Faculdade de Medicina de São José do Rio Preto
Programa de Pós-Graduação em Ciências da Saúde

Gustavo Henrique Marucci

SÍNDROME DE DOWN E POLIMORFISMOS EM GENES ENVOLVIDOS NO METABOLISMO DO FOLATO

Dissertação apresentada à Faculdade de Medicina de São José do Rio preto para obtenção do Título de Mestre no Curso de Pós-Graduação em Ciências da Saúde, Eixo Temático: Medicina e Ciências Correlatas.

São José do Rio Preto
2011

Gustavo Henrique Marucci

Síndrome de Down e polimorfismos em genes envolvidos no metabolismo do folato

Dissertação apresentada à Faculdade de Medicina de São José do Rio preto para obtenção do Título de Mestre no Curso de Pós-Graduação em Ciências da Saúde, Eixo Temático: Medicina e Ciências Correlatas.

Orientadora: Prof[a]. Dr[a]. Érika Cristina Pavarino

São José do Rio Preto
2011

Gustavo Henrique Marucci

Síndrome de Down e polimorfismos em genes envolvidos no metabolismo do folato

BANCA EXAMINADORA

DISSERTAÇÃO PARA OBTENÇÃO DO TÍTULO DE MESTRE

Presidente e Orientador: Érika Cristina Pavarino.

1º Examinador: Ricardo Luiz Dantas Machado

2º Examinador: Mariangela Torreglosa Ruiz Cintra

1º Suplente: Eny Maria Goloni Bertollo

2º Suplente: Izaura dos Santos

São José do Rio Preto, 12/12/2011.

Marucci, Gustavo Henrique
Síndrome de Down e polimorfismos em genes envolvidos no metabolismo do folato
São José do Rio Preto, 2011.
73 p.

Dissertação (Mestrado) – Faculdade de Medicina de São José do Rio Preto – FAMERP
Eixo Temático: Medicina e Ciências Correlatas

Orientadora: Prof[a]. Dr[a]. Érika Cristina Pavarino

1. Síndrome de Down; 2. Trissomia do 21; 3. Polimorfismo genético; 4. Homocisteína

SUMÁRIO

Dedicatória

Aos meus pais, Maria José e Luis Carlos pelo exemplo de vida e por estarem presentes em todos os momentos da minha vida.

AGRADECIMENTOS

A Deus, que me fortalece em todos os momentos.

À Profª Draª Érika Cristina Pavarino

Pelos conhecimentos passados, paciência na orientação e exemplo profissional que muito contribuiu na minha formação.

À Profª Draª Eny Maria Goloni Bertollo

Pelo seu exemplo profissional, seriedade e otimismo em frente às dificuldades.

À toda minha família

Minha mãe Maria José, meu pai Luis Carlos e meu irmão Evandro, pelo apoio, carinho e paciência durante os momentos difíceis. Sem eles eu não chegaria a lugar algum.

À Fabiane

Pelo apoio, companheirismo e compreensão, mesmo nos momentos de ausência.

Às pós-graduandas Joice, Bruna e Cristiane

Pelas contribuições teóricas e práticas que muito me ajudaram no desenvolvimento e finalização desse trabalho.

Aos amigos e colegas da UPGEM.

Pela amizade, apoio e aprendizado mútuo ao longo desses anos.

À Equipe Ding-Down

Pelo apoio e auxílio fundamental para adesão das famílias dos pacientes.

Ao Diretor Geral Prof. Dr. Humberto Liedtke Junior

Pelo grande incentivo e contribuição para o desenvolvimento e fortalecimento desta Instituição.

Ao Programa de Pós-graduação em Ciências da Saúde da FAMERP

Pela constante dedicação na manutenção e fortalecimento do curso de pós-graduação da Instituição.

LISTA DE FIGURAS

LISTA DE TABELAS

LISTA DE TABELAS

LISTA DE ABREVIATURAS E SÍMBOLOS

µmol/L	*Micromol/litre*
5,10-MeTHF	5,10-metilenotetrahidrofolato (*5,10-methylenetetrahydrofolate*)
5,10-CH-THF	5,10-meteniltetrahidrofolato
5-mTHF	5-metiltetrahidrofolato (*5-methyltetrahydrofolate*)
10f-THF	10-formiltetrahidrofolato
A	Adenina (*Adenine*)
B_{12}	Vitamina B_{12}
BHMT	Betaína -homocisteína metiltransferase
bp	*Base pair*
C	Citosina (*Cytosine*)
CEP	Comitê de Ética em Pesquisa
CI	Confidence interval
CNPq	Conselho Nacional de Desenvolvimento Científico e Tecnológico
CONEP	Comitê Nacional de Ética (National Research Commission)
CβS	Cistationina β-sintase *(Cystathionine beta-synthase)*
D	*Allele with deletion*
del	*Allele with deletion*
DHF	Dihidrofolato (*Dihydrofolate*)
DHFR	Dihidrofolato redutase (*Dihydrofolate reductase*)
DNA	Ácido desoxirribonucléico (*Desoxirribonucleic acid*)

DNMTs	DNA metiltransferases (*DNA methyltransferases*)
DS	*Down syndrome*
DTN	Defeitos de tubo neural
Et al.	*Et alia*
FAMERP	Faculdade de Medicina de São José do Rio Preto
FAPESP	Fundação de Amparo à Pesquisa do Estado de São Paulo ver
FUNFARME	Fundação Faculdade Regional de Medicina de São José do Rio Preto
G	Guanina (*Guanine*)
HB	Hospital de Base
Hcy	Homocisteína (*Homocysteine*)
HW	Hardy-Weinberg
I	*Wild allele*
IBGE	Instituto Brasileiro de Geografia e Estatística
IC	Intervalo de confiança
MAT	Metionina adenosyltransferase
Met	Metionina (*metionine*)
MMA	Ácido metilmalônico (*Methylmalonic acid*)
MTCH	5,10-Metilenotetrahidrofolato ciclohidrolase
MTHFD1	Metilenotetrahidrofolato desidrogenase
MTHFR	Metilenotetrahidrofolato redutase (*Methylenetetrahydrofolate reductase*)
MTR	Metionina sintase (*Methionine synthase*)
MTRR	Metionina sintase reductase (*Methionine synthase reductase*)
ng/mL	*Nanogram / microlitre*

NTD	*Neural tube defects*
OR	*Odds ratio*
pb	Pares de base
PCR	Reação em Cadeia da Polimerase (*Polymerase chain reaction*)
q	Braço longo do centrômero ao telômero de um cromossomo
r	Range
RFC1	Carregador de folato reduzido 1
SAH	S-adenosilhomocisteína (*S-adenosylmethionine*)
SAHH	S-adenosilhomocisteína hidrolase
SAM	S-adenosilmetionina
SD	Síndrome de Down
SHMT	Serina hidroximetiltransferase (*Serine hydroxymethyltransferase*)
T	*Timina (Thymine)*
TC2	*Transcobalamina 2*
THF	*Tetrahidrofolato (Tetrahydrofolate)*
TS	Timidilato sintase (*Thymidylate synthase*)
UNICAMP	*Universidade de Campinas*
UPGEM	*Unidade de Pesquisa em Genética e Biologia Molecular*
USP	*Universidade de São Paulo*
$\chi 2$	*Chi-square*

RESUMO

Introdução: A síndrome de Down (SD) é uma doença genética complexa resultante, principalmente, da presença de três cópias do cromossomo 21. É a cromossomopatia humana mais frequente e, na maioria dos casos (cerca de 95%), decorrente da não disjunção cromossômica materna, ocorridas durante a meiose I. Recentes estudos sugerem que a etiologia do risco materno para a SD em mães jovens está relacionada com polimorfismos em genes do metabolismo do folato/homocisteína (Hcy). O funcionamento adequado do metabolismo do folato é essencial para a síntese de grupos metil necessários para a metilação do DNA. A deficiência deste metabólito tem como resultado, a hipometilação do DNA, quebras cromossômicas e aneuploidias. **Objetivos:** Avaliar a influência do polimorfismo C1420T do gene *serina hidroximetiltransferase (SHMT)* no risco materno para a SD e nas concentrações de folato sérico, Hcy e ácido metilmalônico (MMA) plasmáticos ; investigar o impacto dos polimorfismos de deleção de 19 pares de base (pb) do gene *dihidrofolato redutase* (*DHFR*) e de transição C1420T do gene *SHMT* nas concentrações de folato sérico, Hcy e MMA plasmáticos em indivíduos com SD. **Casuística e métodos:** Foram incluídas no estudo 105 mães de indivíduos com trissomia livre do cromossomo 21 e 185 mães de indivíduos sem a síndrome (sem história prévia de aborto espontâneo), e 85 indivíduos com trissomia livre do cromossomo 21. O polimorfismo do gene *DHFR* foi avaliado por meio da Reação em Cadeia da Polimerase (PCR) por diferença de tamanho de fragmentos, e o polimorfismo *SHMT* C1420T foi analisado por PCR em tempo real. **Resultados:** Os genótipos CC e CT do polimorfismo *SHMT* C1420T foram associados à redução do risco materno para SD (CC: P = 0,0002; 95% IC: 0,20 – 0,60; OR: 0,35. CT: P < 0,0001; 95% IC: 0,11 – 0,39; OR: 0,21). Os diferentes genótipos não

influenciaram as concentrações dos metabólitos estudados. No grupo de indivíduos com SD, os genótipos combinados *DHFR* II/*SHMT* TT e *DHFR* DD/*SHMT* TT foram associados, respectivamente, com concentrações aumentadas de Hcy ($P < 0,001$) e de folato ($P < 0,001$). Além disso, indivíduos com os genótipos *DHFR* II/*SHMT* CT apresentaram concentração reduzida de folato ($P = 0,01$). **Conclusão:** Os genótipos CC e CT do polimorfismo *SHMT* C1420T conferem um efeito materno protetor para SD. Este polimorfismo parece não influenciar as concentrações de folato, Hcy e MMA. Os polimorfismos del 19pb *DHFR* e C1420T *SHMT* apresentam um efeito sinérgico na modulação das concentrações de folato e Hcy de indivíduos com SD.

Palavras chave: Síndrome de Down; Trissomia do 21; Polimorfismo genético; Homocisteína

ABSTRACT

Introduction: Down syndrome (DS) is a complex genetic disease resulting mainly from the presence of three copies of chromosome 21. It is the most frequent human chromosomal abnormality and, in most cases (about 95%) results from maternal chromosome nondisjunction, which occurs during meiosis I. Recent studies suggest that the etiology of maternal risk for DS in young mothers is associated with polymorphisms in genes of folate/homocysteine metabolism. The proper function of folate metabolism is essential for the synthesis of methyl groups necessary for DNA methylation. The deficiency of this metabolite has resulted in DNA hypomethylation, chromosomal breakage and aneuploidies. **Objectives:** Evaluate the influence of the C1420T polymorphism in *serine hidroximetiltransferase* (*SHMT*) gene on the maternal risk for DS, and investigate the association between this polymorphism and variation in the concentration of serum folate, plasma Hcy and methylmalonic acid (MMA); investigate the impact of 19 base pairs (pb) deletion polymorphism of the *dihydrofolate reductase* (*DHFR*) gene and the C1420T polymorphism of the *SHMT* gene on serum folate concentrations, plasma Hcy and MMA in individuals with DS. **Material and methods:** 105 mothers of individuals with free trisomy of chromosome 21 and 185 mothers of individuals without the syndrome (no history of miscarriage), and 85 individuals with free trisomy of 21 chromosome were included in this study. The polymorphism of *DHFR* gene was evaluated by Polymerase Chain Reaction (PCR) by fragment size difference, and the polymorphism of *SHMT* gene was analyzed by real-time PCR allelic discrimination. **Results:** The *SHMT* CC and CT genotypes were associated with decreased maternal risk for DS (CC: P= 0.0002; 95% CI: 0.20 – 0.60; OR: 0.35. CT: P < 0.0001; 95% IC: 0.11 - 0.39; OR: 0.21). The different genotypes did not influence the

concentrations of metabolites studied. In individuals with DS, the combined genotypes *DHFR* II/ *SHMT* TT and *DHFR* DD/ *SHMT* TT were associated, respectively, with increased concentrations of Hcy (P<0.001) and folate (P<0.001). Moreover, individuals with *DHFR* II/ *SHMT* CT genotypes presented a reduction of folate concentration (P= 0.01). **Conclusion:** The CC and CT genotypes of *SHMT* C1020T polymorphism has a protector effect for maternal risk for DS. This polymorphism does not seem to influence the folate, Hcy and MMA concentrations. The del 19pb *DHFR* and *SHMT* C1420T polymorphisms present a synergic effect in modulating folate and Hcy concentrations in individuals with DS.

Keys words: Down Syndrome; Trisomy of 21; genetic polymorphism; homocystein; folate

1. INTRODUÇÃO

1. INTRODUÇÃO

A síndrome de Down (SD) é uma cromossomopatia caracterizada, na maioria dos casos, por uma cópia extra do cromossomo 21.[1] A origem desta não disjunção é materna em 95% dos casos e ocorre principalmente durante a meiose I.[2,3]

Esta condição genética, nomeada após a descrição clínica realizada pelo médico inglês John Langdon Haydon Down em 1866, é a aneuploidia humana mais frequente, com incidência aproximada de 1 a 660 nascidos vivos.[4] No Brasil, a estimativa da população foi de cerca de 300.000 indivíduos com SD, de acordo com o censo realizado pelo Instituto Brasileiro de Geografia e Estatística (IBGE) em 2000.[5]

Desde a identificação da trissomia do cromossomo 21 como a principal causa para o desenvolvimento da SD, o único fator de risco bem estabelecido é idade materna avançada.[1,6] Entretanto, os mecanismos que relacionam a idade materna com o surgimento de aneuploidias são pouco compreendidos e, provavelmente, associados com a degradação do processo meiótico.[7, 8, 9]

Apesar da idade materna ser um importante fator de risco para a SD, a ocorrência da síndrome independente da idade sugere a influência de outros fatores etiológicos. Em 1999, James e colaboradores [10] propuseram que alteração no metabolismo do folato observado em mães jovens de indivíduos com SD, secundária a presença do alelo variante 677T do gene metilenotetrahidrofolato reductase (*MTHFR*), pode resultar em modificações no padrão de metilação do DNA e, consequentemente em não disjunção cromossômica. De fato, estudos mostram que a hipometilação do DNA prejudica a formação de heterocromatina e o estabelecimento do cinetocoro, complexo DNA-proteína que garante a divisão precisa de cromossomos entre as células filhas por meio

da ligação do centrômero aos microtúbulos do fuso mitótico e, portanto, está associada à instabilidade genética e à ocorrência de aneuploidias.[8,10]

Desde então, vários estudos são realizados em busca de maior compreensão sobre o impacto do metabolismo anormal do folato no risco materno para SD.[1, 11, 12, 13, 14, 15, 16] Alterações nessa via metabólica podem levar à redução da concentração de S-adenosilmetionina (SAM), o principal doador de grupos metil para as reações de metilação de DNA. [17]

Algumas enzimas desempenham funções importantes na manutenção das concentrações de SAM. A enzima MTHFR catalisa a conversão do 5,10 metilenotetraidrofolato (5,10-MeTHF) para 5-metiltetrahidrofolato (5-mTHF) (Figura 1), a principal forma circulante de folato, exigida para a remetilação da homocisteína (Hcy) para metionina, a partir da qual é sintetizada SAM. Polimorfismos no gene *MTHFR* estão relacionados com a redução da atividade enzimática e, consequentemente, hipometilação do DNA.[10] Estudos demonstram que o polimorfismo *MTHFR* 677C>T está associado com o risco materno aumentado para SD. [10, 13, 18, 19, 20, 21, 22]

Outra enzima importante para o metabolismo do folato é a metionina sintase (MTR), que tem sua atividade modulada pela vitamina B12, representada no plasma pelas concentrações de ácido metilmalônico (MMA).[23] Esta enzima atua na remetilação da Hcy para metionina e seu estado ativo é mantido pela enzima metionina sintase reductase (MTRR).[24] Alguns estudos demonstraram associação entre as variantes *MTR* A2756G e *MTRR* A66G com o risco aumentado para a SD. [1,18, 25,26, 27,28]

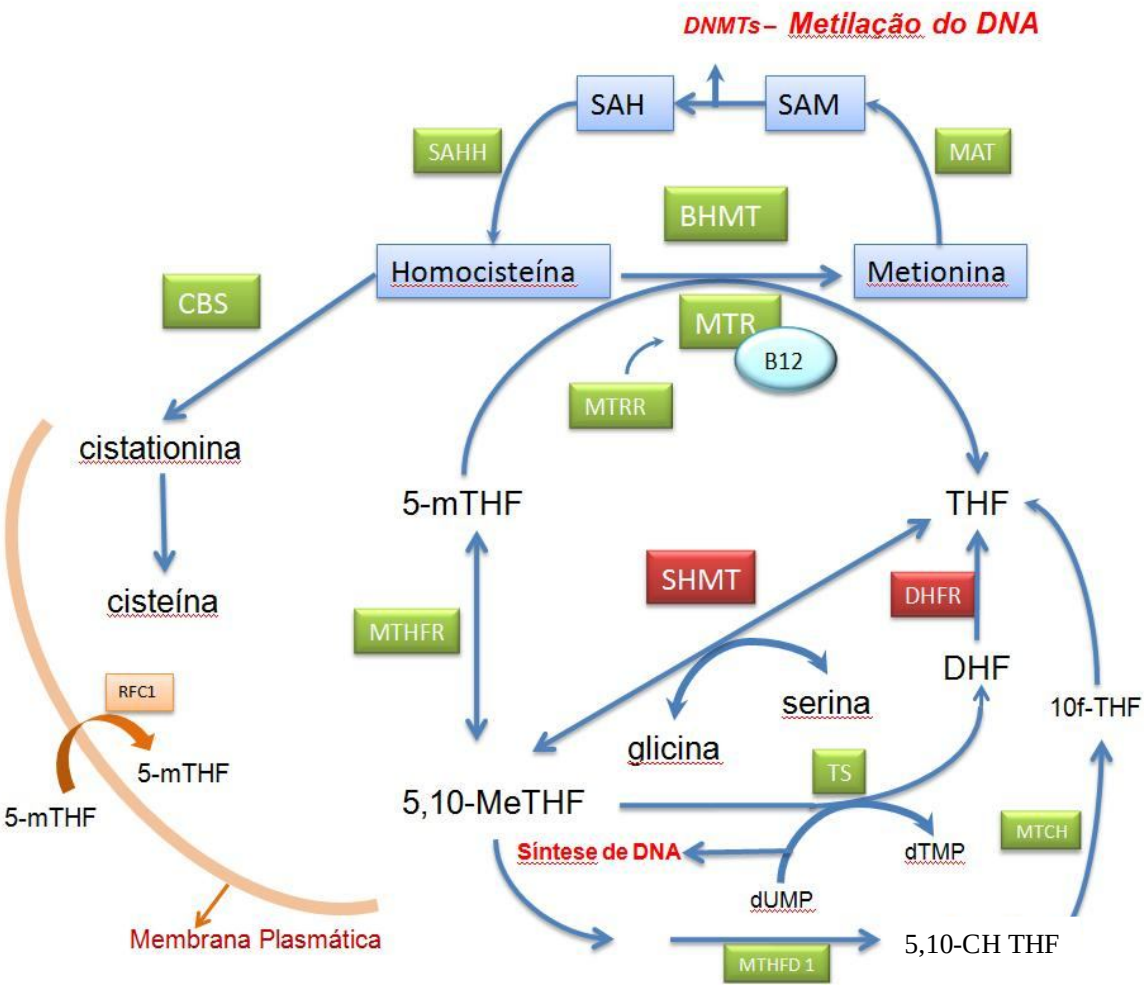

Figura 1. Metabolismo do folato com as principais enzimas envolvidas. CBS = Cistationina β-sintase, DHFR = Dihidrofolato redutase, SHMT = Serina hidroximetiltransferase, BHMT = Betaína -homocisteína metiltransferase, MAT = Metionina adenosyltransferase, MTHFR = Metilenotetrahidrofolato redutase, MTHFD1= Metilenotetrahidrofolato desidrogenase 1, MTCH = 5,10-Metilenotetrahidrofolato ciclohidrolase, 10f-THF = 10-formiltetrahidrofolato, RFC1 = Carregador de folato reduzido 1, MTR = Metionina sintase, MTRR = Metionina sintase reductase, DNMTs = DNA metiltransferases, DHF = Dihidrofolato, THF = Tetrahidrofolato, TS = Timidilato sintase, 5,10-MeTHF = 5,10-metilenotetrahidrofolato, 5,10-CH-THF = 5,10-meteniltetrahidrofolato ,5-mTHF = 5-metiltetrahidrofolato, B_{12} = Vitamina B_{12}, SAM = S-adenosilmetionina, SAH = S-adenosilhomocisteína, SAHH = S-adenosilhomocisteína hidrolase dUMP = Deoxiuridina monofosfato, dTMP = Deoxitimidina monofosfato.

Os estudos que avaliam a contribuição de polimorfismos isolados em genes envolvidos na via metabólica do folato no risco materno para a SD são conflitantes (Tabela 1) e, possivelmente influenciados pelas variações geográficas nas frequências alélicas entre populações, diferenças do número amostral avaliado e fatores ambientais, como a condição nutricional. Todavia, a combinação de genótipos de risco pode modificar o efeito de genes individuais no risco materno para SD (Tabela 2). Além disso, há evidências de que polimorfismos envolvidos nessa via possam modular as concentrações de Hcy, folato e vitamina B_{12}; outros estudos mostram ainda a associação entre Hcy e risco materno para SD (Tabela 3).

É possível que polimorfismo no gene *SHMT* (*Serina hydroxymethyltransferase)*, que foi associado à modulação do risco materno para defeitos de tubo neural (DTN) [29] possa também modular o risco materno para SD, uma vez que ambas as afecções são influenciadas pelos mesmos determinantes genéticos do metabolismo do folato.[30] Reforçando esta hipótese, há evidências de frequência elevada de casos com SD em famílias com risco para DTN e vice-versa.[31]

O gene *Serina hydroxymethyltransferase* (*SHMT*), localizado no cromossomo 17q11.2, apresenta-se polimórfico no nucleotídeo 1420 (C>T), e codifica uma enzima altamente conservada do metabolismo do folato. A sequência da isoenzima humana apresenta 43% de semelhança com a proteína da *Escherichia coli* [51] e catalisa a conversão reversível de serina e tetrahidrofolato (THF) para glicina e 5,10-MeTHF [52] (Figura 1). A enzima SHMT media a competição de grupos metil entre as enzimas metilenotetrahidrofolato redutase (MTHFR) e timidilato sintase (TS), modulando a concentração de 5,10-MeTHF para o timidilato e para a síntese de 5-mTHF (Figura 1). O timidilato é importante para evitar rupturas na molécula de DNA resultante da

Tabela 1. Estudos sobre associação entre polimorfismos em genes do metabolismo do folato no risco materno para a SD (tabela modificada segundo Coppedè, 2009).

Referência	País	MSD/C Nº	MTHFR 677C > T	MTHFR 1298A > C	MTRR 66A > G	MTR 2756A > G	RFC-1 80G > A	CBS 844 ins68	Outros
James *et al.*, 1999[10]	USA	57/50	s	-	-	-	-	-	-
Hobbs *et al.*, 2000[18]	USA/ Canadá	157/144	s	-	s	-	-	-	-
O'Leary *et al.*, 2002[25]	Irlanda	48/192	n	-	s	-	-	-	-
Grillo *et al.*, 2002[32]	Brasil	36/200	s	s	-	-	-	-	-
Stuppia *et al.*, 2002[33]	Itália	64/112	n	-	-	-	-	-	-
Bosco *et al.*, 2003[26]	Itália	63/72	s	n	n	s			
Takamura *et al.*, 2004[34]	Japão	31/60	n	-	-	-	-	-	-
Boduroglu *et al.*, 2004[35]	Turquia	152/91	n	n	-	-	-	-	-
da Silva *et al.*, 2005[11]	Brasil	154/158	s	n	n	n	-	n	-
Chango *et al.*, 2005[36]	França	119/119	n	n	n	n	n	n	-
Acacio *et al.*, 2005[37]	Brasil	70/88	n	n	-	-	-	-	-
Coppedè *et al.*, 2006[12]	Itália	80/111	n	n	-	-	n		
Rai *et al.*, 2006[38]	Índia	149/165	s	s	-	-	-	-	-
Scala *et al.*, 2006[39]	Itália	94/264	n	s	n	n	s	n	n *MTHFD1* 1958G >A
Martínez-Frías *et al.*, 2006[27]	Espanha	91/90	n	s	s	-	-	-	-
Wang *et al.*, 2007[40]	China	100/100	s	-	-	-	-	-	-
Meguid *et al.*, 2008[20]	Egito	42/48	s	s	-	-	-	-	-
Wang *et al.*, 2008[28]	China	64/70	s	-	s	-	-	-	-
Biselli *et al.*, 2008[13]	Brasil	72/194	s	n	-	n	n	-	-
Kohli *et al.*, 2008[41]	Índia	104/109	n	-	-	-	-	-	-
Santos-Rebouças *et al.*, 2008[42]	Brasil	103/108	n	n	n	-	-	-	-
Biselli *et al.*, 2008[43]	Brasil	67/113	-	-	-	-	n	-	n *TC* 776C > G
Coppedè *et al.*, 2009[44]	Itália	94/113	n	n	n	n	-	-	n TS 28bp/ 1494 del6
Pozzi *et al.*, 2009[1]	Itália	74/184	n	-	s	-	-	-	-
Fintelman-Rodrigues *et al.*, 2009 [45]	Brasil	114/110	-	-	-	n	n	n	n *TC*776C>T
Kokotas *et al.*, 2009[46]	Dinamarca	181/1084	n	-	-	-	-	-	-
Brandalize *et al.*, 2010[47]	Brasil	239/197	-	-	n	n	n	n	-
Mendes *et al.*, 2010 [15]	Brasil	105/184	-	-	-	-	-	-	n *DHFR*19-bp deletion
Neagos *et al.*, 2010[48]	Romênia	26/46	-	-	-	-	n	-	
Neagos *et al.*, 2010[49]	Romênia	26/46	-	-	-	-	-	-	n *MTHFD1* 1958G>A
Marucci *et al.*, 2011[16]	Brasil	105/185	-	-	-	-	-	-	s SHMT1420 C>T
Sadiq *et al.*, 2011[22]	Jordânia	53/29	s	n	-	-	-	-	-

Nº indivíduos avaliados; s = associação entre o polimorfismo e risco para a SD; n = ausência de associação entre o polimorfismo e risco para a SD; polimorfismo não avaliado; SD = mães de indivíduos com SD; C = mães controle.

Tabela 2. Combinação de polimorfismos em genes do metabolismo do folato e risco materno para a SD (tabela modificada segundo Coppedè, 2009).

Referência	Alelos ou Genótipos combinados	Associação com o risco materno	
Hobbs et al., 2000[18]	*MTHFR* 677T + *MTRR* 66G	↑	OR = 4,1 (1,9–8,6)
O'Leary et al., 2002[25]	*MTHFR* 677T + *MTRR* 66G	↑	OR = 2,9 (1,2–7,5)
Grillo et al., 2002[32]	*MTHFR* 677CT + *MTHFR* 1298AC	↑	OR = N.A.
Bosco et al., 2003[26]	*MTR* 2756AG + *MTRR* 66AG	↑	OR = 5,0 (1,1–24,1)
da Silva et al. 2005[11]	*CBS* 844I+*MTHFR* 677T +*MTHFR* 1298C +MTR 2756G +MTRR 66G[a]	↑	OR = 1,2 (1,0–1,6)
Acacio et al. 2005[37]	*MTHFR* 677CT + *MTHFR* 1298AC	↑	OR = 5,7 (1,7–18,8)
Rai et al., 2006[38]	*MTHFR* 677CC + *MTHFR* 1298CC	↑	OR = 4,0 (1,2–13,6)
Scala et al. 2006[39]	*MTHFR* 677TT + *MTHFR* 1298CC/CA	↑	OR = 7,2 (1,4–47,2)
	RFC1 80 GG/GA + *MTHFR* 1298CC/CA	↑	OR = 2,6 (1,1–6,3)
	RFC1 80 GG + *MTHFD1* 1958 AA	↑	OR = 4,4 (1,2–17,9)
Coppedè et al. 2006[12]	*RFC1* 80GG + *MTHFR* 677TT	↑	OR = 6,0 (1,0–35,9)
	RFC1 80GA/AA + *MTHFR* 1298AA	↓	OR = 0,4 (0,1–0,9)
Wang et al., 2008[28]	*MTHFR* 677TT/CT + *MTRR* 66GG	↑	OR = 6,0 (2,0–17,5)
Biselli et al., 2008[13]	*MTHFR* 677T + *MTHFR* 1298C + *RFC1* 80G + *MTR* 2756G [a]	↑	OR = 1,7 (1,0–3,0)
Coppedè et al., 2009[44]	*TS* 2R/2R + *MTHFR* 1298AC	↓	OR = 0,11 (0,1–0,5)
Brandalize *et al.*,2009[14]	*MTHFR* 677TT/CT + *MTHFR*1298AA		OR = 1,99(1,1–3,5)*
Brandalize *et al.*,2010[47]	*MTR* 2756 AG+GG e/ou *MTRR* 66 AG+GG e/ou *RFC1*80 AG+GG e/ou *CBS* 844 I/D + I/I)	↑	OR = 4,62 (1,2–17,5) [b] OR = 5,02 (1,4–18,5) [c] OR = 4,25 (1,1–16,2) [d]

↑ = Risco Materno aumentado para a SD; ↓ = Risco Materno reduzido para a SD; OR = Odds ratio (95% - IC); N.A. = Não avaliado; I = Inserção; D=deleção.

* Odds ratio ajustado para idade.

[a] Mães de indivíduos com SD mostraram um número significativamente aumentado de alelos mutantes em relação às mães controle.

[b] presença de um genótipo de risco; [c] presença de dois genótipos de risco; [d] presença de três genótipos de risco.

Tabela 3. Associação das concentrações Hcy e polimorfismos em genes do metabolismo do folato e concentrações de Hcy, folato e vitamina B_{12} em mães de indivíduos com Síndrome de Down (tabela modificada segundo Coppedè, 2009).

Referência	**País**	**Hcy plasmática**	**Outros nutrientes**
James *et al.* ,1999[10]	USA	*MTHFR* 677C > T ⟷ Hcy Aumento de Hcy em MSD	-
O'Leary *et al.*, 2002[25]	Irlanda	*MTHFR* 677C > T ⟷ Hcy	Folato plasmático e vitamina B_{12} - preditores para Hcy
Bosco *et al.*, 2003[26]	Itália	Aumento de Hcy em MSD	-
Sheth and Sheth, 2003[50]	Índia	Aumento de Hcy em MSD	-
Takamura et al. 2004[34]	Japão	Aumento de Hcy em MSD	Redução de folato sérico em MSD
da Silva *et al.* ,2005[11]	Brasil	*MTHFR* 677C > T ⟷ Hcy Aumento de Hcy em MSD	-
Martínez-Frías *et al.*, 2006[27]	Espanha	*MTHFR* 1298A > C *MTRR* 66A > G ⟷ Hcy Aumento de Hcy em MSD	-
Wang *et al.* ,2007[40]	China	*MTHFR* 677C > T ⟷ Hcy Aumento de Hcy em MSD	-
Biselli et al. 2008[13]	Brasil	*MTHFR* 1298A > C ⟷ Hcy Aumento de Hcy em MSD	-
Kohli *et al.*, 2008[41]	Índia	Redução de Hcy em MSD	-
Meguid *et al.*, 2010 [67]	Egito	Aumento de Hcy em MSD	Redução de folato sérico e vitamina B_{12} sérico em MSD

⟷ = Interação; MSD = Mães de filhos com Síndrome de Down.

incorporação errônea de uracila,[53] e o 5-mTHF está diretamente relacionado com as reações de metilação celulares.[3,54] Portanto, o gene *SHMT* tem um papel indireto na manutenção da estrutura do genoma e na metilação do DNA, o que explica seu estudo em diferentes tipos de câncer, [55, 56, 57] como a leucemia aguda linfocítica,[58] doença comum em indivíduos SD. [59]

Embora não há estudos que avaliem a influência do polimorfismo *SHMT* C1420T no risco materno para SD, dois estudos sobre DTN associaram a presença do alelo polimórfico T com a redução do risco materno para DTN [29, 60]. Na literatura, a distribuição da concentração de metabólitos da via do folato em relação aos genótipos do polimorfismo *SHMT* C1420T é contraditória. Alguns estudos não encontraram associação entre os genótipos do polimorfismo *SHMT* C1420T com as concentrações de folato (sérico ou eritrocitário) e de Hcy plasmática em mães de indivíduos com DTN e em pacientes com câncer colorretal [29, 61]. No entanto, Heil et al. [62] relataram que a concentração de Hcy foi significantemente maior em mães de indivíduos com DTN com genótipo *SHMT* 1420 CC em relação àquelas com o genótipo *SHMT* 1420 CT. Portanto, outros estudos são necessários para melhor avaliação da relação entre o polimorfismo *SHMT* C1420T com os metabólitos da via do folato.

Embora estudos sejam necessários para confirmar o efeito do polimorfismo *SHMT* C1420T na modulação do metabolismo do folato, a evidência de associação do mesmo com o risco materno para DNT reforça a necessidade de avaliação dos efeitos dessa variante no risco de mães gerarem filhos com SD.

Metabolismo do folato em indivíduos com SD

A SD resulta principalmente da presença e expressão em triplicata dos genes do cromossomo 21 [63, 64] e alterações no metabolismo do folato em indivíduos com SD pode ser decorre de uma cópia extra do gene *Cistationina β-sintase* (*CβS*), localizado no cromossomo 21 (21q22.3). O gene *CβS* é responsável pela conversão da Hcy em cistationina [64] e se apresenta com expressão elevada em indivíduos com SD.

O aumento das concentrações da enzima CβS promove a redução da concentração de Hcy, e, consequentemente, de metionina, S-adenosilhomocisteína (SAH) e SAM [65, 66, 67]. Com a redução da Hcy, a atuação da enzima MTR, responsável pela conversão da Hcy em metionina, também sofre redução em sua atividade, o que resulta em acúmulo de 5-mTHF e, consequentemente, na redução na formação de THF, requerido para a síntese de DNA e RNA. Estudos mostram que crianças com SD apresentam baixa concentração de Hcy e folato, [65, 67] e a expressão aumentada do gene *CβS* pode alterar as concentrações desses metabólitos. [65, 66]

Algumas variantes genéticas localizadas em genes que não estão presentes no cromossomo 21 também podem modular as concentrações de folato/Hcy em indivíduos com SD. [68,69,70] Estudos mostram que os genótipos *MTHFR* 677TT,[70] *MTR*2756 AG e *MTR*2756GG [26] são mais frequentes em indivíduos com SD em relação àqueles sem a síndrome, e o genótipo *MTHFR* 677TT foi associado com o aumento da Hcy plasmática em indivíduos com SD. [70]

Até o presente, o efeito funcional das variantes de transição C1420T do gene *SHMT* e de deleção de 19 pares de base (pb) no íntron do gene *Dihidrofolato redutase* (*DHFR*) não foram avaliadas em indivíduos com SD. O gene *DHFR* codifica uma enzima que catalisa a conversão de dihidrofolato (DHF) em THF (Figura 1) e há

evidências da presença de um elemento inibitório de transcrição na sequência polimórfica, que ao ser deletado pode resultar em expressão aumentada do alelo variante. [71, 72, 73]

Estudos mostram que o polimorfismo de deleção de 19pb *DHFR* está associado com alterações nas concentrações de Hcy e folato em indivíduos saudáveis [74, 75, 76]. Gellekink et al. [75] relataram que o genótipo *DHFR* homozigoto para a deleção (DD) está associado com concentração reduzida de Hcy plasmática em indivíduos caucasianos, porém nenhuma associação entre este genótipo com as concentrações de folato foi observada. Outro estudo não encontrou efeito desse polimorfismo na concentração de Hcy em adultos, mas o genótipo DD foi associado com aumento das concentrações de folato sérico e o genótipo homozigoto selvagem (II) com o de folato eritrocitário [74]. Kalmbach et al. [76] também não observaram associações entre os genótipos *DHFR* com as concentrações de folato ou Hcy plasmática em adultos jovens; no entanto o genótipo *DHFR* DD foi associado com menor concentração de folato eritrocitário em relação aos genótipos ID e II.

Em relação ao polimorfismo C1420T *SHMT* Heil et al. [62] encontraram uma redução significante nas concentrações de folato sérico e eritrocitário em indivíduos com genótipo homozigoto selvagem (*SHMT* 1420 CC), esses autores incluíram na análise mães de indivíduos com DTN, pacientes com espinha bífida e indivíduos saudáveis. Nesse estudo também foi observado maior concentração de Hcy em mães de indivíduos com DTN com genótipo *SHMT* 1420 CC em relação àquelas com o genótipo *SHMT* 1420 CT, como anteriormente citado.

Assim, considerando a influência dos polimorfismos *SHMT* C1420T e del 19pb *DHFR* nas concentrações dos metabólitos da via do folato/Hcy, a análise desses

polimorfismos na modulação dos produtos resultantes deste metabolismo em indivíduos com SD torna-se relevante.

1.1 OBJETIVOS

1. Avaliar a influência do polimorfismo *SHMT* C1420T como fator de risco materno para a SD e a distribuição das concentrações dos metabólitos para os diferentes genótipos nos grupos caso e controle.
2. Investigar o impacto dos polimorfismos *DHFR* del 19 pb e *SHMT* C1420T nas concentrações de folato sérico, Hcy plasmática e MMA em indivíduos com SD.

2. ARTIGOS CIENTÍFICOS

2. ARTIGOS CIENTÍFICOS

Os resultados referentes a esta dissertação estão apresentados em forma de artigos. São apresentados 02 artigos: um publicado e um submetido para publicação.

Artigo 1

Título: Polymorphism C1420T of Serine hydroxymethyltransferase gene on maternal risk for Down syndrome.

Autores: Gustavo Henrique Marucci, Bruna Lancia Zampieri, Joice Matos Biselli, Sendi Valentin, Eny Maria Goloni Bertollo, Marcos Nogueira Eberlin, Renato Haddad, Maria Francesca Riccio, Hélio Vannucchi, Valdemir Melechco Carvalho, Érika Cristina Pavarino.

Periódico: *Molecular Biology Reports*, 2011 Jun 18. [Epub ahead of print]

Artigo 2

Título: Synergistic effect of *DHFR* 19-bp deletion and *SHMT* C1420T polymorphisms on metabolites concentrations of folate pathway in individuals with Down syndrome

Autores: Cristiani Cortez Mendes, Aline Maria Zanchetta de Aquino Raimundo, Luciana Dutra Oliveira, Bruna Lancia Zampieri, Gustavo Henrique Marucci, Joice Matos Biselli, Eny Maria Goloni-Bertollo, Marcos Nogueira Eberlin, Renato Haddad, Maria Francesca Riccio, Hélio Vannucchi, Valdemir Melechco Carvalho, Érika Cristina Pavarino.

Periódico: *Molecular Biology Reports*, submetido para publicação.

ARTIGO CIENTÍFICO 1

Polymorphism C1420T of Serine hydroxymethyltransferase gene on maternal risk for Down syndrome.

Gustavo Henrique Marucci[1] , Bruna Lancia Zampieri[1] , Joice Matos Biselli[1], Sendi Valentin[1], Eny Maria Goloni Bertollo[1], Marcos Nogueira Eberlin[2], Renato Haddad[2], Francesca Riccio[2], Hélio Vannucchi[3], Valdemir Melechco Carvalho[4], Érika Cristina Pavarino[1].

[1]Unidade de Pesquisa em Genética e Biologia Molecular, Departamento de Biologia Molecular, Faculdade de Medicina de São José do Rio Preto

Address: Avenida Brigadeiro Faria Lima, 5416

Vila São Pedro

CEP:15090-000; São José do Rio Preto, SP, Brazil

[2]Laboratório ThoMSon de Espectrometria de Massas, Instituto de Química, Universidade Estadual de Campinas

Address: Cidade Universitária Zeferino Vaz

CEP: 13083-970; Campinas, SP, Brazil

[3]Laboratório de Nutrição, Departamento de Clínica Médica, Faculdade de Medicina de Ribeirão Preto - USP

Address: Avenida Bandeirantes, 3900

Monte Alegre

CEP: 14049-900; Ribeirão Preto, SP, Brazil

[4]Fleury Medicina e Saúde

Address: Rua dos Otonis, 880, apto. 133

Vila Clementino

CEP: 04025-002; São Paulo, SP, Brazil

Concise title: Folate metabolism and Down syndrome.

Corresponding author: Érika Cristina Pavarino

Av. Brigadeiro Faria Lima, 5416

CEP:15090-000; São José do Rio Preto, SP, Brasil

UPGEM, Bloco U6

Fax: +55-17-3201-5708. Tel: +55-17-3207-5904. E-mail: erika@famerp.br

Abstract

Recent researches have investigated the factors that determine the maternal risk for Down syndrome (DS) in young woman. In this context, some studies have demonstrated the association between polymorphisms in genes involved on folate metabolism and the maternal risk for DS. These polymorphisms may result in abnormal folate metabolism and methyl deficiency, which is associated with aberrant chromosome segregation leading to trisomy 21. In this study, we analyzed the influence of the polymorphism C1420T in *Serine hydroxymethyltransferase* (*SHMT*) gene on maternal risk for DS and on metabolites concentrations of the folate pathway (serum folate and plasma homocysteine and methylmalonic acid). The study group was composed by 105 mothers with DS children (case group) and 185 mothers who had no children with DS (control group). The genotype distribution didn't show significant

statistical difference between case and control mothers ($p = 0.24$) however a protective effect between genotypes CC ($P = 0.0002$) and CT ($P < 0.0001$) and maternal risk for DS was observed. Furthermore, the *SHMT* C1420T polymorphism (rs1979277) does not affect the concentration of metabolites of folate pathway in our DS mothers.

In conclusion, our data showed a protective role for the genotypes *SHMT* CC and CT on maternal risk for DS. The concentrations of metabolites of folate pathway did not differ significantly between the genotypes *SHMT*.

Keywords: Down syndrome, Serine hydroxymethyltransferase, genetic polymorphism, folate metabolism.

Introduction

Down syndrome (DS) or trisomy 21 is resultant, in most cases, from errors during maternal meiotic division [1, 2]. The advanced maternal age is the only well-established risk factor for DS [3, 4] and the risk increases exponentially after 35 years-old [5]. However, the factors that predispose young woman to give birth to children with DS are still unknown, suggesting the influence of others factors on DS etiology.

In 1999, Jill James [6] and colleagues proposed that the abnormal folate metabolism observed in young mothers of DS individuals, secondary to a polymorphism of the Methylenetetrahydrofolate gene, may result in DNA hypomethylation in centromeric and pericentromeric regions, increasing the risk for chromosomal nondisjunction. Since then, several studies have been made aiming to associate functional polymorphisms of genes involved on folate metabolism with the maternal risk for DS [7 – 13].

The principal function of folate pathway is synthesis of S-adenosylmethionine (SAM), the main donor of methyl groups for cellular methylation reactions [14]. Abnormal folate metabolism results in decrease of SAM concentration and DNA hypomethylation [15,16]. Chronic folate/methyl deficiency has been associated, beyond DNA hypomethylation, with DNA strand breaks [17], abnormal gene expression [18] and aberrant chromosome segregation [19].

The *Serine hydroxymethyltransferase* (*SHMT*) gene encodes a highly conserved enzyme from folate metabolism. The human isoenzyme retaining about 43% sequence identity with Escherichia coli protein [20] and catalyzes the reversible conversion of serine and tetrahydrofolate (THF) to glycine and 5,10-methylene THF (5,10-MeTHF) (Figure 1) [21]. The enzyme *SHMT* mediates the competition for methyl groups between the enzymes methylenetetrahydrofolate reductase (MTHFR) and thymidylate synthase (TS), modulating the concentration of 5,10-MeTHF for thymidylate and 5-methyl tetrahydrofolate (5-mTHF) synthesis. Thymidilate is important to prevent uracil misincorporations into DNA, which can lead to DNA strand breaks [22], and 5-mTHF is the main form of circulating folate, which is direct linked to cellular methylation reactions [23]. Therefore, *SHMT* has an indirect role in the maintenance of genome structure and of correct DNA methylation, which explains studies concerning this enzyme in different types of cancer [24-26] inclusive acute lymphocytic leukemia [27] that is a common disease in DS individuals [28]. Skibola et al. [27] found that *SHMT* C1420T polimorphism was associated with risk of adult acute lymphocytic leukemia, however, Lightfoot et al. [29] not found association between *SHMT* C1420T and childhood leukemia. According to our present knowledge, there are no studies that have investigated the influence of *SHMT* C1420T polymorphism (rs1979277) on maternal

risk for DS, although association between this variant and neural tube defect (NTD), which is influenced by the same determinants of the folate metabolism, has been observed [30].

Objective

The aim of this work was to analyze the influence of polymorphism *SHMT* C1420T on maternal risk for DS and analyze the distribution of metabolites concentrations between de genotypes for both groups.

Methods

Two hundred and ninety individuals were included in this study: 105 mothers of individuals with free trisomy 21 (case group) and 185 mothers who had no children with DS and no history of miscarriage (control group). The case group was recruited from the Genetics Outpatient Service of Hospital de Base (HB) of the São José do Rio Preto Medical School (FAMERP), São Paulo, Brazil. Mothers of children with chromosomal translocation or mosaicism were excluded from the case group. Control mothers were enrolled at the FAMERP Campus and at the HB Clinical Analysis Laboratory. The methodology for this study was approved by Research Ethics Committee of the São José do Rio Preto Medical School (CEP-FAMERP, 165/2004), in the State of São Paulo, and by the National Research Commission (CONEP), Brazil. Informed consent for participation in the study was obtained from all participants. Genomic DNA was extracted from mononuclear blood cells [31]. Alternatively, was used a commercial kit for DNA extraction (GFXTM Genomic Blood DNA Purification Kit, GE Healthcare), according to the manufacture instructions. The *SHMT* C1420T polymorphism was

determined by fluorogenic 3'-minor groove binding probes in a real-time polymerase chain reaction (PCR) assay for allelic discrimination. The primers and probes used were as follow: forward primer 5'-CAG AGC CAC CCT GAA AGA GTT C-3', reverse primer, 5'-AGT GGG CCC GCT CCT TTA-3'; probe for wild-type allele (5'-FAM-CGC CTC TCT CTT C-MGB-3') and mutant allele (5'-VIC-CGC CTC TTT CTT C-MGB-3') [27].

Concentrations of plasma homocysteine (Hcy) and methylmalonic acid (MMA) were determined as previously described [32-34] and folate by quimioluminescence (Immulite Kit, DPC Medlab, Brazil), in overnight fasted mothers. These data were determinated in a previous study (unpublished data).

The Hardy-Weinberg (HW) equilibrium was tested by chi-square, using the BioEstat program (Release 5.0). The chi-squared test was performed to investigate differences on *SHMT* genotype frequencies between case and control mothers. Mood's median test was used to investigate the distribution of biochemical parameters of the folate pathaway between the *SHMT* genotypes in each group. Both chi-squared and Mood's median tests were performed using the Minitab software (Release 12.22).

To evaluate if the *SHMT* C1420T polymorphism modulate the maternal risk for DS, multiple logistic regressions were performed by three models: considering the three possible *SHMT* genotypes; the dominant model (wild-type homozygous versus heterozygous and mutant homozygous), assuming that the polymorphism exert its impact on DS risk as in homozygosis as in heterozygosis; and the recessive model (wild-type homozygous and heterozygous versus mutant homozygous), assuming that the polymorphism exert its influence only in homozygosis. In the three models considered, in addition to the genotypes, the maternal age and the concentrations of

serum folate and plasma Hcy and MMA were included in the analysis. Multiple logistic regressions were performed by StatsDirect software (Release 14.0.0.223) and P values ≤ 0.05 were considered statistically significant.

Results

The mean maternal ages of DS mothers was 31.1 ± 8.2 (Range: 13-46) and the control group it was 26.9 ± 5.3 (Range: 15-57). Genotype distribution is in agreement with the HW equilibrium in case (X2 = 1.5983; GL = 1; P = 0.2061) and control groups (X2 = 1.0994; GL = 1; P = 0.2944). The frequency of *SHMT* 1420 T allele was 0.27 in the case mothers and 0.28 in the control mothers. The genotype distribution between the groups and the chi-squared test are presented on Table 1. There was no statistical difference for genotypes frequencies between case and control groups (P = 0.24). No difference between distribution of Hcy, folate and MMA concentration in *SHMT* genotypes was detected in both groups (Table 2).

The results of the multiple logistic regression analyses showed no association between the polymorphism *SHMT* C1420T and the maternal risk for DS in the dominant model (CT and TT: P = 0.34; 95% CI: 0.42 to 1.35) and recessive model (CC and CT: P = 0.10; 95% CI: 0.85 to 6.60; OR: 2.36). When the three genotypes was analyzed independently, we found that genotype CC and CT reduces the maternal risk for DS (CC: P = 0.0002; 95% CI: 0.20 to 0.60; OR: 0.35. CT: P < 0.0001; 95% CI: 0.11 to 0.39; OR: 0.21). The genotype TT showed no association for maternal risk for DS (P = 0.31; 95% CI: 0.31 to 1.45; OR: 0.67).

Discussion

The folate is an important metabolite obtained by diet, and the principal circulate form (5-mTHF) is important to DNA synthesis and conversion of Hcy to SAM, the most important DNA methyl donor [14]. Hcy and MMA concentration are modulated by B12 vitamin [35], that acts as a cofactor for MTR enzyme [36], promoting the conversion of Hcy to methionine. Deficiency of B12 vitamin reduces the MTR activity and results in Hcy accumulation and reduction of methyl groups for DNA methylation. The level of B12 vitamin is usually marker by MMA status, the most sensitive marker for diagnostic vitamin B12 deficiency in adults [37]. Studies have suggested that the impaired folate / Hcy metabolism in mothers of DS individuals can lead to DNA hypomethylation, mainly in pericentromeric region, and can promote abnormal chromosome segregation [6, 38,39].

Advanced maternal age is the only well-established risk factor for DS [40- 42, 4] and our results confirm this literatures dates. However, studies have been made to determine the maternal risk factors for DS in young mothers. Several studies have associated the abnormal folate metabolism with the etiology of the DS [2,7,8,10-12,43-49], and it is likely that some polymorphisms in this pathway might impair DNA methylation with consequences on chromosome segregation and on DS risk [2].

Several polymorphisms of folate pathway have been associated with increased maternal risk for DS, such as MTHFR C667T and A1298C [12, 43, 44, 47] , Methionine synthase A2756G (MTR A2756G) and MTRR A66G [11,45,50]. Combined genotypes of genes involved in folate metabolism were also associated with the modulation of the maternal risk for DS in several studies [2,44, 48, 51].

According to our knowledge, this study is the first one to investigate the influence of *SHMT* C1420T polymorphism on maternal risk for DS. We observed that the presence of allele C (CC and CT) confers a protective effect for maternal risk for DS. This result can be associated with studies about NTD, since that Barkai et al. [52] provided a direct evidence of a relation between DS and NDT, suggesting that the same factors influence both diseases. They observed that families with pregnancies affected by DS have higher incidence of NTD cases and vice-versa. Two studies about NTD related that the presence of the polymorphic allele T confers positive values of folate in the bloodstream and a protective role for NTD [30,53], in contrast with our study, in which the presence of allele C confers a protector factor for maternal risk for DS. These results show that is necessary others studies for evaluation the role of polymorphism C1420T of *SHMT* gene in maternal risk for DS and NTD.

In addition, there was no observed difference in the distribution of metabolites concentrations between the genotypes. In the literature, the distribution of metabolites concentrations of folate metabolism between *SHMT* C1420T genotypes is contradictory. Previous studies didn't associate the *SHMT* C1420T genotypes with folate (erythrocyte or serum) and plasma Hcy concentration in individuals with colorectal cancer and NTD mothers [30,54]. However, Heil et al.[53] found a significantly higher Hcy concentration in individuals with *SHMT* 1420 CC genotype compared to *SHMT* 1420 CT genotype in NTD mothers. They also found a significantly decrease in plasma and red blood cell folate concentration in individuals with the wild-type genotype (*SHMT* 1420 CC). Therefore, others studies are necessary for the better evaluation of the relationship between *SHMT* C1420T polymorphism and the metabolites of folate pathway.

Conclusion

We conclude that CC and CT genotype for *SHMT* C1420T polymorphism confers a protective effect for maternal risk for DS. The polymorphism *SHMT* C1420T seems to have no influence on folate, homocysteine and MMA concentrations.

References

01. Antonarakis SE, Petersen MB, McInnis MG, Adelsberger PA, Schinzel AA, Binkert F et al. (1992) The meiotic stage of nondisjunction in trisomy 21: determination by using DNA polymorphisms. Am J Hum Genet 50:544-50.

02. Coppedè F, Migheli F, Bargagna S, Siciliano G, Antonucci I, Stuppia L et al (2009) Association of maternal polymorphisms in folate metabolizing genes with chromosome damage and risk of Down syndrome offspring. Neurosci Lett 449:15-9.

03. Hassold T, Hunt P (2001) To err (meiotically) is human: the genesis of human aneuploidy. Nat Rev Genet 2:280-91.

04. Allen EG, Freeman SB, Druschel C, Hobbs CA, O'Leary LA, Romitti PA et al. (2009) Maternal age and risk for trisomy 21 assessed by the origin of chromosome nondisjunction: a report from the Atlanta and National Down Syndrome Projects. Hum Genet 125:41-52.

05. Hultén MA, Patel S, Jonasson J, Iwarsson E 2010 On the origin of the maternal age effect in trisomy 21 Down syndrome: the Oocyte Mosaicism Selection model. Reproduction 139:1-9.

06. James SJ, Pogribna M, Pogribny IP, Melnyk S, Hine RJ, Gibson JB et al (1999) Abnormal folate metabolism and mutation in the methylenetetrahydrofolate

reductase gene may be maternal risk factors for Down syndrome. Am J Clin Nutr 70: 495-501.

07. da Silva LR, Vergani N, Galdieri Lde C, Ribeiro Porto MP, Longhitano SB, Brunoni D et al (2005) Relationship between polymorphisms in genes involved in homocysteine metabolism and maternal risk for Down syndrome in Brazil. Am J Med Genet A 135:263-7.

08. Coppedè F, Marini G, Bargagna S, Stuppia L, Minichilli F, Fontana I et al (2006) Folate gene polymorphisms and the risk of Down syndrome pregnancies in young Italian women. Am J Med Genet A 140:1083-91.

09. Biselli JM, Brumati D, Frigeri VF, Zampieri BL, Goloni-Bertollo EM, Pavarino-Bertelli EC (2008) A80G polymorphism of reduced folate carrier 1 (RFC1) and C776G polymorphism of transcobalamin 2 (TC2) genes in Down's syndrome etiology. Sao Paulo Med J 126:329-32. - a

10. Biselli JM, Goloni-Bertollo EM, Zampieri BL, Haddad R, Eberlin MN, Pavarino-Bertelli EC (2008) Genetic polymorphisms involved in folate metabolism and elevated plasma concentrations of homocysteine: maternal risk factors for Down syndrome in Brazil. Genet Mol Res 7:33-42. – b

11. Pozzi E, Vergani P, Dalprà L, Combi R, Silvestri D, Crosti F et al (2009) Maternal polymorphisms for methyltetrahydrofolate reductase and methionine synthetase reductase and risk of children with Down syndrome. Am J Obstet Gynecol 200:636.e1-6.

12. Brandalize AP, Bandinelli E, dos Santos PA, Roisenberg I, Schüler-Faccini L (2009) Evaluation of C677T and A1298C polymorphisms of the MTHFR gene as

maternal risk factors for Down syndrome and congenital heart defects. Am J Med Genet A 149:2080-7.

13. Mendes CC, Biselli JM, Zampieri BL, Goloni-Bertollo EM, Eberlin MN, Haddad R et al (2010) 19-base pair deletion polymorphism of the dihydrofolate reductase (DHFR) gene: maternal risk of Down syndrome and folate metabolism. Sao Paulo Med J 128:215-8.

14. Finkelstein JD, Martin JJ (2000) Homocysteine. Int J Biochem Cell Biol 32:385-9.

15. Pogribny IP, James SJ, Jernigan S, Pogribna M (2004) Genomic hypomethylation is specific for preneoplastic liver in folate/methyl deficient rats and does not occur in non-target tissues. Mutat Res 548:53-9.

16. James SJ, Pogribny IP, Pogribna M, Miller BJ, Jernigan S, Melnyk S (2003) Mechanisms of DNA damage, DNA hypomethylation, and tumor progression in the folate/methyl-deficient rat model of hepatocarcinogenesis. J Nutr 133:3740S-3747S.

17. Pogribny IP, Basnakian AG, Miller BJ, Lopatina NG, Poirier LA, James SJ (1995) Breaks in genomic DNA and within the p53 gene are associated with hypomethylation in livers of folate/methyl-deficient rats. Cancer Res 55:1894-901.

18. Wainfan E, Poirier LA (1992). Methyl groups in carcinogenesis: effects on DNA methylation and gene expression. Cancer Res 52:2071s-2077s.

19. Parry EM, Parry JM, Corso C, Doherty A, Haddad F, Hermine TF et al (2002) Detection and characterization of mechanisms of action of aneugenic chemicals. Mutagenesis 17:509-21.

20. Garrow TA, Brenner AA, Whitehead VM, Chen XN, Duncan RG, Korenberg JR et al (1993). Cloning of human cDNAs encoding mitochondrial and cytosolic serine hydroxymethyltransferases and chromosomal localization. J Biol Chem 268:11910-6.

21. Fowler B. The folate cycle and disease in humans. Kidney Int Suppl. 2001;78:S221-9.

22. Blount BC, Mack MM, Wehr CM, MacGregor JT, Hiatt RA, Wang G et al (1997) Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. Proc Natl Acad Sci U S A 94:3290-5.

23. Bagley PJ, Selhub J (1998) A common mutation in the methylenetetrahydrofolate reductase gene is associated with an accumulation of formylated tetrahydrofolates in red blood cells. Proc Natl Acad Sci U S A.; 95:13217-20.

24. van den Donk M, Pellis L, Crott JW, van Engeland M, Friederich P, Nagengast FM et al (2007) Folic acid and vitamin B-12 supplementation does not favorably influence uracil incorporation and promoter methylation in rectal mucosa DNA of subjects with previous colorectal adenomas. J Nutr 137:2114-20.

25. Niclot S, Pruvot Q, Besson C, Savoy D, Macintyre E, Salles G (2006) et al Implication of the folate-methionine metabolism pathways in susceptibility to follicular lymphomas. Blood 108:278-85.

26. Lightfoot TJ, Skibola CF, Willett EV, Skibola DR, Allan JM, Coppede F et al (2005) Risk of non-Hodgkin lymphoma associated with polymorphisms in folate-metabolizing genes. Cancer Epidemiol Biomarkers Prev 14:2999-3003.

27. Skibola CF, Smith MT, Hubbard A, Shane B, Roberts AC, Law GR et al (2002) Polymorphisms in the thymidylate synthase and serine hydroxymethyltransferase genes and risk of adult acute lymphocytic leukemia.Blood99:3786-91.

28. Whitlock JA (2006) Down syndrome and acute lymphoblastic leukaemia. Br J Haematol 135:595-602.

29. Lightfoot TJ, Johnston WT, Painter D, Simpson J, Roman E, Skibola CF et al (2010) Genetic variation in the folate metabolic pathway and risk of childhood leukemia. Blood 115:3923-9.

30. Relton CL, Wilding CS, Pearce MS, Laffling AJ, Jonas PA, Lynch SA et al (2004) Gene-gene interaction in folate-related genes and risk of neural tube defects in a UK population. J Med Genet 41:256-60.

31. Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 11;16:1215.

32. Haddad R, Mendes MA, Hoehr NF, Eberlin MN (2001) Amino acid quantitation in aqueous matrices via trap and release membrane introduction mass spectrometry: homocysteine in human plasma. Analyst 126:1212 –1215.

33. de Andrade CR, Fukada SY, Olivon VC, de Godoy MA, Haddad R, Eberlin MN (2006) Alpha1D-adrenoceptor-induced relaxation on rat carotid artery is impaired during the endothelial dysfunction evoked in the early stages of hyperhomocysteinemia. Eur J Pharmacol 543:83-91.

34. Carvalho VM, Kok F (2008) Determination of serum methylmalonic acid by alkylative extraction and liquid chromatography coupled to tandem mass spectrometry. Anal Biochem 381:67-73.

35. Herrmann W, Obeid R (2008) Causes and early diagnosis of vitamin B12 deficiency. Dtsch Arztebl Int 105:680-5.

36. Herrmann W, Schorr H, Purschwitz K, Rassoul F, Richter V (2001) Total homocysteine, vitamin B(12), and total antioxidant status in vegetarians Clin Chem 47:1094-101.

37. Hvas AM, Nexo E (2006) Diagnosis and treatment of vitamin B12 deficiency--an update. Haematologica 91:1506-12.

38. Ji W, Hernandez R, Zhang XY, Qu GZ, Frady A, Varela M et al (1997) DNA demethylation and pericentromeric rearrangements of chromosome 1. Mutat Res 379:33-41.

39. Jeanpierre M, Turleau C, Aurias A, Prieur M, Ledeist F, Fischer A et al (1993) An embryonic-like methylation pattern of classical satellite DNA is observed in ICF syndrome. Hum Mol Genet 2:731-5.

40. Jyothy A, Kumar KS, Mallikarjuna GN, Babu Rao V, Uma Devi B, Sujatha M et al (2001) Parental age and the origin of extra chromosome 21 in Down syndrome. J Hum Genet 46:347-50.

41. Beiguelman B, Krieger H, Da Silva LM (1996) Maternal age and Down syndrome in Southeastern Brazil. Rev. Bras. Genet 19:637-40.

42. Gusmão FAF, Tavares EJ, Moreira LMA (2003) Idade materna e síndrome de Down no Nordeste do Brasil. Cadernos de Saúde Pública 19:973-8.

43. Rai AK, Singh S, Mehta S, Kumar A, Pandey LK, Raman R (2006) MTHFR C677T and A1298C polymorphisms are risk factors for Down's syndrome in Indian mothers. J Hum Genet 51:278-83.

44. Scala I, Granese B, Sellitto M, Salomè S, Sammartino A, Pepe A et al (2006) Analysis of seven maternal polymorphisms of genes involved in homocysteine/folate metabolism and risk of Down syndrome offspring. Genet Med 8:409-16.

45. Wang SS, Qiao FY, Feng L, Lv JJ (2008) Polymorphisms in genes involved in folate metabolism as maternal risk factors for Down syndrome in China. J Zhejiang Univ Sci B 9:93-9.

46. Meguid NA, Dardir AA, Khass M, Hossieny LE, Ezzat A, El Awady MK (2008) MTHFR genetic polymorphism as a risk factor in Egyptian mothers with Down syndrome children. Dis Markers 24:19-26.

47. Cyril C, Rai P, Chandra N, Gopinath PM, Satyamoorthy K (2009) MTHFR Gene variants C677T, A1298C and association with Down syndrome: A Case-control study from South India. Indian J Hum Genet 15:60-4.

48. Brandalize AP, Bandinelli E, Dos Santos PA, Schüler-Faccini L (2010) Maternal gene polymorphisms involved in folate metabolism as risk factors for Down syndrome offspring in Southern Brazil. Dis Markers 29:95-101.

49. Sadiq MF, Al-Refai EA, Al-Nasser A, Khassawneh M, Al-Batayneh Q (2011) Methylenetetrahydrofolate Reductase Polymorphisms C677T and A1298C as Maternal Risk Factors for Down Syndrome in Jordan. Genet Test Mol Biomarkers 15:51-7.

50. Martínez-Frías ML, Pérez B, Desviat LR, Castro M, Leal F, Rodríguez L et al (2006) Maternal polymorphisms 677C-T and 1298A-C of MTHFR, and 66A-G MTRR genes: is there any relationship between polymorphisms of the folate pathway, maternal homocysteine levels, and the risk for having a child with Down syndrome? Am J Med Genet A 140:987-97.

51. Fintelman-Rodrigues N, Corrêa JC, Santos JM, Pimentel MM, Santos-Rebouças CB (2009) Investigation of CBS, MTR, RFC-1 and TC polymorphisms as maternal risk factors for Down syndrome. Dis Markers 26:155-61.

52. Barkai G, Arbuzova S, Berkenstadt M, Heifetz S, Cuckle H (2003) Frequency of Down's syndrome and neural-tube defects in the same family. Lancet 361:1331-5.

53. Heil SG, Van der Put NM, Waas ET, den Heijer M, Trijbels FJ, Blom HJ (2001) Is mutated serine hydroxymethyltransferase (SHMT) involved in the etiology of neural tube defects? Mol Genet Metab 73:164-72.

54. Chen J, Kyte C, Valcin M, Chan W, Wetmur JG, Selhub J et al (2004) Polymorphisms in the one-carbon metabolic pathway, plasma folate levels and colorectal cancer in a prospective study. Int J Cancer 110:617-20.

Table 1. Genotype distribution of the *SHMT* C1420T between case (n = 105) and control group (185).

	Case n (%)	**Control n (%)**	χ^2	**P**
Genotypes				
CC	59 (56.2)	92 (49.7)		
CT	36 (34.3)	81 (43.8)	2.85[1]	0.24
TT	10 (9.5)	12 (6.5)		

[1]Pearson Chi-Square with two degrees of freedom.

Table 2. Mood median test for Hcy, folate and MMA concentrations versus genotypes by the three models of analysis.

	Homocysteine			Folate			Methylmalonic acid		
Polymorphism	**Median**	**Range (umol/L)**	**P-value**	**Median**	**Range (ng/mL)**	**P-value**	**Median**	**Range (umol/L)**	**P-value**
Case group									
3 genotypes									
CC	6.06	2.4 – 14.1		11.80	3.7 – 21.4		0.17	0.1 – 1.5	
CT	7.49	4.0 – 21.3	0.40	13.80	6.0 – 36.5	0.36	0.19	0.1 – 1.5	0.25
TT	5.68	3.1 – 8.6		12.45	10.0 – 20.6		0.18	0.1 – 0.4	
Dominant Model									
CC	6.06	2.4 – 14.1	0.41	11.80	3.7 – 21.4	0.18	0.17	0.1 – 1.5	0.1
CT or TT	6.81	3.1 – 21.3		13.50	6.0 – 36.5		0.18	0.1 - 1.5	
Recessive Model									
CC or CT	6.34	2.4 – 21.3	0.50	12.20	3.7 - 36.5	0.91	0.17	0.1 – 1.5	0.68
TT	5.68	3.1 – 8.6		12.45	10.0 – 20.6		0.18	0.1 – 0.4	
Control group									
3 genotypes									
CC	8.25	1.1 – 36.2		14.50	5.0 – 50.0		0.15	0.1 – 0.8	
CT	8.34	1.7 – 36.6	0.97	15.00	6.7 – 57.0	0.82	0.13	0.1 – 0.5	0.16
TT	8.39	4.8 – 12.8		13.85	7.7 – 74.0		0.17	0.1 – 0.3	
Dominant Model									
CC	8.25	1.1 – 36.2	0.82	14.50	5.0 – 50.0	0.94	0.15	0.1 – 0.8	0.88
CT or TT	8.34	1.7 – 36.6		14.70	6.7 – 74.0		0.14	0.1 – 0.5	
Recessive Model									
CC or CT	8.32	1.1 – 36.6	0.98	14.70	5.0 – 57.0	0.56	0.14	0.1 – 0.8	0.07
TT	8.39	4.8 – 12.8		13.85	7.7 – 74.0		0.17	0.1 – 0.3	

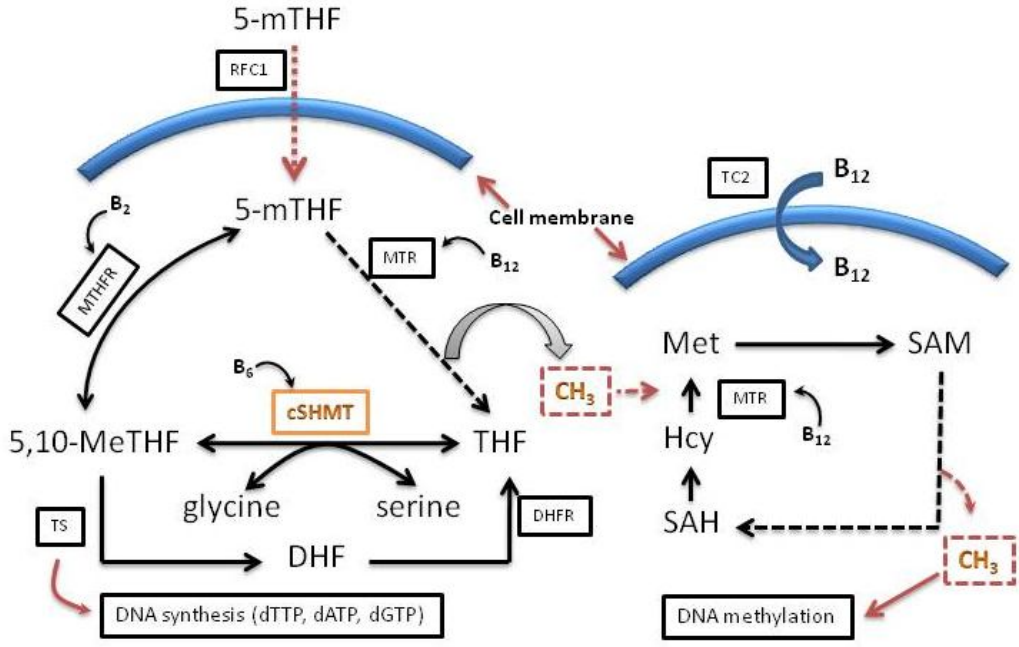

Figure 1. Simplified scheme of folate metabolism. MTHFR*:* methylenetetrahydrofolate reductase; MTR*:* methionine synthase; MTRR*:* methionine synthase reductase; cSHMT*:* cytosolic serine hydroxymethyltransferase; RFC1: reduced folate carrier 1; TC2: transcobalamin 2; TS: thymidylate synthase; DHFR: dihydrofolate reductase; 5,1-MeTHF: 5,10-methylenetetrahydrofolate; THF: tetrahydrofolate; 5mTHF: 5-methyltetrahydrofolate; DHF: dihydrofolate; Hcy: Homocysteine; Met: Metionine; *SAM: S*-adenosylmethionine; *SAH: S*-adenosylhomocysteine.

ARTIGO CIENTÍFICO 2

Synergistic effect of *DHFR* 19-bp deletion and *SHMT* C1420T polymorphisms on metabolites concentrations of folate pathway in individuals with Down syndrome

Cristiani Cortez Mendes[1], Aline Maria Zanchetta de Aquino Raimundo[1], Luciana Dutra Oliveira[1], Bruna Lancia Zampieri[1], Gustavo Henrique Marucci[1], Joice Matos Biselli[1], Eny Maria Goloni-Bertollo[1], Marcos Nogueira Eberlin[2], Renato Haddad[2], Maria Francesca Riccio[2], Hélio Vannucchi[3], Valdemir Melechco Carvalho[4], Érika Cristina Pavarino[1]

[1]Unidade de Pesquisa em Genética e Biologia Molecular, Departamento de Biologia Molecular, Faculdade de Medicina de São José do Rio Preto

Address: Avenida Brigadeiro Faria Lima, 5416

Vila São Pedro

CEP:15090-000; São José do Rio Preto, SP, Brazil

[2]Laboratório ThoMSon de Espectrometria de Massas, Instituto de Química, Universidade Estadual de Campinas

Address: Cidade Universitária Zeferino Vaz

CEP: 13083-970; Campinas, SP, Brazil

[3]Laboratório de Nutrição, Departamento de Clínica Médica, Faculdade de Medicina de Ribeirão Preto - USP

Address: Avenida Bandeirantes, 3900

Monte Alegre

CEP: 14049-900; Ribeirão Preto, SP, Brazil

[4]Fleury Medicina e Saúde

Address: Rua dos Otonis, 880, apto. 133

Vila Clementino

CEP: 04025-002; São Paulo, SP, Brazil

Concise title: Folate metabolism and Down syndrome.

Correspondence: Érika Cristina Pavarino

Av. Brigadeiro Faria Lima, 5416

CEP:15090-000; São José do Rio Preto, SP, Brasil

UPGEM, Bloco U6

Fax: +55-17-3201-5708. Tel: +55-17-3207-5904. E-mail: erika@famerp.br

Abstract

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 over-expression of *Cystathionine β-synthase* (*CβS*) gene, polymorphisms in genes involved in folate/homocysteine (Hcy) metabolism may also influence the concentrations of metabolites of this pathway. Thus, this study evaluated the influence of the *Dihydrofolate reductase* (*DHFR*) 19-base pair (bp) deletion and *Serine hydroxymethyltransferase* (*SHMT*) C1420T polymorphisms on serum folate and plasma Hcy and methylmalonic acid (MMA) concentrations in eighty-five individuals with DS. Genotyping of *DHFR* 19-bp deletion and *SHMT* C1420T polymorphisms were 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. The single polymorphisms of *DHFR* and *SHMT* genes showed no association with folate, Hcy and MMA concentrations. However, the analysis of combined genotypes showed that individuals with *DHFR* II / *SHMT* TT combined genotypes presented higher concentrations of Hcy ($P < 0.001$) in relation to the others genotypes. Moreover, *DHFR* DD / *SHMT* TT combined genotypes presented higher concentrations of folate ($P < 0.001$) and the *DHFR* II / *SHMT* CT genotypes presented lower concentrations of folate ($P = 0.01$). There was no association between MMA concentrations and genotypes. These results demonstrate a synergistic effect of polymorphisms of *DHFR* and *SHMT* genes on the modulation of folate/Hcy pathway in individuals with DS.

Key words: Down syndrome, genetic polymorphism, homocysteine, folate.

Introduction

Down syndrome (DS) results from the presence and expression of three copies of the genes located on chromosome 21 [1,2]. *Cystathionine β-synthase* (*CβS*) gene, located on chromosome 21, is responsible for the condensation of homocysteine (Hcy) and serine to cystathionine and is over-expressed in individuals with DS [2]. Increased concentrations of CβS enzyme results in lower concentrations of Hcy, methionine, S-adenosylhomocysteine, and S-adenosylmethionine [3-5], substrates of folate metabolism.

Studies have shown that, in addition to over-expression of *CβS* gene, polymorphisms in genes involved in folate/Hcy metabolism may also influence metabolites concentrations of this pathway [6-9]. A 19-base pair (bp) deletion polymorphism in intron-1 of the *Dihydrofolate reductase* (*DHFR*) gene, located on chromosome 5q11.2, has been identified [10] and Kalmbach et al. [11] demonstrated that this is a functional polymorphism. Study shows that 19-bp deletion polymorphism is associated with increased expression of *DHFR* gene, responsible for the conversion of dihydrofolate (DHF) in tetrahydrofolate (THF) [12], and changes on the folate/Hcy metabolism [11,13,14].

Other polymorphism, C1420T, which results in the substitution of the amino acid leucine by phenylalanine, was identified in *Serine hydroxymethyltransferase* (*SHMT*) gene, located on chromosome 17p11.2 [15]. This gene encodes the enzyme that catalyzes the reversible conversion of serine and THF to glycine and 5,10-methyleneTHF [16] and Fu et al. [17] showed that the *SHMT* C1420T polymorphism may compromise the formation of the SHMT enzyme.

Both, *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 [11,13-15,18,19]. Thus, the aim of the present study was to evaluate the influence of *DHFR* 19-bp deletion and *SHMT* C1420T polymorphisms on serum folate and plasma Hcy and methylmalonic acid (MMA) concentrations in individuals with DS.

Subjects 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 family.

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. [20] 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 *DHFR* gene was detected using primer sequences described by Dulucq et al. [21] and *SHMT* C1420T was detected using TaqMan® probes and primer sequences described by Skibola et al. [22]. 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 [23-25].

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

Statistical analysis

Hardy-Weinberg equilibrium was tested by chi-square test, using the BioEstat program (version 5.0). Folate and Hcy data presented normal distribution after

logarithmic transformation and were analyzed as mean values in the logarithmic scale. MMA data was not normal, even after Log-transformation, and was analyzed median values in the natural scale. To evaluate the association of *DHFR* 19-bp deletion and *SHMT* C1420T polymorphisms with biochemical parameters, the t-test was used for Hcy and folate data and the Mood´s median test was used for MMA data. The Mood´s median was also used to evaluate the distribution of age in relation to combined genotypes. For multiple logistic regression, the folate concentrations were categorized considering values below or equal/above the percentile 25 (reference: > percentile 25) and Hcy and MMA data were categorized considering values above or equal/below the percentile 75 (reference: < percentile 75). In addition, the genetics data were included in the model considering number of uncommon alleles (0-4 polymorphic alleles). The correlation analysis among folate, Hcy and MMA concentrations and age was performed using the Pearson correlation test. 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.

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 Hardy-Weinberg equilibrium ($\chi 2 = 2.079$; $P = 0.15$; $\chi 2 = 0.004$; $P = 0.95$, respectively). The mean concentrations of folate and Hcy observed in the individuals were 19.69 ± 11.87 ng/mL and 5.83 ± 3.30 µmol/L, respectively, and the median MMA concentration was 0,25 µmol/L (range: 0.09-4.77 µmol/L).

We evaluated the influence of *DHFR* 19-bp deletion and *SHMT* C1420T polymorphisms on folate, Hcy and MMA concentrations in individuals with DS, both alone and in combination with other. The analysis showed no association between genotypes and folate, Hcy and MMA concentrations. However, when we proceed the analysis of combined genotypes, the results showed that individuals with *DHFR* II / *SHMT* TT genotypes presented higher concentrations of Hcy ($P < 0.001$) in relation to the others combined genotypes. In another way, individuals with *DHFR* DD / *SHMT* TT combined genotypes presented higher concentrations of folate ($P < 0.001$) and the *DHFR* II / *SHMT* CT genotypes presented lower concentrations of folate ($P = 0.01$) in relation to the others combined genotypes. The distribution of MMA concentrations and age showed no significant difference between the genotypes. The distribution of the Hcy, folate and MMA concentrations and age according to the combined genotypes are presented in Table 2.

In the logistic regression analysis, was observed no relation between genetics and biochemical parameters. The correlation analysis showed that folate concentrations were negatively correlated with age ($r = -0.223$; $P = 0.05$) and a negative correlation was also observed between MMA concentrations and age ($r = -0.257$; $P = 0.02$). In addition, Hcy concentrations were positively correlated with MMA concentrations ($r = 0.252$; $P = 0.03$).

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 [3]. The presence of three copies of the *CßS*

gene, located on chromosome 21, and of the polymorphisms *Methylenetetrahydrofolate reductase* (*MTHFR*) C677T and *Methionine synthase* (*MTR*) A2756G, involved in the folate/Hcy metabolism, have been associated with variations on the concentrations of metabolites of this pathway [3-9].

Guéant et al. [7] observed that individuals with DS that present *MTHFR* 677T allele and elevated Hcy concentration had low intelligence quotient and Licastro et al. [8] found that the *MTHFR* 677TT genotype increases the concentrations of Hcy in these individuals. However, Fillon-Emery et al. [6] 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 in plasma Hcy concentrations in individuals with DS [9].

To the best of our knowledge, there is no published study that has evaluated the influence of the *DHFR* 19-bp deletion and *SHMT* C1420T polymorphisms on the metabolites concentrations of folate/Hcy pathway in individuals with DS. In the present study, the single *DHFR* 19-bp deletion and *SHMT* C1420T polymorphisms were not associated with the folate, Hcy and MMA concentrations. However, considering that some polymorphisms may interact to produce a synergistic effect, the contribution of the combined genotypes to folate, Hcy and MMA concentrations was evaluated and, although the sample is relatively small, the results show that *DHFR* II / *SHMT* CT combined genotypes were associated with lower folate concentrations and *DHFR* DD / *SHMT* TT genotypes were associated with higher concentration of folate. Moreover, an association between the *DHFR* II / *SHMT* TT combined genotypes and higher Hcy concentration was observed. The sample studied is represented by few individuals with

DHFR II / *SHMT* TT combined genotypes and because of this, further studies are needed to confirm this association.

DHFR is an important folate-metabolizing enzyme responsible for reduction of folic acid into THF [14]. 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 [11,17,18]. Gellekink et al. [13] reported that the *DHFR* DD genotype is associated with lower concentration of plasma Hcy in Caucasian individuals, but no association between this genotype and concentrations of serum and erythrocyte folate was observed. Other 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 [14]. Kalmbach et al. [11] also observed no association between genotypes and plasma Hcy or plasma total folate in young adults, however *DHFR* DD genotype was associated with lower concentration of erythrocyte folate compared to *DHFR* ID and II genotypes.

SHMT enzyme plays a pivotal role in folate/Hcy metabolism by carrying out the reversible conversion of serine and glycine with THF and 5,10-methyleneTHF [16]. Heil et al. [15] identified the *SHMT* C1420T polymorphism and reported that individuals with neural tube defects and *SHMT* CC genotype had decreased concentrations of erythrocyte and plasma folate and increased Hcy concentration. In study involving men with cardiovascular disease, the *SHMT* TT genotype was associated with lower Hcy concentration [19]; yet another study found no significant association between *SHMT* C1420T and plasma folate and Hcy concentrations in colorectal cancer [18].

We also observed a positive correlation between Hcy and MMA concentrations and this association is in accord with results of earlier investigations [26,27] and could be explained by the fact that the vitamin B12 deficiency increases the Hcy concentrations [28,29]. The decrease of the MMA concentrations with age is in contrast with previous studies in healthy elderly people [26,27,30,31] and, although Wolters et al. [27] have observed no association between folate concentrations and age, ours results show a negatively correlation between these parameters.

Our study suggests that there is a synergistic effect of *DHFR* and *SHMT* genes polymorphisms on the modulation of the concentrations of folate/Hcy pathway metabolites in individuals with DS. However, further studies are needed to confirm this effect on the folate/Hcy metabolism.

Acknowledgments

This research was financially supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq – grants n° 302157/2008-5; 119404/2009-5; 119419/2009-2). The authors are grateful to the participants in this study, to the Ding-Down workgroup (multidisciplinary group of health professionals – Faculdade de Medicina de São José do Rio Preto, FAMERP) and to the FAMERP/Fundação Faculdade Regional de Medicina (FUNFARME) for their collaboration in this work.

References

1. Shin JH, Weitzdoerfer R, Fountoulakis M, Lubec G (2004) Expression of cystathionine β-synthase, pyridoxal kinase, and ES1 protein homolog (mitochondrial precursor) in fetal Down syndrome brain. Neurochem Int 45:73–79.

2. Ishinohe A, Kanaumi T, Takashima S, Enokido Y, Nagai Y, Kimura H (2005) Cystathionine b-synthase is enriched in the brains of Down´s patients. Biochem Biophys Res Commun 338:1547–1550.

3. Pogribna M, Melnyk S, Pogribny I, Chango A, Yi P, James SJ (2001) Homocysteine etabolism in children with Down syndrome: in vitro modulation. Am J Hum Genet 69:88-95.

4. Coppus AW, Fekkes D, Verhoeven WMA, Tuinier S, Egger JIM, van Duijn CM (2007) Plasma amino acids and neopterin in healthy persons with Down's syndrome. J Neural Transm 114:1041–1045.

5. Meguid NA, Dardir AA, El-Sayed EM, Ahmed HH, Hashish AF, Ezzat A (2010) Homocysteine and oxidative stress in Egyptian children with Down syndrome. Clinic Biochem 43:963–967.

6. Fillon-Emery N, Chango A, Mircher C, Barbe F, Blehaut H, Herbeth B, et al. (2004) Homocysteine concentrations in adults with trisomy 21: effect of B vitamins and genetic polymorphisms. Am J Clin Nutr 80:1551-1557.

7. Guéant JL, Anello G, Bosco P, Gueant-Rodriguez RM, Romano A, Barone C, et al. (2005) Homocysteine and related genetic polymorphisms in Down's syndrome IQ. J Neurol Neurosurg Psychiatry 76:706-709.

8. Licastro F, Marocchi A, Penco S, Porcellini E, Lio D, Dogliotti G, et al. (2006) Does Down's syndrome support the homocysteine theory of atherogenesis? Experience in elderly subjects with trisomy 21. Arch Gerontol Geriatr 43:381-387.

9. Biselli JM, Goloni-Bertollo EM, Haddad R, Eberlin MN, Pavarino-Bertelli EC (2008) The MTR A2756G polymorphism is associated with na increase of plasma homocysteine concentration in Brazilian individuals with Down syndrome. Braz J Med Biol Res 41:34-40.

10. Johnson WG, Stenroos ES, Spychala JR, Chatkupt S, Ming SX, Buyske S (2004) New 19 bp deletion polymorphism in intron-1 of Dihydrofolate reductase (DHFR): a risk factor for spina bifida acting in mothers during pregnancy? Am J Med Genet 124A(4):339-345.

11. Kalmbach RD, Choumenkovitch SF, Troen AP, Jacques PF, D'Agostino R, Selhub J (2008) A 19-base pair deletion polymorphism in Dihydrofolate reductase is associated with increased unmetabolized folic acid in plasma and decreased red blood cell folate. J Nutr 138(12):2323-2327.

12. Xu X, Gammon MD, Wetmur JG, Rao M, Gaudet MM, Teitelbaum SL, et al. (2007) A functional 19-base pair deletion polymorphism of dihydrofolate reductase (DHFR) and risk of breast cancer in multivitamin users. Am J Clin Nutr 85:1098 –1102.

13. Gellekink H, Blom HJ, van der Linden IJ, den Heijer M (2007) Molecular genetic analysis of the human Dihydrofolate reductase gene: relation with plasma total homocysteine, serum and red blood cell folate levels. Eur J Hum Genet 15(1):103-109.

14. Stanisiawska-Sachadyn A, Brown KS, Mitchell LE, Woodside JV, Young IS, Scott JM, et al. (2008) An insertion/deletion polymorphism of the Dihydrofolate reductase

(DHFR) gene is associated with serum and red blood cell folate concentrations in women. Hum Genet 123(3):289-295.

15. Heil SG, Van der Put NMJ, Waas ET, den Heijer M, Trijbels FJM, Blom HJ (2001) Is mutated Serine hydroxymethyltransferase (SHMT) involved in the etiology of neural tube defects? Mol Genet Metab 73:164-172.

16. Fowler B (2001) The folate cycle and disease in humans. Kidney Int 59(78):221-229.

17. Fu TF, Hunt S, Schirch V, Safo MK, Chen BH (2005) Properties of human and rabbit cytosolic serine hydroxymethyltransferase are changed by single nucleotide polymorphic mutations. Arch Biochem Biophys 442:92-101.

18. Chen J, Kyte C, Valcin M, Chan W, Wetmur JG, Selhub J, et al. (2004) Int J Cancer 110:617–620.

19. Lim U, Peng K, Shane B, Stover PJ, Litonjua AA, Weiss ST, et al. (2005) Polymorphisms in cytoplasmic Serine hydroxymethyltransferase and Methylenetetrahydrofolate reductase affect the risk of cardiovascular disease in men. J Nutr 135(8):1989-1994.

20. Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16(3):1215.

21. Dulucq S, St-Onge G, Gagné V, Ansari M, Sinnett D, Labuda D, et al. (2008) DNA variants in the Dihydrofolate reductase gene and outcome in childhood ALL. Blood 111(7):3692-3700.

22. Skibola CF, Forrest MS, Coppedé F, Agana L, Hubbard A, Smith MT, et al. (2004) Polymorphisms and haplotypes in folate-metabolizing genes and risk of non-Hodgkin lymphoma. Blood 104(7):2155-2162.

23. Haddad R, Mendes MA, Höehr NF, Eberlin MN (2001) Amino acid quantitation in aqueous matrices via trap and release membrane introduction mass spectrometry: homocysteine in human plasma. Analyst 126(8):1212-1215.

24. de Andrade CR, Fukada SY, Olivon VC, de Godoy MA, Haddad R, Eberlin MN, et al. (2006) Alpha1D-adrenoceptor-induced relaxation on rat carotid artery is impared during the endothelial dysfunction evoked in the early stages of hyperhomocysteinemia. Eur J Pharmacol 543(1-3):83-91.

25. Carvalho VM, Kok F (2008) Determination of serum methylmalonic acid by alkylative extraction and liquid chromatography coupled to tandem mass spectrometry. Anal Biochem 381(1):67-73.

26. Bates CJ, Schneede J, Mishra1 G, Prentice A, Mansoor MA (2003) Relationship between methylmalonic acid, homocysteine, vitamin B12 intake and status and socio-economic indices, in a subset of participants in the British National Diet and Nutrition Survey of people aged 65 y and over. Eur J Clin Nutr 57:349–357.

27. Wolters M, Hermann S, Hahn A (2003) B vitamin status and concentrations of homocysteine and

methylmalonic acid in elderly German women. Am J Clin Nutr 78:765–772.

28. Selhub J, Morris MS, Jacques PF (2007) In vitamin B12 deficiency, higher serum folate is associated with increased total homocysteine and methylmalonic acid concentrations. PNAS 104(50):19995–20000.

29. Remacha AF, Souto JC, Piñana JL, Sardà MP, Queraltó JM, Martí-Fabregas J, et al. (2011) Vitamin B12 deficiency, hyperhomocysteinemia and thrombosis: a case and control study. Int J Hematol 93(4):458-464.

30. Loikas S, Koskinen P, Irjala K, Löppönen M, Isoaho R, Kivel SL, et al. (2007) Vitamin B12 deficiency in the aged: a population-based study. Age Ageing 36:177–183.

31. Schneede J, Ueland PM, Kjaerstad SI (2003) Routine determination of serum methylmalonic acid and plasma total homocysteine in Norway. Scand J Clin Lab Invest 63(5):355-367.

Table 1. Allele frequencies of 19-base pair (bp) deletion in intron-1 of *Dihydrofolate reductase* (*DHFR*) gene and *Serine hydroxymethyltransferase* (*SHMT*) C1420T polymorphisms in individuals with Down syndrome.

	Allele frequencies	P-value*
DHFR		
I	0.52	0.15
D	0.48	
SHMT		
C	0.68	0.95
T	0.32	

* Chi-square test.

Table 2. Distribution of serum folate and plasma homocysteine (Hcy) and methylmalonic acid (MMA) concentrations according to combined genotypes of the 19-base pair (bp) deletion in intron-1 of *Dihydrofolate reductase* (*DHFR*) gene and *Serine hydroxymethyltransferase* (*SHMT*) C1420T polymorphisms in individuals with Down syndrome.

Genotypes *DHFR* / *SHMT*	Folate (ng/mL)	Hcy (µmol/L)	MMA (µmol/L)
ID / CT	24.12 ± 16.87	5.52 ± 2.63	0.32 (0.14-4.26)
ID / TT	18.00 ± 11.69	6.63 ± 3.78	0.26 (0.13-0.68)
ID / CC	18.18 ± 8.89	6.74 ± 4.28	0.23 (0.10-3.21)
II / CT	11.88 ± 5.20*	5.06 ± 2.37	0.22 (0.15-1.56)
II / TT	13.50 ± 1.70	8.14 ± 0.25*	2.50 (0.23-4.77)
II / CC	24.95 ± 11.74	4.36 ± 1.54	0.25 (0.09-0.32)
DD / CT	16.02 ± 4.93	5.33 ± 2.99	0.23 (0.14-1.21)
DD / TT	44.25 ± 1.77*	2.48 ± 1.72	0.12 (0.11-0.13)
DD / CC	19.46 ± 12.70	6.95 ± 4.45	0.30 (0.12-2.26)

Folate and Hcy data are reported as means ± SD; MMA data are reported as median and range.

* Statistically significant.

3. CONCLUSÕES

3. CONCLUSÕES

1. Os genótipos CC e CT do polimorfismo *SHMT* C1420T conferem um efeito materno protetor para SD e este polimorfismo não está associado com variações nas concentrações de folato, Hcy e MMA.

2. Os polimorfismos del 19pb *DHFR* e C1420T *SHMT* apresentam um efeito sinérgico na modulação das concentrações de folato e Hcy em indivíduos com SD.

4. REFERÊNCIAS BIBLIOGRÁFICAS

4. Referências Bibliográficas

1. Pozzi E, Vergani P, Dalprà L, Combi R, Silvestri D, Crosti F, et al. Maternal polymorphisms for methyltetrahydrofolate reductase and methionine synthetase reductase and risk of children with Down syndrome. Am J Obstet Gynecol. 2009 ;200:636.e1-6.

2. Antonarakis SE, Petersen MB, McInnis MG, Adelsberger PA, Schinzel AA, Binkert F, et al. The meiotic stage of nondisjunction in trisomy 21: determination by using DNA polymorphisms. Am J Hum Genet. 1992; 50:544-50.

3. Coppedè F. The complex relationship between folate/homocysteine metabolism and risk of Down syndrome. Mutat Res. 2009; 682:54-70

4. Jones KL. Smith's recognizable patterns of human malformation. Philadelphia, Elsevier Saunders, 6th edition, 2006.

5. IBGE Instituto Brasileiro de Geografia e Estatística. Ministério do Planejamento, Orçamento e gestão (2000). Censo Demográfico 2000: educação (pp.1-233). Rio de Janeiro: IBGE

6. Allen EG, Freeman SB, Druschel C, Hobbs CA, O'Leary LA, Romitti PA, et al. Maternal age and risk for trisomy 21 assessed by the origin of chromosome nondisjunction: a report from the Atlanta and National Down Syndrome Projects. Hum Genet. 2009;125:41-52.

7. Hassold T, Hall H, Hunt P. The origin of human aneuploidy: where we have been, where we are going. Human Molecular Genetics 2007;16(2):203-8.

8.Migliore L, Migheli F, Coppedè F. Susceptibility to aneuploidy in young mothers of Down syndrome children. ScientificWorldJournal 2009;9:1052-60.

9. Chiang T, Duncan FE, Schindler K, Schultz RM, Lampson MA. Evidence that weakened centromere cohesion is a leading cause of age-related aneuploidy in oocytes. Curr Biol 2010;20(17):1522-8.

10. James SJ, Pogribna M, Pogribny IP, Melnyk S, Hine RJ, Gibson JB, et al. Abnormal folate metabolism and mutation in the methylenetetrahydrofolate reductase gene may be maternal risk factors for Down syndrome. Am J Clin Nutr. 1999; 70: 495-501.

11. da Silva LR, Vergani N, Galdieri Lde C, Ribeiro Porto MP, Longhitano SB, Brunoni D, et al. Relationship between polymorphisms in genes involved in homocysteine metabolism and maternal risk for Down syndrome in Brazil. Am J Med Genet A. 2005; 135:263-7.

12. Coppedè F, Marini G, Bargagna S, Stuppia L, Minichilli F, Fontana I, et al. Folate gene polymorphisms and the risk of Down syndrome pregnancies in young Italian women. Am J Med Genet A. 2006; 140:1083-91.

13. Biselli JM, Goloni-Bertollo EM, Zampieri BL, Haddad R, Eberlin MN, Pavarino-Bertelli EC. Genetic polymorphisms involved in folate metabolism and elevated plasma concentrations of homocysteine: maternal risk factors for Down syndrome in Brazil. Genet Mol Res. 2008; 7:33-42._a

14. Brandalize AP, Bandinelli E, dos Santos PA, Roisenberg I, Schüler-Faccini L. Evaluation of C677T and A1298C polymorphisms of the MTHFR gene as maternal risk factors for Down syndrome and congenital heart defects. Am J Med Genet A. 2009 ;149:2080-7.

15. Mendes CC, Biselli JM, Zampieri BL, Goloni-Bertollo EM, Eberlin MN, Haddad R et al. 19-base pair deletion polymorphism of the dihydrofolate reductase (DHFR) gene:

maternal risk of Down syndrome and folate metabolism. Sao Paulo Med J 2010; 128:215-8.

16. Marucci GH, Zampieri BL, Biselli JM, Valentin S, Bertollo EM, Eberlin MN, Haddad R, Riccio MF, Vannucchi H, Carvalho VM, Pavarino EC. Polymorphism C1420T of Serine hydroxymethyltransferase gene on maternal risk for Down syndrome. Mol Biol Rep. 2011 Jun 18. [Epub ahead of print]

17. Finkelstein JD, Martin JJ. Homocysteine. Int J Biochem Cell Biol. 2000;32:385-9.

18. Hobbs CA, Sherman SL, Yi P, Hopkins SE, Torfs CP, Hine RJ, et al. Polymorphisms in genes involved in folate metabolism as maternal risk factors for Down syndrome. Am J Hum Genet. 2000;67(3):623-30.

19. Wang W, Xie W, Wang X. The relationship between polymorphism of gene involved in folate metabolism, homocysteine level and risk of Down syndrome. Zhonghua Yi Xue Yi Chuan Xue Za Zhi. 2007;24:533-7.

20. Meguid NA, Dardir AA, Khass M, Hossieny LE, Ezzat A, El Awady MK. Awady, MTHFR genetic polymorphism as a risk factor in Egyptian mothers with Down syndrome children, Dis. Markers. 2008;24:19–26.

21. Wang SS, Qiao FY, Feng L, Lv JJ. Polymorphisms in genes involved in folate metabolism as maternal risk factors for Down syndrome in China. J Zhejiang Univ Sci B. 2008;9(2):93-9.

22. Sadiq MF, Al-Refai EA, Al-Nasser A, Khassawneh M, Al-Batayneh Q. Methylene-tetrahydrofolate Reductase Polymorphisms C677T and A1298C as Maternal Risk Factors for Down Syndrome in Jordan. Genet Test Mol Biomarkers. 2011; 15:51-7.

23. Eskes TK. Abnormal folate metabolism in mothers with Down syndrome offspring: review of the literature. Eur J Obstet Gynecol Reprod Biol. 2006 Feb 1;124(2):130-3.

24. Yamada K, Gravel RA, Toraya T, Matthews RG. Human methionine synthase reductase is a molecular chaperone for human methionine synthase. Proc Natl Acad Sci U S A. 2006;103(25):9476-81.

25. O'Leary VB, Parle-McDermott A, Molloy AM, Kirke PN, Johnson Z, Conley M, et at. MTRR and MTHFR polymorphism: link to Down syndrome? Am, J. Med. Genet. 2002; 107: 151–155.

26. Bosco P, Guéant-Rodriguez RM, Anello G, Barone C, Namour F, Caraci F, Romano A, Romano C, Guéant JL. Methionine synthase (MTR) 2756 (A > G) polymorphism, double heterozygosity methionine synthase 2756 AG/methionine synthase reductase (MTRR) 66 AG, and elevated homocysteinemia are three risk factors for having a child with Down syndrome. Am J Med Genet A. 2003;121A(3):219-24.

27. Martínez-Frías ML, Pérez B, Desviat LR, Castro M, Leal F, Rodríguez L et al. Maternal polymorphisms 677C-T and 1298A-C of MTHFR, and 66A-G MTRR genes: is there any relationship between polymorphisms of the folate pathway, maternal homocysteine levels, and the risk for having a child with Down syndrome? Am J Med Genet A. 2006; 140:987-97.

28. Wang SS, Qiao FY, Feng L, Lv JJ. Polymorphisms in genes involved in folate metabolism as maternal risk factors for Down syndrome in China, J. Zhejiang Univ. Sci. 2008; B9:93–99.

29. Relton CL, Wilding CS, Pearce MS, Laffling AJ, Jonas PA, Lynch SA et al. Gene-gene interaction in folate-related genes and risk of neural tube defects in a UK population. J Med Genet. 2004; 41:256-60.

30. Guéant JL, Guéant-Rodriguez RM, Anello G, Bosco P, Brunaud L, Romano C, et al. Genetic determinants of folate and vitamin B12 metabolism: a common pathway in neural tube defect and Down syndrome? Clin Chem Lab Med. 2003;41(11):1473-7.

31. Barkai G, Arbuzova S, Berkenstadt M, Heifetz S, Cuckle H. Frequency of Down's syndrome and neural-tube defects in the same family. Lancet. 2003; 361:1331-5.

32. Grillo LB, Acácio GL, Barini R, Pinto W Jr, Bertuzzo CS. Mutations in the methylene-tetrahydrofolate reductase gene and Down syndrome. Cad. Cad Saude Publica. 2002;18(6):1795-7.

33. Stuppia L, Gatta V, Gaspari AR, Antonucci I, Morizio E, Calabrese G, Palka G. C677T mutation in the 5.10-MTHFR gene and risk of Down syndrome in Italy. Eur J Hum Genet. 2002 Jun;10(6):388-90.

34. Takamura N, Kondoh T, Ohgi S, Arisawa K, Mine M, Yamashita S, Aoyagi K. Abnormal folic acid-homocysteine metabolism as maternal risk factors for Down syndrome in Japan. Eur J Nutr. 2004;43(5):285-7.

35. Boduroğlu K, Alanay Y, Koldan B, Tunçbilek E. Methylenetetrahydrofolate reductase enzyme polymorphisms as maternal risk for Down syndrome among

36. Chango A, Fillon-Emery N, Mircher C, Bléhaut H, Lambert D, Herbeth B, James SJ, Réthoré MO, Nicolas JP. No association between common polymorphisms in genes of folate and homocysteine metabolism and the risk of Down's syndrome among French mothers. Br J Nutr. 2005;94(2):166-9.

37. Acacio GL, Barini R, Bertuzzo CS, Couto EC, Annichino-Bizzacchi JM, W.P. Methylenetetrahydrofolate reductase gene polymorphisms and their association with trisomy 21. Prenat. Diagn. 2005; 25:1196–1199.

38. Rai AK, Singh S, Mehta S, Kumar A, Pandey LK, Raman R. MTHFR C677T and A1298C polymorphisms are risk factors for Down's syndrome in Indian mothers. J. Hum. Genet. 2006; 51 278–283.

39. Scala I, Granese B, Sellitto M, Salomè S, Sammartino A, Pepe A et al. Analysis of seven maternal polymorphisms of genes involved in homocysteine/folate metabolism and risk of Down syndrome offspring. Genet Med. 2006; 8:409-16.

40. Wang W, Xie W, Wang X. The relationship between polymorphism of gene involved in folate metabolism, homocysteine level and risk of Down syndrome. Zhonghua Yi Xue Yi Chuan Xue Za Zhi. 2007;24:533-7.

41. Kohli U, Arora S, Kabra M, Ramakrishnan L, Gulati S, Pandey RM. Prevalence of MTHFR C677T polymorphism in north Indian mothers having babies with Trisomy 21 Down syndrome. Downs Syndr Res Pract. 2008;12:133–137.

42. Santos-Rebouças CB, Corrêa JC, Bonomo A, Fintelman-Rodrigues N, Moura KC, Rodrigues CS, et al. The impact of folate pathway polymorphisms combined to nutritional deficiency as a maternal predisposition factor for Down syndrome. Dis Markers. 2008:25:149–157.

43. Biselli JM, Goloni-Bertollo EM, Haddad R, Eberlin MN, Pavarino-Bertelli EC. The MTR A2756G polymorphism is associated with na increase of plasma homocysteine concentration in Brazilian individuals with Down syndrome. Braz J Med Biol Res. 2008;41:34-40 - b.

44. Coppedè F, Migheli F, Bargagna S, Siciliano G, Antonucci I, Stuppia L et al. Association of maternal polymorphisms in folate metabolizing genes with chromosome damage and risk of Down syndrome offspring. Neurosci Lett. 2009;449:15-9.

45. Fintelman-Rodrigues N, Corrêa JC, Santos JM, Pimentel MM, Santos-Rebouças CB. Investigation of CBS, MTR, RFC-1 and TC polymorphisms as maternal risk factors for Down syndrome. Dis Markers. 2009; 26:155-61.

46. Kokotas H, Grigoriadou M, Mikkelsen M, Giannoulia-Karantana A, Petersen MB. Investigating the impact of the Down syndrome related common MTHFR 677C>T polymorphism in the Danish population. Dis Markers. 2009;27(6):279-85.

47. Brandalize AP, Bandinelli E, Dos Santos PA, Schüler-Faccini L. Maternal gene polymorphisms involved in folate metabolism as risk factors for Down syndrome offspring in Southern Brazil. Dis Markers. 2010; 29:95-101.

48. Neagos D, Cretu R, Tutulan-Cunita A, Stoian V, Bohiltea LC. RFC - 1 Gene Polymorphism and the Risk of Down Syndrome in Romanian Population. Maedica (Buchar). 2010 Dec;5(4):280-5.

49.Neagos D, Cretu R, Tutulan-Cunita A, Stoian V, Bohiltea LC. Methylenetetrahydrofolate dehydrogenase (MTHFD) enzyme polymorphism as a maternal risk factor for trisomy 21: a clinical study. J Med Life. 2010;3(4):454-7.

50. Sheth JJ, Sheth FJ. Gene polymorphism and folate metabolism: a maternal risk factor for Down syndrome. Indian Pediatr. 2003 ;40(2):115-23.

51. Garrow TA, Brenner AA, Whitehead VM, Chen XN, Duncan RG, Korenberg JR, et al. Cloning of human cDNAs encoding mitochondrial and cytosolic serine hydroxymethyltransferases and chromosomal localization. J Biol Chem.1993;268:11910-6.

52. Fowler B. The folate cycle and disease in humans. Kidney Int Suppl. 2001;78:S221-9.

53. Blount BC, Mack MM, Wehr CM, MacGregor JT, Hiatt RA, Wang G, et al. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. Proc Natl Acad Sci U S A. 1997;94:3290-5.

54. Bagley PJ, Selhub J. A common mutation in the methylenetetrahydrofolate reductase gene is associated with an accumulation of formylated tetrahydrofolates in red blood cells. Proc Natl Acad Sci U S A. 1998; 95:13217-20.

55. van den Donk M, Visker MH, Harryvan JL, Kok FJ, Kampman E. Dietary intake of B-vitamins, polymorphisms in thymidylate synthase and serine hydroxymethyltransferase 1, and colorectal adenoma risk: a Dutch case-control study. Cancer Lett. 2007; 250(1):146-53.

56. Niclot S, Pruvot Q, Besson C, Savoy D, Macintyre E, Salles G, et al. Implication of the folate-methionine metabolism pathways in susceptibility to follicular lymphomas. Blood. 2006 Jul 1;108(1):278-85. Epub 2006 Jan 12.

57. Lightfoot TJ, Skibola CF, Willett EV, Skibola DR, Allan JM, Coppede F, et al. Risk of non-Hodgkin lymphoma associated with polymorphisms in folate-metabolizing genes. Cancer Epidemiol Biomarkers Prev. 2005;14(12):2999-3003.

58. Skibola CF, Smith MT, Hubbard A, Shane B, Roberts AC, Law GR, et al. Polymorphisms in the thymidylate synthase and serine hydroxymethyltransferase genes and risk of adult acute lymphocytic leukemia. Blood. 2002; 99:3786-91.

59. Xavier AC, Taub JW. Acute leukemia in children with Down syndrome. Haematologica. 2010;95(7):1043-5.

60. Pogribny IP, Basnakian AG, Miller BJ, Lopatina NG, Poirier LA, James SJ. Breaks in genomic DNA and within the p53 gene are associated with hypomethylation in livers of folate/methyl-deficient rats. Cancer Res. 1995;55:1894-901.

61. Chen J, Kyte C, Valcin M, Chan W, Wetmur JG, Selhub J et al. Polymorphisms in the one-carbon metabolic pathway, plasma folate levels and colorectal cancer in a prospective study. Int J Cancer. 2004;110:617-20.

62. Heil SG, Van der Put NM, Waas ET, den Heijer M, Trijbels FJ, Blom HJ. Is mutated serine hydroxymethyltransferase (SHMT) involved in the etiology of neural tube defects? Mol Genet Metab. 2001; 73:164-72.

63. Shin JH, Weitzdoerfer R, Fountoulakis M, Lubec G. Expression of cystathionine β-synthase, pyridoxal kinase, and ES1 protein homolog (mitochondrial precursor) in fetal Down syndrome brain. Neurochem Int. 2004; 45:73–79.

64. Ishinohe A, Kanaumi T, Takashima S, Enokido Y, Nagai Y, Kimura H. Cystathionine b-synthase is enriched in the brains of Down´s patients. Biochem Biophys Res Commun. 2005; 338:1547–1550.

65. Pogribna M, Melnyk S, Pogribny IP, Chango A, Yi P, James SJ. Homocysteine metabolism in children with Down syndrome: in vitro modulation. Am J Hum Genet 2001;69:88-95.

66. Coppus AW, Fekkes D, Verhoeven WMA, Tuinier S, Egger JIM, van Duijn CM. Plasma amino acids and neopterin in healthy persons with Down's syndrome. J Neural Transm. 2007; 114:1041–1045.

67. Meguid NA, Dardir AA, El-Sayed EM, Ahmed HH, Hashish AF, Ezzat A. Homocysteine and oxidative stress in Egyptian children with Down syndrome. Clinic Biochem. 2010; 43:963–967.

68. Fillon-Emery N, Chango A, Mircher C, Barbe F, Blehaut H, Herbeth B, et al. Homocysteine concentrations in adults with trisomy 21: effect of B vitamins and genetic polymorphisms. Am J Clin Nutr. 2004;80:1551-1557.

69. Guéant JL, Anello G, Bosco P, Gueant-Rodriguez RM, Romano A, Barone C, et al. Homocysteine and related genetic polymorphisms in Down's syndrome IQ. J Neurol Neurosurg Psychiatry. 2005;76:706-709.

70. Licastro F, Marocchi A, Penco S, Porcellini E, Lio D, Dogliotti G, et al. Does Down's syndrome support the homocysteine theory of atherogenesis? Experience in elderly subjects with trisomy 21. Arch Gerontol Geriatr. 2006;43:381-387.

71. Johnson WG, Stenroos ES, Spychala JR, Chatkupt S, Ming SX, Buyske S. New 19 bp deletion polymorphism in intron-1 of dihydrofolate reductase (DHFR): a risk factor for spina bifida acting in mothers during pregnancy? Am J Med Genet 2004;124(A):339-45.

72. Parle-Mcdermott A, Kirke PN, Mills JL, Molloy AM, Cox C, O'leary VB, et al. Confirmation of the R653Q polymorphism of the trifunctional C1-synthase enzyme as a maternal risk for neural tube defects in the Irish population. Eur J Hum Genet 2006;14(6):768-72.

73. Xu X, Gammon MD, Wetmur JG, Rao M, Gaudet MM, Teitelbaum SL, et al. A functional 19-base pair deletion polymorphism of dihydrofolate reductase (DHFR) and risk of breast cancer in multivitamin users. Am J Clin Nutr. 2007;85(4):1098-102.

74. Stanisiawska-Sachadyn A, Brown KS, Mitchell LE, Woodside JV, Young IS, Scott JM, et al. An insertion/deletion polymorphism of the dihydrofolate reductase (DHFR) gene is associated with serum and red blood cell folate concentrations in women. Hum Genet 2008;123:289-95.

75. Gellekink H, Blom HJ, van der Linden IJ, den Heijer M. Molecular genetic analysis of the human dihydrofolate reductase gene: relation with plasma total homocysteine, serum and red blood cell folate levels. Eur J Hum Genet. 2007;15(1):103-9.

76. Kalmbach RD, Choumenkovitch SF, Troen AP, Jacques PF, D'Agostino R, Selhub J. A 19-base pair deletion polymorphism in Dihydrofolate reductase is associated with increased unmetabolized folic acid in plasma and decreased red blood cell folate. J Nutr 2008; 138(12):2323-2327.

5. ANEXOS

5. ANEXOS

De acordo com Normas Regulamentares de Pesquisa em Seres Humanos, Resolução 196/96 do Ministério da Saúde, esse projeto foi aprovado pelo Comitê de Ética em Pesquisa da Faculdade de Medicina de São José do Rio Preto/SP, CEP–FAMERP (Anexo 1) e pela Comissão Nacional em Pesquisa de Brasília/DF – CONEP (Anexo 2). Para a realização da análise molecular dos polimorfismos dos genes *Dihidrofolato redutase* (*DHFR*) e *Serina hidroximetiltransferase* (*SHMT*), foi aprovada pelo CEP-FAMERP uma extensão na data de 19 de maio de 2008 (Anexo 3).

Esclareço também que foram utilizadas amostras de material biológico provenientes do banco de material biológico aprovado pela CONEP (Anexo 2) e regularizado junto ao CEP-FAMERP.

FACULDADE DE MEDICINA DE SÃO JOSÉ DO RIO PRETO
AUTARQUIA ESTADUAL - LEI Nº 8899 ,de 27/09/94
(Reconhecida pelo Decreto Federal nº 74.179, de 14/06/74)

Parecer n.º 165/2004

COMITÊ DE ÉTICA EM PESQUISA

O Protocolo n.º 3340/2004 sob a responsabilidade de Érika Cristina Pavarino Bertelli com o título "Avaliação Genético-Clínica e Molecular em Síndrome d Down" está de acordo com a Resolução CNS 196/96 e foi **aprovado** por esse CEP. Sujerimos limitar o grupo de mães às mais jovens, a baixo de 35 anos; verificar o hábito alimentar pré-gravidez seria o ideal pois este hábito muda com o tempo. Aguardar aprovação da CONEP para início do estudo.

Lembramos ao senhor(a) pesquisador(a) que, no cumprimento da Resolução 251/97, o Comitê de Ética em Pesquisa (CEP) deverá receber relatórios semestrais sobre o andamento do Estudo, bem como a qualquer tempo e a critério do pesquisador nos casos de relevância, além do envio dos relatos de eventos adversos, para conhecimento deste Comitê. Salientamos ainda, a necessidade de relatório completo ao final do Estudo.

São José do Rio Preto, 12 de julho de 2004.

Prof.ª Dr.ª Patrícia Maluf Cury
Coordenadora do CEP/FAMERP

Av. Brigadeiro Faria Lima, 5416 - Cep 15.090-000 Fone: (017) 210 - 5700
Fax : 227-6201- São José do Rio Preto - São Paulo - Brasil.

MINISTÉRIO DA SAÚDE
Conselho Nacional de Saúde
Comissão Nacional de Ética em Pesquisa - CONEP

PARECER Nº2400/2004

Registro CONEP: 10618 (Este nº deve ser citado nas correspondências referentes a este projeto)

Registro CEP: 3340/04 **Processo nº 25000.106488/2004-41**
Projeto de Pesquisa: *"Avaliação genético clínica e molecular em Síndrome de Down."*
Pesquisador Responsável: Dra. Érika Cristina Pavarino Bertelli
Instituição: Faculdade de Medicina de São José do Rio Preto - FAMERP
Área Temática Especial: Genética Humana

Ao se proceder à análise das respostas ao parecer CONEP nº 2001/2004, relativo ao projeto em questão, considerou-se que:

1) tendo em vista a afirmação da pesquisadora responsável que será estabelecido um banco de material biológico, solicita-se que seja feito um banco de dados junto ao CEP da instituição, informando: quem será o responsável pelo banco, condições de armazenamento, segurança do banco, como será o acesso pelos pesquisadores a esse banco, de que forma será garantida a confidencialidade dos indivíduos que doarem o material para a formação desse banco;
2) as informações enviadas atendem aos aspectos fundamentais da Res. CNS 196/96 sobre diretrizes e normas regulamentadoras de pesquisas envolvendo seres humanos;
3) o projeto foi aprovado pelo Comitê de Ética em Pesquisa – CEP da instituição supracitada.

Diante do exposto, a Comissão Nacional de Ética em Pesquisa - CONEP, de acordo com as atribuições definidas na Resolução CNS 196/96, manifesta-se pela aprovação do projeto de pesquisa proposto com a recomendação 1. acima citada, devendo esta ser acompanhada pelo CEP, para posterior início da pesquisa.

Situação: Projeto aprovado com recomendação.

Brasília, 29 de Novembro de 2004

WILLIAM SAAD HOSSNE
Coordenador da CONEP/CNS/MS

FACULDADE DE MEDICINA DE SÃO JOSÉ DO RIO PRETO
Autarquia Estadual - Lei n.° 8899 de 27/09/94
(Reconhecida pelo Decreto Federal n.° 74.179 de 14/06/74)

COMITÊ DE ÉTICA EM PESQUISA

O Comitê de Ética em Pesquisa em Seres Humanos da Faculdade de Medicina de São José do Rio Preto tomou ciência e aprovou a **prorrogação para agosto de 2009 para extensão da metodologia e tomou ciência do relatório parcial**; referente ao projeto n.° **3340/2004** sob a responsabilidade de **Érika Cristina Pavarino Bertelli** com o título "Avaliação Genético-Clínica e Molecular em Síndrome de Down".

São José do Rio Preto, 19 de maio de 2008.

Prof. Dr. Antonio Carlos Pires
Coordenador do CEP/FAMERP

Av. Brigadeiro Faria Lima, 5416 - 15090-000 - São José do Rio Preto - SP - Brasil
Tel. (17) 3201-5700 - Fax (17) 3229-1777 - *www.famerp.br*