Methylenetetrahydrofolate Reductase Dimer Configuration as a Risk Factor for Maternal Meiosis I-Derived Trisomy 21

Vraneković, Jadranka; Babić Božović, Ivana; Bilić Čače, Iva; Brajenović-Milić, Bojana

Source / Izvornik: **Human heredity, 2021, 30, 1 - 5**

Journal article, Published version
Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

https://doi.org/10.1159/000515121

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:184:451900

Human Heredity

Brief Report

Hum Hered 2020;85:61–65
DOI: 10.1159/000515121

Received: November 30, 2020
Accepted: February 9, 2021
Published online: March 30, 2021

Methylenetetrahydrofolate Reductase Dimer Configuration as a Risk Factor for Maternal Meiosis I-Derived Trisomy 21

Jadranka Vraneković[a] Ivana Babić Božović[b] Iva Bilić Čače[c]
Bojana Brajenović Milić[a]

[a]Department of Biology and Medical Genetics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia; [b]Clinical Institute of Genomic Medicine, University Medical Center Ljubljana, Ljubljana, Slovenia; [c]Department of Pediatrics, Clinical Hospital Center Rijeka, University of Rijeka, Rijeka, Croatia

Keywords
Chromosome nondisjunction · Down syndrome · Folate · Functional interference · *MTHFR* gene polymorphism

Abstract
Background: Evidence suggests that the dimer configuration of methylenetetrahydrofolate reductase (MTHFR) enzyme might be destabilized by polymorphisms in monomers at the positions C677T and A1298C. It has been observed that these polymorphisms may lead to stable (CCAA, CCAC, CCCC) and unstable (CTAA, CTAC, TTAA) enzyme dimer configurations. ***Objective:*** The aim of this study was to evaluate the association of the MTHFR enzyme dimer configuration and folate dietary intake with the stage of meiotic nondisjunction in mothers of children with maternally derived trisomy 21. ***Methods:*** A total of 119 mothers of children with maternally derived free trisomy 21 were included in the study. The mean maternal age at the time of the birth of the child with trisomy 21 was 32.3 ± 6.4 (range 16–43) years. All mothers were Caucasian. Parental origin of trisomy 21 and meiotic stage of nondisjunction was determined using short tandem repeat markers spanning from the centromere to the telomere of chromosome 21q. The *MTHFR* C677T and *A1298C* polymorphism was evaluated by PCR-RFLP. ***Results:*** Increased frequency of the *MTHFR* genotype combinations CTAA, CTAC, and TTAA was found in the group of mothers with meiosis I (MI) nondisjunction ($p = 0.007$). No differences were found between study participants regarding dietary and lifestyles habits. ***Conclusion:*** The risk for MI nondisjunction of chromosome 21 was 4.6-fold higher in cases who had CTAA, CTAC, and TTAA *MTHFR* genotype combinations and who did not used folic acid supplements in the preconception period.

Background

The mammalian active methylenetetrahydrofolate reductase (MTHFR; E.C.1.5.1.20; MIM 236250) enzyme's structure is composed of two monomers with catalytic and regulatory domains. Both monomers contain the noncovalent bound cofactor FAD that could protect the enzyme from destabilization [1]. Destabilization of the enzyme dimer may be caused by altered polypeptide conformations including movement of polypeptide domains or could be induced by the genetic variants [1, 2]. The di-

karger@karger.com
www.karger.com/hhe

Jadranka Vraneković
Department of Biology and Medical Genetics
Faculty of Medicine, University of Rijeka
Braće Branchetta 22, Rijeka 51000 (Croatia)
jadranka.vranekovic@uniri.hr

mer model of MTHFR enzyme of Ulvik et al. [3] postulates that the three common *MTHFR* alleles (C-A, C-C, and T-A) can provided six enzyme configurations that are sensitive to low folate levels. This study showed that individuals with CTAA, CTAC, and TTAA genotype combinations had significantly increased total homocysteine concentration when their serum folate level was low. They proposed that those individuals had unstable enzyme configuration, which reduced the affinity for FAD cofactors. Maternal risk factors for trisomy 21 such as maternal age and single nucleotide polymorphisms of genes in the folate pathway have been of interest to many scientists over the last decade [4–9]. It is well know that most people with Down syndrome (DS) have an extra chromosome 21, some of which are mosaics (2%) or have the Robertsonian translocation (3%), and the majority have free trisomy 21 (95%). In most cases, nondisjunction of chromosome 21 occurred during oogenesis, more often during meiosis I (MI; 73%) than meiosis II (MII; 25%) [10].

Based on the evidence that lower folate dietary intake and maternal *MTHFR* polymorphisms might be risk factors for trisomy 21 and that different mechanisms lead to meiotic stage of nondisjunction (MI and MII), we hypothesized that the risk for trisomy 21 could be different for the particular meiotic stage. Thus, we aimed to assess the association of the MTHFR enzyme dimer configuration and folate dietary intake with the stage of meiotic nondisjunction in mothers of children with maternally derived trisomy 21.

Methods

A total of 119 mothers of children with maternally derived free trisomy 21 were included in the study. The mean maternal age at the time of the birth of the child with trisomy 21 was 32.3 ± 6.4 (range 16–43) years. All mothers were Caucasian. Before the sampling, mothers were asked to complete a specially created questionnaire that included demographic data such as level of education and maternal age at the time of birth of the child with trisomy 21, periconceptional folic acid use, dietary folate intake, cigarette smoking, and alcohol intake. The modified food frequency questionnaire was used for dietary folate intake [11]. Only 105 participants completed the questionnaire.

Genomic DNA was extracted from EDTA-treated blood or saliva using the QIAamp DNA blood FlexiGene DNA Kit or the QIAamp DNA Micro Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions. Parental origin and stage of nondisjunction were determined using the algorithm proposed by Freeman et al. [10]. Briefly, parental origin of trisomy 21 and meiotic stage of nondisjunction were determined using 11 short tandem repeat markers spanning from the centromere to the telomere of chromosome 21q. The order of markers was cen-D21S258, D21S120, D21S1414, D21S1432, D21S11, D21S1435, D21S226, D21S1270, IFNAR, D21S1412, and D21S1411-qter. Short tandem repeat markers were amplified one by one by PCR or together with single-assay QF-PCR as previously described [12]. At least two markers had to be informative to conclude parental origin of extra chromosome 21 in cases where trios (mother, father, child) were available ($n = 93$). Eight informative markers were required in cases where DNA was obtained only from the mother and child ($n = 26$). To determine the type of meiotic error, the pericentromeric markers (D21S258, D21S120, D21S1414, D21S1432, D21S11) of the child were compared with markers in the parents. Namely, when the trisomic offspring had inherited a pericentric marker in the heterozygosity form ("nonreduction"), it meant that nondisjunction of chromosome 21 had occurred during the first meiotic stage in parent gametogenesis. If the inherited marker was reduced to homozygosity in trisomic offspring, disjunction error during the second meiotic stage was documented. A mitotic error was indicated when all informative markers along the chromosome were reduced to homozygosity. Cases with mitotic errors were not included in this study.

The *MTHFR* C677T and *A1298C* polymorphism was evaluated by PCR-RFLP with the *Hinf*I and *Mbo*II restriction endonucleases (Invitrogen) [13].

Statistical analyses were performed with the statistical software package for Windows STATISTICA (StatSoft, Inc., 2019). A p value <0.05 was considered statistically significant.

Results

Of 119 maternally derived trisomy 21 cases, 86% (102/119) were caused by MI nondisjunction and the remaining 14% (17/119) by MII nondisjunction. Mothers of children with MI-derived trisomy 21 were significantly younger compared to mothers whose children had MII-derived trisomy (31.78 ± 6.4 vs. 35.64 ± 5.2 years; $p = 0.021$). No differences were found between those two groups concerning the frequencies of periconceptional folic acid preparation use, cigarette smoking, alcohol consumption, and dietary folate intake (Table 1). The study showed a significantly higher frequency of unstable MTHFR enzyme configuration in mothers of children with MI-derived trisomy 21 compared to mothers of children with MII-derived trisomy 21. The risk for MI nondisjunction was 4.6-fold higher in cases with unstable MTHFR enzyme configuration ($p = 0.007$; Table 2).

Discussion

Based on evidence, this is the first study which investigated the association between the genotype combinations of the two most common polymorphisms in *MTHFR* gene in the population of mothers experiencing

Table 1. Characteristics of mothers with a DS child according to the meiotic stage of nondisjunction

Characteristics	Meiotic stage		*p* value
	MI, *n* = 102 (86%)	MII, *n* = 17 (14%)	
Maternal age, years	31.78±6.4	35.64±5.2	0.021
Level of education			0.673
Primary	5 (5%)	1 (6%)	
Secondary	57 (56%)	11 (65%)	
High school degree or higher	40 (39%)	5 (29%)	
Periconceptional folic acid use			0.489
Consumers	3 (3%)	1 (6%)	
Nonconsumers	86 (84%)	15 (88%)	
Missing data	13 (13%)	1 (6%)	
Folate dietary intake			0.225
High-folate diet	41 (40%)	10 (59%)	
Low-folate diet	48 (47%)	6 (35%)	
Missing data	13 (13%)	1 (6%)	
Alcohol intake (before and during pregnancy)			0.717
Consumers	2 (2%)	0	
Nonconsumers	87 (85%)	16 (94%)	
Missing data	13 (13%)	1 (6%)	
Cigarette smoking (before and during pregnancy)			0.578
Smokers	10 (10%)	2 (12%)	
Nonsmokers	79 (77%)	14 (82%)	
Missing data	13 (13%)	1 (6%)	

Values are presented as mean ± standard deviation or *n* (%). DS, Down syndrome; MI, meiosis I; MII, meiosis II.

Table 2. Frequencies of MTHFR enzyme dimer configuration in mothers of a DS child according to meiotic stage of nondisjunction (*n* = 119)

Configuration	Genotype *MTHFR* C677T/*A1298C*	MI (*n* = 102)	MII (*n* = 17)	OR (95% CI)	*p* value
Stable	CCAA, CCAC, CCCC	42 (41%)	13 (76%)	4.64 (1.4–15.23)	0.007
Unstable	CTAA, CTAC, TTAA	60 (59%)	4 (24%)		

CI, confidence interval; DS, Down syndrome; MI, meiosis I; MII, meiosis II; MTHFR, methylenetetrahydrofolate reductase; OR, odds ratio.

pregnancy with free trisomy 21, according to the model recommended by Ulvik et al. [3]. In a previous case-control study of this population, we did not observe an increased risk for a trisomy 21 pregnancy according to the mentioned model of MTHFR enzyme configuration [12]. In order to test the hypothesis that the risk for trisomy 21 could be different for the particular meiotic stage, we evaluated the association of particular genotype combinations of *MTHFR* gene as well as folate dietary intake according to the maternal meiotic stage of chromosome 21 nondisjunction.

The Ulvik model proposed that enzyme with polymorphism (CTAA, CTAC, and TTAA) in the catalytic domain or in both domains (catalytic and regulatory) is much more unstable and could have some impact on chromosome instability [1, 3]. Evidence showed that the maternal risk for birth of a child with trisomy 21 is increased in mothers who carry both polymorphisms, more than in cases with the single polymorphic site in *MTHFR* gene. Additionally, if the mother had double homozygous TTCC genotype, MTHFR enzyme showed higher instability and inactivity, often resulting in pre-

natal death [14]. It is important to emphasize that neither of those studies analyzed those combinations in association with meiotic stage of nondisjunction or according to enzyme configuration proposed by Ulvik et al. [3]. MI is characterized by chromosome homologue segregation occurring after meiotic recombination. Those events are controlled by both genetic and epigenetic information [15]. Based on evidence and present data, we suggest that decreased activity of unstable enzyme configuration could have a impact on insufficient DNA methylation, as one of epigenetics mark, rather than leading to incorrect recombination events and chromosome 21 segregation [16–19]. Altered recombination events during oogenesis were frequently observed among individuals with free trisomy 21, suggesting that proper recombination events are crucial for chromosome disjunction [10, 12, 19]. Furthermore, we speculate that unstable enzyme configuration could be an oocyte-specific factor that leads to dysregulation of recombination in that single oocyte, since methyl group deficiency could modify the sites where recombination should occur [20, 21]. Additionally, studies showed that the unstable genotype combinations TTAA, CTAA, and CCAA increased total homocysteine levels when folate was reduced, both in mothers of DS children and control mothers [3, 14]. Unfortunately, we had no biochemical data for our participants, but according to the questionnaires it is evident that all participants were nonconsumers of folic acid supplements during the preconception period (Table 1). According to that, we put forward that lack of folic acid supplementation during the preconception period could be one of many environmental factors that change the microenvironment, in particular the oocyte, and have an impact on altered recombination pattern and chromosome segregation [22–24]. Since between study groups no differences regarding dietary and lifestyles habits were found, we realize that impact on chromosome segregation could have particular MTHFR enzyme configuration conditioned by genotype combination. MTHFR is one of the most crucial enzymes in folate or one carbon cycle that regulates DNA replication as well as many methylation reactions in cells, by providing 5-methyl-THF a primary form of circulatory folate. Five-methyl-THF is essential for the production of methionine, an essential amino acid that is further converted to S-adenosylmethionine. The methyl group of S-adenosylmethionine is then delivered to various biological acceptors, including DNA [1, 2]. Based on evidence it is suggested that modifications in the folate metabolic pathway, caused by the presence of MTHFR C677T/A1298C polymorphisms, could result in altered methylation and lead to genomic instability such as a chromosome disjunction error [4–7]. James et al. [16] were the first hypothesize that MTHFR C677T polymorphism is a risk factor for chromosome 21 nondisjunction during oogenesis. They found a significantly higher frequency of *MTHFR* gene C677T polymorphism in mothers of children with DS than in control women. Since then, the accumulated evidence has indicated that reduced activity of MTHFR enzyme, due to polymorphisms C677T and *A1298C*, along with low folate level could be a maternal risk factor for meiotic nondisjunction of chromosome 21. Some studies showed different results, which may reflect different allele frequencies in individual populations or differences in the number of subjects and the influence of environmental factors such as diet [12–15, 25]. Additionally, associations between *MTHFR* gene polymorphisms and formation of micronucleus in peripheral lymphocytes of mothers with a DS child were observed. Such results support the hypothesis that polymorphisms in the *MTHFR* gene could be a maternal risk factor for genetic instability [26, 27].

We are aware that the limitations of the study were the number of participations, lack of biochemical measurements such as total homocysteine in plasma or serum, as well as measurement of the activity of the enzyme. Therefore, the results should be considered preliminarily and suggesting that follow-up is needed to answer the question whether some specific genotype combinations of the *MTHFR* gene have different effects on the configuration of the assembled protein dimers and on biochemical data.

Conclusion

The study highlights for the first time the associations between maternal MTHFR enzyme configuration and meiotic stage of chromosome 21 nondisjunction. The results demonstrate that some genotype configuration, which result in more unstable enzyme configuration according to the Ulvik model, may have some impact on chromosome 21 nondisjunction during the MI stage in women who have not used folic acid supplements in the preconception period.

Acknowledgments

The authors would like to thank all participants in this study.

Statements of Ethics

The Ethics Committee of the Medical School, University of Rijeka approved the study (No. 003-08/1 9-01/114). Written informed consent was obtained prior to participation.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

This study was supported by grant (No. 13.06.1.2.38) of the University of Rijeka, Rijeka, Croatia. The funders had no role in data collection or analysis. The authors received no specific funding for this work.

Author Contributions

J. Vraneković and I. Babić Božović carried out the experiment. J. Vraneković wrote the manuscript with support from I. Babić Božović, I. Bilić Čače, and B. Brajenović Milić. All authors read and approved the manuscript.

References

1 Yamada K, Chen Z, Rozen R, Matthews RG. Effects of common polymorphisms on the properties of recombinant human methylenetetrahydrofolate reductase. Proc Natl Acad Sci USA. 2001 Dec;98(26):14853–8.
2 Guenther BD, Sheppard CA, Tran P, Rozen R, Matthews RG, Ludwig ML. The structure and properties of methylenetetrahydrofolate reductase from Escherichia coli suggest how folate ameliorates human hyperhomocysteinemia. Nat Struct Biol. 1999 Apr;6(4):359–65.
3 Ulvik A, Ueland PM, Fredriksen A, Meyer K, Vollset SE, Hoff G, et al. Functional inference of the methylenetetrahydrofolate reductase 677C > T and 1298A > C polymorphisms from a large-scale epidemiological study. Hum Genet. 2007 Mar;121(1):57–64.
4 Costa-Lima MA, Amorim MR, Orioli IM. Association of methylenetetrahydrofolate reductase gene 677C > T polymorphism and Down syndrome. Mol Biol Rep. 2013 Mar;40(3):2115–25.
5 Wu X, Wang X, Chan Y, Jia S, Luo Y, Tang W. Folate metabolism gene polymorphisms MTHFR C677T and A1298C and risk for Down syndrome offspring: a meta-analysis. Eur J Obstet Gynecol Reprod Biol. 2013 Apr;167(2):154–9.
6 Yang M, Gong T, Lin X, Qi L, Guo Y, Cao Z, et al. Maternal gene polymorphisms involved in folate metabolism and the risk of having a Down syndrome offspring: a meta-analysis. Mutagenesis. 2013 Nov;28(6):661–71.
7 Rai V, Yadav U, Kumar P, Yadav SK, Mishra OP. Maternal methylenetetrahydrofolate reductase C677T polymorphism and Down syndrome risk: a meta-analysis from 34 studies. PLoS One. 2014 Sep;9(9):e108552.
8 Victorino DB, Godoy MF, Goloni-Bertollo EM, Pavarino EC. Meta-analysis of Methylenetetrahydrofolate reductase maternal gene in Down syndrome: increased susceptibility in women carriers of the MTHFR 677T allele. Mol Biol Rep. 2014 Aug;41(8):5491–504.
9 Coppedè F. The genetics of folate metabolism and maternal risk of birth of a child with Down syndrome and associated congenital heart defects. Front Genet. 2015 Jun;6:223.
10 Freeman SB, Allen EG, Oxford-Wright CL, Tinker SW, Druschel C, Hobbs CA, et al. The National Down Syndrome Project: design and implementation. Public Health Rep. 2007 Jan–Feb;122(1):62–72.
11 Colić Barić I, Satalić Z, Pedisić Z, Zizić V, Linarić I. Validation of the folate food frequency questionnaire in vegetarians. Int J Food Sci Nutr. 2009;60(Suppl 5):88–95.
12 Vraneković J, Babić Bozović I, Starcević Cizmarević N, Buretić-Tomljanović A, Ristić S, Petrović O, et al. Functional inference of methylenetetrahydrofolate reductase gene polymorphisms on enzyme stability as a potential risk factor for Down syndrome in Croatia. Dis Markers. 2010;28(5):293–8.
13 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 May;140(10):1083–91.
14 Martínez-Frías ML. The biochemical structure and function of methylenetetrahydrofolate reductase provide the rationale to interpret the epidemiological results on the risk for infants with Down syndrome. Am J Med Genet A. 2008 Jun;146A(11):1477–82.
15 de Massy B. Initiation of meiotic recombination: how and where? Conservation and specificities among eukaryotes. Annu Rev Genet. 2013;47(1):563–99.
16 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 Oct;70(4):495–501.
17 Božović IB, Stanković A, Živković M, Vraneković J, Kapović M, Brajenović-Milić B. Altered LINE-1 methylation in mothers of children with Down syndrome. PLoS One. 2015 May;10(5):e0127423.
18 Vraneković J, Božović IB, Grubić Z, Wagner J, Pavlinić D, Dahoun S, et al. Down syndrome: parental origin, recombination, and maternal age. Genet Test Mol Biomarkers. 2012 Jan;16(1):70–3.
19 Oliver TR, Tinker SW, Allen EG, Hollis N, Locke AE, Bean LJ, et al. Altered patterns of multiple recombinant events are associated with nondisjunction of chromosome 21. Hum Genet. 2012 Jul;131(7):1039–46.
20 Middlebrooks CD, Mukhopadhyay N, Tinker SW, Allen EG, Bean LJH, Begum F, et al. Evidence for dysregulation of genome-wide recombination in oocytes with nondisjoined chromosomes 21. Hum Mol Genet. 2014 Jan;23(2):408–17.
21 Termolino P, Cremona G, Consiglio MF, et al. Insights into epigenetic landscape of recombination-free regions. Chromosoma. 2016 Jun;125(2):301–8.
22 Sukla KK, Jaiswal SK, Rai AK, Mishra OP, Gupta V, Kumar A, et al. Role of folate-homocysteine pathway gene polymorphisms and nutritional cofactors in Down syndrome: A triad study. Hum Reprod. 2015 Aug;30(8):1982–93.
23 Sullivan M, Murray T, Assefa H. Women with Methylenetetrahydrofolate Reductase Gene Polymorphism and the Need for Proper Periconceptional Folate Supplementation. J Pharm Pharmacol. 2015;3:204–22.
24 Hollis ND, Allen EG, Oliver TR, et al. Preconception folic acid supplementation and risk for chromosome 21 nondisjunction: A report from the National Down Syndrome Project. Am J Med Genet A. 2013 Mar;161A(3):438–44.
25 Kaur A, Kaur A. Maternal MTHFR polymorphism (677 C-T) and risk of Down's syndrome child: meta-analysis. J Genet. 2016 Sep;95(3):505–13.
26 Kedar R, Chandel D. MTHFR gene polymorphism and associated nutritional deficiency in the etiology and pathogenesis of Down syndrome. Egypt J Med Hum Genet. 2019;20(1):12.
27 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 Jan;449(1):15–9.