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# Methylene Tetrahydrofolate Reductase Gene and Coronary Artery Disease

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Hyperhomocysteinemia has been identified as a risk factor for coronary artery disease (CAD).<sup>1-9</sup> In a nested case control study in Norway on 21,826 subjects in general population, hyperhomocysteinemia was clearly identified as an independent risk factor for CAD with no threshold level.<sup>9</sup> Methylene tetrahydrofolate reductase (MTHFR) is a key enzyme in the methylation of homocysteine and its role in this pathway is outlined in Figure. Several inherited enzyme defects that can lead to elevated level of plasma homocysteine have been reported. Defective cystathionine b-synthase was the first to be extensively studied.<sup>10</sup> However, defective remethylation of homocysteine due to defective MTHFR was another cause of hyperhomocysteinemia leading to premature vascular disease.<sup>11</sup>

So far 10 mutations have been identified in the gene for MTHFR<sup>12,13</sup> that result into decreased activity of this enzyme, which varies to a great extent. However, a homozygous mutation of the MTHFR gene (677C 1; ala val) alters the highly conserved amino acid alanine to valine, which results in compromised activity of MTHFR and leads to reduced methylation of homocysteine, and hence, to hyperhomocysteinemia.<sup>14</sup> This variant of MTHFR had increased thermolability and was reported to be associated with the development of CAD.<sup>15</sup> Since then the polymorphism of the gene of this enzyme has been a subject of great interest and a number of investigators have reported different results from a number of different populations.

The objective of this review is to highlight the results of some of these studies and to emphasize the need to focus on the genetic architecture of CAD in a country, such as, Pakistan where this disease is quite common in the younger population.<sup>16</sup> As a general rule, the earlier the onset of a complex disease (which results from interactions between genetic and environmental factors), the greater the role of the genetic make-up.

## **Studies on MTHFR Polymorphism in Coronary Artery Disease**

During the past 10 years, there have been a number of studies in all the continents on investigating the effect of MTHFR gene polymorphism on the development of CAD.

Table . Prevalence of thermolabile MTHFR gene among normal healthy controls and patients with coronary artery disease in different populations.

No.	Mutation in MTHFR	Subjects' Country	Nature of Cardiovascular Disease	Study Population		Frequency of Genotype (%)		P-value / odds ratio	Study Group	Reference
				Patient (n)	Control (n)	Patient	Control			
1	C677T	Netherland	Prentature CVD	60	111	TT = 15 TC = 35	TT = 6 TC = 37.5	5	Khajtmans et al., 1996	24
2	C677T	UK	MI	310	222	T allele = 34	T allele = 35	NS	Adams et al., 1996	32
3	C677T	Ireland	CAD	131	105	TT = 17 TC = 43	TT = 7 TC = 43	0.02	Gallagher et al., 1996	33
4	C677T	USA(Boston)	MI	293	290	TT = 11 TC = 42	TT = 13 TC = 40	NS	Ma et al., 1996	26
5	C677T	Australia	CAD	565	225	TT = 11.6	TT = 10.7	NS	Wilcken et al., 1996	22
6	C677T	USA (Washington)	MI (<45 years Females)	79	386	TT = 10	TT = 12.7	NS	Schwartz et al., 1997	34
7	C677T	USA(Texas)	CAD	155	155	TT = 6	TT = 8	NS	Bogada & Marian, 1997	35
8	C677T	Japan	CAD	362	778	TT = 16	TT = 10	0.0067	Morita et al., 1997	36
9	C677T	Canada	CAD	152	123	TT = 14	TT = 10	NS	Christensen et al., 1997	13
10	C677T	Western Australia	CAD	555	143	TT = 9.9	TT = 10.5	NS	van Bockomew et al., 1997	31
11	C677T	Netherland	CAD	735	1250	TT = 9.5 TC = 44.6	TT = 8.5 TC = 42.2	Odd ratio 1.21	Khajtmans et al., 1997	20
12	C677T	USA(Utah)	MI	200	554	TT = 11.5 TC = 43.5	TT = 10.6 TC = 43.0	NS	Anderson et al., 1997	37
13	C677T	USA(Utah)	CAD	510	168	TT = 11.2 TC = 41.6	TT = 13.1 TC = 43.5	NS	Anderson et al., 1997	37
14	C677T	Japan	CHD	214	310	TT = 28.5	TT = 13.5	0.00003	Ou et al., 1998	38
15	C677T	Poland	MI	100	100	TT = 9.0	TT = 11%	NS	Garay et al., 1999	39
16	C677T	China	CAD	100	122	TT = 12.8	TT = 12.3	NS	Zheng et al., 2000	40
17	C677T	Turkey	Prentature MI (<45 years)	96	100	TT = 15.6	TT = 5	0.0016	Gulec et al., 2001	29
18	C677T A1298C	Poland	CAD(<50 year males)	161	211	1298C = 38.4	1298C=19.9	5	Szczeklik et al., 2001	30
19	C677T A1298C	USA	CAD	772	329	TT (677)= 10.9 CC (1298) = 11.7	TT (677)=12.5 CC (1298) = 7.9	NS	Hanson et al., 2001	41
20	C677T A1298C	Germany	CAD	1000	1000	T allele = 30.81 298C = 32.8 allele	T allele = 32.3 1298C=33.3 allele	NS	Meindl et al., 2001	42
21	C677T	Slovak	MI	71	71	TT = 12.1	TT = 5.4%	NS	Raslovaet et al., 2001	43

Table summarizes the results of these studies in terms of prevalence of MTHFR gene variants in CAD patients and normal healthy controls in different populations. In addition to these studies, polymorphism of MTHFR gene has also been recently reported in a Chinese population<sup>17</sup> and in a Russian population in Western Siberia.<sup>18</sup> Both of these studies indicated little or no correlation of MTHFR T677 allele with coronary heart disease. In a recent study by Nakata et al<sup>19</sup>, it has been postulated that the Val allele of MTHFR increases the relative risk for thrombosis homocysteine levels, although a blood pressure attenuates the disease. In a pervious study by Kang et al., the prevalence of thermolabile MTHFR among CAD patients (n=212) compared to controls (n 202) was found to be statistically significant (17% vs 5% and p<sup>15</sup>).

### Effect of MTHFR Mutation on Homocysteine

In view of the above mentioned studies, it becomes quite evident that the frequency of homozygotes (TT) genotype varies among different populations. The overall effect of this mutation on homocysteine concentration in plasma depends on study design, inclusion criteria, ethnic background, age and vitamin intake of the population.<sup>20</sup> With the exception of a few studies<sup>21,22</sup> most studies indicate an association of homozygous genotype (TT) with hyperhomocysteinemia.

Since folate status is considered to be an important modulator of homocysteine level in homozygous individuals<sup>23-26</sup>, it is possible that the different homocysteine levels in homozygotes (TT) reported in different studies may have been due to different intakes of folic acid by the study populations. Therefore, determination of folate level could be an important factor for studying the effect of genotype on homocysteine levels. Taken together, the facts that hyperhomocysteinemia is an established risk factor for the development of CAD and that reduced MTHFR activity is most commonly observed among TT homozygotes suggests that an association exists between the frequency of this genotype and risk of CAD.

#### **MTHFR Mutation and Risk of CAD**

A closer look at some of the studies would reveal that a stringent criteria for the selection of controls might be having a bearing on the outcome of that study. For example, Kang et al.<sup>15</sup>, who have reported a clear association between homozygotes for the variant enzyme and the development of CAD, recruited healthy controls with no history and clinical evidence of arterial occlusive disease. On the other hand, in a study by Kluijtmans et al., that shows only a modest association, control subjects recruited from the general population possibly included some patients with a positive history of CAD.<sup>20</sup> This might have diluted the effect of homozygous TT genotypes on the development of CAD.

Although a number of studies have failed to show any association of homozygous TT genotypes with the risk of CAD, a meta-analysis on 8 different case-controlled studies on the thermolabile MTHFR variant in CAD indicated that the TT genotype is a modest risk factor for CAD.<sup>20</sup>

#### **MTHFR Mutation and Age**

The low frequency of a genetic risk factor in a population can contribute to the population's longevity. If the MTHFR C677T mutation is a risk factor for CAD, then its prevalence should be less in the older population. In a study by Matsushita et al., the frequency of homozygous MTHFR mutation in younger Japanese population (<54 years) was found to be 19% compared to 7% in the older group (<90 years).<sup>27</sup> The difference was statistically significant especially among males (P= 0.006) indicating that this mutation varies with age in the normal population, and that younger people are at a greater risk of developing CAD in such a population.

This trend was also observed by Faure-Delanf et al., who reported a decreased frequency of MTHFR mutated allele among French centenarians (age >100 yr; 13.3%) and nonagenarians (age >90 years; 11.4%) compared to controls (age 20 - 70 yr; 18.5%).<sup>28</sup> Keeping this in view, it would be logical to assume that in studies involving older populations, the frequency of the variant gene would be low both in cases and controls and, therefore, any weak association of the variant allele with CAD would be masked. It is noteworthy that most studies showing an association between the MTHFR C677T mutation and CAD were on relatively younger populations. For example, an investigation on Turkish patients less than 45 years old reported a significant difference in the

frequency of the variant MTHFR amongst cases and controls.<sup>29</sup> Another study reported a similar association on Polish patients who were less than 50 years of age.<sup>30</sup> This notion requires further confirmation by carrying out studies on younger populations of patients.

### **MTHFR Mutation, Folate Status and Risk of CAD**

Frosst et al have identified a region in the human dihydrofolate reductase gene that bears a homology with MTHFR.<sup>14</sup> This region of MTHFR might also be involved in folate binding, and the enzyme may be stabilized in the presence of folate. Therefore, with better folate status, even variant MTHFR T677 may not be associated with hyperhomocysteinemia, suggesting that homozygous IT genotypes may not necessarily be associated with increased risk of CAD in individuals with high folate levels. This possibility has also been highlighted by van Bockxmeer et al. in their study on Western Australian population<sup>31</sup>, as well as by Ma et al. in their study on well-nourished US physicians.<sup>26</sup>

In a third world country like Pakistan, where folate deficiency is quite common and where CAD is quite prevalent in younger population<sup>16</sup>, it would be important to determine the role of the MTHFR gene in the development of CAD.

Such a study will not only be of significance in unraveling the genetic architecture of CAD in our population but may also provide an understanding of the role of folate deficiency (if any) in the development of CAD.

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