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Clinical study regarding the link between cystathionine β-synthase 844ins68 polymorphism and maternal risk for Down syndrome

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Abstract

Objective. *Folic acid is commonly used for the primary prevention of severe congenital malformations and Down syndrome (DS). The objective of this study was to evaluate the frequency of 844ins68 polymorphism of the cystathione beta-synthase (CBS) gene and to verify if the occurrence of this polymorphism is associated with trisomy 21.* ***Methods.*** *In the present study we analyzed 72 mothers (ages between 20 and 42 years old) in which those children presented or not DS. Mothers of children with DS (26 cases) were included as study group and those with children without DS and who had never suffered a miscarriage were enrolled as control group (46 cases). Genomic deoxyribonucleic acid (DNA) was isolated from whole peripheral blood collected on ethylenediaminetetraacetic acid, using peqGOLD blood DNA mini kit. The common CBS polymorphism that causes an insertion of 68 bp at the 844 position was further identified.* ***Results.*** *The frequencies of CBS 844ins68 genotypes (ins-/ins-, ins+/ins-) among case mothers were 84.6 and 15.4%, respectively. The corresponding frequencies among controls were 91.3 and 8.7%, respectively.* ***Conclusions.*** *In our study, we did not find any significant differences in genotype frequencies between the two analyzed groups.*
Keywords: *cystathionine beta-synthase, folate, Down syndrome, polymorphism*

Introduction

Members of the family of B9 vitamins are commonly known as folate, formerly known as folacin. Folate or pteroiloglutamic acid is an essential B-vitamin that occurs naturally in a wide variety of foods, such as spinach, broccoli, asparagus, cabbage, cauliflower, milk, some fruit and nuts. Folic acid is the synthetic form added to foods and found in dietary supplements[1].

Pteroiloglutamic acid plays fundamental role in biosynthesis and methylation of nucleic acids (deoxyribonucleic acid - DNA and ribonucleic acid - RNA) essential for cell division, differentiation, and regulation of gene expression. Therefore it is indispensable for normal growth and functioning of all cells of the human organism[1].

In human cells, folate deficiency is associated with DNA hypomethylation[2], DNA instability (strand breakage, uracil misincorporation)[2,3], aneuploidy of chromosomes 17 and 21[4,5], apoptosis[6], and necrosis[4].

The most important causes of folate deficiency are the inadequate dietary intake, malassimilation or an increased requirement of folates.

Impaired folate metabolism, resulting from the presence of common functional polymorphisms of genes encoding for metabolic enzymes, has been associated with several human diseases including various kinds of cancer[7,8,9], cardiovascular diseases[10,11], degenerative diseases of the nervous system[12,13], depressive disorder and neural tube defects[14,15].

Folates are necessary also for normal embryogenesis. As a result of folate deficiency in the organism of a pregnant woman, the occurrence of congenital malformations in offspring is possible, as well as reduced birth weight of an infant, hypoplasia of placenta, higher incidence of spontaneous abortions and other complications of pregnancy. Research demonstrates that folic acid intake by women before and during the first weeks of pregnancy can reduce the risk of some congenital malformations in offspring.

Folate requires several transport systems to enter the cells and the one best characterized is the reduced folate carrier, a vitamin B6-dependent enzyme, located on intestinal cell membranes.

Human cystathionine β-synthase (CBS) is a hemoprotein which catalyzes the condensation of hemocysteine (Hcy) and serine to form cystathionine. The gene encoding CBS has been localized in chromosome 21 (21q22.3) in a region correlated with Down syndrome (DS) phenotype. The CBS gene is built of 23 exons, but only 1-14 and 16 encode the peptide sequence of 551 amino acids. Many mutations including missense and nonsense ones, as well as some insertion, deletion and splice site variants and several polymorphisms in the CBS gene have been reported[16]. The identification of an 844ins68 insertion

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in the CBS gene was first reported in a patient affected by homocysteinuria due to CBS deficiency[17]. The 844ins68 polymorphism, carried by nearly 10% of the general population, may cause lower total Hcy levels compared to noncarriers, in particular after methionine loading[18].

Methods

The present study includes 72 mothers (aged 20-42 years old). Mothers of children with trisomy 21 (or DS) (26 cases) were included as study group and women whose children were not affected by DS and who had never suffered a miscarriage were enrolled as control group (46 cases). All mothers in this study reside in the same geographic area and have a similar social background. There was no periconceptional use of folic acid. Only 11.53% of the mothers had irregularly taken nutritional supplements in the second or third trimester.

Genomic DNA was isolated from whole peripheral blood collected on ethylenediaminetetraacetic acid, using peqGOLD blood DNA mini kit (Biotech) following the manufacturer's instructions.

The common CBS polymorphism that causes an insertion of 68 bp at the 844 position was identified using the method of Scala and contributors[26]. Primers for amplification were forward and reverse: 5'-CTGCCTTGAGCCCTGAAGCC-3', 3'CTGGACTCGACCTACCGTCCT-5'.

Polymerase chain reaction (PCR) conditions were: denaturation 94°C (1 minute), annealing 60°C (1 minute), elongation 72°C (1 minute) for a total of 39 cycles. When present, the 844ins68 insertion caused a 68bp shifted band of the PCR product (242 bp instead of 174 bp).

Statistical analysis

Statistical analyses were realized with SPSS software, version 16. Differences in allele frequencies and genotype distribution among the different groups were assessed by the chi-square test. Odds ratios (OR) and 95% confidence intervals (CI) were calculated to estimate the risk of the different genotypes. Expected genotype frequencies were calculated from the allele frequencies under the assumption of Hardy-Weinberg equilibrium. Values of $p<0.05$ were considered statistically significant.

Results

In the present study, we examined one polymorphism in CBS gene encoding a folate metabolizing enzyme, as a maternal risk factor for meiotic nondisjunction of chromosomes 21, causing DS, in a cohort of Romanian mothers.

The status of CBS 844ins68 polymorphism was addressed by PCR amplification of genomic DNA. The results of the mutational analysis are shown for few representative cases in Figure 1.

As observed in Figure 1, cases 2, 18 and 43 have the homozygous ins-/ins- genotype and cases 11, 22 and 71, heterozygous ins+/ins- genotype.

The allele frequencies of CBS 844ins68 in DS mothers and control mothers are listed in Table 1. CBS 844ins68

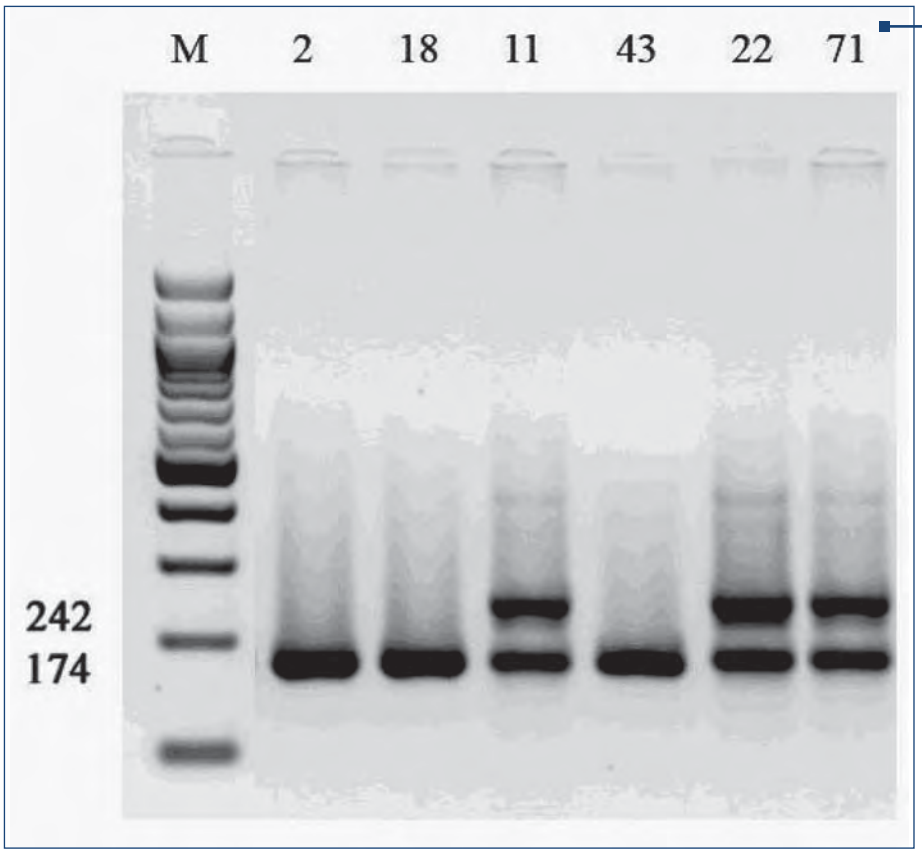

Figure 1. PCR-mutational analysis of CBS 844ins68. Lane 1 - case 2; 2 - case 18; 3 - case 11 ; 4 - case 43; 5 - case 22; 6 - case 71; M - pGEM 100 bp molecular weight marker (Promega)

Table 1 Allele frequencies of CBS 844ins68 in mothers of DS children and control mothers

Genotype	Allele	DS* mothers (%)	Control mothers (%)	χ^2	p value
CBS 844ins 68	Ins -	48 (92.3)	88 (95.7)	0.71	0.40
	Ins +	4 (7.7)	4 (4.3)		
	Total	52	92		

**DS = Down syndrome.*

allele frequency was 7.7% in analyzed patients (χ2: 0.71, p value 0.40).

The frequencies of CBS 844ins68 genotypes (ins-/ins-, ins+/ins-) among case mothers were 84.6 and 15.4%, respectively. The corresponding frequencies among controls were 91.3 and 8.7%, respectively (Table 2, Figure 2). There were no significant differences in genotype frequencies between the two groups (OR 1.91; 95% CI 0.44 - 8.38, p=0.39) and there were no subjects identified with homozygous genotype ins+/ins+ (Table 2).

Discussion

DS, aka trisomy 21, is the most common autosomal disorder in human population and is caused by the inheritance of three chromosomes 21.

An important factor relating DS with one-carbon metabolism is the fact that the CBS gene is located on chromosome 21. This location would explain the functional Hcy deficiency observed in infants with trisomy 21, due to the over-expression of the CBS gene[19]. Impairments in folate metabolism resulting in pericentromeric DNA hypomethylation, which is associated with impaired segregation and aneuploidy[20], have been largely studied as a risk factor for human nondisjunction and folate gene polymorphisms have been associated with an increased risk for trisomy 21 in several such studies[21,22,23]. Folate deficiency has been linked to chromosomal instability and chromosome 21 aneuploidy[4,5].

None of the three published studies aimed at investigating the role of the CBS 844ins68 polymorphism as a possible risk factor for having a child with DS found it to be an independent DS risk factor[24,25,26]. Only Da Silva and contributors observed that the 844ins68 polymorphism, in association with other polymorphisms (MTHFR 677T, MTHFR 1298C, MTR 2756G and MTRR 66G) of the folate pathway, is related to increased risk for DS. The CBS 844ins68 polymorphism has been associated with reduction of Hcy concentration in the presence of the insertion[27,28,29], and it is believed that this insertion is related to increased enzyme activity[27,28]. This variant is always found to be associated in cis with an additional polymorphism in the CBS gene, a thymine-to-cytosine transition at nucleotide position 833, which causes a threonine-to-isoleucine amino acid substitution, and is reported, together with CBS 844ins68, as a 833 T→C/844ins68 in cis double mutation[30].

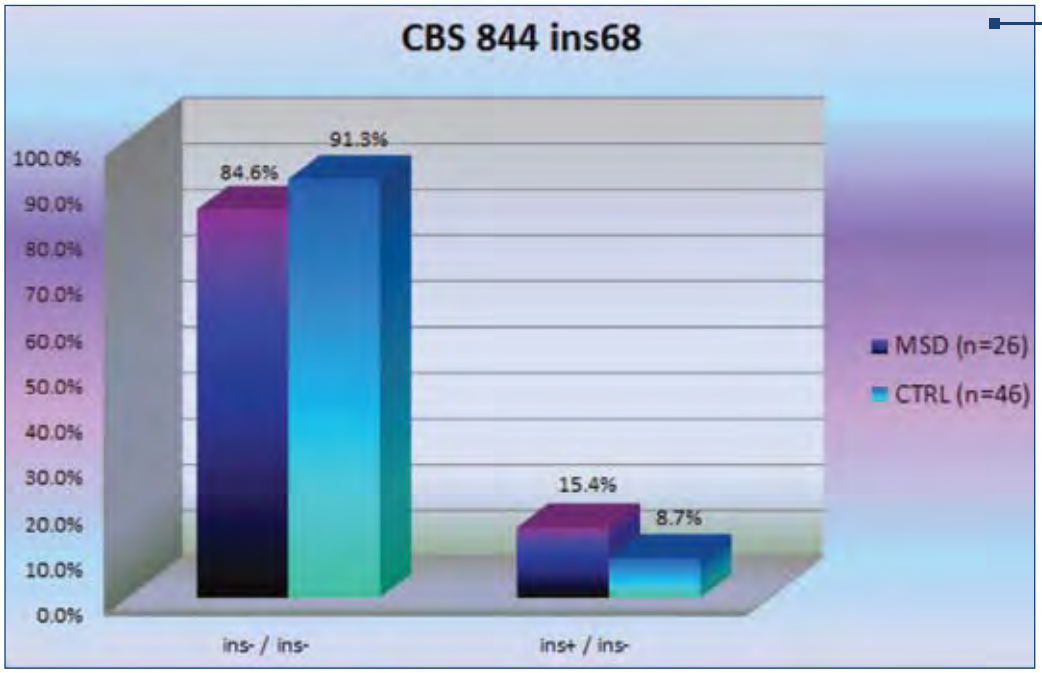

Figure 2. Genotype frequencies of CBS 844ins68 in both groups

Table 2 Genotype frequencies of CBS 844ins68 in mothers of DS children and in control mothers

Genotype	DS* mothers (%)	Control mothers (%)	Odds ratio	95% CI	p value
Ins-/Ins-	22 (84.6)	42 (91.3)	1	Reference	
Ins+/Ins-	4 (15.4)	4 (8.7)	1.91	0.44-8.38	0.39
Ins+/Ins+	0	0	0	0	0

**DS = Down syndrome.*

Interestingly, some studies in which carrier status did not influence fasting homocysteine found that after methionine loading, used experimentally to raise homocysteine concentrations and induce the CBS-mediated trans-sulfuration pathway, the CBS 844ins68 allele is associated with decreased homocysteine levels[18,29,31]. Although the exact mechanism by which the 844ins68 allele affects CBS function is currently unknown, it may show an increase in CBS activity, possibly via up-regulation of the amount of CBS messenger RNA or though the action of another functional polymorphism with which it is in linkage disequilibrium[18].

One explanation of the existing of this type of polymorphism, could required alpha interferon and/or ribavirin treatment, having in the view that one study conducted by Popescu and contributors[32] showed a decreased number of congenital malformations related to alpha interferon and ribavirin exposure during pregnancy.

Conclusions

Allele frequencies of CBS 844ins68 do not point to any preferential association of a particular polymorphism and DS phenotype. Therefore, CBS 844ins68 the polymorphism is not a maternal risk factor for DS.

Nutrigenetics and nutrigenomics are promising areas for evaluating the possibility of DS prevention with folic acid supplementation associated with susceptible genotypes. Thus, further large-scale studies are necessary to better understand the complex association between chromosomal 21 nondisjunction and folate metabolism. ■

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