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ORIGINAL ARTICLE

Hyperhomocysteinemia is an independent predictor of sub-clinical carotid vascular damage in subjects with grade-1 hypertension

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Abstract Although the role of homocysteinemia (Hcy) as a coronary risk factor (RF) has been scaled down, hyper-Hcy and carotid vascular damage (CVD) are still considered as RFs for cerebrovascular events. In 276 grade-1 hypertensives (160 men and 116 women aged 59.6 ± 15.0 years) without known cardiovascular disease and having hyper-Hcy (≥15 μM/L), subclinical CVD was evaluated by ultrasonographic carotid-wall intima media thickness (IMT). Hcy was divided into quartiles and C667→T polymorphism codifying for methylenetetrahydrofolate reductase (MTHFR) was determined. According to the genotype, subjects were divided into CC (wild), CT (heterozygote) and TT (homozygous mutation). Differences between continuous variables were evaluated by analysis of variance, while gender specific odds ratio (OR) and 95 % confidence intervals (CI) of CVD (IMT >0.9 mm or plaque) were calculated by multivariate logistic regression analysis. Blood pressure (BP) values were not different across the quartiles of Hcy. In 46.4 % of cases, sub-clinical CVD was found, with a prevalence increasingly distributed in the quartiles of Hcy (31.9, 42, 52.2, 59.4 %, $p < 0.001$). Prevalence of TT allele of the MTHFR genotype was also significantly distributed in the quartiles of Hcy (13.6, 12.3, 23.5 and 50.6 %, $p < 0.0001$), whereas no relationship was found between genotype and CVD. The last quartile of Hcy predicted CVD (OR 1.32, CI 1.12–2.2, $p = 0.02$) independent of age (OR 1.23, CI 1.002–1.56, $p = 0.0001$), systolic BP (OR 1.52, CI 1.24–2.10), diabetes (OR 2.11, CI 1:32–2.88, $p = 0.01$) and smoking (OR 1.45, CI 1.14–1.98, $p = 0.04$). Adding gender did not modify the model. In hypertensives, Hcy values >36.5 μM/L independently predict CVD and in those who are also diabetic and smokers, Hcy assessment without MTHFR genotype should be recommended to obtain a better stratification of global cerebrovascular risk.

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Introduction

The role of homocysteine, an amino acid with biological functions in methionine metabolism, as a risk factor for coronary heart and more in general for cardiovascular disease has been scaled down in the scientific world [1–5]. This opinion comes from the results of randomized clinical trials performed in subjects with known vascular disease or diabetes where the oral supplementation of folate, B6, and, B12 offered no benefit for the prevention of cardiovascular events [6]. This evidence led to recommendations not to treat cardiovascular disease patients with hyperhomocysteinemia with vitamin supplementation and to avoid routine screening of homocysteine [7] because of serum level variability and for cost-effectiveness [8].

However in the setting of cerebrovascular disease, meta-analysis of prospective observational studies showed that a 25 % lower homocysteine level was associated with a 19 % lower stroke risk [4]. In experimental studies, homocysteine causes oxidative stress, enhances inflammatory processes, and damages vascular endothelium [9, 10] and the addition folic acid supplementation is associated with a reduction in carotid atherosclerosis progression [11, 12] suggesting a potential therapy for stroke prevention in subjects with hyperhomocysteinemia [13].

On the other hand, carotid-wall intima-media thickness (IMT)—a well-known surrogate measure of atherosclerosis [14]—is associated with the classic cardiovascular risk factors [15] and stroke at the population level [16]. Among these risk factors, arterial hypertension is the main condition associated with abnormalities in arterial structure and the IMT measurement by ultrasonography is the most common method to assess the sub-clinical CVD due to HT [17, 18].

The aim of this cross-sectional study was to investigate the role of hyperhomocysteinemia in predicting sub-clinical CVD in a cohort of uncomplicated hypertensive subjects (Fig. 1).

Materials and methods

276 grade-1 hypertensives (160 men and 116 women) aged 59.6 ± 15.0 years, without history of cardiovascular disease and having hyperhomocysteinemia (≥15 μM/L), consecutively referred to our hypertension centre from April 2011 to May 2012, underwent assessment of IMT

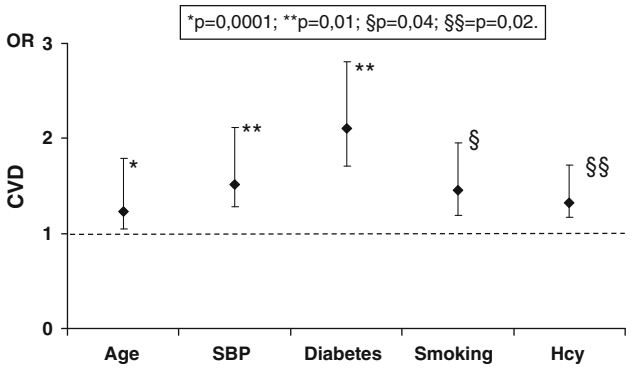

Fig. 1 Adjusted odd ratios (OR) for the independent predictors of sub-clinical carotid vascular damage

(in mm) by ultrasonography. The study protocol was approved by local ethics committee and institutional review boards and the study was conducted in accordance with ICH harmonized tripartite guidelines for good clinical practice and the declaration of Helsinki principles. All subjects gave informed consent.

Study design

All subjects underwent biochemical laboratory examination. Vitamin status and intake were also evaluated, and subjects having low serum levels of folic acid, vitamin B6 and B12 and those with moderate-high alcohol intake (see below) were not included in the study. Homocysteine concentration was determined using an enzymatic assay on the Hitachi 917 analyzer (Roche Diagnostics) using reagents and calibrators from Catch, Inc. Homocysteinemia (Hcy) was divided into quartiles and we examined C667→T polymorphism codifying for methylenetetrahydrofolate reductase (MTHFR). MTHFR genotype determination was performed by a multiplex Polymerase Chain Reaction with Reverse Line Blot (mPCR/RLB) hybridization assay (AB-Thrombo type kit, www.abanalitica.it). In relation to T allele distribution, subjects were divided in normal (CC), heterozygote (CT) and homozygote (TT) for the MTHFR genotype.

Blood pressure (BP) was measured using a standard mercury sphygmomanometer with a 14-cm cuff according to 2013 European Hypertension guidelines [19]. BP was measured in both arms and, if a difference >10 mmHg was found, the arm with the higher BP was used throughout. With subjects seated, systolic (SBP) and phase-5 diastolic (DBP) were taken in triplicate at 10-min intervals, taking special care to avoid any terminal digit preference. The average of the last two measurements was taken into consideration in order to minimize white-coat effects, if any. Pulse pressure was the difference between systolic and diastolic BP. Heart rate was also measured at the same time.

Table 1 General characteristics of the subjects by gender

Items	All (N = 276)	Men (N = 160)	Women (N = 116)	*p* value between gender
Age (yrs)	59.6 ± 15.0	56.2 ± 15.1	64.5 ± 13.3	<0.0001
Serum homocysteine (μM/L)	22.8 ± 10.3	23.1 ± 10.9	22.5 ± 9.5	NS
BMI (kg/m^2)	28.6 ± 5.0	28.1 ± 4.1	29.3 ± 6.0	<0.05
SBP (mmHg)	158.8 ± 17.7	158.9 ± 18.5	158.7 ± 16.7	NS
DBP (mmHg)	95.7 ± 10.7	97.3 ± 11.5	93.6 ± 9.2	<0.005
PP (mmHg)	63.1 ± 15.8	61.6 ± 15.8	65.1 ± 15.7	NS
HR (bpm)	75.9 ± 9.5	74.2 ± 10.2	78.3 ± 7.8	<0.0001
History of HT (years)	9.0 ± 7.1	8.8 ± 7.5	9.1 ± 6.2	NS
Antihypertensive drugs (%)	81.9	79.4	85.4	NS
ACEIs (%)	21.2	21.4	23.6	NS
ARBs (%)	23.5	20.3	24.7	NS
CCBs (%)	19.7	18.7	20.1	NS
Diuretics (%)	11.9	10.8	12.3	NS
β-blockers (%)	4.3	4.7	4.1	NS
Other antihypertensives (%)	1.3	1.2	1.1	NS
Creatinine (mg/dl)	0.9 ± 0.3	1.0 ± 0.2	0.9 ± 0.4	NS
Cl_Cr (ml/min)	102.4 ± 52.3	107.8 ± 39.1	95.6 ± 65.1	NS
Albuminuria (mg/24 h)	57.3 ± 218.5	42.5 ± 103.7	77.5 ± 311.2	NS
Glucose (mg/dl)	105.0 ± 28.7	107.6 ± 31.8	101.5 ± 23.5	NS
TC (mg/dl)	205.6 ± 40.5	202.8 ± 42.0	209.5 ± 38.1	NS
HDL-C (mg/dl)	56.6 ± 15.0	54.2 ± 13.4	60.0 ± 16.5	NS
LDL-C (mg/dl)	124.7 ± 37.1	123.7 ± 38.4	126.0 ± 35.5	NS
TG (mg/dl)	121.9 ± 63.7	124.6 ± 69.3	118.1 ± 55.2	<0.02
Diabetes (%)	17.1	20.1	13.0	NS
History of diabetes (years)	6.5 ± 8.2	7.2 ± 6.9	5.9 ± 9.7	NS
Smoking (%)	16.3	20.0	11.2	<0.04
History of smoking (years)	20.4 ± 3.2	27.2 ± 4.8	16.8 ± 5.4	<0.05
CVD (%)	46.4	43.8	50.0	NS
MTHFR (TT vs. Non-TT, %)	29.3	30.6	27.6	NS

BMI body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *PP* pulse pressure, *HR* heart rate, *HT* hypertension, *ClCr* creatinine clearance, *TC* total cholesterol, *HDL-C* high-density lipoproteins cholesterol, *LDL* low-density lipoproteins cholesterol, *TG* triglycerides, *ACEI* angiotensin-converting enzyme inhibitors, *ARBs* angiotensin-I receptor blockers, *CCBs* calcium channel blockers, *CVD* carotid vascular damage, *MTHFR* methylenetetrahydrofolate reductase

BP was also measured after 1 and 5 min of orthostatism. Arterial hypertension was defined as SBP ≥140 mm Hg or DBP ≥90 mm Hg or current treatment with antihypertensive medications. Body mass index (BMI) was calculated as the ratio of weight (in kilograms) to squared height (in meters). Subjects with repeated fasting blood glucose of ≥126 mg/dL, a history of diabetes, or treatment with antidiabetic drugs were considered to be diabetic.

Weekly alcohol intake was recorded by means of a questionnaire. Subjects were divided into drinkers and non-drinkers, and those assuming >15 drinks weekly were excluded from the study. Subjects were also classified into never and prior or current (≥1 cigarette daily) smokers.

Fasting serum glucose, potassium and lipids were analyzed by enzymatic method. Serum creatinine (SCr) was measured in mg/dl using an auto-analyzer (Cobas Mira Plus 8000, Roche diagnostic, USA) with the alkaline picrate-kinetic Jaffé method [20], and albuminuria was detected from a 24 h urine specimen with the turbidimetric method (Cobas Mira Plus 8000, Roche diagnostic, USA). Subjects were carefully instructed to collect 24 h urine specimen for creatinine clearance (CrCl, in ml/min) estimation, calculated using the following formula: CrCl = Urine SCr × Urine volume/creatinuria × minutes.

Assessment of intima-media thickness

Ultrasonographic images were acquired at end diastole-defined as the R wave of an electrocardiogram-by a Philiphs IE33 sonographer using a 5–10 MHz multifrequency high-resolution linear transducer. Measurements were carried out in the common carotid artery, with the subject supine, neck extended and head slightly turned in the direction opposite to the carotid artery being examined. A 10-mm longitudinal section located at a distance of 1 cm from the bifurcation was studied, and measurements were performed in the proximal and distal walls in lateral, anterior and posterior projections, along an axis perpendicular to the artery, to establish two

Table 2 General characteristics of the population across the homocysteinemia quartiles

Items	1st quartile (15.6 ± 0.4)	2nd quartile (17.4 ± 0.7)	3rd quartile (21.4 ± 1.5)	4th quartile (36.5 ± 1.2)	*p*-value
Age (yrs)	57.1 ± 14.6	61.0 ± 14.1	60.6 ± 15.2	59.9 ± 15.9	NS
Men gender (%)	55.1	56.5	58.0	58	NS
BMI (kg/m^2)	28.0 ± 3.7	28.7 ± 5.5	28.7 ± 5.0	28.8 ± 5.7	NS
SBP (mmHg)	160.5 ± 17.1	158.9 ± 16.8	159.5 ± 17.9	156.4 ± 16.1	NS
DBP (mmHg)	96.9 ± 11.0	95.5 ± 11.4	95.5 ± 9.7	95.1 ± 11.0	NS
PP (mmHg)	63.6 ± 14.6	61.0 ± 14.4	64.0 ± 19.1	63.9 ± 15.0	NS
HR (bpm)	75.7 ± 8.7	75.6 ± 9.1	75.3 ± 8.5	78.0 ± 11.3	NS
Creatinine (mg/dl)	0.9 ± 0.3	0.9 ± 0.2	1.0 ± 0.2	1.0 ± 0.3	NS
Cl_Cr (ml/min)	110.1 ± 32.6	112.3 ± 33.5	94.5 ± 30.7	95.4 ± 23.1	NS
Albuminuria (mg/24 h)	44.7 ± 108.4	49.3 ± 119.2	52.3 ± 117.7	81.6 ± 94.8	<0.001
Glucose (mg/dl)	102.3 ± 29.7	109.4 ± ±39.1	103.1 ± 19.9	105.4 ± 22.4	NS
TC (mg/dl)	201.4 ± 42.1	207.3 ± 39.6	203.0 ± 37.5	211.0 ± 42.7	NS
HDL-C (mg/dl)	57.8 ± 16.4	54.3 ± 17.1	56.5 ± 12.8	57.8 ± 13.5	NS
LDL-C (mg/dl)	110.0 ± 57.0	136.5 ± 76.9	111.1 ± 60.9	130.0 ± 54.5	NS
TG (mg/dl)	121.6 ± 35.9	124.3 ± 38.8	125.7 ± 36.7	127.2 ± 37.6	<0.001
Diabetes (%)	10.1	14.5	14.7	29.0	<0.02
Smoking (%)	7.2	10.2	20.3	27.5	<0.004
CVD (%)	31.9	42.0	52.2	59.4	<0.001
MTHFR (TT vs. Non-TT, %)	12.3	13.6	23.5	50.6 %	<0.0001

BMI body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *PP* pulse pressure, *HR* heart rate, *Cl_Cr* creatinine clearance, *TC* total cholesterol, *HDL-C* high-density lipoproteins cholesterol, *LDL* low-density lipoproteins cholesterol, *TG* triglycerides, *CVD* carotid vascular damage, *MTHFR* methylenetetra-hydrofolate reductase

lines: the intima-media interface and the media-adventitia interface. A total of six measurements were obtained in each carotid artery, and the average mean and maximum values were recorded [21]. We considered CVD to be present when the mean carotid IMT was greater than 0.9 mm [19] or arteriosclerotic plaques (carotid IMT >1.5 mm) were detected.

Statistical analysis

Categorical variables were expressed as percentage rates and were compared with the Pearson's χ^2 test. Continuous variables were expressed as mean and standard deviation, and the differences values were evaluated by analysis of variance, while gender-specific odds ratio (OR) and 95 % confidence intervals (CI) of CVD were calculated for independent variables by multivariate logistic regression analysis. The null hypothesis was rejected for $p < 0.05$. Statistical analyses were performed using SPSS package version 17.0

Results

The general characteristics of the subjects are summarized in Table 1, also showing gender stratification. Men were younger than women (56.2 ± 15.1 and 64.5 ± 13.3, $p < 0.0001$) and have compared to women significantly higher diastolic BP, triglycerides values and prevalence of smoking habits and smoking duration. On the contrary BMI and heart rate values were higher in women than in men. In 46.4 % of cases, sub-clinical CVD was found, without difference between genders (43.8 vs. 50.0, NS). No significant differences were found in the prevalence of the antihypertensive treatment, of different antihypertensive drugs taken and of the history of arterial hypertension and diabetes between genders (Table 1). The prevalence of the antidiabetic therapy (mainly with oral antidiabetic drugs) was not different in men than women (data not shown). However the duration of hypertension and diabetes were higher in subjects with then without CVD, respectively 13.4 ± 5.8 versus. 7.5 ± 4.2 ($p < 0.05$) in former and 10.3 ± 6.2 versus. 6.7 ± 5.8 ($p < 0.05$) in the latter.

As shown in Table 2, age, BP, Scr, TC, HDL-C and LDC-C values were not different across the quartiles of Hcy. On the contrary, albuminuria and triglycerides were significantly ($p < 0.001$) higher in upper versus lower Hcy quartiles. Prevalence of diabetes (10.1, 14.5, 14.7 and 29 %, $p < 0.02$), smoking (7.2, 10.2, 20.3, 27.5 %, $p < 0.004$) and sub-clinical CVD (31.9, 42, 52.2, 59.4 %, $p < 0.001$) was increasingly distributed in the quartiles of Hcy.

Prevalence of TT allele of the MTHFR genotype was significantly distributed in the quartiles of Hcy (13.6, 12.3, 23.5 and 50.6 %, $p < 0.0001$), whereas no relationship was found between sub-clinical CVD and the genotype (48.6 vs. 34.3, NS).

In the multivariate logistic analysis the last quartile of Hcy predicted sub-clinical CVD (OR 1.32, CI 1.12–2.2,

$p = 0.02$) independent of age (OR 1.23, CI 1.002–1.56, $p = 0.0001$), systolic BP (OR 1.52, CI 1.24–2.10), diabetes (OR 2.11, CI 1:32–2.88, $p = 0.01$) and smoking (OR 1.45, CI 1.14–1.98, $p = 0.04$). Adding gender did not modify the model.

Discussion

In our study, performed in uncomplicated hypertensive patients without previous cardiovascular events, age, systolic BP, diabetes, smoking and serum homocysteine each independently predict sub-clinical CVD.

Age is one of the most important determinants of carotid IMT so that IMT is a well-established marker of early atherosclerosis and arterial stiffness associated with advancing age. Our results have been also confirmed in a population-based study, involving people with unknown cardiovascular diseases demonstrating that age was an independent predictor for increased carotid IMT [22].

BP levels have also been associated with increased carotid IMT. The large elastic arteries, such as the carotid artery, represent the most distensible part of the arterial system and are vulnerable to systolic stress. In particular, the systolic BP load stimulates the growth of vascular smooth muscle cells that in long term cause the increase of IMT [23].

In clinical practice, the ultrasonographic assessment of carotid IMT is commonly used as a surrogate marker of cardiovascular disease in people with diabetes. Several research groups have found a strong association between diabetes and IMT, and according with Yamasaki et al. [24], the positive association between diabetes and IMT found in our study is in part due to higher duration of diabetes observed in subjects with than those without sub-clinical CVD. Although the incidence of cardiovascular disease is increasing among patients with diabetes, the role of glycemia in this process remains uncertain, and most of the data on the evolution of atherosclerosis in young uncomplicated diabetic subjects are driven by autopsy studies.

Cigarette smoking is an established causal factor for early atherosclerosis. However, the exact mechanism of smoking-induced damage to the arterial wall and its relation to the atherosclerotic process is still largely not established. The Framingham study demonstrated that smoking has a specific fibrogenic effect which causes increase of IMT [25], but that degree of atherosclerosis seems most related with male gender, the intensity and duration of smoking [26] like in our study.

In our experience, the predictors of sub-clinical CVS were similar in men than in women, confirming the data from the literature where, in people with slight global cardiovascular risk gender differences on IMT disappeared with advanced age [27, 28].

As so far discussed, we confirmed the data from the literature in which the roles of age, systolic BP, diabetes and smoking as independent predictors of sub-clinical CVD are undisputed [22–28]. On the contrary, the relationship between serum Hcy and IMT as independent predictor of sub-clinical CVD in uncomplicated hypertensive subjects (HTs) was poorly investigated.

Although Hcy is not even mentioned in the new 2013 European hypertension guidelines for the management of arterial hypertension [19], accumulating evidences suggest that hyper-Hcy may play a role in increasing IMT in white-coat and sustained hypertensive adolescents and in newly diagnosed HTs [29, 30]. Our study was not addressed to investigate the mechanism by which homocysteine plays an independent role as predictor of sub-clinical CVD [31]. Moreover, we speculate that in HTs hyper-Hcy might increase carotid artery IMT by reducing the amount of the endothelial progenitor cells involved in the repair of the injured endothelium and in modulating the oxidant/antioxidant balance of the body [29]. In keeping with this finding, plasma nitrogen oxides (NO) were lower in hypertensives with hyper-Hcy compared with normotensive controls [30]. Furthermore, homocysteine has atherogenic and prothrombotic properties, and histopathologic examinations demonstrated that it induces vascular injury as intimal thickening, elastic lamina disruption, smooth muscle hypertrophy, marked platelet accumulation and, thrombus formation [32].

Conflicting opinions are in the literature about the role of the MTHFR genotype in relation to Hcy and folate metabolism, the risk of sub-clinical CVD and hypertension. Previous studies have shown that the MTHFR variants C677T have been directly associated with both hyper-Hcy [33] and hypertension risk [34]. In our experience, although in univariate analysis the TT allele of MTHFR genotype was directly associated with higher Hcy levels, no association with this genotype and sub-clinical CVD was observed in multivariate regression analysis. This result confirms the current opinion that identifying patients with the TT genotype of MTHFR is unlikely to be cost effective [35].

Limitation of the study

The design of our cross-sectional study does not permit us to address the temporal relationships between early atherosclerosis and the IMT. However, longitudinal studies suggest that arterial remodelling accompanies significant progression of atherosclerosis in the carotid arteries. Moreover, we speculate on the possible mechanisms by which homocysteine independently predicts sub-clinical CVD. Finally, our study was not addressed to evaluate the effects on IMT and cerebrovascular events by homocysteine lowering through vitamin supplementation.

In conclusion, in uncomplicated hypertensives, homocysteine values >36.5 mM/L independently predict subclinical CVD and in those who are also diabetic and smokers, Hcy determination should be recommended to better stratify the global cerebrovascular risk. We also speculate that hyper-Hcy is (or might be) a mild risk factor for cerebrovascular events exerting an action on IMT probably through influencing the NO-endothelin axis [36]. However, in light of the mild potential benefits with folate and combined B vitamins observed in primary stroke prevention [37], control-randomized trials are needed in uncomplicated hypertensive subjects having hyper-Hcy that represents over the 40 % of the overall hypertensive population and in which high BP levels and sub-clinical CVD still remain the main cerebrovascular risk factors.

Conflict of interest The authors declare they have any conflict of interest with the present study.

References

1. T.J. Cleophas, N. Hornstra, B. van Hoogstraten, J. van der Meulen, Homocysteine, a risk factor for coronary artery disease or not? A meta-analysis. Am. J. Cardiol. **86**, 1005 (2000)
2. U.B. Fallon, Y. Ben-Shlomo, P. Elwood, Ubbink J.B., Smith G.D.: homocysteine and coronary heart disease in the Caerphilly cohort: a 10 year follow up. Heart **85**, 153 (2001)
3. P. Knekt, A. Reunanen, G. Alfthan, M. Heliövaara, H. Rissanen, J. Marniemi et al., Hyperhomocystinemia: a risk factor or a consequence of coronary heart disease? Arch. Intern. Med. **161**, 1589 (2001)
4. Homocysteine Studies Collaboration. Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis. JAMA **288**, 2015–2022 (2002)
5. M. Klerk, P. Verhoef, R. Clarke, H.J. Blom, F.J. Kok, E.G. Schouten, MTHFR Studies Collaboration Group.: mTHFR 677C– > T polymorphism and risk of coronary heart disease: a meta-analysis. JAMA **288**, 2023 (2002)
6. Martí-Carvajal A.J., Solà I., Lathyris D., Karakitsiou D.E., Simancas-Racines D.: Homocysteine-lowering interventions for preventing cardiovascular events. Cochrane Database Syst Rev 1:CD006612 (2013) doi:10.1002/14651858
7. S.C. Smith Jr, R.V. Milani, D.K. Arnett, J.R. Crouse 3rd, M.M. McDermott, P.M. Ridker et al., Atherosclerotic Vascular Disease Conference: Writing Group II: risk factors. Circulation **109**, 2613 (2004)
8. R.S. Rosenson, C.C. Tangney, Preanalytical sources of measurement error: the conundrum of the homocysteine hypothesis. Atherosclerosis **194**, 520 (2007)
9. M. Curro, S. Condello, D. Caccamo, R. Ientile, Homocysteine-induced toxicity increases TG2 expression in neuro2a cells. Amino Acids **36**, 725–730 (2009)
10. G.N. Welch, J. Loscalzo, Homocysteine and atherothrombosis. N. Engl. J. Med. **338**, 1042–1050 (1998)
11. U. Till, P. Rohl, A. Jentsch, H. Till, A. Muller, R. Riezler, Decrease of carotid intima-media thickness in patients at risk to cerebral ischemia after supplementation with folic acid, vitamins B6 and B12. Atherosclerosis **181**, 131–135 (2005)
12. H.N. Hodis, W.J. Mack, L. Dustin, R.H. Selzer, High-dose B vitamin supplementation and progression of subclinical atherosclerosis: a randomized controlled trial. Stroke **40**, 730–736 (2009)
13. M. Lee, K.S. Hong, S.C. Chang, J.L. Saver, Efficacy of homocysteine-lowering therapy with folic Acid in stroke prevention: a meta-analysis. Stroke **41**, 1205–1212 (2010)
14. H.N. Hodis, W.J. Mack, L. LaBree, R.H. Selzer, Liu C, Liu C. et al.: Reduction in carotid arterial wall thickness using lovastatin and dietary therapy: a randomized controlled clinical trial. Ann. Intern. Med. **124**, 548–556 (1996)
15. D.H. O'Leary, J.F. Polak, R.A. Kronmal, P.J. Savage, N.O. Borhani, S.J. Kittner et al., Thickening of the carotid wall: a marker for atherosclerosis in the elderly? Stroke **27**, 224–231 (1996)
16. P.J. Touboul, J. Labreuche, E. Vicaut, P. Amarenco, Carotid intima-media thickness, plaques, and Framingham risk score as independent determinants of stroke risk. Stroke **36**, 1741–1745 (2005)
17. J.F. Polak, M.J. Pencina, K.M. Pencina, C.J. O'Donnell, P.A. Wolf, R.B. D'Agostino Sr, Carotid-wall intima-media thickness and cardiovascular events. N. Engl. J. Med. **365**, 213–221 (2011)
18. M.W. Lorenz, H.S. Markus, M.L. Bots, M. Rosvall, M. Sitzer, Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis. Circulation **115**, 459–467 (2007)
19. ESH/ESC guidelines for the management of arterial hypertension: The Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Eur Heart J. (2013) [Epub ahead of print]
20. B.C. Mazzachi, M.J. Peake, V. Ehrhardt, Reference range and method comparison studies for enzymatic and Jaffé creatinine assays in plasma and serum and early morning urine. Clin. Lab. **46**, 53–55 (2000)
21. P.J. Touboul, M.G. Hennerici, S. Meairs, H. Adams, P. Amarenco, N. Bornstein, et al Mannheim carotid intima-media thickness consensus (2004-2006). An update on behalf of the Advisory Board of the 3rd and 4th Watching the Risk Symposium, 13th and 15th European Stroke Conferences, Mannheim, Germany, 2004, and Brussels, Belgium, 2006. Cerebrovasc. Dis. **23**, 75–80 (2007)
22. C. Pastorius, J. Medina-Lezama, F. Corrales-Medina, A. Bernabe-Ortiz, R. Paz-Manrique, B. Salinas-Najarro et al., Normative values and correlates of carotid artery intima-media thickness and carotid atherosclerosis in Andean-Hispanics: The Prevencion Study. Atherosclerosis **211**, 499–505 (2010)
23. D.Y. Leung, S. Glagov, M.B. Mathews, Cyclic stretching stimulates synthesis of matrix components by arterial smooth muscle cells in vitro. Science **191**, 475–477 (1976)
24. Y. Yamasaki, R. Kawamori, H. Matsushima et al., Atherosclerosis in carotid artery of young IDDM patients monitored by ultrasound high resolution B-mode imaging. Diabetes **43**, 634–639 (1994)
25. W.B. Kannel, P. Sorlie, F. Brand, W.P. Castelli, P.M. McNamara, G.J. Gheradi, Epidemiology of coronary atherosclerosis: Post Mortem vs. clinical risk factor correlations: The Framingham Study, in *Atherosclerosis*, ed. by A.M. Gotto, L.C. Smith, B. Allen (Springer, New York, 1980), pp. 54–56
26. A.R. Sharrett, J. Ding, M.H. Criqui, M.F. Saad, K. Liu, J.F. Polak, A.R. Folsom, M.Y. Tsai, G.L. Burke, M. Szklo, Smoking, diabetes, and blood cholesterol differ in their associations with subclinical atherosclerosis: The Multiethnic Study of Atherosclerosis (MESA). Atherosclerosis **186**, 441–447 (2005)
27. C. Sinning, P.S. Wild, F.M. Echevarria, S. Wilde, R. Schnabel, E. Lubos, S. Herkenhoff, C. Bickel, S. Klimpe, T. Gori, T.F. Münzel, S. Blankenberg, Espinola-Klein C; Gutenberg-Heart Study. Sex differences in early carotid atherosclerosis (from the community-based Gutenberg-Heart Study). Am. J. Cardiol. **107**, 1841–1847 (2011)
28. J. Ferrieres, A. Elias, J.B. Ruidavets et al., Carotid intima-media thickness and coronary heart disease risk factors in a low-risk population. J. Hypertens. **17**, 743–748 (1999)

29. P. Bogdanski, E. Miller-Kasprzak, D. Pupek-Musialik, A. Jablecka, M. Lacinski, P.P. Jagodzinski, H. Jakubowski, Plasma total homocysteine is a determinant of carotid intima-media thickness and circulating endothelial progenitor cells in patients with newly diagnosed hypertension. Clin. Chem. Lab. Med. **50**, 1107–1113 (2012)
30. S. Lengyel, E. Katona, J. Zatik, C. Molnár, G. Paragh, B. Fülesdi, D. Páll, The impact of serum homocysteine on intima-media thickness in normotensive, white-coat and sustained hypertensive adolescents. Blood Press. **21**, 39–44 (2012)
31. K.S. McCully, Vascular pathology of homocysteinemia: implications for the pathogenesis of arteriosclerosis. Am. J. Pathol. **56**, 111 (1969)
32. J.C. Tsai, M.A. Perrella, M. Yoshizumi et al., Promotion of vascular smooth muscle cell growth by homocysteine: a link to atherosclerosis. Proc. Natl. Acad. Sci. USA **91**, 6369 (1194)
33. A.K. Bottiger, A. Hurtig-Wennlof, M. Sjostrom, A. Yngve, T.K. Nilsson, Association of total plasma homocysteine with methylenetetrahydrofolate reductase genotypes 677C > T, 1298A > C, and 1793G > A and the corresponding haplotypes in Swedish children and adolescents. Int. J. Mol. Med. **19**, 659–665 (2007)
34. X. Qian, Z. Lu, M. Tan, H. Liu, D. Lu, A meta- analysis of association between C677T polymorphism in the methylenetetrahydrofolate reductase gene and hypertension. Eur. J. Hum. Genet. **15**, 1239–1245 (2007)
35. P.W. Wilson, Homocysteine and coronary heart disease: how great is the hazard? JAMA **288**, 2042 (2002)
36. E. Katona, G. Settakis, Z. Varga, G. Paragh, D. Bereczki, B. Fülesdi, D. Páll, Target-organ damage in adolescent hypertension. Analysis of potential influencing factors, especially nitric oxide and endothelin-1. J. Neurol. Sci. **247**, 138–143 (2006)
37. L.B. Goldstein, R. Adams, M.J. Alberts et al., Primary prevention of ischemic stroke: a guideline from the American Heart Association/American Stroke Association. Stroke **37**, 1538–1633 (2006)