

HYPERHOMOCYSTEINEMIA: GENETIC POLYMORPHISMS AND
RISK OF CORONARY ARTERY DISEASE

by

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A handwritten signature in black ink, appearing to read "Hayashi Satomi". The signature is written in a cursive style with a horizontal line underneath.

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STATEMENT BY AUTHOR

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DEDICATION

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ABSTRACT

This comprehensive literature review focuses on homocysteine, gene polymorphisms related to homocysteine metabolism and their relationship to coronary artery disease (CAD). Currently, CAD is known as a multifactorial genetic disease, resulting from complex interactions between genetic factors and various environmental influences. In recent years, tremendous knowledge about the hereditary aspect of CAD has been gained, including an understanding of CAD as a multifactorial condition resulting from complex interactions between genetic factors and various environmental influences that trigger, accelerate, or exacerbate the disease process. Among the risk factors for CAD, hyperhomocysteinemia has been recognized for its relation to atherosclerotic alterations in the vessels. In addition, gene polymorphisms in methylenetetrahydrofolate reductase (MTHFR), methionine synthase reductase (MTRR), methionine synthase (MS), and cystathionine β - synthase (CBS), which are involved in homocysteine metabolism, have been identified as a result of advances in genetic research related to cardiovascular pathophysiology. In particular, the results of recent salient studies have provided evidence of significant association of these genetic polymorphisms and CAD in Japanese and part of European populations but not in the United States, Australian, and part of European populations. This disparity may explain the variation of prevalence of CAD among different populations. Potential gene-environment interactions may elevate homocysteine levels and increase the risk of CAD. This discussion includes the pathogenesis of hyperhomocysteinemia, definitions of normal and elevated homocysteine levels, the physiological background of homocysteine metabolism, polymorphisms of genes involved in homocysteine metabolism from the perspective of CAD risk, and implications for nursing practice

based on emerging information regarding hyperhomocysteinemia as a risk factor for CAD. Findings from these recent studies are important for nurses, clinicians, and researchers to be able to incorporate cardiovascular genetic information in their practice and research and provide more adequate care to reduce the risk for CAD and improve patient outcomes.

INTRODUCTION

Coronary artery disease (CAD) is the leading cause of mortality and morbidity for both men and women in western countries (American Heart Association, 2002). We now know that CAD is a multifactorial genetic disease, resulting from complex interactions between genetic factors and various environmental influences (Hagberg, Wilund, & Ferrell, 2000; Hayden, Liu, & Ma, 1994; Humphries, Talmud, Hawe, Bolla, Day, & Miller, 2001; Jeerooburkhan, Jones, Bujac, Cooper, Miller, Vallance et al., 2001; Kluff, 2002; Talmud, Hawe, & Miller, 2002; Vischetti, Zito, Donati, & Iacoviello, 2002), and that hypertension, obesity, diabetes and hyperlipidemia are known to be major risk factors for CAD. However, only about two-thirds of cardiovascular events are related to well-established major risk factors (Abby, Harris, & Harris, 1998; Graham & O'Callaghan, 2000; Lefkowitz & Willerson, 2001; Stampfer & Malinow, 1995; Wilcken, 1998).

There is an ongoing search for new markers of cardiovascular risks. Currently, the landscape of research regarding CAD risks includes multiple genes regulating vascular physiology and factors contributing to atherosclerosis, the molecular basis of atherogenesis, identification of vulnerable plaques, and coronary revascularization (Francis, Raizada, Mangi, Melo, Dzau, Vale et al., 2001; Lefkowitz & Willerson, 2001). The results of these studies are more likely to identify populations susceptible to CAD before they develop the disease and enable the use of more effective therapeutic strategies leading to reduction of morbidity and mortality of CAD. In particular, the results emerging from studies of genetic factors support the hypothesis that multiple genetic factors contribute to all aspects of pathogenesis of CAD (Francis et al., 2001; Lefkowitz & Willerson, 2001).

Among risk factors for CAD, recent research has shown that hyperhomocysteinemia is considered a strong and independent risk factor for CAD because homocysteine is highly correlated to vascular injury resulting in atherosclerosis (Foody, Milberg, Robinson, Pearce, Jacobsen, & Sprecher, 2000; Morris, Jacques, Rosenberg, Selhub, Bowman, Gunter et al., 2000; Vollset, Refsum, Tverdal, Nygard, Nordrehaug, Tell et al., 2001). In addition, the results of genetic studies related to homocysteine metabolism support the hypothesis that genetic polymorphisms in factors involved in homocysteine metabolism may contribute to more adequate identification of individuals at risk and provide more specific preventative intervention directed to factors related to the pathogenesis of atherosclerosis (Francis et al., 2001; Lefkowitz & Willerson, 2001). Therefore, it is important to identify how hyperhomocysteinemia and genetic polymorphisms involved in homocysteine metabolism contribute to CAD risk. This comprehensive research review aims to discuss normal homocysteine levels, the relationship between hyperhomocysteinemia and CAD mortality, the background of homocysteine metabolism, the pathogenesis of homocysteine and mechanisms of vascular injury, and polymorphisms of genes involved in homocysteine metabolism from the perspective of CAD risk based on the research findings, and concludes with nursing implications.

NORMAL HOMOCYSTEINE LEVELS AND

THE ASSOCIATION OF HOMOCYSTEINE WITH CAD MORTALITY

Homocysteine is a normal metabolite of methionine, which is one of the essential amino acids, and is derived from animal and plant protein. Homocysteine is a sulfur-containing (thiol) amino acid and is usually present in plasma in four forms. One percent

of homocysteine circulates as the free thiol, 70 to 80 % as disulfide bound to plasma proteins, and 20 to 30 % combines with itself or with other thiols (Hankey & Eikelboom, 1999). Despite the various forms, total plasma homocysteine levels usually refer to the total of all four forms of homocysteine in plasma, and are expressed in units of $\mu\text{mol/L}$ (Hankey & Eikelboom, 1999). The normal total plasma homocysteine level is commonly defined as 5 to 15 $\mu\text{mol/L}$ under fasting conditions and higher homocysteine levels are classified arbitrarily as moderate 16 to 30, intermediate 31 to 100, and severe greater than 100 $\mu\text{mol/L}$ (Kang, Wong, & Malinow, 1992).

However, data from large European population studies indicated that as homocysteine levels increase over 12 $\mu\text{mol/L}$, there was an increase in cardiovascular risk (Nygard, Nordrehaug, Refsum, Ueland, Farstad, & Vollset, 1997; Vollset et al, 2001). After adjusting for age, gender, left ventricular ejection fraction, serum creatinine, total cholesterol, and severity of CAD, the mortality ratio in those with total homocysteine levels of 15 $\mu\text{mol/L}$ was 1.6 times that of individuals with levels of 10 $\mu\text{mol/L}$ (Nygard et al, 1997). A meta-analysis study (Boushey, Beresford, Omenn, & Motulsky, 1995) also indicated a similar result. The risk for CAD increases about 1.6 times in men and 1.8 times in women with a 5 $\mu\text{mol/L}$ increment in homocysteine level (Boushey et al., 1995). Therefore, it is suggested to use 11.4 $\mu\text{mol/L}$ for men and 10.4 $\mu\text{mol/L}$ for women at which an increased risk of CAD may begin (Robinson, Mayer, Miller, Green, Van Lente, Gupta et al., 1995; Selhub, Jacques, Rosenberg, Rogers, Bowman, Gunter et al., 1999).

Plasma homocysteine level is also related to mortality from CAD. Data from a large population study (Nygard et al., 1997) suggested that at 4-year follow-up, the estimated survival rate was 96.2 % in patients with homocysteine levels below 9 $\mu\text{mol/L}$, 91.4 % for those with homocysteine of 9 to 14.9 $\mu\text{mol/L}$, and 75.3 % for those with levels

of 15 $\mu\text{mol/L}$ or higher. In addition, the results of a retrospective study (Wald, Watt, Law, Weir, McPartlin, & Scott, 1998), which examined 229 men without a history of CAD at study entry and who subsequently died from CAD and 1,126 age-matched counterparts, indicated that men who died from CAD had significant higher homocysteine levels than those who did not ($p < 0.001$). The results of this study also indicated that men with homocysteine levels greater than 15.17 $\mu\text{mol/L}$ had 2.9 times higher CAD mortality than those with homocysteine levels less than 10.25 $\mu\text{mol/L}$ after adjustment for serum apolipoprotein B levels and systolic blood pressure (Wald et al., 1998). The results of these above studies (Nygard et al, 1997; Vollset et al. 2001; Boushey et al., 1995; Robinson et al., 1999) showed that increased homocysteine levels are significantly associated with CAD mortality.

PHYSIOLOGICAL BACKGROUND OF HOMOCYSTEINE METABOLISM

Homocysteine is metabolized from methionine after dietary intake of animal and plant protein, and is metabolized by two pathways including remethylation and trans-sulfuration (Finkelstein, 1998; Selhub, & Miller, 1992) (See Figure 1 illustrating events in homocysteine metabolism). In the transsulfuration pathway, homocysteine is metabolized to cystathionine then cysteine, and finally excreted or detoxified in the liver, kidney, small intestine, and pancreas (Finkelstein, 1998; Selhub & Miller, 1992). This pathway requires a vitamin B₆ dependent enzyme, the cystathionine β - synthase (C β S), to catabolize homocysteine (Finkelstein, 1998; Selhub & Miller, 1992). The remethylation pathway is the other pathway in which homocysteine is remethylated to methionine by receiving methyl from 5-methyltetrahydrofolate when converting to tetrahydrofolate. The remethylation pathway takes place in all tissues and requires a vitamin B₁₂ dependent enzyme, called methionine synthase (MS) as a cofactor. The

remethylation pathway also requires involvement of a vitamin B₂ dependent enzyme, called the methylene-tetrahydrofolate reductase enzyme (MTHFR), and methionine synthase reductase (MTRR). MTHFR catalyzes the changing process from tetrahydrofolate, which is converted from folate, to 5-methyltetrahydrofolate and MTRR maintains the active state of the MS enzyme (Finkelstein, 1998; Selhub & Miller, 1992).

In summary, homocysteine metabolism requires several major enzymes, such as CBS, MS, MTRR, and MTHFR (Finkelstein, 1998; Selhub & Miller, 1992). In addition, vitamin B₂, vitamin B₆, vitamin B₁₂, and folate are involved in homocysteine metabolism pathways (Finkelstein, 1998; Selhub & Miller, 1992). Defects in these four enzymes and deficiencies in vitamin B₂, vitamin B₆, vitamin B₁₂, or folate can increase homocysteine levels and may contribute to risk of CAD (Finkelstein, 1998; Selhub & Miller, 1992).

PATHOGENESIS OF HOMOCYSTEINE AND MECHANISMS OF VASCULAR INJURY

Hyperhomocysteinemia is highly correlated with vascular injury resulting in atherosclerosis (McCully & Wilson, 1975; Abby et al., 1998; Wilcken, 1998). The possible contribution of elevated plasma homocysteine levels to the pathogenesis of vascular diseases has been recognized since the 1960s (McCully, 1969).

Atherosclerosis is an inflammatory disease and the response-to-injury hypothesis of atherosclerosis is currently accepted to examine its occurrence (Ross, 1999). This hypothesis holds that endothelial injury and dysfunction alter normal homeostasis in the endothelium and increase endothelial adhesiveness and permeability as a part of the inflammatory response (Ross, 1999). The injury also induces a procoagulant condition in the endothelium, resulting in expression and secretion of vasoactive molecules, cytokines, and growth factors (Ross, 1999). Production of these substances stimulates migration and proliferation of smooth muscle cells to form an intermediate lesion and

thicken the artery wall (Ross, 1999). Continued inflammation results in emigration and increase of macrophages and lymphocytes within the lesion (Ross, 1999).

Macrophages and lymphocytes then attach to the under side of endothelial cells (Ross, 1999). Foam cells form and contribute to early lesions, called fatty streaks (Ross, 1999).

This, in turn, leads to atherosclerosis (Ross, 1999). Hyperhomocysteinemia may contribute to this proposed mechanism of atherogenesis by being a source of injury.

The following studies illustrate the major mechanisms of vascular injury underlying hyperhomocysteinemia (Upchurch, Welch, Fabian, Freedman, Johnson, Keaney, et al., 1997; Tsai, Perrella, Yoshizumi, Hsieh, Haber, Schlegel et al., 1994; Lentz & Sadler, 1991).

Upchurch et al. (1997) examined if homocysteine affects the bioavailability rather than production of endothelial-derived relaxing factor, nitric oxide (NO). The investigator also examined if homocysteine decreases intracellular glutathione peroxidase activity and steady-state glutathione peroxidase messenger ribonucleic acid (mRNA) levels (Upchurch et al., 1997). Intracellular glutathione peroxidase is an enzyme to detoxify hydrogen peroxide and protect cells from injury by catalyzing free radical breakdown. Upchurch et al. (1997) measured NO production, endothelial nitric oxide synthase (eNOS) levels, NOS 3 mRNA levels, intracellular glutathione peroxidase activity in bovine aortic endothelium treated with a range of homocysteine concentrations (Upchurch et al., 1997). Cells treated with homocysteine showed a dose-dependent decrease in NO significantly (Upchurch et al., 1997). However, homocysteine did not change either eNOS or NOS3 mRNA levels, which means that homocysteine may not affect NOS synthesis or transcription (Upchurch et al., 1997). Cells treated with homocysteine showed a significant decline of glutathione peroxidase activity (Upchurch

et al., 1997). In addition, steady-state glutathione peroxidase mRNA in cells treated with homocysteine was decreased significantly compared with the control cells, which means that transcription of intra-cellular glutathione peroxidase was decreased after homocysteine treatment (Upchurch et al., 1997). These cell culture results indicate that homocysteine limited the bioavailability of nitric oxide and glutathione peroxidase to detoxify oxidative damage from hydrogen peroxide leading to an increase of oxidative stress and endothelial cell injury in bovine (Upchurch et al., 1997).

Another study examined the effects of homocysteine on the growth of vascular smooth muscle cells and endothelial cells in a model system (Tsai et al., 1994). This study was undertaken because it was not clear if homocysteine induces smooth muscle cell proliferation directly or indirectly resulting in induction of endothelium injury. Tsai et al. (1994) measured [^3H] thymidine incorporation, which represents deoxyribonucleic acid (DNA) synthesis, in rat aortic smooth muscle cells and human umbilical vein endothelial cells after adding varying levels of homocysteine to examine the effects of homocysteine on DNA synthesis. The result indicated that there was a dose response in homocysteine-induced increase in [^3H] thymidine incorporation in smooth muscles cell, whereas there was a dose response of homocysteine-induced decrease in endothelial cells (Tsai et al., 1994). Tsai et al. (1994) also examined if homocysteine stimulated DNA synthesis and cell proliferation in smooth muscle cells after addition of homocysteine. The results indicated that homocysteine increased DNA synthesis in vascular smooth muscle cells in rats resulting in stimulation of smooth cell proliferation, whereas homocysteine decreased DNA synthesis in endothelial cells (Tsai et al., 1994). This result can be interpreted to indicate that homocysteine may present growth-

promoting effect on vascular smooth muscle while atherosclerotic progress through the interaction with other growth factors or cytokines (Tsai et al., 1994).

Lentz and Sadler (1991) examined the effects of homocysteine on thrombomodulin expression and anticoagulant protein C activation in human umbilical vein endothelial cells. Thrombomodulin is a glycoprotein located on the surface of endothelium acting as a specific thrombin receptor. It promotes activation of the anti-coagulant protein C, which in turn, inhibits the procoagulant activities of thrombin (Lentz & Sadler, 1991). The authors examined effects of homocysteine on thrombomodulin synthesis, on thrombomodulin glycosylation, on thrombomodulin surface expression, and on protein C activation. The results showed that thrombomodulin mRNA and thrombomodulin synthesis increased slightly after the addition of homocysteine (Lentz & Sadler, 1991). Although thrombomodulin was synthesized and remained sensitive, thrombomodulin failed to appear on the cell surface in the presence of homocysteine (Lentz, & Sadler, 1991). Both thrombomodulin and protein C were irreversibly inactivated with homocysteine treatment (Lentz & Sadler, 1991). The results indicate that homocysteine inhibited cell-surface thrombomodulin expression and inactivated both thrombomodulin and protein C irreversibly. Thus, it is possible that homocysteine may contribute to the pro-coagulant tendency of the endothelium by decreasing cell-surface thrombomodulin expression and inactivating protein C (Lentz & Sadler, 1991).

The results of the cell culture studies discussed suggested that homocysteine may limit the bioavailability of nitric oxide, increases oxidative stress, stimulates smooth cell proliferation, and suppress the anticoagulant effects. Consequently, it may be proposed that the mechanism for homocysteine involvement in vascular injury causes oxidative injury to the endothelial cells by limiting bioavailability of nitric oxide (Upchurch

et al., 1997). In addition, it may decrease cell-surface thrombomodulin expression and inactivates protein C (Lentz & Sadler, 1991), resulting in procoagulant properties. Hyperhomocysteinemia also stimulates smooth cell proliferation to form an intermediate lesion and thicken the artery wall. These mechanisms underlying hyperhomocysteinemia result in endothelial injury and dysfunction, arterial thickness and stiffness, and increased blood coagulation leading to atherogenesis and thrombogenesis. The proposed mechanisms of vascular injury induced by hyperhomocysteinemia were developed based on the evidences of cell culture studies, and thus, more studies are needed to confirm if these proposed mechanisms may be applicable to human cells. The mechanisms underlying the relationship between hyperhomocysteinemia and endothelial injury are summarized and illustrated in Figure 2.

EFFECTS OF GENETIC POLYMORPHISMS ON HOMOCYSTEINE LEVELS AND RISK OF CAD

Although it has been hypothesized that hyperhomocysteinemia leads to atherosclerotic alterations, altered enzymatic activities involved in homocysteine metabolism may actually underlie high homocysteine concentrations resulting in atherogenesis (McCully, 1969). As a result of advances in genetic research regarding CAD, studies have been able to focus on the identification of genetic defects and polymorphisms, which means naturally occurring variations in a DNA sequence at a given locus (Ott, 1999), involved in homocysteine metabolism (Fohr, Prinz-Langenohl, Bronstrup, Bohlmann, Nau, Berthold et al., 2002; Herrmann & Paul, 2001). In the last 10 years, numerous variations in genes in homocysteine metabolism have been investigated. In particular, gene polymorphisms in enzymes involved in homocysteine metabolism, such as MS, MTRR, CBS, and MTHFR, have been studied for their

association with homocysteine levels and risk of CAD. In general, all variations within a gene may not result in abnormal expression of abnormal protein products (Ott, 1999; Snustad, Simmons, & Jenkins, 1997). However, polymorphism of the MTHFR, for example, leads to reduced enzymatic activity of MTHFR (Kang, Wong, Bock, Horwitz, & Grix, 1991; Frosst, Blom, Milos, Goyette, Sheppard, Matthews et al., 1995; DeBusk, 2003). Genes coding for CBS, MS, MTHFR, and MTRR may contribute to hyperhomocysteinemia leading to the risk for CAD (Lucock, 2000; Hankey & Eikelboom, 1999; Graham & O'Callaghan, 2000; Young & Woodside, 2000). Many studies have supported the association of polymorphisms of the MTHFR gene with homocysteine levels and/or risk of CAD (Christensen, Frosst, Lussier-Cacan, Selhub, Goyette, Rosenblatt et al., 1997; Izumi, Iwai, Ohmichi, Nakamura, Shimoike, & Kinoshita, 1996; Jacques, Kalmbach, Bagley, Russo, Rogers, Wilson et al., 2002; Kluijtmans, Kastelein, Lindemans, Boers, Heil, Brusckke et al., 1997; Ma, Stampfer, Hennekens, Frosst, Selhub, Horsford et al., 1996; Madonna, De Stefano, Coppola, Cirillo, Cerbone, Orefice et al., 2002; Meleady, Ueland, Blom, Whitehead, Refsum, Daly et al., 2003; Morita, Taguchi, Kurihara, Kitaoka, Kaneda, Kurihara et al., 1997; Ou, Yamakawa-Kobayashi, Arinami, Amemiya, Fujiwara, Kawata et al., 1998; Passaro, Vanini, Calzoni, Alberti, Zamboni, Fellin et al., 2001; Saw, Yuan, Ong, Arakawa, Lee, Coetzee et al., 2001; Schmitz, Lindpaintner, Verhoef, Gaziano, & Buring, 1996; Todesco, Angst, Litynski, Loehrer, Fowler & Haefeli, 1999; Tokgozoglu, Alikasifoglu, Unsal, Atalar, Aytemir, Ozer et al., 1999). On the other hand, inconclusive results have been found in MS, MTRR, and CBS genes (Gaughan, Kluijtmans, Barbaux, McMaster, Young, Yarnell et al., 2001; Zhang & Dai, 2001; Kluijtmans, Boers, Kraus, Van Den Heuvel, Cruysberg, Trijbels et al., 1999; Dilley, Hooper, El-Jamil, Renshaw, Wenger, & Evatt, 2001; De Stefano,

Dekou, Nicaud, Chasse, London, Stansbie et al., 1998). Effects of genetic polymorphisms in enzymes involved in homocysteine metabolism including MTRR, CBS, and MTHFR, on homocysteine levels and risk for CAD will be discussed in the following sections.

POLYMORPHISM IN METHIONINE SYNTHASE REDUCTASE GENE

Methionine synthase reductase (MTRR) maintains the active state of the methionine synthase (MS), and thus, it is one of the key enzymes in homocysteine metabolism. A substitution of adenine (A) to guanine (G) at the 66th nucleotide of the MTRR gene (A66G) is a common polymorphism. Gaughan et al. (2001) studied 601 Northern-Irish men ages 30 to 49 to investigate the effects of the A66G polymorphism of the MTRR gene on homocysteine concentration (Gaughan et al., 2001). The results showed that 29 % of this population had the AA allele (one of two or more alternative forms of a gene), 53.6 % had the AG allele, and 17.5 % of those had the GG allele (Gaughan et al., 2001). The results indicated that the AA allele was more likely to have increased homocysteine levels than other alleles of the MTRR gene and the effect of the AA allele on homocysteine concentration was independent of serum folate, vitamin B₁₂, and vitamin B₆ (Gaughan et al., 2001). The authors estimated that individuals with the AA allele have an approximately 4 % increase in CAD risk compared to those with the GG allele based on the results of a previous study (Danesh & Lewington, 1998) which suggested that a 5 µmol/L increase in homocysteine levels conferred a 30 % increased risk for CAD (Gaughan et al., 2001).

However, the results of this study can not be generalized to other ethnic populations, women, or other age groups because the subjects included only Northern-Irish men ages 30 to 49. In addition, this study examined the relationship between

MTRR gene polymorphisms and homocysteine levels but not in association with CAD mortality or morbidity even though increased risk for CAD was estimated. For these reasons, the results provide equivocal support for the relationship between the A66G polymorphism of the MTRR gene, homocysteine levels, and the risk of CAD.

POLYMORPHISM IN CYSTATHIONINE β - SYNTHASE GENE

Cystathionine β - synthase (CBS) is a vitamin B₆ dependent enzyme that plays an essential role in homocysteine metabolism. Deficiency of CBS has shown to be a recessively inherited inborn error of methionine metabolism resulting in high concentrations of homocysteine in plasma and urine, and currently known as hyperhomocystinuria (McCully, 1969). The mutation and polymorphisms in the CBS gene reduce enzymatic activity of CBS leading to increase homocysteine levels (Kraus, Janosik, Kozich, Mandell, Shih, Sperandeo et al., 1999). Investigators have cloned and sequenced the entire human CBS gene and more than 60 mutations and seven polymorphisms in the CBS gene have been identified (Kraus et al., 1999). However, recent genetic studies have failed to detect any involvement of the CBS gene in hyperhomocystinemia and premature vascular disease (Zhang & Dai, 2001; Kluijtmans et al., 1999; Dilley et al., 2001; De Stefano et al., 1998).

Dilley et al. (2001) examined the frequency of a polymorphism that substituted cytosine (C) for thymine (T) at the 833rd nucleotide (T833C) and the frequency of a polymorphism that substituted adenine (A) for guanine (G) at the 919th nucleotide of the CBS gene (G919A) and the association of these polymorphisms with myocardial infarction (MI) in African Americans. This study enrolled 110 subjects with MI cases and 185 race and age matched controls age 65 or younger (Dilley et al., 2001). The results showed that the G919A polymorphism of the CBS gene was not found in either MI cases

or controls (Dilley et al., 2001). One-third of total subjects had the TC allele and 4.6 % of controls and 6.5 % of MI cases had the CC allele of the T833C polymorphisms of the CBS gene (Dilley et al., 2001). The results showed that the T833C and G919A polymorphisms were not associated with MI in this population (Dilley et al., 2001).

Kluijtmans et al. (1999) investigated preference of 10 CBS gene polymorphisms in 29 Dutch patients with hyperhomocystinuria. The results showed that the T833C polymorphism of the CBS gene was the most prevalent in patients with hyperhomocystinuria; however, the subjects were not significantly different in their clinical and biochemical expressions regardless of polymorphisms of the CBS gene (Kluijtmans et al., 1999).

Zhang and Dai (2001) examined if a 68-base-pairs (bp) insertion at the 8th exon of the 844th nucleotide of the CBS gene (844 ins 68) is an independent risk factor for ischemic stroke and/or MI among a Chinese population. This study enrolled 102 patients with ischemic stroke (age range 30 to 78), 71 patients with MI (age range 28 to 76), and 100 healthy individuals for the control group (age range 22 to 70) (Zhang & Dai, 2001). The results showed that 1.0 % of ischemic stroke cases, 1.4 % of MI cases, and 5.0 % of controls had the different alleles, called heterozygote for CBS 844 ins 68 (Zhang & Dai, 2001). Thus, it appeared no significant difference in the frequency of heterozygote for the CBS 844 ins 68 among the sample groups (Zhang & Dai, 2001). The CBS 844 ins 68 mutation failed to represent risk for neither ischemic stroke nor MI among a Chinese population (Zhang & Dai, 2001).

De Stefano et al. (1998) examined the 844 ins 68 mutation, a polymorphism that substituted thymine (T) for cytosine (C) at the 699th nucleotide (C699T), a polymorphism at the 1080th nucleotide (C1080T), and a polymorphism at the 1985th nucleotide

(C1985T) of the CBS gene, to identify the association between these markers and homocysteine concentration. This study included 785 men ages 18 to 28 whose fathers had proven MI before the age of 55 and age matched counterparts recruited from 11 European countries (De Stefano et al., 1998). The results also failed to represent the association between polymorphisms of the CBS gene and homocysteine concentration (De Stefano et al., 1998).

In summary, research fails to support the association between CBS gene polymorphisms and CAD risk in African American and Chinese MI patients.

POLYMORPHISM IN METHYLENETERTRAHYDROFOLATE REDUCTASE GENE

Methylenetetrahydrofolate reductase (MTHFR) is one of the key enzymes involved in catalyzing remethylation of homocysteine and is essential for homocysteine metabolism. The C677T polymorphism of the MTHFR gene occurs when cytosine (C) is replaced by thymine (T) at the 677th nucleotide of the MTHFR gene (Frosst et al., 1995; DeBusk, 2003). The C677T polymorphism of the MTHFR gene results in reduced catalytic activity and thermolability *in vitro* (Kang et al., 1991), and thus, it increases homocysteine levels if folate intake is low (Frosst et al., 1995). Much research has investigated the association of the C677T polymorphism with hyperhomocysteinemia and CAD risk. Thus, the following discussion will be focused on the C677T polymorphism. Table 1 provides a summary of studies that investigated the relationship between the C677T polymorphism of the MTHFR gene and hyperhomocysteinemia and/or CAD risks.

MTHFR C677T Polymorphisms and Homocysteine Levels

Four population-based studies (Jacques et al., 2002; Saw et al., 2001; Passaro et al., 2001; Fohr et al., 2002) and 10 case-control studies (Kluijtmans et al., 1997; Ou et al., 1998; Meleady et al., 2003; Christensen et al., 1997; Ma et al., 1996; Todesco et al., 1999; Schmitz et al., 1996; Madonna et al., 2002; Tokgozoglu et al., 1999; Van Bockxmeer, Mamotte, Vasikaran, & Taylor, 1997) examined the relationship between the C677T polymorphism of the MTHFR gene and hyperhomocysteinemia. Among these studies, 11 supported that those with the TT allele had significantly higher homocysteine levels than those with the CT or the CC alleles (Jacques et al., 2002; Saw et al., 2001; Passaro et al., 2001; Kluijtmans et al., 1997; Ou et al., 1998; Meleady et al., 2003; Christensen et al., 1997; Ma et al., 1996; Todesco et al., 1999; Madonna et al., 2002; Tokgozoglu et al., 1999). The results of two case-control studies (Morita et al., 1997; Ma et al., 1996) indicated that the TT allele was significantly associated with homocysteine levels in both cases and controls. The results of four case-control studies (Christensen et al., 1997; Ma et al., 1996; Todesco et al., 1999; Tokgozoglu et al., 1999) indicated that CAD cases with the TT allele had significantly higher homocysteine levels than controls with the TT allele. On the other hand, one population-based study (Fohr et al., 2002) and two case-control studies (Meisel, Cascorbi, Gerloff, Stangl, Laule, Muller et al., 2001; Schmitz et al., 1996) concluded no association between the C677T polymorphism of the MTHFR gene and homocysteine levels.

Each study had a large sample size, ranged from 118 to 1,000. Some studies (Passaro et al., 2001; Kluijtmans et al., 1997; Meleady et al., 2003; Schmitz et al., 1996; Ma et al., 1996; Madonna et al., 2002; Fohr et al., 2002; Folsom et al., 1998; Tokgozoglu et al., 1999; Meisel et al., 2001) considered some of the factors that can influence the

total plasma homocysteine levels, including fasting status (Ubbink, Vermaak, Van Der Merwe, & Becker, 1992; Guttormsen, Schneede, Fiskerstrand, Ueland, & Refsum, 1994), age (Kario et al., 2001; Jacques, Rosenberg, Rogers, Selhub, Bowman, Gunter et al., 1999; Rasmussen, Ovesen, Bulow, Knudsen, Laurberg, & Perrild, 2000; Jacques, Bostom, Wilson, Rich, Rosenberg, & Selhub 2001), gender (Foody, Milberg, Robinson, Pearce, Jacobsen, & Sprecher, 2000; Jacques et al., 2001), smoking status (Vollset, Refsum, & Ueland, 2001; Jacques et al., 2001), alcohol use (De La Vega, Santolaria, Gonzalez-Reimers, Aleman, Milena, Martinez-Riera et al., 2001; Jacques et al., 2001), or co-morbidity of other diseases (Diekman, Van Der Put, Blom, Tijssen, & Wiersinga, 2001). However, none of these studies adjusted all factors influencing homocysteine levels including age, gender, renal impairment or reduced glomerular filtration rate (Warren, 1999; Graham, & O'Callaghan, 2000; Hankey & Eikelboom, 1999), smoking, the amount of coffee consumption (Olthof, Hollman, Zock, & Katan, 2001; Urgert, Van Vliet, Zock, & Katan, 2000; Grubben, Boers, Blom, Broekhuizen, De Jong, Van Rijt et al., 2000; Vollset, Nygard, Refsum, & Ueland, 2000; Graham & O'Callaghan, 2000; Jacques et al., 2001), alcohol consumption, hyper- and hypothyroidism, and use of drugs, such as methotrexate, phenytoin, carbamazepine, theophylline, and oral contraceptives, which interfere with folate or vitamin B₆ metabolism (Warren, 1999; Graham & O'Callaghan, 2000; Hankey & Eikelboom, 1999). For this reason, it is premature to conclude that the T677T allele of the MTHFR gene is independently associated with homocysteine levels although the results of most studies suggested that the T677T allele may be associated with hyperhomocysteinemia.

MTHFR C677T Polymorphisms and CAD

Among 14 case-control studies, seven concluded that the T677T allele of the MTHFR gene had significantly increased risk of CAD (Kluijtmans et al., 1997; Izumi et al., 1996; Passaro et al., 2001; Ou et al., 1998; Morita et al., 1997; Meleady et al., 2003; Schmitz et al., 1996), whereas the results of seven other studies had no significant association between polymorphisms of the MTHFR gene and CAD risk (Christensen et al., 1997; Ma et al., 1996; Madonna et al., 2002; Folsom et al., 1998; van Bockxmeer et al., 1997; Tokgozoglu et al., 1999; Meisel et al., 2001).

The sample size in each study was large. However, Kluijtmans et al. (1997) and Morita et al. (1997) examined only men, and Passaro et al. (2001) examined only women. In addition, age range of cases and controls were not matched in the study by Morita et al. (1997). The mean age of CAD cases was 62 ± 9 years, whereas the mean age of the controls was 48 ± 11 years (Morita et al., 1997). Because of the effect of age on homocysteine levels, CAD cases were more likely to have higher homocysteine levels than controls. Passaro et al. (2001) found that the T677T allele was significantly related to the intima-media thickness in carotid arteries. Although the level of intima-media thickness in carotid arteries may indicate atherosclerotic vascular alteration and a precursor of CAD, it is not considered to be CAD. Furthermore, some studies (Kluijtmans et al., 1997; Izumi et al., 1996; Passaro et al., 2001; Ou et al., 1998; Morita et al., 1997) did not adjust other factors influencing CAD risk, such as hyperlipidemia, hypertension, smoking, and diabetes. Therefore, the odds ratio of risk of CAD for the T677T allele of the MTHFR gene obtained in these studies may be biased because of lack of adjustment. Although the evidence may suggest that the T677T allele has

significantly increased risk of CAD, it is still unclear whether this polymorphism is an independent predictor of CAD.

Meleady et al. (2003) and Schmitz et al. (1996) obtained adjusted odds ratio and the results supported that the T677T allele of the MTHFR gene was a risk of CAD. However, seven other studies also obtained adjusted odds ratio but failed to support the association between the risk of CAD and the T677T allele of the MTHFR gene (Christensen et al., 1997; Ma et al., 1996; Madonna et al., 2002; Folsom et al., 1998; van Bockxmeer et al., 1997; Tokgozoglu et al., 1999; Meisel et al., 2001). It is hard to deny the possibility of association between the C677T polymorphism of the MTHFR gene and CAD risk, and the difference of the association may be attributed to variation in the population. The stronger association (the odds ratio was 1.21 to 30.8) was found in Japanese and part of European population including Dutch, Italians, Irish, Spanish, French, Belgians, Swedish, Portuguese, and Norwegian, (Morita et al., 1997; Ou et al., 1998; Izumi et al., 1996; Kluijtmans et al., 1997; Passaro et al., 2001; Meleady et al., 2003). On the contrary, negative or weak association (the odds ratio below 1.1) was found among Americans, Australians, French Canadians, and part of European populations including Germans, Italians, and Turkish (Christensen et al., 1997; Ma et al., 1996; Madonna et al., 2002; Folsom et al., 1998; Tokgozoglu et al., 1999; van Bockxmeer et al., 1997; Meisel et al., 2001).

According to a meta-analysis (Jee, Beaty, Suh, Yoon, & Appel, 2000) from 12 studies conducted in six different countries, Japanese, Dutch, Italians, and Germans had higher odds ratio of CAD for the TT allele of the MTHFR gene compared with the CC allele (Jee et al., 2000). This was not true in Australians and Americans (Jee et al., 2000). The odds ratio of CAD for the TT allele compared with the CC allele across 12

studies was 1.4 (1.2 to 1.6 with 95 % confidence intervals). In Japan and the Netherlands, the odds ratio of CAD for the TT allele compared with the CC allele was high. However, this was not true for other countries. In particular, the results of studies in Japan had 2.0 and 1.6 of the odds ratio of CAD for the TT allele compared with the CC allele and for the CT allele compared with the CC allele respectively (Jee et al., 2000). On the other hand, the results from other countries indicated that the odds ratio of CAD for the TT allele compared with the CC allele and those for the CT allele compared with the CC allele were 1.1 for both comparisons and it was not statistically significant (Jee et al., 2000). Thus, the data from this meta-analysis suggested that the T677T allele of the MTHFR gene is associated with increased CAD risk in Japanese, but not in other populations (Jee et al., 2000). Consequently, it is possible that the association of the C677T polymorphism of the MTHFR gene with risk for CAD is related to ethnicity.

Gene-Environment Interactions in MTHFR Gene

Among 18 studies reviewed, seven examined effects of plasma folate levels on the relationship between MTHFR gene polymorphisms and homocysteine levels (Saw et al., 2001; Meleady et al., 2003; Christensen et al., 1997; Ma et al., 1996; Fohr et al., 2002; van Bockxmeer et al., 1997; Tokgozoglu et al., 1999). The results of four studies indicated that the T677T allele of the MTHFR gene had a significantly elevated mean total plasma homocysteine levels when plasma folate levels were below the median in the sample population (Saw et al., 2001; Christensen et al., 1997; Ma et al., 1996; Tokgozoglu et al., 1999). In addition, the results of one study indicated that women with the T677T allele of the MTHFR gene had a significant homocysteine concentration

lowering effect after four weeks of folic acid supplementation ($p < 0.05$) (Fohr et al., 2002).

Furthermore, the results of a large population study investigated the effects of folic acid supplementation on homocysteine levels for each allele of the C677T polymorphisms (Malinow, Nieto, Kruger, Duell, Hess, Gluckman et al., 1997) indicated that the reduced homocysteine after folic acid supplementation is 10.6 % in the CC allele, 14.3 % in the CT allele, and 23.9 % in the TT allele. This result illustrated the gene-environment interaction between the C677T polymorphism of the MTHFR gene and efficacy of folic acid supplement. Individuals with the TT allele benefited most from folic acid supplementation. The results appeared to indicate that interaction between the C677T polymorphism and folate levels might affect homocysteine levels, and thus, gene-environment interactions might play a pivotal role in regulating homocysteine levels.

GENE-GENE INTERACTIONS

Most studies have focused on a single gene polymorphism. However, some recent studies have investigated the relationship between homocysteine levels and a combination of multiple gene polymorphisms (Tsai, Bignell, Yang, Welge, Graham, & Hanson, 2000; Dekou, Gudnason, Hawe, Miller, Stansbie, & Humphries, 2001; Feix, Fritsche-Polanz, Kletzmayr, Vychytil, Horl, Sunder-Plassmann et al., 2001). One study sought an additive effect of the C677T polymorphism of the MTHFR gene, the 68bp insertion in exon 8 of the CBS gene (844 ins 68), and the A2756G polymorphism of the MS gene on homocysteine levels (Dekou et al., 2001). The A2756G polymorphism of the MS gene occurs when adenine (A) is replaced by guanine (G) at the 2756th nucleotide of the MS gene resulting in change of the place coded for aspartic acid (Asp) to glycine (Gly) at the 919th amino acid (Asp919Gly) (Dekou et al., 2001). Dekou et al.

(2001) examined 1,470 healthy middle aged men between 50 and 61 years of age recruited from the second Northwick Park Heart Study (NPHS-II). After adjustment for age and smoking status, the results showed that there was no raising effect on homocysteine levels in 23 men who carried both the T677T allele of the MTHFR gene and the CBS 844 ins 68 ($p = 0.03$) (Dekou et al., 2001). Men carrying the A2756A allele of the MS gene and the CBS 844 ins 68 had a 1 $\mu\text{mol/L}$ lower median homocysteine levels in the sample population than those carrying the A2756A allele but not the CBS 844 ins 68 ($p = 0.09$) (Dekou et al., 2001). In addition, when men carrying both the T677T allele of the MTHFR gene and the G2756G allele of the MS gene, their homocysteine levels were additive resulting in a 2.15 $\mu\text{mol/L}$ higher homocysteine level than those carrying the C677C allele of the MTHFR gene and the A2756A allele of the MS gene (Dekou et al., 2001). The results indicated that the increasing effects of the T677T allele of the MTHFR gene and the A2756A allele of the MS gene on homocysteine levels were additive and the lowering effect of the CBS 844 ins 68 on homocysteine levels may exist (Dekou et al., 2001).

Another study investigated the effects of the 68bp insertion in exon 8 of the CBS gene (844 ins 68), the A2756G polymorphism of the MS gene, and the C677T polymorphism of the MTHFR gene on fasting and four hour post methionine loading homocysteine levels (Tsai et al., 2000). This study included 761 men ages 24 to 82 and 270 women ages 21 to 77 (Tsai et al., 2000). Six hundred sixty of those had premature CAD and 289 participants had undergone primary prevention or diagnostic evaluation for cardiovascular disease (Tsai et al., 2000). Eighty-two healthy controls matched for sex, age, and race were included (Tsai et al., 2000). The results showed that the interaction among the CBS 844 ins 68, the A2756G polymorphism of the MS gene, and the C677T

polymorphism of the MTHFR gene were additive (Tsai et al., 2000). A 3-way ANOVA model showed no interaction among three variants which means that effects on homocysteine levels of each factor were not altered by other factors (Tsai et al., 2000). Thus, the results of this study indicated a multiple genetic polymorphisms may affect homocysteine levels (Tsai et al., 2000).

Another study examined the prevalence of combined polymorphisms of the A2756G polymorphism of the MS gene, the C677T polymorphism of the MTHFR gene, and the A1298C polymorphism of the MTHFR gene with hyperhomocysteinemia among patients with renal failure and healthy counterparts (Feix et al., 2001). The A1298C polymorphism of the MTHFR gene means adenine (A) is replaced by cytosine (C) at the 1298th nucleotide. This study included 733 kidney graft recipients, 415 hemodialysis patients, 179 peritoneal dialysis patients, and 389 healthy counterparts (Feix et al., 2001). The results of this study showed that the A2756G polymorphism of the MS gene in combination with the T677T/A1298A alleles of the MTHFR gene or the C677T/A1298C alleles of the MTHFR gene were associated with hyperhomocysteinemia (Feix et al., 2001).

Consequently, the combined effects of CBS, MTHFR, and MS genes on homocysteine levels were additive, and thus, gene-gene interaction may affect homocysteine levels (Dekou et al., 2001; Tsai et al., 2000; Feix et al., 2001). Studies that use multiple genetic markers will be of great interest for investigating the genetic basis of hyperhomocysteinemia.

NURSING IMPLICATIONS

CAD is a significant cause of death and its morbidity and mortality impact individuals' health and quality of life. Among emerging research for new markers of

cardiovascular risks, hyperhomocysteinemia is considered to be strongly related to risk for CAD. Thus, understanding homocysteine as a risk factor of CAD and current research related to this topic can contribute significantly to the field of nursing and help provide more appropriate care for the prevention and management of CAD.

Homocysteine is highly associated with vascular injury leading to atherogenesis and thrombogenesis, and thus it is considered a risk factor for CAD. While normal plasma homocysteine levels are currently defined as 5 to 15 $\mu\text{mol/L}$, it is suggested that levels be redefined as 11.4 $\mu\text{mol/L}$ for men and 10.4 $\mu\text{mol/L}$ for women as the normal cut-off point for increasing CAD risk. Homocysteine levels can be influenced by multiple genes coding for the enzymes involving in the regulation of homocysteine, such as CBS, MS, MTHFR, and MTRR. Findings from previous research (Gaughan et al., 2001; Zhang & Dai, 2001; Kluijtmans et al., 1999; Dilley et al., 2001; De Stefano et al., 1998; Jacques et al., 2002; Saw et al., 2001; Kluijtmans et al., 1997; Izumi et al., 1996; Passaro et al., 2001; Ou et al., 1998; Morita et al., 1997; Meleady et al., 2003; Christensen et al., 1997; Todesco et al., 1999; Ma et al., 1996; Schmitz et al., 1996; Fohr et al., 2002; Madonna et al., 2002; Folsom et al., 1998; Van Bockxmeer et al., 1997; Tokgozoglu et al., 1999; Meisel et al., 2001) has shown the association of these genes with homocysteine levels and risk of CAD. However, the association between MS, MTRR, and CBS genes and hyperhomocysteinemia was inconclusive (Gaughan et al., 2001; Zhang & Dai, 2001; Kluijtmans et al., 1999; Dilley et al., 2001; De Stefano et al., 1998), whereas polymorphisms of the MTHFR gene may be associated with homocysteine levels and/or risk of CAD (Christensen et al., 1997; Izumi et al., 1996; Jacques et al., 2002; Kluijtmans et al., 1997; Ma et al., 1996; Madonna et al., 2002; Meleady et al., 2003; Morita et al., 1997; Ou et al., 1998; Passaro et al., 2001; Saw et

al., 2001; Schmitz et al., 1996; Todesco et al., 1999; Tokgozoglu et al., 1999).

Individuals with the T677T allele of the MTHFR gene had significantly higher plasma homocysteine levels and higher risk for CAD. Although some literature indicated that hyper-homocysteinemia is considered an independent risk factor for CAD (Vollset et al., 2001; Morris, Jacques, Rosenberg, Selhub, Bowman, Gunter et al., 2000; Foody et al., 2000), it is premature to conclude that hyperhomocysteinemia is an independent risk for CAD based on this literature review. The T677T allele of the MTHFR gene may be associated with risk for CAD, and the relationship between the T677T allele of the MTHFR gene and risk for CAD may vary depending on ethnicity. The interaction between the C677T polymorphism of the MTHFR gene and folate levels affects homocysteine levels, and folic acid supplementation may be beneficial for individuals with the T677T allele of the MTHFR gene. In addition, the effects of the combination of gene polymorphisms in CBS, MTHFR, and MS on homocysteine levels are additive. Thus, the gene-environment interaction and gene-gene interaction can be important in determining the efficacy of therapeutic effects.

Consequently, the nurse can apply knowledge of hyperhomocysteinemia and the C677T polymorphism of the MTHFR gene as an additional indicator for CAD risk to nursing practice. When taking patient's health history and engaging in health assessment, it is important to assess if the patient has health problems that increase risk of hyperhomocysteinemia. It is more likely to have hyperhomocysteinemia if individuals have increasing age, male gender, renal impairment, increased coffee consumption, alcohol consumption, hypothyroidism, and use of drugs interfering with folate or vitamin B₆ metabolism. On the contrary, homocysteine levels decrease with the presence of hyperthyroidism. In addition, the association between homocysteine levels, the C677T

polymorphism of the MTHFR gene, and CAD risk varies depending on ethnicity. Individuals with malnutrition or gastrointestinal impairment resulting in malabsorption may have hyperhomocysteinemia. Thus, it is important to identify if the patient is at risk for hyper-homocysteinemia while conducting assessment and develop an appropriate plan to reduce and control modifiable risks.

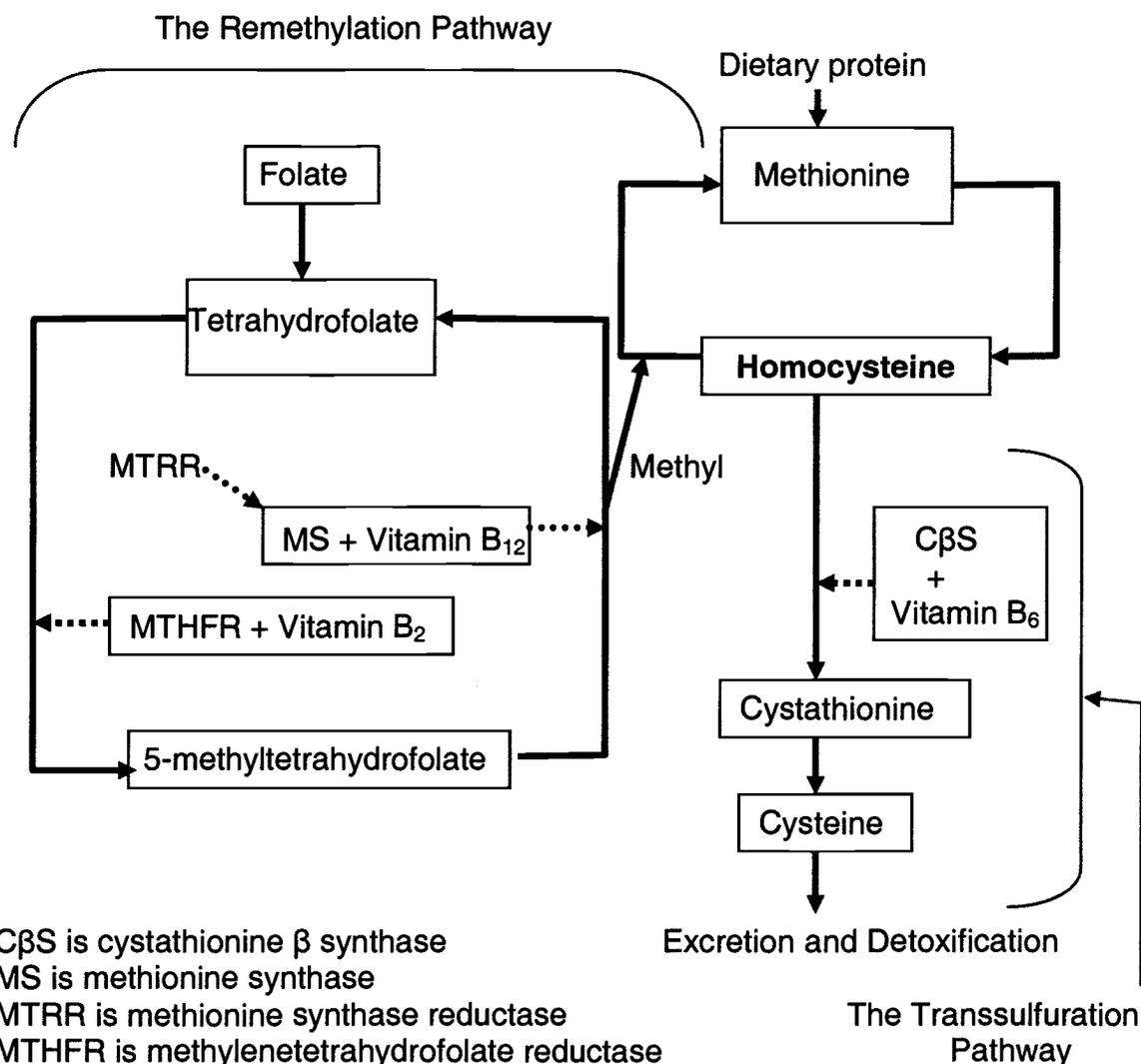
Dietary recommendations for folate intake are 180 to 200 μg a day in the United States (Food and Nutrition Board, National Academy of Science, 1989). Centers for Disease Control and Prevention (CDC) reported that the average daily intake of folate in the United States from 1988 to 1994 was 317 μg for men and 236 μg for women (CDC, October 2003). In 1996, food fortification with folic acid was mandated in the United States but updated statistical data regarding the average daily intake of folate are not available (CDC, 2003). Thus, it would be appropriate to carefully assess patient's diet and advise increased intake of vitamin B and/or folate fortified food, such as whole grain cereals, or use of supplementation if the patient has health problems that may contribute to hyperhomocysteinemia. It is also appropriate to recommend taking liver and green leafy vegetables as folate rich food resources.

It is premature to conclude that hyperhomocysteinemia and the C677T polymorphism of the MTHFR gene are an independent risk factor for CAD. Rather, it appears they are additive risk factors for CAD. However, early screening for homocysteine levels and genetic tests for C677T MTHFR gene polymorphisms would be helpful to provide a proper intervention if the patient has a familial history of premature CAD, health problems promoting hyperhomocysteinemia, or multiple risk factors for CAD. In addition, if the patient were identified to have C677T MTHFR gene polymorphisms, it would be appropriate to provide more frequent screening to prevent

progress of atherosclerotic alteration. Nurses should provide adequate information about the interpretation of clinical data of homocysteine levels and genetic testing in terms of CAD risk to help patients engage in appropriate therapeutic regimens and prevent patient's misconceptions. In addition, ongoing genetic research may provide new information about multiple genetic markers that contribute to hyperhomocysteinemia. Therefore, it will be necessary to monitor new research findings to update knowledge on hyper-homocysteinemia, genetic polymorphisms regulating homocysteine metabolism, and risks for CAD.

FIGURE 1

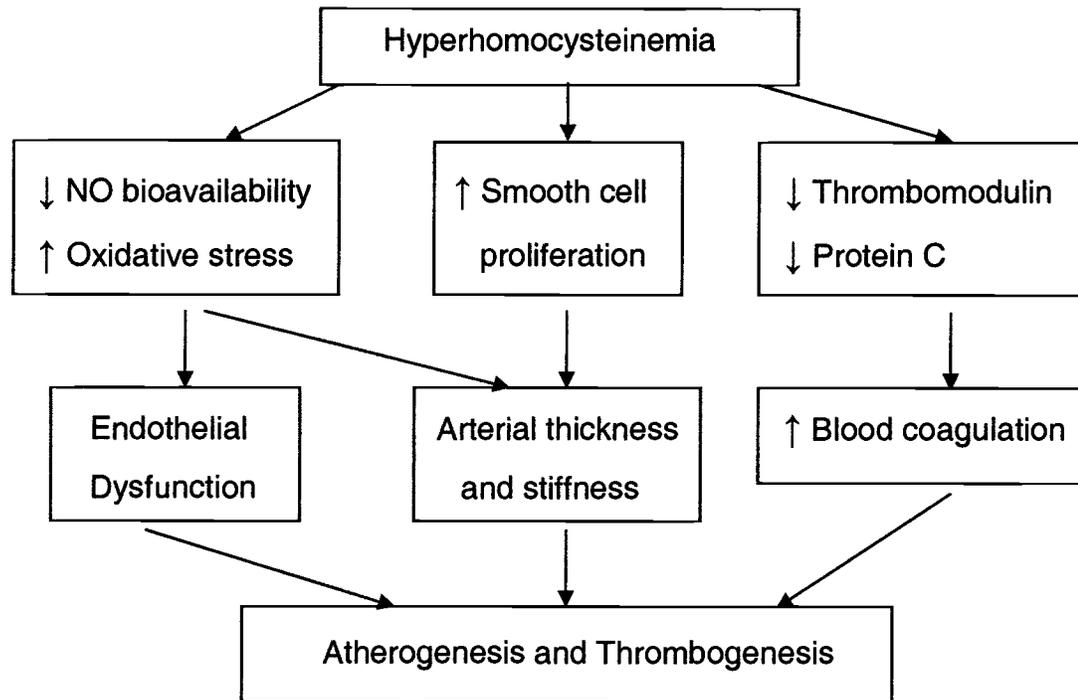
Homocysteine Metabolism



The figure illustrates homocysteine metabolism. In the transsulfuration pathway, homocysteine is metabolized to cystathionine and cysteine, and finally excreted and detoxified. Cystathionine β -synthase (CBS), which is a vitamin B₆-dependent-enzyme, catalyzes this reaction. In the remethylation pathway, homocysteine is remethylated to methionine. Methionine synthase (MS), which requires vitamin B₁₂ as a cofactor, is involved in this pathway to enable donating methyl from 5-methyltetrahydrofolate when it converts to tetrahydrofolate. Methionine synthase reductase (MTRR) activates methionine synthase (MS). This process also requires involvement of the methylenetetrahydrofolate reductase (MTHFR), which is a vitamin B₂ dependent enzyme, when tetrahydrofolate converts to 5-methyltetrahydrofolate to catalyze this reaction.

FIGURE 2

Pathogenesis of Hyperhomocysteinemia and Mechanisms of Vascular Injury



NO = nitric oxide

↓ means decreasing or limiting effects

↑ means increasing effects

This figure illustrates pathogenesis of hyperhomocysteinemia and mechanisms of vascular injury. Hyperhomocysteinemia limits the bioavailability of nitric oxide, increases oxidative stress, stimulates smooth cell proliferation, and decreases cell-surface thrombomodulin expression and inactivates protein C. Limited bioavailability of nitric oxide and increased oxidative stress result in endothelial dysfunction and arterial thickness and stiffness. Stimulated smooth cell proliferation also contributes to arterial thickness and stiffness. Reduced thrombomodulin expression and inactivated protein C result in increased blood coagulation. These conditions lead to atherogenesis and thrombogenesis.

TABLE 1

Polymorphisms in Methylenetetrahydrofolate Reductase (MTHFR) Gene and Plasma Homocysteine Levels and CAD Risk

Authors	Year	Purpose	Methods			Results			Effects of folate levels on the relationship between MTHFR gene polymorphisms & tHcy levels
			Sample size & gender	Age	Ethnicity	tHcy levels	Risk for CAD		
Jacques et al.	2002	C677T MTHFR gene polymorphisms vs. tHcy levels	450 men & women from the 5 th examination of Framingham	Mean (SD) 56.6 (0.8)	US population	TT allele had significantly higher tHcy levels than CC allele (p = 0.01) or CT alleles (p = 0.04).	NA	NA	TT allele had significantly higher (30%) tHcy levels than CC & CT alleles when plasma folate levels were below the median in the sample population
Saw et al.	2001	C677T MTHFR gene polymorphisms vs. tHcy levels.	216 men & 270 women (total 486)	45-74 years	Chinese in Singapore	TT allele had significantly higher (by 25 %) mean tHcy levels than CC & CT alleles (p < 0.0001)	NA	NA	TT allele had significantly higher (30%) tHcy levels than CC & CT alleles when plasma folate levels were below the median in the sample population

Table continued : Polymorphisms in MTHFR Gene and Plasma Homocysteine Levels and CAD Risk

Methods					Results			
Authors	Year	Purpose	Sample size & gender	Age	Ethnicity	tHcy levels	Risk for CAD	Effects of folate levels on the relationship between MTHFR gene polymorphisms & tHcy levels
Kluijtmans et al.	1997	C677T MTHFR gene polymorphisms vs. tHcy levels & risk of CAD	735 CAD cases & 1250 controls Men	Not indicated	Dutch population	TT allele & CT allele had significantly higher tHcy levels than CC allele	The OR of risk of CAD for TT allele vs. CC allele was 1.21 (0.87-1.68)	NA
Izumi et al.	1995	C677T MTHFR gene polymorphisms vs. CAD	250 CAD cases vs. 201 controls Men & women	Mean (SD) 60.4 (9.9) for cases 59.3 (8.1) for control	Japanese	NA	The OR of CAD for TT allele vs. CC & CT alleles was 2.13, (p = 0.0091)	NA
Passaro et al.	2001	C677T MTHFR polymorphisms vs. tHcy levels & the level of IMT in carotid arteries.	120 healthy women	55-80 years	Italian	TT allele had significantly higher tHcy levels than CC & CT alleles (p < 0.001).	The OR of ITM for TT allele vs. CC allele was 30.8 (p = 0.005)	NA

Table continued : Polymorphisms in MTHFR Gene and Plasma Homocysteine Levels and CAD Risk

		Methods				Results			
Authors	Year	Purpose	Sample size & gender	Age	Ethnicity	tHcy levels	Risk for CAD	Effects of folate levels on the relationship between MTHFR gene polymorphisms & tHcy levels	
Ou et al	1997	C677T MTHFR gene polymorphisms vs. tHcy levels & risk for CAD	214 CAD cases & 310 controls Men (86 %) & women (14 %)	33-77 years	Japanese	TT allele had significantly higher tHcy levels.	TT allele was significantly more frequent in cases than controls (p < 0.00003) The OR of CAD for TT allele in the MTHFR gene was 1.95	NA	
Morita et al.	1997	C677T MTHFR gene polymorphisms vs. CAD	362 CAD cases & 778 healthy controls Men	Mean (SD) 62 (9) for cases & 48 (11) for controls	Japanese	NA	TT allele was significantly more frequent in cases than controls (p = 0.0067). The OR of CAD for TT allele was 1.65	NA	
Meleady et al.	2003	C677T MTHFR gene polymorphisms vs. tHcy levels & risk for vascular diseases	711 CAD, CVD, & PVD cases vs. 747 controls	Less than 60 years	Dutch, Italians, Irish, Spanish, French, Belgian, Swedish, Portuguese, Norwegian	TT allele had significantly higher tHcy levels in cases & controls (p < 0.001)	Frequency of TT allele was not significantly different between cases & controls (p = 0.36) The adjusted OR of CAD for TT vs. CC alleles was 1.48 (1.0 -2.2)	TT allele & CAD risk were not significantly associated regardless of folate levels	

Table continued : Polymorphisms in MTHFR Gene and Plasma Homocysteine Levels and CAD Risk

		Methods				Results		
Authors	Year	Purpose	Sample size & gender	Age	Ethnicity	tHcy levels	Risk for CAD	Effects of folate levels on the relationship between MTHFR gene polymorphisms & tHcy levels
Christensen et al.	1997	C677T MTHFR gene polymorphisms vs. tHcy levels	152 patients with CAD & 121 controls 207 men & 66 women	less than 60 years	French Canadian	TT allele had significantly higher tHcy levels in men with CAD (p < 0.05)	Frequency of MTHFR polymorphisms was not significantly different between cases & controls	TT allele had significantly higher tHcy levels than CC allele in CAD cases when folate levels were below the median in the sample population (p < 0.05)
Todesco et al.	1999	C677T MTHFR gene polymorphisms vs. tHcy levels	138 CAD & PAOD cases & 118 controls men & women	21-95 years	Swiss	MTHFR gene polymorphisms had no significant different tHcy levels in total subjects. TT allele had significantly higher tHcy levels than other alleles in CAD & PAOD cases	Relationship between MTHFR gene polymorphisms & tHcy levels was not compared between cases & controls.	NA

Table continued : Polymorphisms in MTHFR Gene and Plasma homocysteine Levels and CAD Risk

		Methods				Results			
Authors	Year	Purpose	Sample size & gender	Age	Ethnicity	tHcy levels	Risk for CAD	Effects of folate levels on the relationship between MTHFR gene polymorphisms & tHcy levels	
Shmitz et al.	1996	C677T MTHFR gene polymorphisms vs. tHcy levels & risk for MI	190 MI cases & 188 control (age & sex matched) Men & women	less than 76 years	White, middle class US population	C677T MTHFR gene polymorphisms had no significant influence on tHcy levels in both cases & controls.	The adjusted OR of MI for TT allele vs. CC & CT alleles was 1.1 (0.6 – 2.2), (p = 0.8)	NA	
Ma et al.	1996	C677T MTHFR gene polymorphisms vs. tHcy levels & risk of MI	293 MI cases & 290 controls Men	40- 84 years	US population	TT allele had significantly higher age adjusted tHcy levels in both cases & controls.	The adjusted OR of risk of MI for CT allele vs. CC allele was 1.07 & The adjusted OR of risk of MI for TT allele vs. CC allele was 0.84	TT allele had a significantly elevated mean tHcy levels only when plasma folate levels were low	
Four et al.	2002	C677T MTHFR gene polymorphisms vs. tHcy levels	160 healthy, non-pregnant women	19-39 years.	German	No significant difference between MTHFR gene polymorphisms & tHcy levels	NA	TT allele had a significant tHcy lowering effect (20 %) after 4 weeks of folic acid supplementation (p < 0.05)	

Table continued : Polymorphisms in MTHFR Gene and Plasma Homocysteine Levels and CAD Risk

Methods				Results				
Authors	Year	Purpose	Sample size & gender	Age	Ethnicity	tHcy levels	Risk for CAD	Effects of folate levels on the relationship between MTHFR gene polymorphisms & tHcy levels
Madonna et al.	2001	C677T MTHFR gene polymorphisms vs. tHcy levels & risk for ischemic stroke	125 young adult ischemic stroke cases 262 controls men & women	Mean (SD) 38.4 (11.7) for cases & 36 (13.2) for controls	Italian	TT allele had significantly higher tHcy levels in cases than controls (p = 0.02)	Frequency of TT allele was not significantly different between cases & controls (p > 0.05)	NA
Folsom et al.	1998	C677T MTHFR gene polymorphisms vs. CAD	232 CAD cases & 537 controls men (75%) & women (25%)	45-64 years	US population	NA	The adjusted OR of risk of CAD for TT allele was 0.59 & the adjusted OR of risk of CAD for CT allele was 1.48. (p > 0.10)	NA
								C677T MTHFR gene polymorphisms had no significant influence on CAD incidence

Table continued : Polymorphisms in MTHFR Gene and Plasma Homocysteine Levels and CAD Risk

Methods					Results			
Authors	Year	Purpose	Sample size & gender	Age	Ethnicity	tHcy levels	Risk for CAD	Effects of folate levels on the relationship between MTHFR gene polymorphisms & tHcy levels
Van Bockxmeer et al.	1997	C677T MTHFR gene polymorphisms vs. risk of CAD & re-stenosis	555 cases (358 CAD cases & 197 past angio-plasty cases) & 143 controls Men & women	Less than 50 years for CAD cases & controls Mean (SD) 56 (8) for past angio-plasty cases	White Australian	NA	MTHFR gene polymorphisms had no significant association with risk for premature CAD or for re-stenosis after angioplasty	MTHFR gene polymorphisms had no association with tHcy levels in cases regardless of folate levels
Tokgozoglu et al.	1999	C677T, MTHFR gene polymorphisms vs. CAD	151 CAD cases vs. 91 controls No gender information was indicated	Mean (SD) 57 (11) for cases & 52 (11) for controls	Turkish	TT allele had significantly higher tHcy levels in cases than other alleles (p = 0.001) Relationship between genotype & tHcy levels was not examined in controls	Frequency of TT allele was not significantly different between cases & controls (p = 0.3) Multiple logistic regression indicated that TT allele was not an independent predictor of CAD	TT allele had a significantly elevated mean tHcy levels in CAD cases only when plasma folate levels were below the median in the sample population (p = 0.01)

Table continued : Polymorphisms in MTHFR Gene and Plasma Homocysteine Levels and CAD Risk

Methods				Results				
Authors	Year	Purpose	Sample size & gender	Age	Ethnicity	tHcy levels	Risk for CAD	Effects of folate levels on the relationship between MTHFR gene polymorphisms & tHcy levels
Meisel et al.	2001	C677T, A1298C, & T1317C MTHFR gene polymorphisms vs. tHcy levels & CAD	1000 CAD cases vs. 1000 controls	Age range was not addressed	German Caucasians	None MTHFR gene polymorphisms had significant influence on tHcy levels in both cases & controls	None MTHFR gene polymorphisms had significant influence on risk	NA

MTHFR = methylenetetrahydrofolate reductase
 MI = myocardial infarction
 CAD = coronary artery disease
 CVD = cerebral vascular disease
 PVD = peripheral vascular disease
 PAOD = Peripheral arterial occlusive disease

IMT = intima-media thickness
 tHcy total homocysteine
 OR = odds ratio
 SD = standard deviation
 US = the United States
 & = and
 Authors indicates only first author for each study

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