Gene-Gene and Gene-Environment Interactions in Mild Hyperhomocysteinemia

Armando D'Angelo, Giuseppina Mazzola, Isabella Fermo

Coagulation Service and Thrombosis Research Unit, IRCCS H S.Raffaele, Milano, Italy

Key Words

Hyperhomocysteinemia · Thrombosis · Polymorphisms · Methionine-synthase · Methylenetetrahydrofolate reductase · Methionine-synthase reductase · Cystathionine synthase · Transcobalamin

Abstract

Mild/moderate hyperhomocysteinemia (HHcy), a highly prevalent condition, is independently associated with an increased risk of arterial and venous thromboembolic diseases. Early reports of the association of mild/moderate HHcy with juvenile venous thromboembolism have shown familiarity for HHcy in relatives of index cases with thrombosis. Similar to inherited thrombophilia defects, inheritance of the HHcy phenotype was accordingly retained important for the definition of HHcy as an independent risk factor for thrombosis. A number of common polymorphisms in genes coding for methylenetetrahydrofolate reductase (MTHFR), methionine-synthase, methionine-synthase reductase and cystathionine β -synthase (CBS) have been explored for their association with homocysteine levels, fasting and post-methionine load, and with thrombotic diseases. MTHFR thermolability accounts for a 10-fold increase in the risk of mild/moderate HHcy. With the possible exception of the CBS 844ins68 insertion, there is no evidence for an increased risk of HHcy for any of these polymor-

phisms, isolated or in association with MTHFR thermolability. Environmental factors and MTHFR thermolability are main determinants of the HHcy phenotype. If mild/moderate HHcy is a pathogenetic risk factor for thrombosis, intervention aimed to improve the vitamin status appears of major importance, irrespective of common gene polymorphisms of the homocysteine metabolism.

Copyright © 2004 S. Karger AG, Base

Mild/moderate hyperhomocysteinemia (HHcy), a highly prevalent condition, is independently associated with an increased risk of arterial and venous thromboembolic diseases [1-3] and is increasingly recognized as a potential risk factor for additional pathological conditions [4-6].

Early reports of the association of mild/moderate HHcy with juvenile venous thromboembolism have shown familiarity for HHcy in relatives of index cases with thrombosis [7-9]. Similar to inherited thrombophilia defects, inheritance of the HHcy phenotype was accordingly retained important for the definition of HHcy as an independent risk factor for thrombosis.

Current knowledge recognizes a number of determinants of the mild HHcy phenotype, including genetic as well as environmental factors. Homocystinuria is an inherited disease due to homozygous or compound heterozygous defects in enzymes regulating the methionine metabolism. In patients affected by homocystinuria, over 90 mutations of

KARGER

Fax +41 61 306 12 34
E-Mail: karger@karger.ch
www.karger.com

© 2004 S. Karger AG, Basel
1424-8832/04/0336-0337\$21.0/0

Accessible online at:
www.karger.com/pht

Armando D'Angelo, MD
Coagulation Service and Thrombosis Research Unit
IRCCS H S.Raffaele,
Via Olgettina 60
20132 Milano, Italy

E-mail: armando.dangelo@hsr.it

the cystathionine- β -synthase (CBS) gene have been reported [10], most of which are private. Defects in the 5,10-methylene tetrahydrofolate reductase (MTHFR) and methionine-synthase (MTR) genes, albeit less frequent, are also responsible for homocystinuria [11]. Hence, mild HHcy has been initially attributed to heterozygous mutations in the allele responsible for homocystinuria in the homozygous setting. This was held particularly true for heterozygous CBS mutations in the setting of methionine intolerance in the presence of normal fasting tHcy levels [12]. Intracellular S-adenosyl-methionine exerts a stimulatory effect on CBS activity [13], and regions in the C-terminal domain of CBS are responsible for this effect [14].

CBS activity is detectable in liver, pancreas, kidney and brain [15], but not in endothelial cells, where remethylation of homocysteine plays a major role [16]. Thus, while post methionine-load hyperhomocysteinemia may be sustained by such defects in a significant proportion of cases [17,18] mild fasting hyperhomocysteinemia may recognize additional inherited causes. Argumentation regarding genetic causes of hyperhomocysteinemia, and as a consequence, the prevalence of hyperhomocysteinemia may markedly vary depending on geographical areas [19,20].

A number of relatively common polymorphisms of the genes coding for enzymes of the homocysteine metabolic pathway have been investigated with respect to their influence on tHcy levels and the risk of athero-thrombosis. MTHFR, the enzyme responsible for methylene tetrahydrofolate reduction, is ubiquitous and plays a key role in homocysteine remethylation. A common polymorphism of this gene results in a thermolabile variant of the enzyme with impaired activity and reduced 5-methyltetrahydrofolate (5-MTHF) availability [21,22]. MTHFR thermolability is the most common inherited cause of mild/moderate hyperhomocysteinemia (HHcy), with a frequency ranging from 5% to 20% in the general population [1-3,20,23]. Phenotype expression of HHcy in subjects with MTHFR thermolability is a function of 5-MTHF bioavailability [24,25] and hence of the nutrient status [23-30]. Plasma folate levels are borderline low more frequently in 677TT subjects than in subjects with the MTHFR 677CT or CC genotype [31]. In a number of population studies the risk of hyperhomocysteinemia contributed by MTHFR thermolability (677TT) ranged from 2 to 10. This risk is much greater for men than for women [31]. In addition to geographical differences in the prevalence of the mutation, this large variation is at least partially explained by the vitamin status. There is some evidence for a potential interaction of the MTHFR variant with both folate and vitamin B12 levels [23]. As a result of the gene-environment interaction in the production of the HHcy phenotype, while the association of HHcy with venous and arterial thrombosis has been established in many studies,

MTHFR thermolability has been found a significant, but weak risk factor for both arterial and venous thrombosis [32-34]. A second common polymorphism of the MTHFR gene, A1298C, which rarely occurs in cis with the C677T mutation [35], was found to result in decreased MTHFR activity [36,37], more pronounced in the homozygous than heterozygous state. At variance with the C677T mutation, neither the homozygous nor the heterozygous state of this transition is associated with higher tHcy or a lower plasma folate concentration; however individuals heterozygous for both mutations appeared initially to have elevated tHcy levels and decreased folate levels [36]. In spite of the high frequency of the mutated allele in several populations (0.28-0.33), these findings have not been confirmed in subsequent studies [31,38-40].

It is common experience that subjects with MTHFR thermolability, normal kidney function and similar vitamin plasma levels may differ markedly in their tHcy levels. This suggests the possibility that additional enzyme variants of the homocysteine metabolic pathway either protective or aggravating may act in concert with MTHFR thermolability in determining tHcy levels. In the last few years a number of additional relatively common polymorphisms of the MTR, MTRR and CBS genes have been reported and investigated as risk factors for hyperhomocysteinemia and for thrombosis, with contradictory results.

Methionine-synthase (MTR) is a vitamin B12-dependent enzyme catalyzing the remethylation of homocysteine to methionine. A A2756G transition in the MTR gene was originally identified in patients with deficiencies in methionine-synthase activity [41]. An early report attributed an increased risk for late-onset vascular disease to the presence of the mutation [42], but more recent studies did not confirm this hypothesis, with this relatively frequent polymorphism (allele frequency 0.16-0.18) being a neutral or mild protective factor against the development of hyperhomocysteinemia [23, 39,43-49].

Methionine synthase reductase (MTRR) is required for the reductive activation of methionine-synthase. A polymorphic mutation has also been identified for MTRR [50]. This polymorphism is also an A to G transition that occurs at codon 66 of the MTRR DNA sequence and has an allele frequency of 0.50 or higher. Although this variant was not associated with fasting tHcy concentrations in three studies examining this relationship [49,51,52], an association between the maternal MTRR 66G allele, low maternal cobalamin, and spina bifida was observed in one study [51].

Our understanding of the relationship between gene polymorphisms and phenotype is complicated by the possibility that reported risks contributed by these polymorphisms may be mediated by mechanisms other than homocysteine levels [49] and that both protective and negative

effects on tHcy levels have been reported in the literature for the same polymorphism. This latter issue is best exemplified by the findings related to the 68 base pairs insertion (844ins68) in the coding region of exon 8 of the CBS gene. This polymorphism has been identified as a common mutation among normal individuals, with prevalence ranging from 7.5% in Italians to 18.8% in Irish [53,54-57]. The 844ins68 variant is an exact duplication of the intron-exon boundary of exon 8 and is consistently associated in cis with an additional mutation in the CBS gene, a T to C transition at nucleotide position 833 [54]. In the homozygous state the T833C substitution is responsible for homocystinuria [10,58]. The 68bp insertion creates an alternative splice site, which rescues the wild type CBS sequence from the mutated allele. mRNA data have provided evidence that the allele carrying the insertion is poorly transcribed [54]. This mutation was investigated as a risk factor for arterial occlusive disease and/or for hyperhomocysteinemia in studies which have produced conflicting results [43,53,55,59-63]. While the insertion was neutral [53,57,59] or even protective [43,55,61,62] with respect to homocysteine levels, a slightly higher prevalence of the mutation was reported in patients with premature arterial disease than in controls in one study [59], and it was found more often associated with the 677TT mutation of the MTHFR gene in patients with peripheral artery occlusive disease than in controls in other studies [60,63]. The CBS insertion was found a risk factor for early-onset venous or arterial occlusive disease and a determinant of fasting hyperhomocysteinemia only when associated to the thermolabile variant of MTHFR [64] and the tHcy elevating effect of this association was also reported in patients with intermediate hyperhomocysteinemia [65,66]. These findings have not been confirmed in large studies [31,39,44,46,47,67-70] mainly suggestive an additive, protective effect of the insertion on tHcy levels when associated to MTHFR thermolability. However, a study investigating the distribution of functional polymorphisms in genes involved in homocysteine/folate metabolism in children with high IQ and in children with average IQ found no differences in the frequencies of genetic variants in the MTR or MTHFR genes, but the CBS 844ins68 allele was significantly underrepresented in children with high IQ [71].

Two silent third codon changes of the CBS gene (C699T and C1080T) are significantly associated with lower fasting and/or post-methionine load tHcy levels and may be in linkage disequilibrium with regulatory elements that upregulate CBS gene transcription [72]. They are apparently associated with a decreased risk of coronary artery disease and with increased responsiveness to homocysteine lowering by folic acid [73]. Increased responsiveness of tHcy levels to high intake of natural folate from food has been also described for subjects with MTHFR thermolability or carrying the

G2756 allele of the MTR gene [74].

Interestingly, three CBS missense mutations have been recently identified in the non catalytic C terminal region of the enzyme, yielding enzymes which are catalytically active but deficient in their response to S-adenosylmethionine. Two such mutations were found in patients suffering premature thrombosis and homocystinuric tHcy levels, but lacking any of the connective tissue disorders typical of homocystinuria due to CBS deficiency [75]. A 31-bp variable number tandem repeat (VNTR) in the CBS gene extends from exon 13 into intron 13 and can theoretically create multiple alternate splice sites [76]. This VNTR consists of 16 to 21 repeat units and shows a significant increase in plasma homocysteine concentrations with an increasing number of repeat elements, in particular after methionine loading and in patients with arterial occlusive disease [77].

The apparently higher vitamin B12 requirement of patients with early-onset vascular occlusive events and the interaction of cobalamin levels with MTHFR thermolability in phenotype expression of hyperhomocysteinemia [23] suggested the possibility that defects in carriers or receptors participating in the synthesis of methylated vitamin B12 may lead to a reduced utilization of vitamin B12. Transcobalamin (TC) is the critical transporter that delivers vitamin B12 to peripheral tissues. Heterozygosity for a polymorphism in TC (C776G), resulting in the substitution of proline for arginine, is the major determinant of the TC phenotypic variability in the population and has been associated with hyperhomocysteinemia [78,79]. However, the frequency of the mutated allele was similar in Italian patients with early-onset arterial or venous occlusive diseases and in controls (0.38) and was not associated with plasma tHcy, folate and vitamin B12 levels [40].

Current evidence suggests that environmental factors and MTHFR thermolability are main determinants of the HHcy phenotype. The genetic contribution of common polymorphisms to the variance in tHcy levels is however modest, varying from less than 2% to 9% [31,46]. Among environmental factors, vitamin status and renal function appear to play a major role, with the folate and vitamin B12 levels explaining about 35% of the variance in tHcy [31]. Gender and age are also major determinants of tHcy levels, but the gender effect tends to disappear with increasing age. If mild/moderate HHcy is a pathogenetic risk factor for thrombosis, intervention aimed to improve the vitamin status appears of major importance, irrespective of common gene polymorphisms of the homocysteine metabolism.

Acknowledgements

Supported in part by a grant from the Italian Ministry of Health (Ricerca Finalizzata 2003).

References

- D'Angelo A, Selhub J. Homocysteine and thrombotic disease. *Blood* 1997;90:1-11.
- Cattaneo M. Hyperhomocysteinemia, atherosclerosis and thrombosis. *Thromb Haemost* 1999;81:165-176.
- Refsum H, Ueland PM, Nygard O, Vollset SE. Homocysteine and cardiovascular disease. *Annu Rev Medicine* 1998;49:31-62.
- Seshadri S, Breiser A, Selhub J, Jacques PF, Rosenberg IH, D'Agostino RB, Wilson PW, Wolf PA. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. *N Engl J Med* 2002;346:476-483.
- Arroliga AC, Sandur S, Jacobsen DW, Tewari S, Mustafa M, Mascha EJ, Robinson K. Association between hyperhomocysteinemia and primary pulmonary hypertension. *Respir Med* 2003;97:825-9.
- McLean RR, Jacques PF, Selhub J, Tucker KL, Samelson EJ, Broe KE, Hannan MT, Cupples LA, Kiel DP. Homocysteine as a predictive factor for hip fracture in older persons. *N Engl J Med* 2004;350:2042-2049.
- Falcon CR, Cattaneo M, Panzeri D, Martinelli I, Mannucci PM. High prevalence of hyperhomocysteinemia in patients with juvenile venous thrombosis. *Arterioscler Thromb* 1994;14:1080-1083.
- Fermo I, Viganò D'Angelo S, Paroni R, Mazzola G, Calori G, D'Angelo A. Prevalence of moderate hyperhomocysteinemia in patients with early-onset venous and arterial occlusive disease. *Ann Intern Med* 1995;123:747-753.
- Franken DG, Boers GHJ, Blom HJ, Cruysberg JRM, Trijbels FJM, Hamel BCJ. Prevalence of familial mild hyperhomocysteinemia. *Atherosclerosis* 1996;125:71-80.
- Kraus JP, Kozich V. Cystathionine- β -synthase and its deficiency. In "Homocysteine in Health and Disease" (R. Carmel and D.W. Jacobsen eds), Cambridge University Press, Cambridge, UK, 2001, pp 223-243.
- Olteanu H, Banerjee R. Cobalamin-dependent remethylation. In "Homocysteine in Health and Disease" (R. Carmel and D.W. Jacobsen eds), Cambridge University Press, Cambridge, UK, 2001, pp 135-144.
- Boers GHJ, Smals AGH, Trijbels FJM, Fowler B, Bakkeren JAJM, Schoonderwaldt HC, Klijer WJ, Kloppenborg PWC. Heterozygosity for homocystinuria in premature peripheral and cerebral occlusive arterial disease. *N Engl J Med* 1985;313:709-715.
- Selhub J, Miller JW. The pathogenesis of homocysteinemia: interruption of the coordinate regulation by S-adenosylmethionine of the remethylation and transsulfuration of homocysteine. *Am J Clin Nutr* 1991;55:131-138.
- Shan X, Kruger WD. Correction of disease-causing CBS mutations in yeast. *Nat Genet* 1998;19:91-93.
- Bao L, Vlcek C, Paces V, Kraus JP. Identification and tissue distribution of human cystathionine beta-synthase mRNA isoforms. *Arch Biochem Biophys* 1998;350:95-103.
- Jacobsen DW, Savon SR, Stewart RW, Robinson K, Green R, Kottke-Merchant K, Di Corleto PE. Limited capacity for homocysteine catabolism in vascular cells and tissues: a pathophysiologic mechanism for arterial damage in hyperhomocysteinemia? *Circulation* 1995;91:29.
- Guttormsen AB, Ueland PM, Kruger WD, Kim CE, Ose L, Folling I, Refsum H. Disposition of homocysteine in subjects heterozygous for homocystinuria due to cystathionine beta-synthase deficiency: relationship between genotype and phenotype. *Am J Med Genet* 2001;100:204-213.
- Tsai MY, Garg U, Key NS, Hanson NQ, Suh A, Schwichtenberg K. Molecular and biochemical approaches in the identification of heterozygotes for homocystinuria. *Atherosclerosis* 1996;122:69-77.
- Kraus JP. Biochemistry and molecular genetics of cystathionine beta-synthase deficiency. *Eur J Pediatr* 1998;157(Suppl 2):S50-53.
- Gudnason V, Stansbie D, Scott J, Bowron A, Nicaud V, Humphries S, on behalf of the EARS group. C677T (thermolabile alanine/valine) polymorphism in methylenetetrahydrofolate reductase (MTHFR): its frequency and impact on plasma homocysteine concentration in different European populations. *Atherosclerosis* 1998;136:347-354.
- Kang SS, Zhou J, Wong PWK, Kowalyszyn J, Strokosch G. Intermediate homocysteinemia: a thermolabile variant of methylenetetrahydrofolate reductase. *Am J Hum Genet* 1988;43:414-421.
- Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJH, den Heijer M, Kluijtmans LAJ, van den Heuvel LP, Rozen R. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nature Genetics* 1995;10:111-113.
- D'Angelo A, Coppola A, Madonna P, Fermo I, Pagano A, Mazzola G, Galli L, Cerbone AM. The role of vitamin B12 in fasting hyperhomocysteinemia and its interaction with the homozygous C677T mutation of the methylenetetrahydrofolate reductase (MTHFR) gene. A case-control study of patients with early-onset thrombotic events. *Thromb Haemost* 2000;83:563-570.
- Jacques PF, Bostom AG, Williams RR, Ellison RC, Eckfeldt JH, Rosenberg IH, Selhub J, Rozen R. Relation between folate status, a common mutation in methylenetetrahydrofolate reductase and plasma homocysteine concentration. *Circulation* 1996;93:7-9.
- Guttormsen AB, Ueland PM, Nesthus I, Nygard O, Schneede J, Vollset SE, Refsum H. Determinants and vitamin responsiveness of intermediate hyperhomocysteinemia ($>40 \mu\text{mol/liter}$): The Hordaland Homocysteine Study. *J Clin Invest* 1996;98:2174-2183.
- Selhub J, Jacques PF, Wilson PWF, Rush D, Rosenberg IH. Vitamin status and intake as primary determinants of homocysteinemia in the elderly. *JAMA* 1993;270:2693-2698.
- Lindenbaum J, Rosenberg IH, Wilson PWF, Stabler SP, Allen RH. Prevalence of cobalamin deficiency in the Framingham elderly population. *Am J Clin Nutr* 1994;60:2-11.
- Hopkins PN, Wu LL, Hunt SC, James BC, Vincent GM, Williams RR. Higher plasma homocyst(e)ine and increased susceptibility to adverse effects of low folate in early familial coronary artery disease. *Arterioscler Thromb Vasc Biol* 1995;15:1314-1320.
- Verhoef P, Kok FJ, Kruysen DACM, Schouten EG, Witteman CM, Grobbee DE, Ueland PM, Refsum H. Plasma total homocysteine, B vitamins and risk of coronary atherosclerosis. *Arterioscler Thromb Vasc Biol* 1997;17:989-995.
- Girelli D, Friso S, Trabetti E, Olivieri O, Russo C, Pessotto R, Faccini G, Pignatti PF, Mazzucco A, Corrocher R. Methylenetetrahydrofolate reductase C677T mutation, plasma homocysteine, and folate in subjects from Northern Italy with or without angiographically documented severe coronary atherosclerotic disease: evidence for an important genetic-environmental interaction. *Blood* 1998;91:4158-4163.
- Kluijtmans LA, Young IS, Boreham CA, Murray L, McMaster D, McNully H, Strain JJ, McPartin J, Scott JM, Whitehead AS. Genetic and nutritional factors contributing to hyperhomocysteinemia in young adults. *Blood* 2003;101:2483-2488.
- Klerk M, Verhoef P, Clarke R, Blom HJ, Kok FJ, Schouten EG, and the MTHFR Studies Collaboration Group. MTHFR 677C T polymorphism and risk of coronary heart disease. A meta-analysis. *JAMA* 2002;288:2023-2031.
- Wald DS, Law M, Morris JK. Homocysteine and cardiovascular disease: evidence on causality form a meta-analysis. *Br Med J* 2002;325:1202-1208.
- Ray JG, Shmorgun D, Chan WS. Common C677T polymorphism of the methylenetetrahydrofolate reductase gene and the risk of venous thromboembolism: meta-analysis of 31 studies. *Pathophysiol Haemost Thromb* 2002;32:51-58.
- Zetterberg H, Rymo L, Coppola A, D'Angelo A, Spandidos D, Blennow K. MTHFR C677T and A1298C polymorphisms and mutated sequences occurring in cis. *Eur J Hum Genet* 2002;10:579-582.
- van der Put NMJ, Gabreels F, Stevens EM, Smeitink JA, Trijbels FJ, Eskes TK, van den Heuvel LP, Blom HJ. A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? *Am J Hum Genet* 1998;62:1044-1051.
- 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.
- 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.
- Geisel J, Zimbelmann I, Schorr H, Knapp JP, Bodis M, Hubner U, Herrmann W. Genetic defects as important factors for moderate hyperhomocysteinemia. *Clin Chem Lab Med* 2001;39:698-704.
- H. Zetterberg, A. Coppola, A. D'Angelo, M. Palmer, L. Rymo, K. Blennow. No association between the MTHFR A1298C and transcobalamin C776G genetic polymorphisms and hyperhomocysteinemia in thrombotic disease. *Thromb Res* 2002;108:127-131.
- Leclerc D, Campeau E, Goyette P, Adjalla CE, Christensen B, Ross M, Eydoux P, Rosenblatt DS, Rozen R, Gravel RA. Human methionine synthase: cDNA cloning and identification of mutations in patients of the cblG complementation group of folate/cobalamin disorders. *Hum Mol Genet* 1996;5:1867-1874.

- 42 Morita H, Kurihara H, Sugiyama T, Hamada C, Kurihara Y, Shindo T, Oh-hashii Y, Yazaki Y. Polymorphism of the methionine synthase gene: association with homocysteine metabolism and late-onset vascular disease in the Japanese population. *Arterioscl Thromb Vasc Biol* 1999;19:298-302.
- 43 Tsai MY, Welge BG, Hanson NQ, Bignell MK, Vessey J, Schwichtenberg K, Yang F, Bullemer FE, Rasmussen R, Graham KJ. Genetic causes of mild hyperhomocysteinemia in patients with premature occlusive coronary artery diseases. *Atherosclerosis* 1999;143:63-70.
- 44 Wang XL, Duarte N, Cai H, Adachi T, Sim AS, Cranney G, Wilcken DE. Relationship between total plasma homocysteine, polymorphisms of homocysteine metabolism related enzymes, risk factors and coronary artery disease in the Australian hospital-based population. *Atherosclerosis* 1999;146:133-140.
- 45 Harmon DL, Shields DC, Woodside JV, McMaster D, Yarnell JWG, Young IS, Peng K, Shane B, Evans AU, Whitehead AS. Methionine synthase D919G polymorphism is a significant but modest determinant of circulating homocysteine concentrations. *Genet Epidemiol* 1999;17:298-309.
- 46 Tsai MY, Bignell M, Yang F, Welge BG, Graham KJ, Hanson NQ. Polygenic influence on plasma homocysteine: association of two prevalent mutations, the 844ins68 of cystathionine beta-synthase and A(2756)G of methionine synthase, with lowered plasma homocysteine levels. *Atherosclerosis* 2000;149:131-137.
- 47 Dekou V, Gudnason V, Hawe E, Miller GJ, Stansbie D, Humphries SE. Gene-environment and gene-gene interaction in the determination of plasma homocysteine levels in healthy middle-aged men. *Thromb Haemost* 2001;85:67-74.
- 48 Chen J, Stampfer MJ, Ma J, Selhub J, Malinow MR, Hennekens CH. Influence of a methionine synthase (D919G) polymorphism on plasma homocysteine and folate levels and relation to risk of myocardial infarction. *Atherosclerosis* 2001;154:667-672.
- 49 Jacques PF, Bostom AG, Selhub J, Rich S, Ellison RC, Eckfeldt JH, Gravel RA, Rozen R. Effects of polymorphism of methionine synthase and methionine synthase reductase on total plasma homocysteine in the NHLBI Family Heart Study. *Atherosclerosis* 2003;166:49-55.
- 50 Leclerc D, Odievre M, Wu Q, Wilson A, Huizenga JJ, Rozen R, Scher SW, Gravel RA. Molecular cloning, expression and physical mapping of the human methionine synthase reductase gene. *Gene* 1999;240:75-88.
- 51 Wilson A, Platt R, Wu Q, Leclerc D, Christensen B, Yang H, Gravel RA, Rozen R. A common variant in methionine synthase reductase combined with low cobalamin (vitamin B12) increases risk of spina bifida. *Mol Genet Metab* 1999;67:317-323.
- 52 Brown CA, McKinney KQ, Kaufman JS, Rozen R. A common polymorphism in methionine synthase reductase increases risk of premature coronary artery disease. *J Cardiovasc Risk* 2000;7:197-200.
- 53 Tsai MY, Bignell M, Schwichtenberg K, Hanson NQ. High prevalence of a mutation in the cystathionine synthase gene. *Am J Hum Genet* 1996;59:1262-1267.
- 54 Sperandio MP, de Franchis R, Andria G, Sebastio G. A 68bp insertion found in a homocystinuric patient is a common variant and is skipped by alternative splicing of the cystathionine synthase mRNA. *Am J Hum Genet* 1996;59:1391-1393.
- 55 Kluijtmans LAJ, Boers GHJ, Trijbels FJM, van Lith-Zanders HMA, van den Heuvel LPWJ, Blom HJ. A common 844ins68 insertion variant in the cystathionine synthase gene. *Biochem Mol Med* 1997;62:23-25.
- 56 Giusti B, Comeglio P, Attanasio M, Gori AM, Brunelli T, Prisco D, Pepe G, Gensini F, Abbate R. Different distribution of the double mutant "T833C/68bp insertion" in cystathionine synthase gene in Northern and Southern Italian populations. *Thromb Haemost* 1997;78:1293.
- 57 Ramsbottom D, Scott JM, Molloy A, Weir DG, Kirke PN, Mills JL. Are common mutations of cystathionine synthase involved in the aetiology of neural tube defects? *Clin Genet* 1997;51:39-42.
- 58 Gaustadnes M, Rudiger N, Rasmussen K, Ingerslev J. Familial thrombophilia associated with homocystinuria for the cystathionine beta-synthase 833T->C mutation. *Arterioscler Thromb Vasc Biol* 2000;20:1392-1395.
- 59 Franco RF, Trip MD, ten Cate H, Prins MH, Kastelein JJP, Reitsma PH. The prevalence of the 68bp insertion in the cystathionine beta synthase gene in patients with premature arterial vascular disease: correlation with homocysteine levels and vitamin status. *Blood* 1997;90 (Suppl 1):153a.
- 60 Orendac M, Muskova B, Richterova E, Zvarova J, Stefek M, Zaykova E, Stribny J, Hyanek J, Kraus JP, Kozich V. Mutation C677T in the MTHFR gene and polymorphism 844ins68bp in the CBS gene: risk factors for peripheral arterial occlusive disease? *The Netherlands J Med* 1998;52(Suppl):S47.
- 61 De Stefano V, Dekou V, Nicaud V, Chasse JF, London J, Stansbie D, Humphries SE, Gudnason V. Linkage disequilibrium at the cystathionine beta synthase (CBS) locus and the association between genetic variation at the CBS locus and plasma levels of homocysteine. *Ann Hum Genet* 1998;62:481-490.
- 62 Tsai MY, Yang F, Bignell M, Aras O, Hanson NQ. Relation between plasma homocysteine concentration, the 844ins68 variant of the cystathionine beta-synthase gene, and pyridoxal-5'-phosphate concentration. *Mol Genet Metab* 1999;67:352-356.
- 63 Orendac M, Muskova B, Richterova E, Zvarova J, Stefek M, Zaykova E, Kraus JP, Stribny J, Hyanek J, Kozich V. Is the common 844ins68 polymorphism in the cystathionine -synthase gene associated with atherosclerosis? *J Inher Metab Dis* 1999;22:674-675.
- 64 de Franchis R, Fermo I, Mazzola G, Sebastio G, Di Minno G, Coppola A, Buoniconti A, Andria G, D'Angelo A. A common mutation of the cystathionine -synthase gene (844ins68) is a risk factor for early-onset venous and arterial occlusive disease and a determinant of fasting hyperhomocysteinemia only when associated to the thermolabile variant of methylenetetrahydrofolate reductase. *Thromb Haemost* 2000;84:576-582.
- 65 Andria G, Buoniconti A, Sperandio MP, de Franchis R, D'Angelo A, Sebastio G, Guttormsen AB, Refsum H, Ueland PM. Intermediate hyperhomocysteinemia and the 844ins68 mutation of the cystathionine -synthase gene. *Am J Hum Genet* 1998;63(Suppl):A207(#1186).
- 66 Guastadnes M, Rudiger N, Rasmussen K, Ingerslev J. Intermediate and severe hyperhomocysteinemia with thrombosis: a study of genetic determinants. *Thromb Haemost* 2000;83:554-558.
- 67 De Stefano V, Dekou V, Nicaud V, Chasse JF, London J, Stansbie D, Humphries SE, Gudnason V. Linkage disequilibrium at the cystathionine beta synthase (CBS) locus and the association between genetic variation at the CBS locus and plasma levels of homocysteine. *Ann Hum Genet* 1998;62:481-490.
- 68 Tsai MY, Yang F, Bignell M, Aras O, Hanson NQ. Relation between plasma homocysteine concentration, the 844ins68 variant of the cystathionine beta-synthase gene, and pyridoxal-5'-phosphate concentration. *Mol Genet Metab* 1999;67:352-356.
- 69 Zhang G, Dai C. Gene polymorphisms of homocysteine metabolism-related enzymes in Chinese patients with occlusive coronary artery or cerebral vascular diseases. *Thromb Res* 2001;104:187-195.
- 70 Dilley A, Hooper WC, El-Jamil M, Renshaw M, Wenger NK, Evatt BL. Mutations in the genes regulating methylene tetrahydrofolate reductase (MTHFR C->T677) and cystathionine beta-synthase (CBS G->A919, CBS T->C833) are not associated with myocardial infarction in African Americans. *Thromb Res* 2001;103:109-115.
- 71 Barbaux S, Plomin R, Whitehead AS. Polymorphisms of genes controlling homocysteine/folate metabolism and cognitive function. *Neuroreport* 2000;11:1133-1136.
- 72 Aras O, Hanson NQ, Yang F, Tsai MY. Influence of 699C T and 1080C T polymorphisms of the cystathionine beta-synthase gene on plasma homocysteine levels. *Clin Genet* 2000;58:455-459.
- 73 Kruger WD, Evans AA, Wang L, Malinow MR, Duell PB, Anderson PH, Block PC, Hess DL, Graf EE, Upson B. Polymorphisms in the CBS gene associated with decreased risk of coronary artery disease and increased responsiveness to total homocysteine lowering by folic acid. *Mol Genet Metab* 2000;70:53-60.
- 74 Silaste ML, Rantala M, Sampi M, Alftan G, Aro A, Kesaniemi YA. Polymorphisms of key enzymes in homocysteine metabolism affect diet responsiveness of plasma homocysteine in healthy women. *J Nutr* 2001;131:2643-2647.
- 75 Maclean KN, Guastadnes M, Oliveriusova J, Janosik K, Kraus E, Kozich V, Kery V, Skovby F, Rudiger N, Ingerslev J, Stabler SP, Allen RH, Kraus JP. High homocysteine and thrombosis without connective tissue disorders are associated with a novel class of cystathionine beta-synthase (CBS) mutations. *Hum Mutat* 2002;19:641-655.
- 76 Yang F, Hanson NQ, Schwichtenberg K, Tsai MY. Variable number tandem repeat in exon/intron border of the cystathionine -synthase gene: a single nucleotide substitution in the second repeat prevents multiple alternate splicing. *Am J Hum Genet* 2000;65:385-390.
- 77 Lievers KJ, Kluijtmans LA, Heil SG, Boers GH, Verhoef P, van Oppenraay-Emmerzaal D, den Heijer M, Trijbels FJ, Blom HJ. A 31 bp VNTR in the cystathionine beta-synthase (CBS) gene is associated with reduced CBS activity and elevated post-load homocysteine levels. *Eur J Hum Genet* 2001;9:583-589.
- 78 McCaddon A, Blennow K, Hudson P, Regland B, Hill D. Transcobalamin polymorphism and homocysteine. *Blood* 2001;98:3497-3499.
- 79 Namour F, Olivier JL, Abdelmoutaleb I, Adjalla C, Debarb R, Salvat C, Guéant JL. Transcobalamin codon 259 polymorphism in HT-29 and Caco-2 cells and in Caucasians: relation to transcobalamin and homocysteine concentration in blood. *Blood* 2001;97:1092-8.