

ACMG Practice Guideline: lack of evidence for *MTHFR* polymorphism testing

Scott E. Hickey, MD, FACMG¹, Cynthia J. Curry, MD, FACMG² and Helga V. Toriello, PhD, FACMG³

MTHFR polymorphism testing is frequently ordered by physicians as part of the clinical evaluation for thrombophilia. It was previously hypothesized that reduced enzyme activity of *MTHFR* led to mild hyperhomocysteinemia which led to an increased risk for venous thromboembolism, coronary heart disease, and recurrent pregnancy loss. Recent meta-analyses have disproven an association between hyperhomocysteinemia and risk for coronary heart disease and between *MTHFR* polymorphism status and risk for venous

thromboembolism. There is growing evidence that *MTHFR* polymorphism testing has minimal clinical utility and, therefore should not be ordered as a part of a routine evaluation for thrombophilia.

Genet Med 2013;15(2):153–156

Key Words: folic acid; homocysteine; *MTHFR* polymorphism; thermolabile variant; thrombophilia; thrombosis

The 5,10-methylenetetrahydrofolate reductase (*MTHFR*) enzyme catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the primary circulatory form of folate, and a cosubstrate for homocysteine remethylation to methionine. Methionine is subsequently converted to S-adenosylmethionine, which serves as an essential methyl donor in reactions involving nucleic acids, proteins, and many other biological compounds. There are two commonly recognized polymorphic variants in the gene encoding for this enzyme: the “thermolabile” variant c.665C→T (p.Ala222Val), historically more commonly referred to as C677T, and the c.1286A→C (p.Glu429Ala) variant; both are missense changes that are known to decrease enzyme activity.^{1,2} It is estimated that >25% of Hispanics and between 10 and 15% of North American Caucasians are homozygous for the “thermolabile” variant.³ Variants c.665C→T and c.1286A→C are in linkage disequilibrium with each other, and therefore a combination of both variants is usually seen only in individuals who are compound heterozygotes in *trans*.⁴ Homozygosity for one variant in combination with heterozygosity for the other variant is rare.⁵ Targeted mutation analysis for the c.665C→T and c.1286A→C variants is available in more than 50 Clinical Laboratory Improvement Amendments–certified laboratories in the United States.

Reduced enzyme activity of *MTHFR* is a genetic risk factor for hyperhomocysteinemia, especially in the presence of low serum folate levels.^{6–8} Mild to moderate hyperhomocysteinemia has been identified as a risk factor for venous thrombosis^{9,10} and has been associated with other cardiovascular diseases, such as coronary artery disease.^{11–13} Hyperhomocysteinemia is multifactorial, involving a combination of genetic, physiologic, and environmental factors.^{3,14} Several enzymes with vitamin B

cofactors—including vitamin B6, vitamin B12, and folate—are involved in regulating homocysteine levels. Individuals who are *MTHFR* polymorphism homozygotes may have hyperhomocysteinemia, usually to a mild or moderate degree of uncertain clinical significance. As mentioned, homocysteine is associated with coronary artery disease, although this appears to be independent of *MTHFR* genotype status.¹⁵ Although B vitamin supplementation has been shown to decrease plasma homocysteine levels, the effect on cardiovascular end points has been mostly negative.^{16–18} Some authors have found mild significant effects on stroke;^{16,18} however, a meta-analysis of homocysteine-lowering trials did not find evidence that supplementation with B vitamins, including folic acid, resulted in any decrease in cardiovascular events or mortality.¹⁹ Furthermore, a more recent meta-analysis of unpublished data sets has cast doubts on the hypothesis that lifelong moderate homocysteine elevation has any effect on cardiovascular disease, raising the possibility that publication bias accounted for the previously observed aggregate association.²⁰

The potential associations between *MTHFR* genotype status and a number of medical complications have been evaluated using methodologies such as case–control, cohort, Mendelian randomization, and meta-analysis. A modest positive association has been found between the *MTHFR* “thermolabile” polymorphism and many different medical complications, including, but not limited to, thromboembolic disease (in non-North-American populations only),^{21,22} stroke,^{23–27} aneurysm,²⁸ peripheral artery disease,²⁹ migraine,³⁰ hypertension,^{31,32} recurrent pregnancy loss,^{33,34} male infertility,^{35,36} risk for offspring with neural tube defects,^{37,38} certain cancers,^{39–41} neuropsychiatric disease,⁴² and chemotherapy toxicity.^{43,44}

¹Department of Pediatrics, The Ohio State University College of Medicine, Columbus, Ohio, USA; ²Genetic Medicine Central California, University of California, San Francisco–Fresno, Fresno, California, USA; ³Department of Pediatrics/Human Development, Spectrum Health Hospitals and College of Medicine, Michigan State University, Grand Rapids, Michigan, USA. Correspondence: Scott E. Hickey (Scott.Hickey@nationwidechildrens.org)

Submitted 7 November 2012; accepted 12 November 2012; advance online publication 3 January 2013. doi:10.1038/gim.2012.165

Conversely, many other studies looking at similar complications found no statistical association.^{45–52} The c.1286A→C variant has been studied less, but current evidence suggests that it is milder than the “thermolabile” variant.^{53–56} Preliminary findings in combined genotypes have found that they are not significantly different from controls.^{57,58}

Because *MTHFR* polymorphism is only one of many factors contributing to the overall clinical picture, the utility of this testing is currently ambiguous. Furthermore, US-mandated fortification of grain products with folic acid to decrease the incidence of neural tube defects has resulted in increased serum folate concentrations and lowered serum total homocysteine levels in the general population.⁵⁹ This public health initiative may be incidentally reducing some of the perceived risk associated with *MTHFR* polymorphisms.^{60,61} This is hypothesized to be one reason that an association between the “thermolabile” variant and venous thromboembolism is no longer observed in the North-American population.²¹

The American Congress of Obstetricians and Gynecologists does not recommend the measurement of homocysteine or *MTHFR* polymorphisms in the evaluation of the etiology of venous thromboembolism.⁶² The British Committee for Standards in Haematology and the British Society for Haematology do not include *MTHFR* polymorphism testing as part of their clinical guidelines for heritable thrombophilia testing.⁶³ The ACMG consensus statement on factor V Leiden testing briefly references the limited clinical utility of *MTHFR* polymorphism testing and that homocysteine measurement may be more informative.⁶⁴

A medical geneticist may be asked to evaluate a patient who has tested positive, either heterozygous or homozygous, for an *MTHFR* polymorphism (Box 1). The geneticist should assess the information given to the family by the previous provider, including the interpretation pertaining to causality for presenting symptoms. It is imperative that the geneticist ensure that patients have received thorough and appropriate evaluations for their symptoms because it is not uncommon that medical problems are incorrectly attributed to positive *MTHFR* status. Often, referral to a hematologist or maternal–fetal medicine specialist for further evaluation of their symptoms is indicated.

Once a patient has been found to carry one or more *MTHFR* polymorphisms, genetic counseling is very difficult, given the vast medical literature exploring possible associations with a wide variety of diseases. In general, the following genotypes currently appear unlikely to be of clinical significance: “thermolabile” variant c.665C→T heterozygote, c.1286A→C homozygote, or (c.665C→T); (c.1286A→C) compound heterozygote. There is theoretical reason to be concerned that the rare individuals with triple variant *MTHFR* genotypes (i.e., individuals who are homozygous for one variant and heterozygous for the other) may have resulting clinical risks, although that is currently speculative.

A fasting total plasma homocysteine level may be obtained in any patient who is homozygous for the “thermolabile” variant, in order to provide more information for counseling. For the purpose of laboratory interpretation, it should be noted that

total homocysteine levels increase with age and are lower in the pregnant population.^{65,66} Genetic counseling should take into account the clinical reason for which the test was performed. Many studies have revealed discrepant findings between Caucasians and Asians.^{21,35,51} It seems most likely that this is related to dietary factors, such as folic acid intake; however, caution should be applied when generalizing the following recommendations to the Asian-American population.

Patients who are homozygous for the “thermolabile” variant with normal plasma homocysteine can be reassured that there is currently no evidence of increased risk for venous thromboembolism^{21,45} or recurrent pregnancy loss⁵¹ related to their *MTHFR* status, common reasons for which clinical testing is done. A patient who is homozygous for the c.665C→T “thermolabile” variant but also has elevated homocysteine, however, may be at mildly increased risk for both of these events (venous thromboembolism odds ratio 1.27 and recurrent pregnancy loss pooled risk 2.7).^{20,32} The patient can also be reassured that there is no evidence of any association with *MTHFR* “thermolabile” variant homozygosity and mortality, from cardiovascular disease or otherwise.^{67,68}

Once it is known that an individual is homozygous for the “thermolabile” variant, it is appropriate to review some of the known associations and possible risks. It should be emphasized to the patient that the observed effects have been modest and the absolute risks are likely low. With some associations, it may be found in the future that there is no increase above population risk.

Women homozygous for c.665C→T should be counseled that they have a modestly increased risk (odds ratio 1.6) to have offspring with a neural tube defect.^{37,38} This risk is increased further if the fetus is also homozygous. There is possibly a weak correlation with stroke (odds ratio 1.26),²⁷ but it has not been as extensively studied as cardiovascular disease in general. A more

BOX 1: ACMG RECOMMENDATIONS

- *MTHFR* polymorphism genotyping should not be ordered as part of the clinical evaluation for thrombophilia or recurrent pregnancy loss
- *MTHFR* polymorphism genotyping should not be ordered for at-risk family members
- A clinical geneticist who serves as a consultant for a patient in whom an *MTHFR* polymorphism(s) is found should ensure that the patient has received a thorough and appropriate evaluation for his or her symptoms
- If the patient is homozygous for the “thermolabile” variant c.665C→T, the geneticist may order a fasting total plasma homocysteine, if not previously ordered, to provide more accurate counseling
- *MTHFR* status does not change the recommendation that women of childbearing age should take the standard dose of folic acid supplementation to reduce the risk of neural tube defects as per the general population guidelines^{71–77}

recent study suggests that the effect is ameliorated in populations with folate supplementation.⁶¹ There is no known contraindication to taking oral contraceptives. Neonates who have had strokes are underrepresented in current studies, so the interpretation of *MTHFR* genotyping in this setting is particularly challenging.²⁴ *MTHFR* genotype status has been associated with an increased risk of some cancers and a decreased risk of other cancers.^{69,70} The overall cancer risk does not appear to be changed.⁷¹ Patients should be counseled that it is important to provide their *MTHFR* genotype status to any physician who is considering starting them on types of chemotherapy whose activity depends on intracellular concentration of folate (e.g., methotrexate). In individuals who have a known thrombophilia, such as factor V Leiden or prothrombin c.*97G→A, most available studies support the contention that *MTHFR* genotype status does not alter their thrombotic risk to a clinically significant degree.⁷²

An at-risk individual may elect to take a daily vitamin B supplement, such as a multivitamin or prenatal vitamin, although there is currently no evidence that specific treatments reduce risks associated with hyperhomocysteinemia or *MTHFR* genotype status. Because folic acid and vitamin B12 toxicities are rare, the risks associated with daily supplementation are low. An individual who elects to take supplemental pyridoxine, however, should be aware of the risk for ataxia and sensory neuropathy.⁷⁰

DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

- Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet* 1995;10:111–113.
- Weisberg I, Tran P, Christensen B, Sibani S, Rozen R. A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. *Mol Genet Metab* 1998;64:169–172.
- Eldibany MM, Caprini JA. Hyperhomocysteinemia and thrombosis: an overview. *Arch Pathol Lab Med* 2007;131:872–884.
- Ogino S, Wilson RB. Genotype and haplotype distributions of MTHFR677C>T and 1298A>C single nucleotide polymorphisms: a meta-analysis. *J Hum Genet* 2003;48:1–7.
- Brown NM, Pratt VM, Buller A, et al. Detection of 677CT/1298AC “double variant” chromosomes: implications for interpretation of MTHFR genotyping results. *Genet Med* 2005;7:278–282.
- Christensen B, Frosst P, Lussier-Cacan S, et al. Correlation of a common mutation in the methylenetetrahydrofolate reductase gene with plasma homocysteine in patients with premature coronary artery disease. *Arterioscler Thromb Vasc Biol* 1997;17:569–573.
- Harmon DL, Woodside JV, Yarnell JW, et al. The common ‘thermolabile’ variant of methylene tetrahydrofolate reductase is a major determinant of mild hyperhomocysteinemia. *QJM* 1996;89:571–577.
- Crider KS, Zhu JH, Hao L, et al. MTHFR 677C→T genotype is associated with folate and homocysteine concentrations in a large, population-based, double-blind trial of folic acid supplementation. *Am J Clin Nutr* 2011;93:1365–1372.
- den Heijer M, Koster T, Blom HJ, et al. Hyperhomocysteinemia as a risk factor for deep-vein thrombosis. *N Engl J Med* 1996;334:759–762.
- den Heijer M, Rosendaal FR, Blom HJ, Gerrits WB, Bos GM. Hyperhomocysteinemia and venous thrombosis: a meta-analysis. *Thromb Haemost* 1998;80:874–877.
- The Homocysteine Studies Collaboration. Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis. *JAMA* 2002;288(16):2015–2022.
- de Ruijter W, Westendorp RG, Assendelft WJ, et al. Use of Framingham risk score and new biomarkers to predict cardiovascular mortality in older people: population based observational cohort study. *BMJ* 2009;338:a3083.
- Wald DS, Morris JK, Wald NJ. Reconciling the evidence on serum homocysteine and ischaemic heart disease: a meta-analysis. *PLoS ONE* 2011;6:e16473.
- Nagele P, Meissner K, Francis A, Födinger M, Saccone NL. Genetic and environmental determinants of plasma total homocysteine levels: impact of population-wide folate fortification. *Pharmacogenet Genomics* 2011;21:426–431.
- Lin PT, Huang MC, Lee BJ, Cheng CH, Tsai TP, Huang YC. High plasma homocysteine is associated with the risk of coronary artery disease independent of methylenetetrahydrofolate reductase 677C→T genotypes. *Asia Pac J Clin Nutr* 2008;17:330–338.
- Lonn E, Yusuf S, Arnold MJ, et al. Homocysteine lowering with folic acid and B vitamins in vascular disease. *N Engl J Med* 2006;354:1567–1577.
- Børnaa KH, Njølstad I, Ueland PM, et al. Homocysteine lowering and cardiovascular events after acute myocardial infarction. *N Engl J Med* 2006;354:1578–1588.
- Wang X, Qin X, Demirtas H, et al. Efficacy of folic acid supplementation in stroke prevention: a meta-analysis. *Lancet* 2007;369:1876–1882.
- Clarke R, Halsey J, Lewington S, et al. Effects of lowering homocysteine levels with B vitamins on cardiovascular disease, cancer, and cause-specific mortality: Meta-analysis of 8 randomized trials involving 37 485 individuals. *Arch Intern Med* 2010;170:1622–1631.
- Clarke R, Bennett DA, Parish S, et al. Homocysteine and coronary heart disease: meta-analysis of MTHFR case-control studies, avoiding publication bias. *PLoS Med* 2012;9:e1001177.
- Den Heijer M, Lewington S, Clarke R. Homocysteine, MTHFR and risk of venous thrombosis: a meta-analysis of published epidemiological studies. *J Thromb Haemost* 2005;3:292–299.
- Gohil R, Peck G, Sharma P. The genetics of venous thromboembolism. A meta-analysis involving approximately 120,000 cases and 180,000 controls. *Thromb Haemost* 2009;102:360–370.
- Nowak-Göttl U, Sträter R, Heinecke A, et al. Lipoprotein (a) and genetic polymorphisms of clotting factor V, prothrombin, and methylenetetrahydrofolate reductase are risk factors of spontaneous ischemic stroke in childhood. *Blood* 1999;94:3678–3682.
- Kenet G, Lütkehoff LK, Albisetti M, et al. Impact of thrombophilia on risk of arterial ischemic stroke or cerebral sinovenous thrombosis in neonates and children: a systematic review and meta-analysis of observational studies. *Circulation* 2010;121:1838–1847.
- Xin XY, Song YY, Ma JF, et al. Gene polymorphisms and risk of adult early-onset ischemic stroke: A meta-analysis. *Thromb Res* 2009;124:619–624.
- Marjot T, Yadav S, Hasan N, Bentley P, Sharma P. Genes associated with adult cerebral venous thrombosis. *Stroke* 2011;42:913–918.
- Casas JP, Bautista LE, Smeeth L, Sharma P, Hingorani AD. Homocysteine and stroke: evidence on a causal link from mendelian randomisation. *Lancet* 2005;365:224–232.
- McColgan P, Peck GE, Greenhalgh RM, Sharma P. The genetics of abdominal aortic aneurysms: a comprehensive meta-analysis involving eight candidate genes in over 16,700 patients. *Int Surg* 2009;94:350–358.
- Khandanpour N, Willis G, Meyer FJ, et al. Peripheral arterial disease and methylenetetrahydrofolate reductase (MTHFR) C677T mutations: A case-control study and meta-analysis. *J Vasc Surg* 2009;49:711–718.
- Schürks M, Rist PM, Kurth T. MTHFR 677C>T and ACE D/I polymorphisms in migraine: a systematic review and meta-analysis. *Headache* 2010;50:588–599.
- Niu WQ, You YG, Qi Y. Strong association of methylenetetrahydrofolate reductase gene C677T polymorphism with hypertension and hypertension-in-pregnancy in Chinese: a meta-analysis. *J Hum Hypertens* 2012;26:259–267.
- Kosmas IP, Tatsioni A, Ioannidis JP. Association of C677T polymorphism in the methylenetetrahydrofolate reductase gene with hypertension in pregnancy and pre-eclampsia: a meta-analysis. *J Hypertens* 2004;22:1655–1662.
- Nelen WL, Blom HJ, Steegers EA, den Heijer M, Eskes TK. Hyperhomocysteinemia and recurrent early pregnancy loss: a meta-analysis. *Fertil Steril* 2000;74:1196–1199.
- Govindaiah V, Naushad SM, Prabhakara K, Krishna PC, Radha Rama Devi A. Association of parental hyperhomocysteinemia and C677T Methylene tetrahydrofolate reductase (MTHFR) polymorphism with recurrent pregnancy loss. *Clin Biochem* 2009;42:380–386.
- Gupta N, Gupta S, Dama M, et al. Strong association of 677 C>T substitution in the *MTHFR* gene with male infertility—a study on an indian population and a meta-analysis. *PLoS ONE* 2011;6:e22277.

36. Wu W, Shen O, Qin Y, et al. Methylenetetrahydrofolate reductase C677T polymorphism and the risk of male infertility: a meta-analysis. *Int J Androl* 2012;35:18–24.
37. Liu TC, Wang ZP, Zhao ZT. (Meta analysis on the association between parental 5,10-methylenetetrahydrofolate reductase C677T polymorphism and the neural tube defects of their offspring). *Zhonghua Liu Xing Bing Xue Za Zhi* 2011;32:60–67.
38. Blom HJ, Shaw GM, den Heijer M, Finnell RH. Neural tube defects and folate: case far from closed. *Nat Rev Neurosci* 2006;7:724–731.
39. Boccia S, Hung R, Ricciardi G, et al. Meta- and pooled analyses of the methylenetetrahydrofolate reductase C677T and A1298C polymorphisms and gastric cancer risk: a huge-GSEC review. *Am J Epidemiol* 2008;167:505–516.
40. Langevin SM, Lin D, Matsuo K, et al. Review and pooled analysis of studies on MTHFR C677T polymorphism and esophageal cancer. *Toxicol Lett* 2009;184:73–80.
41. Qi X, Ma X, Yang X, et al. Methylenetetrahydrofolate reductase polymorphisms and breast cancer risk: a meta-analysis from 41 studies with 16,480 cases and 22,388 controls. *Breast Cancer Res Treat* 2010;123:499–506.
42. Peerbooms OL, van Os J, Drukker M, et al. Meta-analysis of *MTHFR* gene variants in schizophrenia, bipolar disorder and unipolar depressive disorder: evidence for a common genetic vulnerability? *Brain Behav Immun* 2011;25:1530–1543.
43. Fisher MC, Cronstein BN. Metaanalysis of methylenetetrahydrofolate reductase (*MTHFR*) polymorphisms affecting methotrexate toxicity. *J Rheumatol* 2009;36:539–545.
44. Spyridopoulou KP, Dimou NL, Hamodrakas SJ, Bagos PG. Methylenetetrahydrofolate reductase gene polymorphisms and their association with methotrexate toxicity: a meta-analysis. *Pharmacogenet Genomics* 2012;22:117–133.
45. Zintzaras E, Zdoukopoulos N. A field synopsis and meta-analysis of genetic association studies in peripheral arterial disease: The CUMAGAS-PAD database. *Am J Epidemiol* 2009;170:1–11.
46. Gouveia LO, Canhão P. *MTHFR* and the risk for cerebral venous thrombosis—a meta-analysis. *Thromb Res* 2010;125:e153–e158.
47. Rao R, Tah V, Casas JP, et al. Ischaemic stroke subtypes and their genetic basis: a comprehensive meta-analysis of small and large vessel stroke. *Eur Neurol* 2009;61:76–86.
48. Peck G, Smeeth L, Whittaker J, Casas JP, Hingorani A, Sharma P. The genetics of primary haemorrhagic stroke, subarachnoid haemorrhage and ruptured intracranial aneurysms in adults. *PLoS ONE* 2008;3:e3691.
49. Vetrivel V, Vijayalakshmi K, Paul SF, Venkatachalam P. ACE and *MTHFR* gene polymorphisms in unexplained recurrent pregnancy loss. *J Obstet Gynaecol Res* 2008;34:301–306.
50. González-Herrera L, García-Escalante G, Castillo-Zapata I, et al. Frequency of the thermolabile variant C677T in the *MTHFR* gene and lack of association with neural tube defects in the State of Yucatan, Mexico. *Clin Genet* 2002;62:394–398.
51. Godbole K, Gayathri P, Ghule S, et al. Maternal one-carbon metabolism, *MTHFR* and *TCN2* genotypes and neural tube defects in India. *Birth Defects Res Part A Clin Mol Teratol* 2011;91:848–856.
52. Ren A, Wang J. Methylenetetrahydrofolate reductase C677T polymorphism and the risk of unexplained recurrent pregnancy loss: a meta-analysis. *Fertil Steril* 2006;86:1716–1722.
53. Ulvik A, Ueland PM, Fredriksen A, et al. Functional inference of the methylenetetrahydrofolate reductase 677C > T and 1298A > C polymorphisms from a large-scale epidemiological study. *Hum Genet* 2007;121:57–64.
54. Domagala TB, Adamek L, Nizankowska E, Sanak M, Szczeklik A. Mutations C677T and A1298C of the 5,10-methylenetetrahydrofolate reductase gene and fasting plasma homocysteine levels are not associated with the increased risk of venous thromboembolic disease. *Blood Coagul Fibrinolysis* 2002;13:423–431.
55. Zetterberg H, Coppola A, D'Angelo A, Palmér M, Rymo L, Blennow K. No association between the *MTHFR* A1298C and transcobalamin C776G genetic polymorphisms and hyperhomocysteinemia in thrombotic disease. *Thromb Res* 2002;108:127–131.
56. Wang XW, Luo YL, Wang W, Zhang Y, Chen Q, Cheng YL. Association between *MTHFR* A1298C polymorphism and neural tube defect susceptibility: a metaanalysis. *Am J Obstet Gynecol* 2012;206:251.e1–251.e7.
57. Hanson NQ, Aras O, Yang F, Tsai MY. C677T and A1298C polymorphisms of the methylenetetrahydrofolate reductase gene: incidence and effect of combined genotypes on plasma fasting and post-methionine load homocysteine in vascular disease. *Clin Chem* 2001;47:661–666.
58. Kölling K, Ndrepepa G, Koch W, et al. Methylenetetrahydrofolate reductase gene C677T and A1298C polymorphisms, plasma homocysteine, folate, and vitamin B12 levels and the extent of coronary artery disease. *Am J Cardiol* 2004;93:1201–1206.
59. Jacques PF, Selhub J, Bostom AG, Wilson PW, Rosenberg IH. The effect of folic acid fortification on plasma folate and total homocysteine concentrations. *N Engl J Med* 1999;340:1449–1454.
60. Tsai MY, Loria CM, Cao J, et al. Clinical utility of genotyping the 677C>T variant of methylenetetrahydrofolate reductase in humans is decreased in the post-folic acid fortification era. *J Nutr* 2009;139:33–37.
61. Holmes MV, Newcombe P, Hubacek JA, et al. Effect modification by population dietary folate on the association between *MTHFR* genotype, homocysteine, and stroke risk: a meta-analysis of genetic studies and randomised trials. *Lancet* 2011;378:584–594.
62. Lockwood C, Wendel G; Committee on Practice Bulletins—Obstetrics. Practice Bulletin no. 124: Inherited thrombophilias in pregnancy. *Obstet Gynecol* 2011;118(3):730–740.
63. Baglin T, Gray E, Greaves M, et al. Clinical guidelines for testing for heritable thrombophilia. *Br J Haematol* 2010;149:209–220.
64. Grody WW, Griffin JH, Taylor AK, Korf BR, Heit JA. American College of Medical Genetics consensus statement on factor V Leiden mutation testing. *Genet Med* 2001;3:139–148.
65. Dominguez LJ, Galioto A, Pineo A, et al. Age, homocysteine, and oxidative stress: relation to hypertension and type 2 diabetes mellitus. *J Am Coll Nutr* 2010;29:1–6.
66. Ueland PM, Vollset SE. Homocysteine and folate in pregnancy. *Clin Chem* 2004;50:1293–1295.
67. Yang Q, Bailey L, Clarke R, et al. Prospective study of methylenetetrahydrofolate reductase (*MTHFR*) variant C677T and risk of all-cause and cardiovascular disease mortality among 6000 US adults. *Am J Clin Nutr* 2012;95:1245–1253.
68. Roest M, van der Schouw YT, Grobbee DE, et al. Methylenetetrahydrofolate reductase 677 C/T genotype and cardiovascular disease mortality in postmenopausal women. *Am J Epidemiol* 2001;153:673–679.
69. Taioli E, Garza MA, Ahn YO, et al. Meta- and pooled analyses of the methylenetetrahydrofolate reductase (*MTHFR*) C677T polymorphism and colorectal cancer: a HuGE-GSEC review. *Am J Epidemiol* 2009;170:1207–1221.
70. Tong N, Sheng X, Wang M, et al. Methylenetetrahydrofolate reductase gene polymorphisms and acute lymphoblastic leukemia risk: a meta-analysis based on 28 case-control studies. *Leuk Lymphoma* 2011;52:1949–1960.
71. Zacho J, Yazdanyar S, Bojesen SE, Tybjaerg-Hansen A, Nordestgaard BG. Hyperhomocysteinemia, methylenetetrahydrofolate reductase c.677C>T polymorphism and risk of cancer: cross-sectional and prospective studies and meta-analyses of 75,000 cases and 93,000 controls. *Int J Cancer* 2011;128:644–652.
72. De Stefano V, Casorelli I, Rossi E, Zappacosta B, Leone G. Interaction between hyperhomocysteinemia and inherited thrombophilic factors in venous thromboembolism. *Semin Thromb Hemost* 2000;26:305–311.
73. Institute of Medicine. Food and Nutrition Board. *Dietary Reference Intakes: Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline*. National Academy Press: Washington, DC, 1998.
74. Vitamin Study Research Group. Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. MRC Vitamin Study Research Group. *Lancet* 1991;338:131–137.
75. Czeizel AE, Dudás I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *N Engl J Med* 1992;327:1832–1835.
76. Centers for Disease Control and Prevention. 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* 1992;41:1–7.
77. Toriello HV; Policy and Practice Guideline Committee of the American College of Medical Genetics. Policy statement on folic acid and neural tube defects. *Genet Med* 2011;13:593–596.