Increased fasting total homocysteine plasma levels as a risk factor for thromboembolism in children

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Summary

Elevated total homocysteine (tHcy) concentrations are an independent risk factor for thromboembolic events in adults. In children with moderate hyperhomocysteinemia data are sparse. Therefore, between 1995 and 2002 we consecutively recruited 163 white pediatric patients with a first symptomatic thromboembolic event and 255 healthy controls (mean age: 6.4 years in patients vs. 6.6 years in controls, range: 3 months to 18 years) and measured fasting tHcy levels. Median tHcy levels in patients were significantly higher (6.6 µmol/l, range 2.9-20.4 µmol/l) than in controls (5.7 µmol/l, 2.0-14.0 µmol/l, $p<0.0001$). 48 of the 163 patients with thromboembolism (29.5%) versus 26 of the 255 controls (10.2%) had tHcy levels above the age-specific normal 90th percentile (OR 2.9, 95%CI: 1.7-4.8). The odds ratio for children in the highest quintile compared to children with levels in the lowest quintile was 4.3 (1.6-8.1; highest quintile: median tHcy level 9.6 µmol, range 8.0-20.4), showing a significantly increased risk for thromboembolic disease with even mild hyperhomocysteinemia. We conclude that hyperhomocysteinemia above the age-specific cut-off values is a risk factor for thromboembolic events in children. Therefore, screening for elevated fasting tHcy levels of patients with thromboembolism is recommended to stratify the risk of thromboembolism.

Keywords

Homocysteine, hyperhomocysteinemia, thromboembolism, children

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Introduction

Although venous and arterial thromboses are rare diseases in infancy and childhood, symptomatic thrombotic manifestations are recorded in 0.07/10,000 children and in 5.3/10,000 admissions of children (1). In many children the etiopathology of the thromboembolic event remains unclear; however, an elevated homocysteine concentration has been suggested as one relevant risk factor in these patients (2).

Disorders of homocysteine metabolism resulting in homocystinuria or hyperhomocysteinemia are often caused by defects in the genes of either the cystathionine β-synthase (CBS) or the 5,10 methylenetetrahydrofolate-reductase (MTHFR) resulting in reduced metabolism of homocysteine. The most common

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causes of severe hyperhomocysteinemia - or homocystinuria - are defects in the transsulfuration pathway due to an autosomal-recessively inherited CBS-deficiency. Patients with this rare congenital defect suffer from premature cardiovascular disease and venous thrombosis at a very young age (3). Moderate or mild hyperhomocysteinemia is more often observed and is frequently associated with defects in the remethylation pathway, e.g. homozygosity for the MTHFR-677T allele especially in individuals with low plasma folate levels (4-6). The prevalence of a homozygous state of this variant in the German population is estimated at ~10% (7).

Severe hyperhomocysteinemia is an established and independent risk factor for cardiovascular complications in adults and children (2, 8). In the past decade it has become increasingly evident that even moderate hyperhomocysteinemia is a risk factor for premature arteriosclerosis and venous thromboembolic events as well as strokes in adults (9, 10). More than 50% of the cardiovascular complications observed in patients with severe hyperhomocysteinemia are thromboembolic events (3). However, the relation between moderately elevated homocysteine levels and venous thromboembolism is still a matter of discussion.

Although this issue has been repeatedly studied in adults, data concerning children with hyperhomocysteinemia are sparse. Whether moderate hyperhomocysteinemia is a risk factor for symptomatic thromboembolism in children is not clear.

Therefore, we performed a controlled study to assess a possible relation between elevated tHcy levels and thromboembolic events in children in Germany.

Materials and methods

Ethics

The present study was performed in accordance with the ethical standards laid down in the updated relevant version of Declaration of Helsinki and approved by the medical ethics committee at the Westfälische Wilhelms-University, Münster, Germany.

Study period

In total 971 pediatric patients aged neonate to 18 years with a first symptomatic thromboembolic event were consecutively recruited and screened for hereditary prothrombotic risk factors between January 1995 and June 2002 by the participating centers in the catchment areas of Hamburg, Kiel, Lübeck, Münster, Bielefeld, Düsseldorf, Berlin, Magdeburg, Halle and Munich.

Inclusion criteria

Inclusion criteria were a thromboembolic event confirmed objectively by standard imaging methods, i.e., duplex sonography, venography, CT or MR imaging for the diagnosis of venous thromboembolism, and cerebral CT scanning, MR imaging, MR angiography, or transcranial Doppler ultrasonography for the diagnosis of thromboembolic ischemic stroke. We included only white children because of published data concerning ethnic differences in tHcy levels (11). Additionally, the children had to be on normal diets.

Exclusion criteria

Patients with concomitant diseases, e.g. renal insufficiency (12, 13), medication, e.g. antiepileptic drugs (14, 15), folate- or vitamin B12-deficiency (16), and vitamin supplementation were excluded. It is notable that in Germany - unlike the situation in the US - food is subject to no routine folate supplementation. Furthermore, patients with inherited CBS-deficiency leading to homocystinuria were excluded. Additionally, patients for whom parental consent was refused were not enrolled into the study.

We did not exclude patients with additional exogenous or inherited thromboembolic risk factors, because den Heijer et al. reported in a recent study of 269 patients that the exclusion of subjects with other established risk factors for thrombosis - e.g. a deficiency of protein C, protein S, antithrombin, pregnancy, recent childbirth or oral contraceptive use - do not materially affect the estimated risk ratios for patients with hyperhomocysteinemia (10). Although these data of den Heijer et al. were derived from adult patients and may therefore be of limited value in pediatric patients, we assume that our findings were not substantially influenced by other putative prothrombotic risk factors.

Final study population

From the ongoing multicenter study a randomly selected subgroup of 163 white patients (mean age: 6.4 years, range: 3 months to 18 years) from all study centers of the different catchment areas of Germany was analyzed in a subgroup analysis. Randomization into the substudy - and therefore reduction of patient number - was performed to reduce laboratory costs of homocysteine, vitamin B12 and folate measurements. Blood collection from these patients was performed in Münster.

The thrombotic manifestation included deep vein thrombosis (n=34), cerebral venous thrombosis (n=14), renal venous thrombosis (n=7), axillary and subclavian vein thrombosis (n=3), caval vein thrombosis (n=1), portal and hepatic vein thrombosis (n=5), pulmonary embolism (n=4) and thromboembolic stroke of venous origin (n=95).

Control group

Patients were compared with 255 healthy, age-related white children from different geographic areas of Germany (mean age: 6.6 years, range: 3 months to 18 years).

Samples from controls were collected with informed parental consent. Controls were recruited between February 1996 and June 2002. They comprised children with no history of chronic disease or of thromboembolic events and medication-free at the

time of recruitment, who presented as outpatients for evaluation before minor surgery (planned circumcisions and hernias) or as potential bone-marrow donors.

Blood sampling

Fasting tHcy concentrations were investigated in the subjects according to a standardized protocol. In all included patients peripheral venipuncture was performed in the morning between 8 and 10 a.m. after a standardized fasting period: at least 4 hours for infants below one year of age including breastfed infants, and more than 8 hours for children above one year of age and for adolescents.

In order to avoid influences of the acute phase of thromboembolism, the minimum time between onset of thromboembolic symptoms in patients and tHcy determination was 3 months. Additionally, the results obtained at 3 months were confirmed 6 and 12 months after the thromboembolic event. For statistical analysis, values of the measurement taken after 6 months were used; no relevant difference was found after 3, 6 and 12 months. None of the patients or controls received routine supplementation with folate and/or vitamin preparations before blood sampling.

There have been reports that measurement of tHcy after a methionine loading test is more sensitive for diagnosis of hyperhomocysteinemia. However, in our study a methionine loading test was not performed since we assumed that this test would be less manageable in children. Thus, we cannot exclude the possibility that a methionine loading test may be even more sensitive in identifying children at risk for thromboembolism.

Homocysteine assays

Total plasma tHcy levels were measured in EDTA-plasma by high-performance liquid chromatography (HPLC) with reverse phase separation and fluorescent detection based on the method of Araki and Sako (17). Separation conditions were modified using 0.24 mM acetate, flow rate 1ml/min, and a reverse phase column C18 Xterra (150 x 39 mm, Waters, Eschborn). CVs within/between days were 2.2%/3.5%. After blood collection in EDTA-containing tubes, samples were transported on ice to the laboratory, centrifuged, separated and frozen at -20°C within 30 minutes after venipuncture in order to avoid artificially increased tHcy concentrations. In all children with elevated tHcy levels, plasma folate and vitamin B12 levels were measured (age-related limits for vitamin B12: 243 - 894 pg/ml, and for folate: 4.2-19.9 ng/ml).

Statistical analysis

All statistical analyses were performed using the StatView 5 software package (SAS Institute Inc.) and the MedCalc software package (MedCalc, Mariakerke, Belgium).

Upper 90th percentiles of tHcy levels were determined in the 255 healthy pediatric controls in the different age groups. An unmatched odds ratio was calculated for tHcy levels > 90th percentile in the 163 patients and 255 controls.

Moreover, the distribution of fasting homocysteine concentrations in patients and controls was compared using quintile distributions in this group. Thus, odds ratios adjusted for age and sex by logistic regression comparing the distribution of patients/controls in the 2nd to 5th quintiles versus the distribution in the lowest (1st) quintile were determined with a 95% confidence interval (CI) as described previously (18-20).

For variables with a non-Gaussian frequency distribution, data were presented as medians and ranges, otherwise as means. All evaluations and all comparisons of the group of patients and controls were conducted using the Mann-Whitney test, χ-square-test and the odds ratio (p-values < 0.05 were considered significant).

Results

The median level of tHcy was 6.6 µmol/l (range 2.9-20.4 µmol/l) in patients with thromboembolic events compared with 5.7 µmol/l (range 2.0-14.0 µmol/l) in control subjects. Median levels were significantly different between these two groups ($p < 0.0001$).

Figures 1 and 2 show the raw data of total fasting homocysteine levels in relation to age. The correlation coefficients were 0.2 (p=0.02) in the patient group versus 0.13 (p=0.04) in the control group. Thus, a trend to somewhat higher tHcy concentrations with increasing age was observed in both groups.

Table 1 shows tHcy concentrations in the different age groups separated for gender in patients and controls. There was no significant difference in tHcy levels in any age group with respect to gender.

Upper age-specific 90th percentiles of tHcy levels in the 255 healthy pediatric controls showed a tendency towards two age-dependent peaks: In infancy (children < 12 months, n=39) the upper 90th percentile was 8.5 µmol/l, and during puberty (10-18 years, n=57) 9.0 µmol/l. In children between 1-6 years (n=94) the upper 90th percentile was 8.0 µmol/l, and between 6-10 years (n=65) 7.3 µmol/l. Taken together, the upper 90th percentile in the healthy pediatric control group was 8.4 µmol/l.

In the group of children with thromboembolism, 48 of the 163 patients (29.5%) versus 26 of the 255 children (10.2%) in the control group had tHcy levels above the age-specific 90th percentile (OR 2.9, 95% CI: 1.7 - 4.8).

Table 2 shows the quintiles of these tHcy plasma concentrations. For calculation of quintiles and odds ratios, the group of 255 controls was analyzed together with the 163 patients adjusted for age and sex. The ORs demonstrate that patients with tHcy levels in the highest quintile had a significantly increased risk of thromboembolic events compared with patients with levels in the lowest quintile (highest quintile: median tHcy level 9.6 µmol, range 8.0-20.4, OR 4.3, CI 1.6-8.1). Thus the risk of a

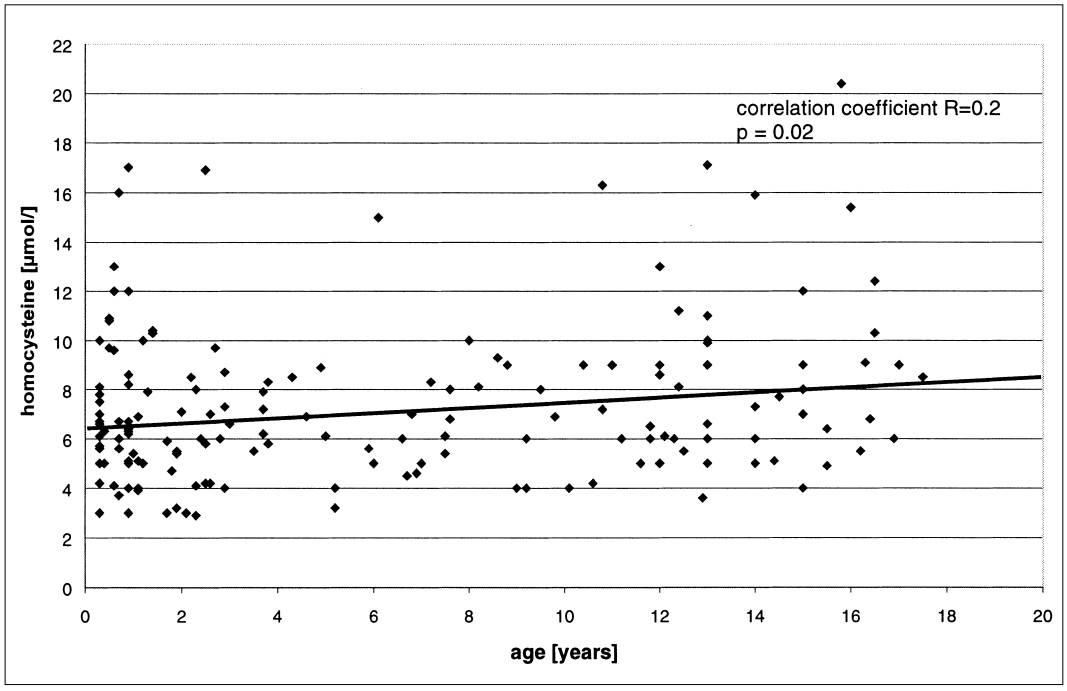

Figure 1: Fasting total homocysteine concentrations and age in patients (n=163).

thromboembolic event was 4.3 times higher with tHcy levels in the highest quintile.

In all patients with elevated tHcy levels, folate and vitamin B12 plasma concentrations were measured and found to be within the age-related limits.

Interestingly, none of the patients suffering from protein C deficiency (n=2), protein S deficiency (n=4) or antithrombin deficiency (n=2) showed elevated homocysteine concentrations. The latter also applied to the 3 control subjects with protein C deficiency, protein S deficiency and antithrombin deficiency (one case each).

Table 3 shows homocysteine concentrations and MTHFR C677T genotype in patients and controls. No dependency of tHcy concentration on genotype, in particular no elevated levels in subjects with the MTHFR TT-genotype compared with children with the CC or CT-genotype, was observed in either group

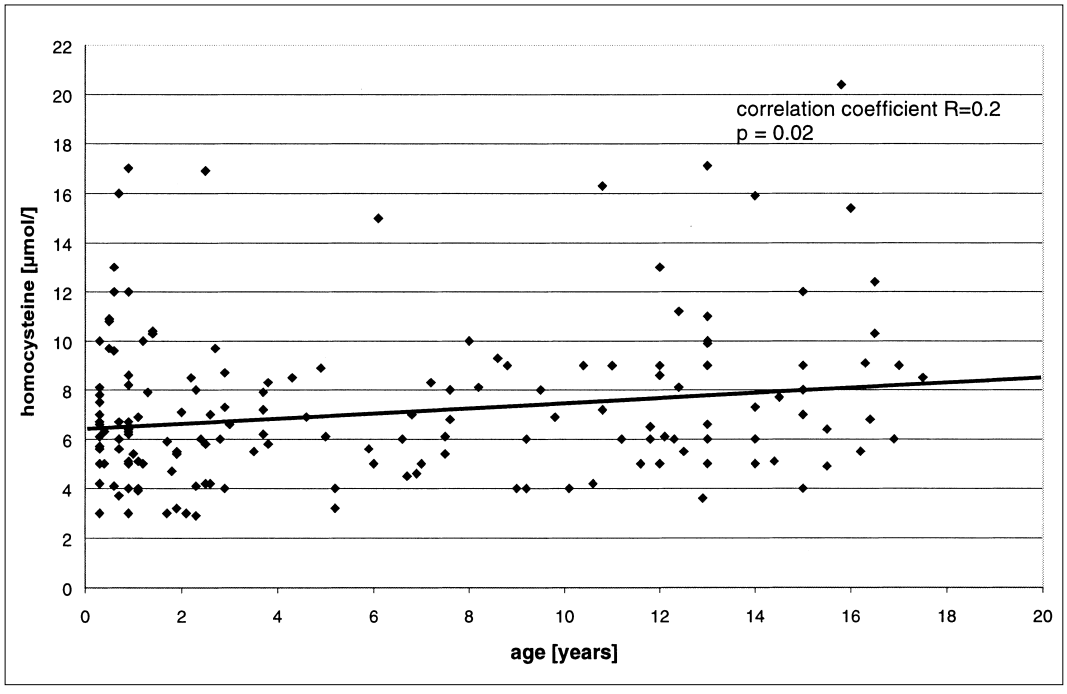

Figure 2: Fasting total homocysteine concentrations and age in controls (n=255).

Table 1: Fasting total homocysteine concentrations dependent on gender and age (median and range) in patients and controls.

Age	Patients		Controls	
<12 months	6.6 (3.0-17.0) n=41	male: 6.6 (3.7-13.0) female:6.7 (3.0-17.0)	6.0 (2.0-12.0) n=39	male: 6.0 (3.6-10.7) female:5.9 (2.0-12.0)
1 to 6 years	6.0 (2.9-16.9) n=47	male: 5.6 (2.9-10.4) female:6.5 (3.0-16.9)	5.0 (2.4-14.0) n=94	male: 5.1 (2.4-10.3) female:5.0 (3.0-14.0)
6 to 10 years	6.9 (4.0-16.3) n=26	male: 6.1 (4.0-17.0) female:8.0 (4.2-16.3)	6.0 (2.0-10.0) n=65	male: 6.0 (2.5-10.0) female:6.0 (2.0-8.6)
10 to 18 years	7.7 (3.6-20.4) n=49	male: 7.8 (4.9-15.9) female:7.3 (3.6-20.4)	6.3 (2.3-12.5) n=57	male: 6.9 (3.0-9.0) female:6.1 (2.3-12.5)
Total	6.6 (2.9-20.4) n=163	male: 6.5 (2.9-15.9) female:7.0 (3.0-20.4)	5.7 (2.0-14.0) n=255	male: 5.7 (2.4-10.7) female:5.7 (2.0-14.0)

There was no significant intergroup difference in age and gender.

Table 2: Median values and ranges of quintiles of total fasting homocysteine concentrations. ORs calculated by unmatched logistic regression adjusted for age and gender (2nd to 5th quintile compared to the lowest quintile).

THcy levels [µmol/l]	Median (range)	Cases (n=163)	Controls (n= 255)	Odds ratios
1st quintile	4.0 (2.0-4.4)	25	59	-
2nd quintile	5.0 (4.5-5.4)	22	61	0.5 (0.2-1.7)
3rd quintile	6.0 (5.5-6.4)	31	52	1.1 (0.5-2.8)
4th quintile	7.0 (6.5-7.9)	31	51	1.1 (0.5-2.8)
5th quintile	9.6 (8.0-20.4)	54	32	4.3 (1.6-8.1)

Table 3: Fasting total homocysteine concentrations dependent on the MTHFR C677T-genotype in patients and controls. (p-value in the patient group p=0.59, in the control group p=0.95, Mann-Whitney). The OR (CI 95%) for the prevalence of the MTHFR TT-genotype in patients is 0.62 (0.3 –1.3), p=0.25.

MTHFR C677T genotype	Hcy levels (median and range) in patients [µmol/l]	Hcy levels (median and range) in controls [µmol/l]
CC + CT	6.7 (2.9-20.4) n=151	5.7 (2.0-14.0) n=226
TT	6.4 (3.9-13.0) n=12	5.8 (3.5-9.9) n=29

(p=0.59 and 0.95, Table 3). The prevalence of the MTHFR TT-genotype in patients was lower than in controls (Odd´s ratio 0.62, p=0.25), indicating no increased risk for thromboembolic events in subjects with the TT variant.

Discussion

Data on homocysteine plasma concentrations in children with thromboembolic complications are sparse and equivocal. Most studies investigating tHcy as a possible risk factor for thromboembolic events focus on adults (21). In our cohort of 163 children with thromboembolic complications, tHcy levels were significantly higher than in the group of 255 control children. The latter was demonstrated in an analysis by stratification into quintiles of homocysteine concentrations and calculation of odds ratios adjusted for age and sex by logistic regression as a valid statistical tool in large-scale populations (18). This multivariate methodology allows an estimation of increased relative risk with even mildly elevated homocysteine concentrations (2). Comparison of the 163 patients and the 255 controls revealed that tHcy levels exceeding the highest quintile were associated with a significantly increased risk for thromboembolic events

(OR 4.3). The median tHcy concentration of 9.6 µmol/l with a range of 8.0-20.4 in the highest quintile indicates moderate hyperhomocysteinemia and therefore suggests that a tHcy concentration in this range is already a risk factor for thromboembolic events in children.

As the vitamin status of all patients with hyperhomocysteinemia in our study was within the normal range, abnormalities in folate or vitamin B12 metabolism are unlikely to have contributed to hyperhomocysteinemia in our patients. Nevertheless, folate supplementation was used as a therapeutic approach in these patients with the aim of lowering tHcy levels (22).

Moreover, in Germany – from where our patient and control populations were derived – routine folate supplementation in daily food is not practiced. In contrast, folate supplementation of daily food is very common in the United States (23). Therefore, comparison of folate status and homocysteine levels between European patients and patients from the US is suggested as a target of an international multicenter study in children.

Most published studies on hyperhomocysteinemia as a thrombophilic risk factor were performed in adults and elderly patients. Patients with deep-vein thrombosis showed an OR for thrombosis of 2.5 with plasma tHcy levels above the 95th percentile (10). Therefore, den Heijer et al. suggested that high plasma tHcy levels were a risk factor for deep-vein thrombosis in the population investigated (10). The pooled OR of a recent meta-analysis performed by Ray et al. was 2.95 for elevated fasting plasma tHcy and venous thromboembolism (VTE) (24). In addition, Falcon and coworkers reported a rate of 18.8% for elevated homocysteine concentration in patients with venous thrombosis before the age of 40 years compared with 1.9% in healthy controls (25). These results in adults are in accordance with our data in children with symptomatic thromboembolism studied after the acute phase of thromboembolism (OR of 3.6 in children with tHcy levels in the highest quintile).

Interest in hyperhomocysteinemia in children has increased in recent years and several research groups have published reference levels for tHcy for children from birth until puberty. In children below 12 years the mean tHcy concentration is about 4-8 µmol/l, which is about 60% of the values expected in adults (26, 27). In the literature total homocysteine levels are reported to increase moderately as a function of age (28, 29), a finding which is in accordance with our data concerning adolescents. The raw data displayed in the scatterplots show a tendency to higher tHcy concentrations with increasing age in children older than neonates and infants. This dependency of Hcy levels on age was statistically significant; however, the correlation was rather weak. The trend to increasing homocysteine concentrations with increasing age is in accordance with the literature (28, 29) and with the observation of lower tHcy levels in children than in adolescents and adults. An unexpected and additional finding was the moderate peak in tHcy levels in infants younger than 12 months observed in our patients.

Similar to published data in adults, some pediatric studies (12, 30), but not all (26, 29, 31, 32) reported a slightly higher tHcy level in male than in female children. In our study, no significant difference between genders was found in any age group. However, children over 15 years of age were relatively underrepresented in our population. Therefore our data may underestimate a possible trend to higher tHcy levels in boys since this gender effect has been suggested to be enhanced during and after puberty (> 15 years) (33).

The interaction between hyperhomocysteinemia and protein C is controversially discussed (34). Lentz and co-workers showed that activation of protein C by thrombin and inactivation of FVa by activated protein C are not impaired during moderate hyperhomocysteinemia (35). Moreover, den Heijer at al. showed that exclusion of patients with exogenous and inherited prothrombotic risk factors did not materially alter the risk estimates for hyperhomocysteinemia in their large patient population (10). We did not include the statistical analysis of protein C, protein S and antithrombin deficiencies in our patients/controls since only 8 of the 163 patients were affected compared with 3 control subjects. None of these patients and controls suffered additionally from increased homocysteine concentrations. Therefore it is unlikely that protein C, protein S or antithrombin deficiency had an important influence in the cohort presented here.

Moreover, we studied the possible relation between fasting homocysteine concentrations and the MTHFR-genotype, since increased Hcy levels have been reported in adults who are homozygous for the MTHFR-677T allele (5, 7). However, it is not clear whether a tendency to higher Hcy levels in subjects with homozygosity for the MTHFR-677T allele can be already found in children. In contrast to our former data published by Koch et al. in a smaller cohort of children, we cannot confirm the previously described interaction between homocysteine concentrations and MTHFR-genotypes (36). In our study, the prevalence of the homozygous TT-variant observed in the present analysis was even lower in patients compared with controls and was found to be in accordance with literature data (5, 7). However, the latter difference did not reach statistical significance.

Although some studies have addressed tHcy levels in children, only few studies are available concerning the issue of hyperhomocysteinemia as an independent risk factor for thromboembolic events in children (2, 14). We considered tHcy levels above the 90th age-specific percentile of fasting tHcy levels to be elevated. Based on these cut-off values of tHcy levels, 29.5% of the patients and 10.2% of the controls had values above the 90th age-corrected percentiles. This corresponds to an odds ratio of 2.9 for thromboembolic events, suggesting that a value above the 90th percentile is a risk factor for thromboembolism.

In summary, our data suggest that hyperhomocysteinemia above the age-specific cut-off value according to the highest

quintile is a risk factor for thromboembolic events in the children investigated. According to the data presented here, screening for elevated fasting tHcy levels in children with thromboembolism is justified. Further studies are recommended to evaluate the role of fasting homocysteine in children from different ethnical backgrounds suffering from thrombosis.

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References

1. Andrew M, David M, Adams M, et al. Venous thromboembolic complications (VTE) in children: first analyses of the Canadian Registry of VTE. Blood 1994; 83: 1251-7.
2. Van Beynum IM, Smeitink JAM, den Heijer M, et al. Hyperhomocysteinemia - A risk factor for ischemic stroke in children. Circulation 1999; 99: 2070-2.
3. Mudd SH, Skovby F, Levy HL, et al. The natural history of homocystinuria due to cystathionine beta-synthase deficiency. Am J Hum Genet 1985; 37: 1-31.
4. 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: 111-3.
5. Junker R, Nowak-Göttl U, Fobker M. The Methylenetetrahydrofolate-reductase (MTHFR) C677T-polymorphism and other genetic causes of hyperhomocysteinemia in venous thrombosis. J Lab Med 2001; 25: 239-53.
6. Jacques PF, Rosenberg IH, Rogers G, et al. Serum total homocysteine concentrations in adolescent and adult Americans: results from the third National Health and Nutrition Examination Survey. Am J Clin Nutr 1999; 69: 482-9.
7. Nowak-Göttl U, Wermes C, Junker R, et al. Prospective evaluation of the thrombotic risk in children with acute lymphoblastic leukemia carrying the MTHFR TT 677 genotype, the prothrombin G20210A variant, and further prothrombotic risk factors. Blood 1999; 93: 1595-9.
8. Karg JD, Sinnreich R, Rosenberg IH, et al. Plasma homocysteine and parental myocardial infarction in young adults in Jerusalem. Circulation 2002; 105: 2725-9.
9. Boushey CJ, Beresford SAA, Omenn GS, et al. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. JAMA 1995; 274: 1049-57.
10. Den Heijer M, Koster T, Blom HJ, et al. Hyperhomocysteinemia as a risk factor for deep-vein thrombosis. N Engl J Med 1996; 334: 759-62.
11. Lin JS, Shen MC, Cheng WC, et al. Age, sex and vitamin status affect plasma level of homocysteine, but hyperhomocysteinaemia is possibly not an important risk factor for venous thrombophilia in Taiwanese Chinese. Br J Haematol 2002; 117: 159-63.
12. Osganian SK, Stampfer MJ, Spiegelman D, et al. Distribution of and factors associated with serum homocysteine levels in children: Child and Adolescent Trial for Cardiovascular Health. JAMA 1999; 281: 1189-96.
13. Merouani A, Lambert M, Delvin EE, et al. Plasma homocysteine concentration in children with chronic renal failure. Pediatr Nephrol 2001; 16: 805-11.
14. Cardo E, Vilaseca MA, Campistol J, et al. Evaluation of hyperhomocysteinaemia in children with stroke. Eur J Paediatr Neurol 1999; 3: 113-7.
15. Verrotti A, Pascarella R, Trotta D, et al. Hyperhomocysteinemia in children treated with sodium valproate and carbamazepine. Epilepsy Res 2000; 42: 253-7.
16. Selhub J, Jacques PF, Bostom AG, et al. Relationship between plasma homocysteine and vitamin status in the Framingham study population. Impact of folic acid fortification. Public Health Rev 2000; 28: 117-45.
17. Araki A, Sako Y. Determination of free and total homocysteine in human plasma by high performance liquid chromatography with fluorescence detection. J Chromatogr 1987; 422: 43-52.
18. Nowak-Göttl U, Junker R, Hartmeier M, et al. Increased lipoprotein (a) is an important risk factor for venous thromboembolism in childhood. Circulation 1999; 100: 743-8.
19. Schlesselman, JJ. Case control studies: design, conduct, analysis. Oxford University Press. Oxford, New York. 1982.
20. Hosmer DW, Lemeshow S. Applied logistic regression. New York: Wiley. 1989.
21. Key NS, McGlennen RC. Hyperhomocyst (e)inemia and Thrombophilia. Arch Pathol Lab Med 2002; 126:1367-75.
22. Brouwer IA, van Dusseldorp M, Thomas CMG, et al. Low-dose folic acid supplementation decreases plasma homocysteine concentrations: a randomised trial. Indian Heart J 2000; 52: S53-8.
23. Rothenberg SP. Increasing the dietary intake of folate: pros and cons. Semin Hematol 1999; 36: 65-74.
24. Ray JG. Meta-analysis of hyperhomocysteinemia as a risk factor for venous thromboembolic disease. Arch Intern Med 1998; 158: 2101-6.
25. Falcon CR, Cattaneo M, Panzeri D, et al. High prevalence of hyperhomocyst(e)inemia in patients with juvenile venous thrombosis. Arterioscler Thromb 1994; 14: 1080-3.
26. Tonstad S, Refsum H, Sivertsen M, et al. Relation of total homocysteine and lipid levels in children to premature cardiovascular death in male relatives. Pediatr Res 1996; 40: 47-52.
27. Balasa VV, Gruppo RA, Glueck CJ, et al. The relationship of mutations in the MTHFR, prothrombin, and PAI-1 genes to plasma levels of homocysteine, prothrombin and PAI1 in children and adults. Thromb Haemost 1999; 81: 739-44.
28. Delvin EE, Rozen R, Merouani, et al. Influence of methylenetetrahydrofolate reductase genotype, age, vitamin B-12, and folate status on plasma homocysteine in children. Am J Clin Nutr 2000; 72: 1469-73.
29. Rauh M, Verwied S, Knerr I, et al. Homocysteine concentrations in a German cohort of 5000 individuals: reference ranges and determinants of plasma levels in healthy children and their parents. Amino Acids 2001; 20: 409-18.
30. Minniti G, Cerone R, Piana A, et al. Plasma and serum total homocysteine concentrations in paediatric patients, evaluated by high-performance liquid chromatography with fluorescence. Clin Chem Lab Med 2000; 38: 675-6.
31. Reddy MN. Reference ranges for total homocysteine in children. Clin Chim Acta 1997; 262: 153-5.
32. Vilaseca MA, Moyano D, Ferrer I, et al. Total homcysteine in pediatric patients. Clin Chem 1997; 43: 690-2.
33. De Laet C, Wautrecht JC, Brasseur D, et al. Plasma homocysteine concentration in a Belgian school-age population. Am J Clin Nutr 1999; 69: 968-72.
34. Undas A, Williams EB, Butenas S, et al. Homocysteine inhibits inactivation of factor Va by activated protein C. J Biol Chem 2001; 276: 4389-97.
35. Lentz SR, Piegors DJ, Fernandez JA, et al. Effect of hyperhomocysteinemia on protein C activation and activity. Blood 2002; 100: 2108-12.
36. Koch HG, Nabel P, Junker R, et al. The 677T genotype of the common MTHFR thermolabile variant and fasting homocysteine in childhood venous thrombosis. Eur J Pediatr 1999; 158: 113-6.