

Methylenetetrahydrofolate Reductase: A genetic marker for CVD?

by

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Abstract: As the analysis of the genome continues, today's medical students will soon be confronted with an increasing number of available genetic tests. One currently being offered is the cardiogenomic profile, which includes several genetic measures. I researched the MTHFR 677 C->T base pair substitution as an example of the complexity of analyzing the data from such tests. I found that in this case the data does not support testing for this genetic marker in the US population and that the public could be better served through promotion of folate intake and testing of serum homocysteine concentrations in at-risk populations.

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Background:

On October 1, 1990 the Human Genome project officially began. This was begun as a great adventure, with great hopes. Indeed, at the time the project began, the technology did not yet exist to perform such a great task, certainly not within the 15-year timetable proposed. But as scientists collaborated the project finished way ahead of schedule- in April of 2003. All held great hopes for the medical advances that could eventually come from this wealth of knowledge; in the July 1, 1999 issue of the New England Journal of Medicine, Dr. Francis S. Collins wrote that "gene isolation provides the best hope for

understanding disease at its most fundamental level" (Fig. 1).

As the leading cause of death in the US, and a pathology affecting 62 million Americans, cardiovascular disease is an obvious focus of further investigation using this new genomic knowledge. In fact, companies such as Genovations are already offering CardioGenomic Profiles (Fig. 2), which tout a new personalized preventive medicine based on effectiveness for the individual rather than preventive measures based on obtaining positive results for the greatest number of people. Undoubtedly, these tests will become more frequent with each year as the medical students of today move into their careers.

Rather than investigate all of the identified genetic markers for cardiovascular disease (CVD), I chose to study one in depth- the substitution of a thymine for a cytosine in base number 677 in the methylenetetrahydrofolate reductase (MTHFR) gene, which is theorized to cause an increase in blood homocysteine levels. I sought to take a critical look at the possible clinical applications for such a genetic finding in an individual.

Homocysteine:

The first indication of high homocysteine levels as a cardiovascular risk factor came in 1964 with Mudd's research into defects in cystathionine B-synthase (CBS). In 1975, McCully and Wilson formed the hypothesis that moderate homocysteine elevations could put a person at risk for atherosclerosis after finding similar arterial damage in patients with two different diseases characterized by hyperhomocysteinemia. In 1975, Wilcken and Wilcken showed that coronary artery patients were more likely to have problems metabolizing homocysteine than a control group. Since then, homocysteine levels have been studied more extensively as indicators of increased heart disease risk, although the exact mechanism is unknown.

One leading theory is based on the fact that H_2O_2 is produced when homocysteine is oxidized to form homocystine. Glutathione peroxidase, the enzyme which serves as the primary mechanism for protecting endothelial cells from reactive oxygen species, has reduced concentrations in the presence of high tHcy, resulting in greater oxidative stress on blood vessels. In fact, the negative effects of exposure to high homocysteine levels were

minimized when bovine aortas were first pretreated with vitamins C and E, potent antioxidants.

This theory has not been conclusively proven definitive, but we do know that today up to 40% of all patients with coronary atherosclerosis have hyperhomocysteinemia. Therefore, if a base pair defect was found to reliably predict high tHcy, this would indeed be a significant finding, particularly if a drug or dietary change could circumvent the affected enzyme and return homocysteine levels to normal.

Homocysteine metabolism:

When homocysteine is produced by the metabolism of methionine, it can then follow 3 possible pathways (Fig. 3). It can be irreversibly degraded to cysteine in the liver and kidneys using the enzyme CBS. A second option is also restricted to the liver and kidneys; the remethylation of homocysteine to methionine by betaine and the action of betaine-homocysteine methyltransferase. The third pathway, which is not restricted to specific organs, is the remethylation of homocysteine to methionine by methionine synthase. This pathway will be the focus of the present treatise, because in order for this pathway to take place, 5-methyltetrahydrofolate must be produced from 5,10-methylene-tetrahydrofolate by methylenetetrahydrofolate reductase (MTHFR).

677 C->T and its effect on MTHFR function:

The most direct change in MTHFR caused by the base pair defect is the substitution of an alanine amino acid with a valine, but what implications does this have for enzyme function?

According to several studies in the late 1990s, patients with 677CT maintain 71% of MTHFR function, while 677TT patients have 34% of the MTHFR function of a 677CC patient. These are significant percentages, but the difference in blood homocysteine concentration (tHcy) between a homozygous 677CC and a 677TT patient is only an increase of 2.6uM. This is a small amount when one considers that the normal range is up to 12uM and a moderate tHcy is 12-30uM. The small change in concentration after such a significant loss in enzyme functionality can probably be explained by increased action by the two other possible pathways.

The question that remained was if this defect alone could cause a statistically significant difference in cardiovascular health. Due to the small difference in tHcy, this would require a study involving about 12,000 cases and controls.

Although an independent study of this magnitude is hard to conduct and certainly involves its own complications, a comparable quantity of data from diverse studies was synthesized by Klerk et al. in a meta-analysis released in JAMA in October 2003. Their results indicated that an individual with the 677 TT genotype has a 16% higher risk of developing coronary heart disease (CHD) than someone who has the 677 CC genotype.

Another interesting aspect of this study was that this seemed to affect only those populations with low folate status, meaning a low mean folic acid intake. The final analysis was that “the present study provides some indirect evidence of the likely benefits of increasing population mean levels of folate” but that “provided that folate status is

adequate, there is little value of screening for MTHFR 677 C->T.”

Other factors affecting tHcy levels:

When looking at the levels of tHcy, one must not forget about the many other variables involved. For instance, genetic abnormalities in other enzymes are often involved, including the aforementioned CBS enzyme.

There are several epidemiological and lifestyle factors as well. Males, postmenopausal females, and older populations were all found to have a higher risk of hyperhomocysteinemia. The tHcy levels were also higher in those who consume coffee and smokers. Alcohol consumption was shown to have a J-shaped correlation to high tHcy levels; nondrinkers had higher average levels than those whose consumption of alcohol was moderate, but heavy drinkers were the most likely to have the highest levels.

Drugs are also a factor. Antifolates such as methotrexate cause increased tHcy because they inhibit the conversion of dihydrofolates to tetrahydrofolates by dihydrofolate reductase. Nitrous oxide inactivates methionine synthase, which prevents its use to facilitate the conversion of homocysteine to methionine. There are also indications that some antiepileptic medications could contribute to high tHcy levels, but this has been researched to a lesser extent.

Finally, several diseases are causative of high tHcy. These include intestinal diseases such as ulcerative colitis, which could cause a folate or B12 deficiency, depriving the body of necessary coenzymes to process homocysteine. Cancer is also a contributing factor, which is partially due to methotrexate use, as well as the increased demand for

methyl groups. Kidney disease is also an important consideration, since it interferes with the renal excretion and resorption of non-protein bound homocysteine.

Conclusions:

While there are many possibilities for future breakthroughs with genomic profiling, the currently established research shows little evidence behind testing the general population for the 677 C->T base pair substitution on the MTHFR gene. In addition, one must remember that hyperhomocysteinemia is a multifactorial condition. Judging by the current evidence, it makes more sense to encourage smoking cessation and discourage heavy drinking. For older persons and those taking the aforementioned drugs or with the above diseases, the direct measurement of tHcy would be much more effective than testing for risk factors on a genomic level.

From this information, one clearly cannot draw conclusions about the other markers included in the cardiogenomic

profile or judge its overall clinical utility. However, the MTHFR gene serves as a useful example of the complexity of analyzing data from such profiles.

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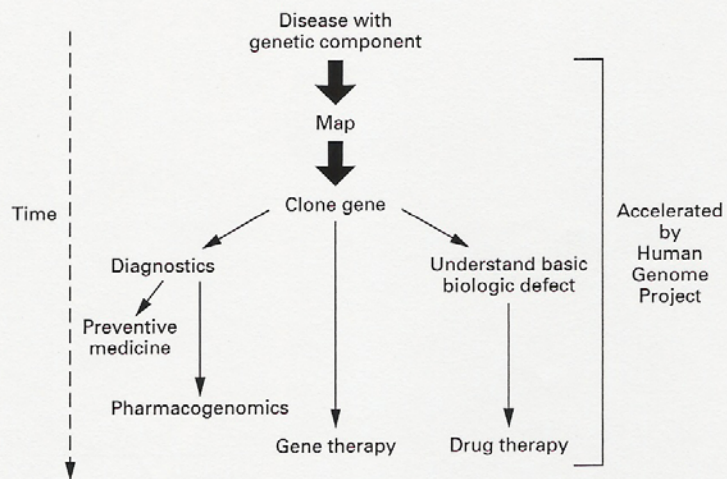


Figure 1. Steps Involved in the Genetic Revolution in Medicine.

Uncovering the genetic contributions to an illness is accomplished by cloning the gene for the disease, with the use of the tools of the Human Genome Project. Once the contributing genes and their disease-predisposing variants have been identified, diagnostic tests can be developed to predict future risk — but these tests are most effective when a preventive strategy is available to reduce the risk in persons found to be predisposed to a particular disease. Another rapidly developing application of diagnostics is pharmacogenomics, the prediction of responsiveness to drugs. Ultimately, the real payoff of genetic research will be the development of new gene therapies and drug therapies, but they will generally require many more years of intensive research.


Figure 1- credit: Collins, F. S. *New England Journal of Medicine*. **341**, 31 (1999)


GENOVATIONS


Optimizing your Genomic Potential

Cardio Genomic summary - part 1

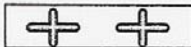
Cholesterol Regulation & Atherosclerosis

APO E 

CETP 

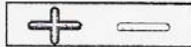
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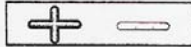
Methylation


MTHFR 

Cardio Genomic summary - part 2


Hypertension


GNB3 

AGT 

AGTR1 

Coagulation

Factor 2 

Factor 5 

Reduction-Oxidation Balance


CYBA*8 

Figure 2- credit: Genovations, Inc., www.genovations.com

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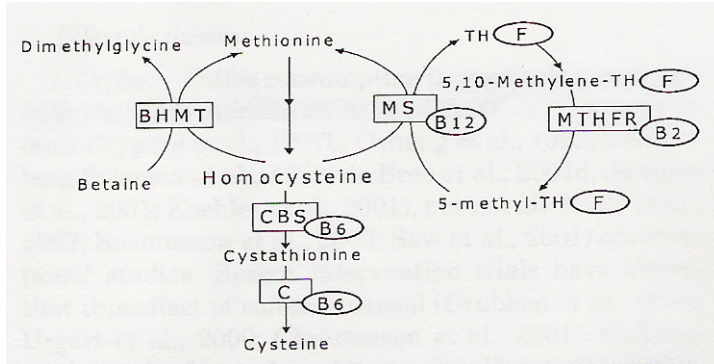


Figure 3- credit: De Bree, A.V. et al. *Pharmacological Reviews*. **54**, 601 (2002)

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