Am. J. Hum. Genet. 58:17–20, 1996

INVITED EDITORIAL
Nutritional Ecogenetics: Homocysteine-Related Arteriosclerotic Vascular Disease, Neural Tube Defects, and Folic Acid

Arno G. Motulsky

Departments of Medicine and Genetics, University of Washington, Seattle

In the search for genetic factors underlying premature coronary heart disease, most attention has been given to genes affecting lipids. However, coronary artery disease often cannot be explained by known lipid genes. Since the 1970s, an increasing number of reports have suggested that elevated levels of the amino acid homocysteine (hyperhomocysteinemia) were associated with various kinds of arteriosclerotic vascular disease. Our recent meta-analysis of 27 relevant publications summarizes these findings (Boushey et al. 1995). Homocysteine is an independent risk factor for arteriosclerotic vascular disease unrelated to hyperlipidemia, hypertension, diabetes, and smoking. As with cholesterol levels, the risk is graded; that is, the risk rises with increasing homocysteine levels. We calculated that 10% of the population's risk for coronary artery disease appears to be attributable to elevated homocysteine levels. With each 5 μmol/liter rise in total homocysteine levels, the risk for coronary artery disease was increased by 60% for men and by 80% for women. (males—relative risk 1.6, 95% confidence intervals 1.4–1.7; females—relative risk 1.8, 95% confidence intervals 1.4–2.3). These numbers were of the same order of magnitude as the risk figures for coronary artery disease associated with a 20 mg/dl increase in total cholesterol levels (Willett 1990).

Are Elevated Homocysteine Levels Caused by Heterozygote Status for Homocystinuria?

A well-known genetic cause of very high homocysteine levels is the autosomal recessive inborn error of homocystinuria, which is associated with both thrombosis and arteriosclerosis (Mudd et al. 1995). It has been postulated, therefore, that heterozygotes for homocystinuria (frequency ~1/200–1/300 in the United States) with slightly elevated homocysteine levels might have a higher frequency of arteriosclerotic vascular disease (McCully 1969; Wilcken and Wilcken 1976). Homocystinuria is caused by deficiency of cystathionine synthase. Enzyme assays among vascular disease patients did in fact often reveal a lower activity, suggesting heterozygosity (Boers et al. 1985; Clarke et al. 1991). However, many aged, ostensibly healthy individuals (who presumably were not heterozygotes for homocystinuria) frequently had quite low cystathionine synthase levels, making it difficult to be certain about heterozygote status (Gartler et al. 1981). More recently, the earlier-reported finding of lower cystathionine synthase levels could not be replicated by retesting of the same individuals (Engbersen et al. 1995). Furthermore, in a large-scale study of parents and grandparents of homocystinuria patients, there was no increased frequency of strokes or heart attacks, as compared with appropriate controls (Mudd et al. 1981). However, a less-than-threefold relative risk would not have been detectable.

The isolation of the cystathionine synthase gene and its mutations permits the molecular detection of heterozygote status for homocystinuria (Mudd et al. 1995). At least 17 different mutations have been found (Kluijtmans et al. 1995). However, a single unique mutation may account for most cases in well-defined populations with a restricted number of ancestors. Thus, 70% (Whitehead et al. 1994) and 50% (Kluijtmans et al. 1996) of the homocystinuria mutations in Ireland and Holland, respectively, were represented by a single type that differed in these countries. One hundred Irish patients with premature vascular disease were tested for the common Irish mutation of cystathionine synthase, and not a single heterozygote could be detected (Whitehead et al. 1994). As reported in this issue of the *Journal* (Kluijtmans et al. 1996), no heterozygotes for the common Dutch mutation for homocystinuria were found by molecular testing of 60 patients with various types of premature vascular diseases. Additional data reporting failure to find heterozygotes for homocystinuria among patients with elevated homocysteine levels are accumulating (see Kozich et al. 1995; Kluijtmans et al. 1996).

Received November 13, 1995; accepted for publication November 13, 1995.

Address for correspondence and reprints: Dr. Arno G. Motulsky, Departments of Medicine and Genetics, University of Washington, Box 356423, Seattle, WA 98195.

This article represents the opinion of the author and has not been peer reviewed.

0002-9297/96/5801-0003$02.00

A Polymorphism of a Folic Acid–Related Enzyme as a Cause of Homocysteine Elevations

What, then, are the reasons for the hyperhomocysteinemia seen in patients with vascular disease? Even marginal nutritional deficiency of folic acid and, possibly, of vitamin B_{12} or vitamin B_6 might be involved, and other genetic causes of hyperhomocysteinemia exist. Severe deficiency of methylene tetrahydrofolate reductase (MTHFR)—an enzyme involved in folic-acid metabolism—causes elevated homocysteine levels, appears to be inherited as a very rare autosomal recessive disease, and is usually associated with developmental delay (Rosenblatt 1995). However, even heterozygotes for this defect are uncommon. Of greater pertinence was the finding of a common thermolabile polymorphism of MTHFR in the homozygous state in 5% of Caucasian controls (Kang et al. 1988*a*, 1988*b*). The gene for MTHFR has recently been cloned (Goyette et al. 1994), and the DNA mutation responsible for the heat-labile variant has been identified (Frosst et al. 1995). The frequency of homozygotes for this polymorphism was 12% among French Canadians (Frosst et al. 1995), and the range was 12%–15% in populations of European, Middle Eastern, and Japanese origin (S. Deeb and A. G. Motulsky, unpublished observations) but was distinctly lower in Finnish (5.4%) (S. Deeb and A. G. Motulsky, unpublished observations) and Dutch populations (5.2%) (Kluijtmans et al. 1996). African Americans had a very low frequency (1.4%) (S. Deeb and A. G. Motulsky, unpublished observations). Fasting homocysteine levels were almost twice as high in homozygotes for this variant, compared with those not carrying the polymorphic allele (Frosst et al. 1995). Homocysteine levels in heterozygotes were indistinguishable from those in noncarriers.

Kang et al. (1988*a*) first reported a more-than-threefold increase in the homozygous frequency of the MTHFR polymorphism among patients with coronary artery disease, compared with controls (17% vs. 5%), and demonstrated that the elevated homocysteine levels associated with the MTHFR variant could be reduced by folic-acid administration (Kang et al. 1988*b*, 1991). These investigators later showed an increased frequency of this variant in patients with more severe degrees of coronary artery stenosis (Kang et al. 1993). In a recent study, 28% of hyperhomocysteinemic patients with premature vascular disease were shown to be homozygous carriers of the thermolabile MTHFR variant as determined by biochemical methods (Engbersen et al. 1995). Kluijtmans (1996) reported on 60 Dutch patients with premature vascular disease (myocardial infarction, cerebral vascular disease, and peripheral vascular disease) who did *not* have any of the known risk factors for coronary heart disease, such as hyperlipidemia, hypertension, and diabetes. Fifteen percent of this group, as compared with 5.2% of controls, were homozygote carriers for the variant, on the basis of molecular testing. However, our ongoing studies do not show an increased frequency of the homozygous MTHFR variant among unselected coronary artery disease patients of unknown folate status (S. Deeb and A. G. Motulsky's laboratory's unpublished observations).

Recent work on a large U.S. population (Rozen et al. 1995) demonstrates that, although mean homocysteine levels were statistically increased when all homozygote carriers of the MTHFR variant were considered, this increase was limited to those homozygotes whose plasma folic-acid levels were below the median, and it was not seen in the 50% of the population with folic-acid levels above the median. This finding is an important example of nutritional/genetic interaction, in showing that homocysteine elevations occurred only when folic-acid nutrition was less than optimal—that is, among persons in the lower one-half of the distribution of plasma folic-acid levels.

The methylenetetrahydrofolate variant is not likely to account for all instances of genetically determined hyperhomocysteinemia. Search for additional polymorphisms at the MTHFR locus, as well as for polymorphisms and heterozygote states of other enzymes whose malfunction may lead to homocysteinemia, will be of great interest. Such enzymes include another folic acid–related enzyme, 5-methyl tetrahydrofolate-homocysteine methyltransferase (methionine synthase) (Rosenblatt 1995), the 5-methyl tetrahydrofolate receptor (R. Hinnell, personal communication), and various vitamin B_{12}–related enzymes, such as those involved in absorption and cellular uptake of vitamin B_{12}, release of vitamin B_{12} from lysosomes, failure to convert vitamin B_{12} to methyl or adenosyl vitamin B_{12}, and failure to convert vitamin B_{12} to methyl-B_{12} (Fenton and Rosenberg 1995). All these conditions in the homozygote state are associated with elevated homocysteine levels. However, much additional biochemical and molecular work will need to be done before a specific search for polymorphisms affecting these reactions can be instituted.

Prevention of Vascular Disease by Folic Acid?

A variety of studies have shown that homocysteine elevations are reversible by increasing folic-acid intake. Our meta-analysis of 11 studies of folic-acid effects on homocysteine levels suggests that 200 μg folic acid/d will reduce homocysteine levels by ~4 μmol/liter (Boushey et al. 1995). Various biologic data and the epidemiologic evidence suggest that homocysteine itself is the cause, rather than an epiphenomenon, of arteriosclerosis (Boushey et al. 1995). It is therefore likely (but has not been demonstrated) that reduction of homocysteine lev-

els will decrease the morbidity and mortality of arteriosclerotic vascular disease. On the basis of our assessment of the role of folic acid in reduction of raised homocysteine levels, and on the basis of the quantitative effects of homocysteine elevations on the pathogenesis of coronary artery disease, we have calculated that 9% of male and 54% of female coronary artery deaths in the United States (~50,000 total deaths/year) could be prevented by fortification of flour and cereal products by using 350 μg folic acid/100 g food. Further benefits would of course be expected in reduced morbidity of coronary artery disease, as well as in lessened mortality and morbidity of strokes and peripheral vascular disease. Somewhat smaller reductions in vascular disease (e.g., prevention of 40,000 coronary artery disease–related deaths/year) would occur with fortification at 140 μg (Boushey et al. 1995, and in press). Supplementation with folic-acid tablets (400 μg/d)—or simply encouraging better dietary intake of folate by the eating of appropriate foods, such as green vegetables, beans, and orange juice—would have lesser but still sizable effects, depending on the proportion of the population complying with such recommendations.

Homocysteine and Neural Tube Defects

The folic acid–homocysteine relationship is of additional great interest because of its relevance for the etiology and prevention of neural tube defects. There is excellent evidence that folic acid given before and during the first 4 wk of pregnancy can prevent ≥50% of neural tube defects (Centers for Disease Control 1992). Recently, mothers of infants with neural tube defects were shown to have increased homocysteine levels (Mills et al. 1995). Furthermore, the frequency of the homozygous MTHFR polymorphism was two to three times increased among Dutch mothers, fathers, and patients with neural tube defect (5% of controls, vs. 16% of mothers, 10% of fathers, and 13% of patients) (van der Put et al. 1995). Homozygotes for the MTHFR variant from this population had elevated mean homocysteine levels (17.1 vs. 13.3 μmol/liter) and lower folic-acid levels (9.5 vs. 12.8 μmol/liter). The lower frequency (~1% homozygotes) of the MTHFR polymorphism among the African American population (S. Deeb and A. G. Motulsky, unpublished observations) is of some interest, in view of the lower incidence of neural tube defects among Blacks, and provides indirect evidence for the likely etiologic relationship of the MTHFR polymorphism and neural tube defects.

Policy Implications

With the development of genetic and other biomarker tests, strategies of disease prevention directed at those at high risk for a given disease are increasingly under discussion. Thus, by screening for cholesterol and other lipid factors, individuals at high risk for coronary artery disease can be identified for appropriate dietary or medicinal intervention. At the same time, population strategies that counsel everyone to eat a prudent diet with attention to lower intake of saturated fat and cholesterol have merit. Appropriately controlled trials on the role of folic acid in preventing vascular diseases should have a very high priority. Population screening to identify homozygote carriers for the MTHFR polymorphism are premature at this time, but testing among affected families should be considered as soon as additional studies have demonstrated the validity of such approaches. Fortunately, the molecular tests for this MTHFR polymorphism are simple and inexpensive. Research on other genetic variants that may raise homocysteine levels, such as folic-acid and vitamin B_{12}–related enzymes and receptors, are strongly recommended, to elucidate the role of various genetic factors in raising homocysteine levels and in interacting with vitamin nutrition.

At the present time, a population approach—in which everyone, particularly women of childbearing age, is encouraged to increase folic-acid intake—makes good sense. Food fortification at 350 μg/100 g food would be ideal, but fortification at 140 μg/100 g would be acceptable. Implementation of food fortification with folic acid has been slow because folic acid may mask the hematologic manifestations of pernicious anemia due to vitamin B_{12} deficiency while the neurologic symptoms, which may include spinal cord damage, can progress (Savage and Lindenbaum 1995). With the 140-μg fortification scheme, such damage is highly unlikely. Furthermore, the addition of 1 mg of vitamin B_{12} to all vitamin supplements containing 400 μg folic acid would almost surely prevent such potential problems. The folic acid–homocysteine relationship emerges as a key example of nutritional ecogenetics and points to a time when a unique and specific genetic profile may be established as a basis to advise about an individual's optimal diet for prevention of disease.

References

Boers GHJ, Smals AGH, Trijbels JMF, Fowler B, Bakkeren JAJM, Schoonderwaldt HC, Kleijer WJ, et al (1985) Heterozygosity for homocystinuria in premature peripheral and cerebral occlusive arterial disease. N Engl J Med 313:709–715

Boushey CJ, Beresford SSA, Omenn GS, Motulsky AG (1995) A quantitative assessment of plasma homocysteine as a risk factor for vascular disease: probable benefits of increasing folic acid intakes. JAMA 274:1049–1057

———. A meta-analysis of plasma homocysteine as a risk factor for arteriosclerotic vascular disease and the potential preventive role of folic acid. In: International Congress on

Homocysteine Metabolism: From Basic Science to Clinical Medicine. Kluwer Academic, Norwell, MA (in press)

Centers for Disease Control (1992) Recommendations for the use of folic acid to reduce the number of cases of spina bifida and other neural tube defects. MMWR Morb Mortal Wkly Rep 41:1–7

Clarke R, Daly L, Robinson K, Naughten E, Cahalane S, Fowler B, Graham I (1991) Hyperhomocysteinemia: an independent risk factor for vascular disease. N Engl J Med 324:1149–1155

Engbersen AMT, Franken DG, Boers GHJ, Stevens EMB, Trijbels FJM, Blom HJ (1995) Thermolabile 5,10-methylenetetrahydrofolate reductase as a cause of mild hyperhomocysteinemia. Am J Hum Genet 56:142–150

Fenton WA, Rosenberg LE (1995) Inherited disorders of cobalamin transport and metabolism. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic and molecular bases of inherited disease. McGraw-Hill, New York, pp 3129–3149

Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GHJ, et al (1995) A candidate genetic risk factor for vascular disease: a common mutation in methylene tetrahydrofolate reductase: isolation of cDNA, mapping and mutation identification. Nat Genet 7:195–200

Gartler SM, Hornung SK, Motulsky AG (1981) Effect of chronologic age on induction of cystathionine synthase, uroporphyrinogen I synthase, and glucose-6-phosphate dehydrogenase activities in lymphocytes. Proc Natl Acad Sci USA 78:1916–1919

Goyette P, Sumner JS, Milos R, Duncan AMV, Rosenblatt DS, Matthews RG, Rozen R (1994) Human methylenetetrahydrofolate reductase: isolation of cDNA, mapping and mutation identification. Nat Genet 7:195–200

Kang S-S, Passen EL, Ruggie N, Wong PWK, Sora H (1993) Thermolabile defect of methylenetetrahydrofolate reductase in coronary artery disease. Circulation 88:1463–1469

Kang S-S, Wong PWK, Susmano A, Sora J, Norusis M, Ruggie N (1991) Thermolabile methylenetetrahydrofolate reductase: an inherited risk factor for coronary artery disease. Am J Hum Genet 48:536–545

Kang S-S, Wong PWK, Zhou J, Sora J, Lessick M, Ruggie N, Grcevich G (1988*a*) Thermolabile methylenetetrahydrofolate reductase in patients with coronary artery disease. Metabolism 37:611–613

Kang S-S, Zhou J, Wong PWK, Kowalisyn J, Strokosch G (1988*b*) Intermediate homocysteinemia: a thermolabile variant of methylenetetrahydrofolate reductase. Am J Hum Genet 43:414–421

Kluijtmans LAJ, Blom HJ, Boers GHJ, Van Oost BA, Trijbels JMF, van den Heuvel LPWJ (1995) Two novel missense mutations in the cystathionine β-synthase gene in homocystinuric patients. Hum Genet 96:249–250

Kluijtmans, LAJ, van den Heuvel LPWJ, Boers GHJ, Frosst P, Stevens EMB, van Oost BA, den Heijer M, et al (1996) Molecular genetic analysis in mild hyperhomocysteinemia: a common mutation in the methylenetetrahydrofolate reductase gene is a genetic risk factor for cardiovascular disease. Am J Hum Genet 58:35–41 (in this issue)

Kozich V, Draus E, De Franchis R, Fowler B, Boers GHJ, Graham I, Kraus JP (1995) Hyperhomocysteinemia in premature arterial disease: examination of cystathionine β-synthase alleles at the molecular level. Hum Mol Genet 4:623–629

McCully KS (1969) Vascular pathology of homocysteinemia. Am J Pathol 56:111–128

Mills JL, McPartlin JM, Kirke PN, Lee YJ, Conley MR, Weir DG, Scott JM (1995) Homocysteine metabolism in pregnancies complicated by neural-tube defects. Lancet 345:149–151

Mudd SH, Havlik R, Levy HL, McKusick VA, Feinleib M (1981) A study of cardiovascular risk in heterozygotes for homocystinuria. Am J Hum Genet 33:883–893

Mudd SH, Levy HL, Skovby F (1995) Disorders of transsulfuration. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic and molecular bases of inherited disease. McGraw-Hill, New York, pp 1279–1327

Rosenblatt DS (1995) Inherited disorders of folate transport and metabolism. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic and molecular bases of inherited disease. McGraw-Hill, New York, pp 3111–3128

Rozen R, Jacques P, Bostom A, Ellison C, Williams R, Rosenberg I, Selhub J (1995) Interaction of a common mutation in methylenetetrahydrofolate reductase (MTHFR) with low folate status in hyperhomocysteinemia. Am J Hum Genet Suppl 57:A250

Savage DG, Lindenbaum J (1995) Folate-cobalamin interactions. In: Bailey LB (ed) Folate in health and disease. Marcel Dekker, New York, pp 237–285

van der Put, Steegers-Theunissen RPM, Frosst P, Trijbels FJM, Eskes TKAB, van den Heuval LP, Mariman ECM, et al (1995) Mutated methylenetetrahydrofolate reductase as a risk factor for spina bifida. Lancet 356:1070–1071

Whitehead AS, Ward P, Tan S, Naugthen E, Kraus JP, Sellar GC, McConnell DJ, et al (1994) The molecular genetics of homocystinuria, hyperhomocysteinemia, and premature vascular disease in Ireland. In: Mato JM, Caballero A (eds) Methionine metabolism: molecular mechanisms and clinical implications. Bouncopy, Madrid, pp 79–83

Wilcken DEL, Wilcken B (1976) The pathogenesis of coronary artery disease: a possible role for methionine metabolism. J Clin Invest 57:1079–1082

Willett W (1990) Diet and coronary heart disease. In: Nutritional epidemiology. Oxford University Press, New York, pp 341–379