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DHFR 19-bp Deletion and *SHMT* C1420T Polymorphisms and Metabolite Concentrations of the Folate Pathway in Individuals with Down Syndrome

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Background: Down syndrome (DS) results from the presence and expression of three copies of the genes located on chromosome 21. Studies have shown that, in addition to overexpression of the *Cystathionine β-synthase* (*CBS*) gene, polymorphisms in genes involved in folate/homocysteine (Hcy) metabolism may also influence the concentrations of metabolites of this pathway. *Aim:* Investigate the association between *Dihydrofolate reductase* (*DHFR*) 19-base pair (bp) deletion and *Serine hydroxymethyltransferase* (*SHMT*) C1420T polymorphisms and serum folate and plasma Hcy and methylmalonic acid (MMA) concentrations in 85 individuals with DS. *Methods:* Molecular analysis of the *DHFR* 19-bp deletion and *SHMT* C1420T polymorphisms was performed by polymerase chain reaction (PCR) by difference in the size of fragments and real-time PCR allelic discrimination, respectively. Serum folate was quantified by chemiluminescence and plasma Hcy and MMA by liquid chromatography–tandem mass spectrometry. *Results:* Individuals with *DHFR* DD/*SHMT* TT genotypes presented increased folate concentrations ($p=0.004$) and the *DHFR* II/*SHMT* TT genotypes were associated with increased MMA concentrations ($p=0.008$). In addition, the MMA concentrations were negatively associated with age ($p=0.04$). *Conclusion:* There is an association between *DHFR* DD/*SHMT* TT and *DHFR* II/*SHMT* TT combined genotypes and folate and MMA concentrations in individuals with DS.

Introduction

Down syndrome (DS) results from the presence and expression of three copies of the genes located on chromosome 21 (Shin *et al.*, 2004; Ishinohe *et al.*, 2005). The *Cystathionine β-synthase* (*CBS*) gene, located on chromosome 21, is responsible for the condensation of homocysteine (Hcy) and serine to cystathionine and is overexpressed in individuals with DS (Ishinohe *et al.*, 2005). Increased concentrations of the CBS enzyme results in lower concentrations of Hcy, methionine, S-adenosylhomocysteine, and S-adenosylmethionine (Pogribna *et al.*, 2001; Coppus *et al.*, 2007; Meguid *et al.*, 2010), substrates of folate metabolism.

Studies have shown that, in addition to overexpression of the *CBS* gene, polymorphisms in genes involved in folate/Hcy metabolism may also influence metabolite concentrations of this pathway (Fillon-Emery *et al.*, 2004; Guéant *et al.*, 2005; Licastro *et al.*, 2006; Biselli *et al.*, 2008; Biselli *et al.*, 2012). A 19-base pair (bp) deletion polymorphism in intron-1 of the *Dihydrofolate reductase* (*DHFR*) gene, located on chromosome 5q11.2, has been identified (Johnson *et al.*, 2004) and Kalmbach *et al.* (2008) demonstrated that this is a functional polymorphism. Study shows that the 19-bp deletion polymorphism is associated with increased expression of the *DHFR* gene, responsible for the conversion of dihydrofolate in tetrahydrofolate (THF) (Xu *et al.*, 2007), and changes of folate/Hcy metabolism (Gellekink *et al.*, 2007; Kalmbach *et al.*, 2008; Stanisiawska-Sachadyn *et al.*, 2008; Mendes *et al.*, 2010).

Another polymorphism, C1420T, which results in substitution of leucine by phenylalanine, was identified in the *Serine hydroxymethyltransferase* (*SHMT*) gene, located on chromosome 17p11.2 (Heil *et al.*, 2001). This gene encodes the enzyme that catalyzes the reversible conversion of serine and THF to glycine and 5,10-methylene THF (Fowler, 2001) and Fu *et al.*

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(2005) showed that the *SHMT* C1420T polymorphism may compromise the formation of the SHMT enzyme.

Both the *DHFR* 19-bp deletion and *SHMT* C1420T polymorphisms involved in folate/Hcy metabolism have been associated with variations in the concentrations of Hcy and folate in several populations (Heil *et al.*, 2001; Chen *et al.*, 2004; Lim *et al.*, 2005; Gellekink *et al.*, 2007; Kalmbach *et al.*, 2008; Stanisiawska-Sachadyn *et al.*, 2008; Mendes *et al.*, 2010; Marucci *et al.*, 2012). Thus, the aim of the present study was to investigate the association between the *DHFR* 19-bp deletion and *SHMT* C1420T polymorphisms and the serum folate and plasma Hcy and methylmalonic acid (MMA) concentrations in individuals with DS.

Materials and Methods

This study was composed by eighty-five individuals with full trisomy 21 confirmed by karyotype (median age 1.36, range 0.07–30.35 years old; 47 male and 38 female) recruited at the General Genetics Outpatient Service of Hospital de Base, São José do Rio Preto, SP, Brazil. The study protocol was approved by the Research Ethics Committee of São José do Rio Preto Medical School (CEP-FAMERP, 165/2004), in São Paulo state, and informed consent was obtained for all families.

Fasting blood samples were collected for molecular and biochemical analysis (serum folate and plasma Hcy and MMA). DNA extraction was performed as previously described by Miller *et al.* (1988) and polymorphisms in *DHFR* and *SHMT* genes were analyzed by polymerase chain reaction (PCR) using difference in the size of fragments and real-time PCR allelic discrimination, respectively. The 19-bp deletion polymorphism in the *DHFR* gene was detected using primer sequences described by Dulucq *et al.* (2008) and *SHMT* C1420T was detected using TaqMan® probes and primer sequences described by Skibola *et al.* (2004). Serum folate was quantified by chemiluminescence (*Immulite Kit, DPC Medlab,* Brazil) and liquid chromatography–tandem mass spectrometry was used to determine concentrations of plasma Hcy and MMA, as previously described (Haddad *et al.*, 2001; de Andrade *et al.*, 2006; Carvalho and Kok, 2008).

The Hardy–Weinberg equilibrium was tested by the chi-square test, using the BioEstat program (version 5.0). The folate, Hcy, and MMA concentrations among different genotypes were compared, after adjustment for age, using multiple linear regression. The computer-assisted statistical analyses were carried out using the Minitab for Windows program (Release 14). Values of $p \leq 0.05$ were considered significant.

In this study, the allele with a 19-bp deletion in the *DHFR* gene was denominated D and the allele without the deletion was named I.

Results

Table 1 presents genotype frequencies of *DHFR* 19-bp deletion and *SHMT* C1420T polymorphisms in individuals with DS and both the genotype distributions were in the Hardy–Weinberg equilibrium ($\chi^2 = 2.079$; $p = 0.15$; $\chi^2 = 0.004$; $p = 0.95$, respectively). The mean concentrations of folate, Hcy, and MMA observed in the individuals were 19.69 ± 11.87 ng/mL, 5.83 ± 3.30 μM, and 0.54 ± 0.84 μM, respectively. The distribution of the Hcy, folate, and MMA concentrations and age according to the combined genotypes are presented in Table 2.

Table 1. Genotype Frequencies of *Dihydrofolate Reductase* 19-Base Pair Deletion and *Serine Hydroxymethyltransferase* C1420T Polymorphisms in Individuals with Down Syndrome

	Genotype frequencies n (%)
DHFR	
II	20 (23.3)
ID	48 (55.8)
DD	18 (20.9)
SHMT	
CC	41 (46.1)
CT	39 (43.8)
TT	9 (10.1)

I, allele without 19-bp deletion; D, allele with 19-bp deletion; C, wild-type allele; T, polymorphic allele.

The results did not show any individual association between these polymorphisms and folate and Hcy and MMA concentrations. However, the *DHFR* DD/*SHMT* TT combined genotypes were associated with elevated folate concentrations (Coefficient = 25.69; $p = 0.004$) and the *DHFR* II/*SHMT* TT combined genotypes were associated with increased MMA concentrations (Coefficient = 1.78; $p = 0.008$). In addition, the MMA concentrations were associated with age (Coefficient = −0.04; $p = 0.04$).

Discussion

The overexpression of genes results in biochemical alterations that affect the multiple interacting metabolic pathways culminating in cellular dysfunction and contributing to the pathogenesis of DS (Pogribna *et al.*, 2001). The presence of three copies of the *CBS* gene, located on chromosome 21, and *Methylenetetrahydrofolate reductase* (*MTHFR*) C677T, *Methionine synthase* (*MTR*) A2756G, *Transcobalamin 2* (*TC2*) C776G, and *Betaine homocysteine methyltransferase* (*BHMT*) G742A polymorphisms, involved in folate/Hcy metabolism, have been associated with variations on the concentrations of metabolites of this pathway (Pogribna *et al.*, 2001; Fillon-Emery *et al.*, 2004; Guéant *et al.*, 2005; Licastro *et al.*, 2006; Coppus *et al.*, 2007; Biselli *et al.*, 2008; Meguid *et al.*, 2010; Biselli *et al.*, 2012).

Guéant *et al.* (2005) observed that individuals with DS who present the *MTHFR* 677T allele and an elevated Hcy concentration had a low intelligence quotient and Licastro *et al.* (2006) found that the *MTHFR* 677TT genotype increases the concentrations of Hcy in these individuals. However, Fillon-Emery *et al.* (2004) found no difference in Hcy concentrations according to the *MTHFR* C677T genotype in adults with DS. In another study, the heterozygous genotype *MTR* 2756AG was associated with increased plasma Hcy concentrations in individuals with DS (Biselli *et al.*, 2008). Recent study that evaluated the association between twelve polymorphisms in genes involved in the folate/Hcy metabolism and folate, Hcy and MMA concentrations indicated that the *MTHFR* C677T, *MTR* A2756G, *TC2* C776G, and *BHMT* G742A polymorphisms are predictors of the Hcy concentration. In this study, individuals with DS and *MTR* 2756 AG or GG genotype presented an increased Hcy concentration and *MTHFR* 677 TT, *TC2* 776 GG, and *BHMT* 742 AA genotypes were associated with a decreased Hcy concentration. (Biselli *et al.*, 2012).

Table 2. Distribution of Serum Folate and Plasma Homocysteine and Methylmalonic Acid Concentrations According to Combined Genotypes of the *Dihydrofolate Reductase* 19-Base Pair Deletion and *Serine Hydroxymethyltransferase* C1420T Polymorphisms in Individuals with Down Syndrome

Genotypes DHFR/SHMT	*Age (years)*	*Folate (ng/ml)*	*Hcy (μM)*	*MMA (μM)*
II/CT	4.04 ± 8.84	12.46 ± 5.23	4.95 ± 2.28	0.44 ± 0.46
II/TT	0.24 ± 0.16	13.50 ± 1.70	8.14 ± 0.25	2.50 ± 3.22
II/CC	3.74 ± 3.07	24.95 ± 11.74	4.36 ± 1.54	0.23 ± 0.08
ID/CT	3.51 ± 6.42	24.08 ± 16.37	5.52 ± 2.56	0.67 ± 1.01
ID/TT	2.48 ± 1.97	18.00 ± 11.69	6.63 ± 3.78	0.36 ± 0.29
ID/CC	2.95 ± 3.50	17.95 ± 9.02	6.79 ± 4.37	0.48 ± 0.72
DD/CT	7.51 ± 7.06	16.20 ± 4.28	5.26 ± 2.53	0.44 ± 0.40
DD/TT	2.25 ± 1.51	44.25 ± 1.77	2.48 ± 1.72	0.12 ± 0.01
DD/CC	4.28 ± 4.12	19.46 ± 11.65	6.68 ± 4.19	0.57 ± 0.71

I, allele without 19-bp deletion; D, allele with 19-bp deletion; C, wild-type allele; T, polymorphic allele.

In the present study, we observed that the *DHFR* DD/*SHMT* TT combined genotypes were associated with increased folate concentrations and *DHFR* II/*SHMT* TT genotypes were associated with elevated MMA concentrations. The finding concerning the association between genotypes and the MMA concentrations must be interpreted with some caution considering that we observed a negative association between MMA concentrations and age and that the individuals with *DHFR* II/*SHMT* TT genotypes presented the lower mean age than the individuals with other combined genotypes. Other special attention should be paid to the sample size.

DHFR is an important folate-metabolizing enzyme responsible for reduction of folic acid into THF (Stanisiawska-Sachadyn *et al.*, 2008). A common polymorphism in this gene, a 19-bp deletion polymorphism in intron-1, was associated with alterations on the concentration of metabolites involved in the folate/Hcy pathway (Gellekink *et al.*, 2007; Kalmbach *et al.*, 2008; Stanisiawska-Sachadyn *et al.*, 2008; Mendes *et al.*, 2010). Gellekink *et al.* (2007) reported that the *DHFR* DD genotype is associated with a lower concentration of plasma Hcy in Caucasian individuals, but no association between this genotype and concentrations of serum and erythrocyte folate was observed. Another study found no effect of this polymorphism on Hcy concentration in healthy adults, but the DD genotype was associated with increased concentrations of serum and erythrocyte folate relative to the II genotype in women (Stanisiawska-Sachadyn *et al.*, 2008). Kalmbach *et al.* (2008) also observed no association between genotypes and plasma Hcy or plasma total folate in young adults; however, the *DHFR* DD genotype was associated with a lower concentration of erythrocyte folate compared to *DHFR* ID and II genotypes. A recent study with women also demonstrated that folate, Hcy, and MMA concentrations did not differ between the genotypes (Mendes *et al.*, 2010).

The SHMT enzyme plays a pivotal role in the folate/Hcy metabolism by carrying out the reversible conversion of serine and glycine with THF and 5, 10-methyleneTHF (Fowler, 2001). Heil *et al.* (2001) identified the *SHMT* C1420T polymorphism and reported that individuals with neural tube defects and the *SHMT* CC genotype had decreased concentrations of erythrocyte and plasma folate and an increased Hcy concentration. In a study involving men with cardiovascular disease, the *SHMT* TT genotype was associated with the lower Hcy concentration (Lim *et al.*, 2005); yet, Chen *et al.* (2004) found no significant association between *SHMT* C1420T and plasma folate and Hcy concentrations in colorectal cancer. In addition, another study observed that the *SHMT* C1420T polymorphism does not affect the folate, Hcy, and MMA concentrations in women (Marucci *et al.*, 2012).

This is the first study that evaluated the association between the *DHFR* 19-bp deletion and *SHMT* C1420T polymorphisms and metabolite concentrations of the folate pathway. Despite the small sample size, our results suggest that there is an association between *DHFR* DD/*SHMT* TT and *DHFR* II/*SHMT* TT combined genotypes and the folate and MMA concentrations, respectively, in individuals with DS. However, further studies are needed to confirm these associations.

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Author Disclosure Statement

No competing financial interests exist.

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