. There may be other factors in the CTX population, other than diet and genetic background (i.e. immunosuppression, renal failure), that increase tHcy, because neither MTRR A66G nor associated differences in cobalamin or folate associated with differences in tHcy. Finally, the MTRR polymorphism was not associated with the development of TxCAD, although we have previously shown an association between high Hcy levels and TxCAD [6].

We did not confirm the findings of Pethig *et al.* [9] or Potena *et al.* [10], who observed significantly higher tHcy levels in patients with the MTHFR 677 TT genotype, and association with folate levels. Indeed, none of the SNP was associated tHcy. This discordance may be related to differences in the study groups. Recently, a meta-analysis of published studies about the impact of MTHFR C677T on Hcy levels confirmed a significant difference in the impact of this SNP on Hcy levels only when study heterogeneity was accounted for [26]. There is little doubt that sufficiency of folate intake is important in determining tHcy and its association with MTHFR C677T genotype. Folate intake has been greatly increased in the North American population since 1998, when folic acid fortification of flour and related products became mandatory, significantly increasing folate levels in the general population, as we reported [15].

Analysis of other SNP (MTHFR A1256C and MS A2756G) in genes involved in Hcy metabolism did not reveal any differences between study subjects and controls. This is not surprising, however, given the variability of previous reports [8,21]. Dietary factors such as vitamin intake may be important confounders, limiting the power to observe associations with genetic differences. Cobalamin levels were significantly different, however, among different MS A2756G genotypes. We found that the presence of at least one A allele was significantly associated with decreased cobalamin. The impact of the AA genotype in this population was also evident in that all patients with cobalamin deficiency (<118 pmol/l) or with indeterminate cobalamin levels (>118 but <148 pmol/l) were in this group. A similar finding in non-CTX subjects was recently reported by Yates *et al.* [27]. We hypothesize that CTX recipients have a stressed Hcy–cobalamin–folate axis, and therefore have greater requirements for vitamins like cobalamin and folate, depending on genotype. It may be that altered MS activity is associated with increased cobalamin turnover, and depletes circulating cobalamin in those with lower vitamin reserves. Similar arguments have been put forward for folate in the case of the MTHFR C677T SNP [28].

Analysis of the MTRR A66G mutation yielded similar results. Circulating cobalamin was significantly different among genotypes, but in this case, folate levels were also different. The MTRR 66 AA genotype was significantly less frequent in CTX than in the control population, and was associated with significantly higher levels of cobalamin and folate, but was not correlated with TxCAD.

The fact that MS A2756G and MTRR A66G genotypes were significantly associated with cobalamin levels in our study has particular importance as heart transplant patients are at risk for low cobalamin levels. Unmeasured alterations in Hcy metabolism may be present. As these

alterations may have an impact on long-term outcomes for at least some of the CTX population, a careful prospective study of cobalamin metabolism in relation to Hcy metabolism seems warranted. In the meantime, it seems prudent to ensure that there is adequate B vitamin intake in all CTX subjects.

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