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Molecular Genetic Analysis in Mild Hyperhomocysteinemia: A Common Mutation in the Methylenetetrahydrofolate Reductase Gene Associated with Recurrent Cerebrovascular Strokes

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Elevated plasma homocysteine is an independent risk factor for arteriosclerotic heart disease and thrombosis. One of the most common genetic defects in homocysteine metabolism is a mutation in the enzyme methylenetetrahydrofolate reductase (MTHFR) that creates a thermolabile enzyme with reduced activity. We report a case of 12-year old male child who presented with three episodes of strokes over the last three years. Laboratory evaluation showed only mild hyperhomocysteinemia (11.3 μmol L^{-1}). Further testing revealed that he was homozygous for the MTHFR 677 C-to-T mutation. Considering that 10% of the population's risk for arterial vascular disease may be attributable to elevated homocysteine levels, the high prevalence of the MTHFR mutation may represent an important genetic risk factor. We recommend MTHFR mutation analysis for patients with variable or ambiguous homocysteine levels, since plasma levels are dependent on other factors as sample handling, which is not the case with DNA results.

Key words: Methylenetetrahydrofolate reductase (MTHFR), homocysteine, stroke

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INTRODUCTION

Ischemic stroke is a leading cause of death in developed countries; its prevalence among young adults ranges from 3 to 5%[1,2]. Over the last years, several studies have been performed to elucidate the mechanisms of this ischemic event. Homocysteine (Hcy) is a sulfur amino acid that is metabolized to cysteine by trans-sulfuration or to methionine by remethylation. Homocysteine circulates in plasma in 3 forms: as a single free amino acid (1%), as homocysteine or cysteine-homocysteine disulfides (20 to 30%), or bound to plasma proteins (70 to 80%). Together, these account for total plasma homocysteine (tHcy). Inborn errors of metabolism arising from a deficiency of Hcy-metabolizing enzymes result in extremely high tHcy concentrations (severe hyperhomocysteinemia) and are associated with premature thrombosis, possibly as a result of oxidative damage mediated by the sulfhydryl group of free single-chain homocystine[3].

Accumulating evidences showing that a marginal increase in plasma homocysteine is associated with a higher risk of coronary artery or cerebrovascular disease have revived interest in the metabolism of this amino acid[4-6]. Mild hyperhomocysteinemia results from both nutritional and more subtle genetic influences. Subclinical deficiencies of folate, vitamins B12 and B6 and inheritance of the thermolabile variant of methylenetetrahydrofolate reductase (MTHFR) are associated with modest elevations above the 90th percentile of the normal range. Other conditions, including renal impairment, hypothyroidism and drug therapy (e.g. folate antimetabolites, theophylline, smoking, or oral contraceptives), are also associated with mild hyperhomocysteinemia[7].

MATERIALS AND METHODS

We report the case of a twelve- year old male child. He is the third offspring of nonconsanguinos Egyptian parents with two healthy living female sibs (14 and 7 years old). He was born as a full term by elective Caesarean section. He developed hemolytic jaundice in the second day (Rh incompatibility) and was admitted to NICU for one week, where exchange transfusion and phototherapy were the adopted lines of treatment. His milestones of development were achieved at appropriate age.

The condition started on May 2000 with a sudden onset of right-sided hemichorea, hemiparesis, hemihypothesia, upper facial nerve palsy together with aphasia. He gave a history of trauma to the head one day before. There was no history of recent infection, drugs or irradiation. There were no attacks of recurrent headache, vomiting or seizures. There were no hearing or vision problems, no academic problems and no exercise intolerances. There was no family history of angina, early-unexplained deaths, diabetes mellitus, deafness, stroke or seizures.

Of interest is a past history of anuria at the age of 1. 25 years, where he was diagnosed then as acute renal failure, underwent peritoneal dialysis and investigations at that period revealed multiple stones for which he underwent bilateral pyelolithotomy. Stone analysis revealed calcium oxalate stone. Follow up ultrasound revealed still a right renal stone in the pelvis and two in the urinary bladder, for which one year later he under went a second pyelolithotomy and a suprapubic cystolithotomy. Follow up pelvi-abdominal ultrasound revealed hydronephrotic changes in the right kidney with diltation of the pevicalyceal system due to postsurgical pelviureteric obstruction. He underwent diltation of the pelvicalyceal system. In his follow up, he did not have recurrence of stone and kidney function tests are within normal values.

RESULTS

Examination (May, 2000) revealed that his skull circumference, weight and height for age were at the 50th percentile, with normal body proportions. The heart rate, respiratory rate and blood pressure were normal for his age. I.Q. assessment (by kiskey Nebraska test of learning aptitude) was 96. Examination of facies, hands, teeth, skin and hair revealed no abnormality. Eye examination revealed normal visual acuity, intraoccular pressure, slit lamp and fundi examination. Audiometry assessment revealed bilateral normal hearing sensitivity with excellent speech discrimination.

His neurologic examination revealed upper motor neuron lesion of the right facial nerve, with weakness of the right side of the body showing a pyramidal distribution together with hypotonia, exaggerated reflexes, ankle clonus and positive Babiniski on the right side. Choreoathetotic movements were noticed in the first few days of illness. Abdominal, chest and cardiologic examination showed no abnormality. Skeletal examination revealed normal examination of the spine, thoracic cage and limbs.

A battery of investigations was done at that period which included blood picture, erythrocyte sedimentation rate, kidney and liver function tests, coagulation profile, lipid profile, sickling test, Antinuclear antibodies and Anti nDNA, Anticardiolipin (IgG and IgM), plasma and urine aminogram, blood homocysteine, blood lactate and pyruvate, (Table 1) together with radiographic investigations which included echocardiography

Table 1: Results of the initial laboratory data

CBC:	TLC: 4.5x 10^6. cm m^{-1} PNL: 69.8%, Lymph: 12.7%, Monocytes:12% Eosinophils:3.5% Basophils:1.7% Hemoglobin: 13.1gm%, HCV: 37.8 MCV: 80.9 fl, MCH: 28.1 pg, MCHC: 34.7 gm d L^{-1}. Platlets: 246 000.cm m^{-1}
ESR:	14 mm. h^{-1}
Sodium:	134 mEq L^{-1}
Potassium:	3.8 mEq L^{-1}
Partial Thromboplastin Time:	32.9 sec (Range 26- 45)
Prothrombin Time:	13.1 sec with a conc. 90% (INR 1.06).
Lipid profile:	
Cholesterol:	178 mg dL^{-1} (Range 109-189; 80th percentile)
Triglycerides:	71 mg dL^{-1} (Range 35-108)
LDL:	120 mg dL^{-1} (Range 60-140)
HDL:	44 mg dL^{-1} (Range 35-84)
VLDL:	14 mg dL^{-1} (Range 8-32).
Anti thrombin III:	108% (Range: 80- 120)
Protein C:	93% (Range: 70- 140)
Protein S:	91% (Range: 70- 140)
ANA:	negative
Anti DNA:	negative
Anticardiolypin:	Ig G: 19 (-ve < 23), Ig M: 5 (-ve < 11)
Sickling test:	negative.
Blood Lactate:	25.8 mg dL^{-1} (Normal: <20)
Blood Pyruvate:	0.97 mg dL^{-1} (Range: 0.36- 0.59).
Plasma and Urine aminogram:	Normal
Assessment of blood Homocysteine:	11.3 µmol L^{-1} (Normal:< 10.30)

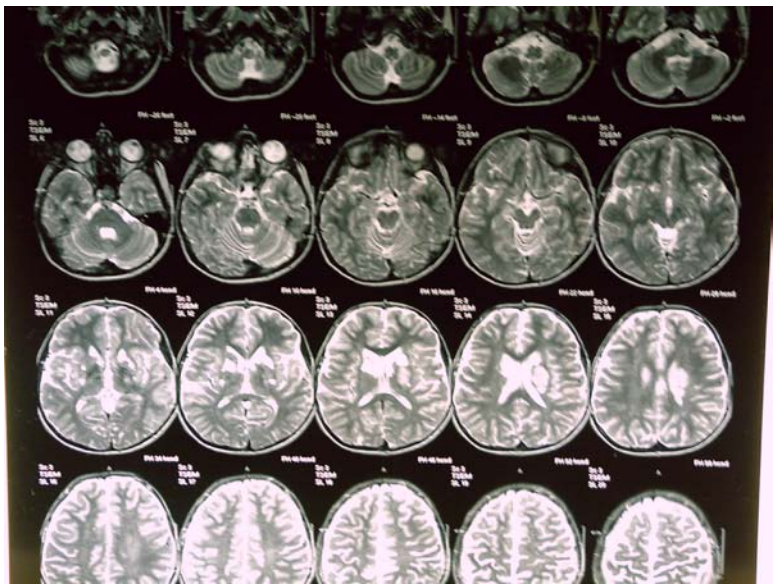

Fig. 1a: MRI showing a moderate size zone of signal abnormality at the left basal ganglionic/ corona radiata region exhibiting intense bright signal on different weighted images, represents late acute/ early subacute infarcts of left middle cerebral artery territory. This zone shows high signal intensity on T2-W (Fig. 1a) images. Also, variable zones of signal abnormality with both ganglionic region, mainly the caudate nuclei and left putamenal regions. These exhibit signal intensity which follows CSF on all sequences and as such represent encephalomalacic zones of old infarcts

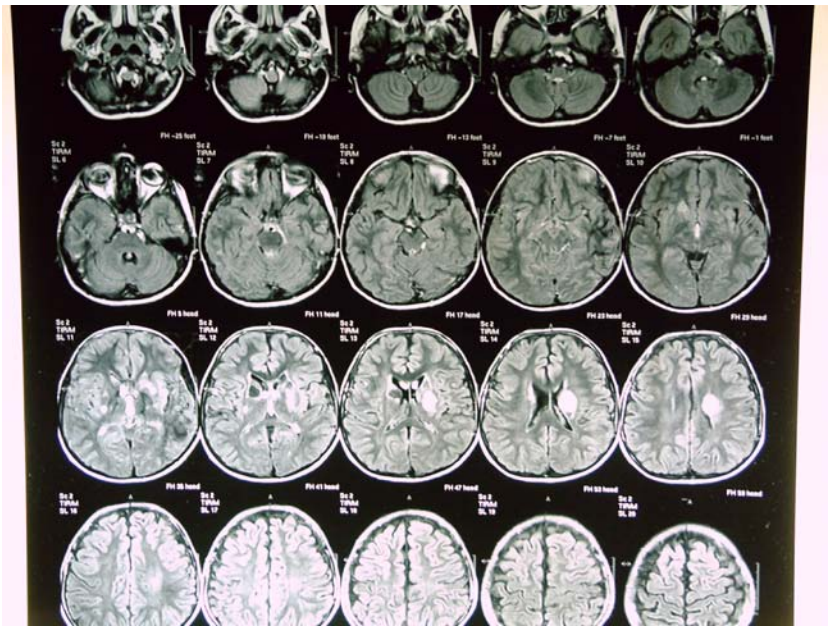

Fig. 1b: MRI showing a moderate size zone of signal abnormality at the left basal ganglionic/ corona radiata region exhibiting intense bright signal on different weighted images, represents late acute/ early subacute infarcts of left middle cerebral artery territory. This zone shows high signal intensity on FLAIR (Fig. 1b) images. Also, variable zones of signal abnormality with both ganglionic region, mainly the caudate nuclei and left putamenal regions. These exhibit signal intensity which follows CSF on all sequences and as such represent encephalomalacic zones of old infarcts

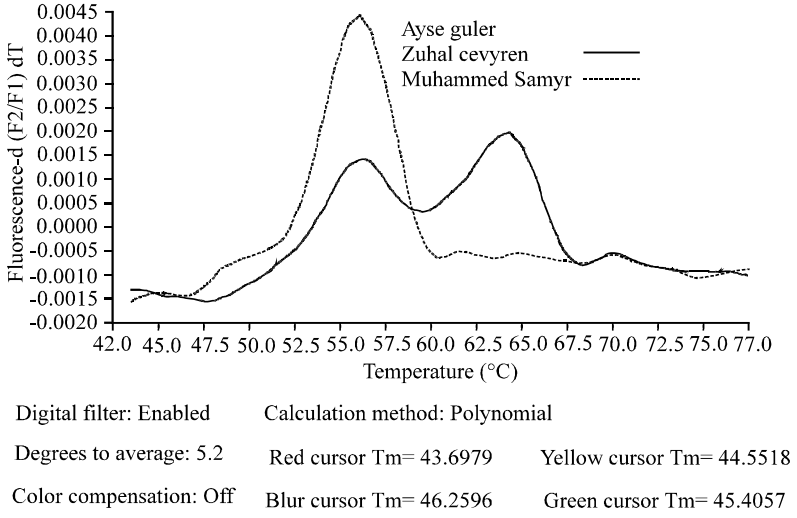

Fig. 2: Melting curve after polymerase chain reaction to detect the mutant allele Methylene tetrahydrofolate reductase (MTHFR C677T) mutation in our case (3, the red cursor)

(transthoracic and transesophageal), transcranial Doppler, MRI and MRA of the brain. Except for only mild elevations of the blood lactate (25.8 mg dL^{-1}) and blood homocysteine (11.3 μmol L^{-1}), all the laboratory parameters were within normal. The echocardiography showed mild mitral valve prolapse.

The MRI of the brain showed two moderate sized cerebral infarctions in different stages of evolution:

1 Globus Pallidus of right lentiform nucleus extending to right periventricular region and involving the genu of right internal capsule, the image was consistent with chronic insult.
2 Posterior aspect of left lentiform nucleus and corona radiata regions, the image was consistent with subacute insult.

Other radiologic investigations did not reveal any abnormality.

The child was referred to the Stroke Unit and received heparin, then was maintained on salicylates (75 mg day^{-1}) and the condition resolved completely in a year's time.

In the period from the beginning of December 2002 to February 2003, the child developed two other strokes and the MRI confirmed the presence of acute infarction of the left middle cerebral artery territory and several bilateral old infarcts of both middle cerebral arteries territories (Fig. 1 a and b).

Reevaluating the case, the following investigations were done including levels of complement components (C3 and C4), Rheumatoid factor, screening for hemoglobinopathies, together with a repeat of serum lactate, arterial blood gases, anticardiolipin (IgM and IgG), aminoacid profile together with a repeat of homocysteine level in blood, all the previous investigations revealed normal results, but the blood homocysteine showed again a mild elevation of 13.6 μmol L^{-1}.

After reviewing the clinical history and the previous investigations, the following causes of recurrent strokes were investigated:

Factor V Leiden G1691 A mutation,
Factor II (Prothrombin) G20210 A mutation
Methylene tetrahydrofolate reductase (MTHFR C677T) mutation.

The first two tests were normal, but the third revealed homozygous mutations (Fig. 2), which makes homocystinuria type III (Methylene tetrahydrofolate reductase deficiency) the most probable diagnosis.

DISCUSSION

Previous reports have shown that hyperhomocysteinemia is closely associated with the occurrence of stroke[8-10]. The common thermolabile MTHFR variant results from a C-to-T point mutation at nucleotide 677 (changing Ala to Val), which significantly reduces the basal activity of the enzyme, leading to hyperhomocysteinemia, particularly in folate-deficient states[11]. The prevalence of this MTHFR mutation is extraordinarily high, with homozygotes representing 5 to 12% of several normal cohorts[11,12]. Homozygosity for this common MTHFR mutation has been shown to be associated with both higher homocysteine levels[10,11,13] and a 3-fold increased risk of premature cardiovascular diseases[12]. Furthermore, carriers of this defective enzyme have higher homocysteine levels than control subjects and are at increased risk for premature coronary artery disease[14]. In their study, Kang *et al.*[15] reported that the mean total plasma homocysteine concentration in patients with thermolabile MTHFR was 13.19 μmol L^{-1}, a significantly different value from the normal mean of 8.50 μmol L^{-1}. Folate acts to stabilize the thermolabile enzyme with the 677C-T mutation. Serum folate levels greater than 15.4 nM appeared to neutralize the effects of 677C-T mutations[16].

In the present case only mild elevation of the homocysteine level was noted (11.3 μmol L^{-1} at one setting and 13.6 μmol L^{-1} at another). These values are just above the 90th percentile range for homocysteine reported by Delvin *et al.*[17] in their study of healthy children below 12 years of age (3.2- 7.8 μmol L^{-1}) and by Tonstad *et al.*[18] in their study on the same age group (3.5- 6.5 μmol L^{-1}).

Case-control studies have reported stronger associations[4,10,19] than prospective studies, in which some[Abbate *et al.*[6], Giles *et al.*[20]) but not others (Alfthan *et al.*[21]) claim that hyperhomocysteinemia is a risk factor for future stroke development. However, metaanalysis of the data confirmed the association between mild to moderate hyperhomocysteinemia and ischemic stroke[22]. In the case of CVA, two further issues are relevant: first, strokes arise from numerous pathophysiological processes, including intracranial hemorrhage, cardiac embolization, atherothrombosis (rupture of either large-vessel atheroma with cerebral embolism or of small-vessel atheroma with occlusion) and vasculitis. Most studies have failed to distinguish between these diverse stroke types and any individual

risk factor might influence only one of these processes[23]. Other methodological limitations include inadequate statistical power, the use of nonfasting blood samples to measure homocysteine[24], delayed measurement of homocysteine until several months after the acute event and failure to adjust for potential confounders such as age, vitamin status and MTHFR genotype[25].

Studies in humans have shown that acute hyperhomocysteinemia causes endothelial dysfunction, which might promote atheroma development[26]. Furthermore, raised homocysteine concentrations are associated with asymptomatic carotid artery wall thickening and stenosis[27]. and correlate with the severity of cerebral artery stenosis[19] It could therefore be postulated that elevated tHcy is a risk factor for atherothrombotic stroke in particular[8].

In conclusion, considering that 10% of the population's risk for arterial vascular disease may be attributable to elevated homocysteine levels, the high prevalence of the MTHFR 677 C-to-T mutation may represent an important genetic risk factor for this common disease. The identification of these genetically at-risk individuals may be particularly relevant in light of the ability to lower homocysteine-associated vascular risk with folic acid therapy. We recommend MTHFR mutation analysis for patients with variable or ambiguous homocysteine levels, since plasma levels are dependent on other factors as nutritional status and sample handling, which is not the case with DNA results.

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