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Original article

Heterozygous carriers of classical homocystinuria tend to have higher fasting serum homocysteine concentrations than non-carriers in the presence of folate deficiency

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SUMMARY

Background & aims: Many studies have reported that serum total homocysteine (tHcy) levels in cystathionine-beta-synthase (CBS) carriers are usually normal and only elevated after a methionine load. However, the amount of methionine required for a loading test is non-physiological and is never reached with regular feeding. Therefore, CBS carriers do not seem to be at an increased risk of cardiovascular diseases. However, the risk of cardiovascular diseases of CBS carriers with folate deficiency has not been studied. We recently found an extraordinarily high carrier rate (1/7.78) of a novel CBS mutation (p.D47E, c.T141A) in an Austronesian Taiwanese Tao tribe who live in a geographic area with folate deficiency. We evaluated if the CBS carriers tend to have higher fasting serum tHcy concentrations than non-carriers in presence of folate deficiency.

Methods: The serum tHcy and folate levels before and after folate replacement were measured in 48 adult Tao carriers, 40 age-matched Tao non-carriers and 40 age-matched Han Taiwanese controls.

Results: The serum tHcy level of the Tao CBS carriers (17.9 ± 3.8 μmol/l) was significantly higher than in Tao non-carriers (15.7 ± 3.5 μmol/l; $p < 0.008$) and Taiwanese controls (11.8 ± 2.9 μmol/l; $p < 0.001$). Furthermore, a high prevalence of folate deficiency in the Tao compared with the Taiwanese controls (4.9 ± 1.8 ng/ml vs. 10.6 ± 5.5 ng/ml; $p < 0.001$) was also noted. Of note, the difference in tHcy levels between the carriers and non-carriers was eliminated by folate supplementation. (carriers: 13.65 ± 2.13 μmol/l; non-carriers:12.39 ± 3.25 μmol/l, $p = 0.321$).

Conclusions: CBS carriers tend to have a higher tHcy level in the presence of folate deficiency than non-carriers. Although many reports have indicated that CBS carriers are not associated with cardiovascular disease, the risk for CBS carriers with folate deficiency has not been well studied. Owing to a significantly elevated level of fasting tHcy without methionine loading, it is important to evaluate the risk of cardiovascular disease in CBS carriers with folate deficiency.

1. Introduction

It has been reported for decades that markedly elevated plasma levels of total homocysteine (tHcy) in patients with cystathionine-beta-synthase (CBS) deficiency can lead to premature arteriosclerotic cerebrovascular disease [1]. Recent studies reveal that even mildly elevated plasma levels of tHcy (mild hyperhomocysteinemia) appear to be an independent risk factor for cardiovascular disease (CVD) [2,3]. One prospective study reported a 3.4-fold higher risk of

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myocardial infarction for men with a plasma tHcy level above 15.8 μmol/l [4]. Clinically, mild hyperhomocysteinemia cases who are caused by genetic variants may affect folate metabolism deficiency or dietary deficiency of folate and/or vitamin B_{12}. Whether carriers of CBS deficiency tend to have hyperhomocysteinemia or increased risk of cardiovascular disorders has attracted much attention in recent decades. Most studies have reported that the concentration of homocysteine in fasting carriers of CBS mutations is normal, although a systematic increase tHcy after a methionine load [5,6]. However, a typical 100 mg/kg body weight load of methionine is non-physiological and is never reached with regular feeding. Therefore, the heterozygous carriers of CBS mutations do not seem to be at an increased risk of CVD. Without methionine loading, elevated fasting concentrations of homocysteine have occasionally been reported in the carriers of CBS mutations [7]. Several studies documented that carriers of CBS mutations are at an increased risk of CVD [8,9], in contrast with other studies which indicated that obligate carriers of CBS mutations are not at an increased risk of CVDs [1,10].

1 We recently found an extraordinarily high prevalence (1/240) of homocystinuria in an Austronesian Taiwanese Tao tribe living on Orchid Island, 39 miles east of the southern tip of mainland Taiwan. A hotspot novel mutation (p.D47E) was found, with a carrier rate of 1 in 7.78 islanders. This p.D47E mutation is B6 non-responsive and interferes with the function and the stability of the CBS protein *in vivo*. It can decrease in both enzymatic activity and amount of enzyme to 20% resulting in a final residual enzymatic activity of 4% in cultured fibroblasts of controls [11]. We also found that the mean serum tHcy level of the Tao CBS carriers and was significantly higher than in the non-carriers and mainland Taiwanese controls. Furthermore, a high prevalence of folate deficiency in the Tao subjects compared with the mainland Taiwanese controls was also noted. Although many reports have shown that CBS carriers are not associated with CVD [1,10], the risk of CVD for the individuals with one CBS mutation and folate deficiency has not been well studied. Owing to a significantly elevated fasting serum tHcy level without methionine loading in CBS carriers with folate deficiency, it is important to evaluate the risk of CVD in the CBS carriers who are in the geographic areas where folate deficiency is common.

2. Materials and methods

2.1. Before folate replacement

2.1.1. Participants

A total of 48 adult Tao carriers with p.D47E mutations (23 males and 25 females, age range 48–76 years), 40 age-matched adult Tao inhabitants of Orchid Island without the p.D47E mutation (Tao controls, 14 males and 26 females, age range 48–76 years), and 40 age-matched normal adult Taiwanese (Han Taiwanese controls, 20 males and 20 females, age range 47–73 years) were enrolled in this study. Mutation analysis to confirm the carrier status was performed for all participants, and all participants had normal liver and renal functions.

2.1.2. Methods

After an overnight fast, blood was collected for measurements of serum concentrations of homocysteine. Possible confounding factors such as serum concentrations of vitamin B_{12}, folate and methylenetetrahydrofolate reductase (*MTHFR*) were also analyzed. The total concentration of plasma homocysteine was measured using a Bayer ADVIA® Centaur™ automated chemiluminescent assay (Bayer HealthCare, Tarrytown, NY). Plasma vitamin B_{12} and folate were measured using a competitive chemiluminescent enzyme immunoassay (Immulite 2000-BIODPC, USA).

2.2. After folate replacement

Since most of the Tao participants were folate deficient, they received a 5 mg/day replacement regimen of folate for 6 months, although only 10 carriers and 10 non-carriers completed the course of replacement. Fasting homocysteine and folate concentrations were then measured after the replacement period. The measurements of serum concentrations of homocysteine and folate were performed as described in the previous paragraph.

All of the procedures of this study were approved by the Institutional Review Board of Taipei Veterans General Hospital, and all participants were informed of the details of the procedures and provided written consent to participate in this study.

2.3. MTHFR genotyping

Single-nucleotide polymorphism analysis of C677T was performed in all participants in whom serum homocysteine concentrations were measured, including 48 adult Tao carriers of the p.D47E mutation and 40 adult Tao islanders without the p.D47E mutation. The primers, polymerase chain reactions and denaturing high performance liquid chromatography conditions were the same as described in an earlier report [12]. All amplicons with abnormal denaturing high performance liquid chromatography elution profiles were analyzed using previously described sequencing procedures [12].

2.4. Statistical analysis

We calculated the mean (±standard deviation) concentrations of homocysteine, folate, and vitamin B_{12} among the different groups. We used the Student's *t*-test to compare normally distributed continuous variables between groups, and the non-parametric Mann–Whitney test for confirmation. SPSS statistical software version 17.0 (SPSS Inc., an IBM Company, Chicago, IL) was used for all analyses. Statistical significance was considered when the p value was <0.05.

3. Results

3.1. MTHFR genotyping

Out of these 88 Tao inhabitants, including 48 p.D47E carriers and 40 non-carriers, 3 p.D47E carriers and 2 non-carriers were found to be heterozygous for the *MTHFR* C677T genotype. No homozygote for C677T was identified in this cohort. The frequency of T allele in Tao was around 2.8%, which is much lower than that in our Han Taiwanese (31.25%) [13]. The homocysteine levels of these c.677C > T heterozygous individuals from Tao carriers or controls have no significant difference from their affiliated groups (p.D47E carrier: $p = 0.34$; non-carrier: $p = 0.49$).

3.2. Biochemical measurements

The serum concentrations of homocysteine, vitamin B_{12} and folate of the p.D47E carriers, Tao controls and Taiwanese controls are shown in Table 1. Folate was significantly lower in the p.D47E carriers (4.9 ± 1.6 ng/ml) and Tao non-carriers (5.0 ± 1.9 ng/ml) than in the Taiwanese controls (10.6 ± 5.5 ng/ml, both $p < 0.001$), and similar in the carriers and the Tao non-carriers ($p = 0.941$). The mean fasting serum concentration of homocysteine was significantly higher in the p.D47E carriers (17.9 ± 3.8 μmol/l) and Tao non-carriers (15.7 ± 3.5 μmol/l) than in the Taiwanese controls (11.8 ± 2.9 μmol/l; both $p < 0.001$), and significantly higher in the carriers than in the Tao non-carriers (17.9 ± 3.8 μmol/l vs.