The Relationship Between Maternal 5,10-Methylenetetrahydrofolate Reductase C677T Polymorphism and the Development of Neural Tube Defects: A 5-year study in Aegean Obstetrics and Gynecology Training and Research Hospital

NÖRAL TÜP DEFEKTİ GELİŞİMİ İLE MATERNAL 5,10-METİLENTETRAHİDROFOLAT REDÜKTAZ C677T POLİMORFİZMİ ARASINDAKİ İLİŞKİ: EGE DOĞUMEVİ VE KADIN HASTALIKLARI EĞİTİM VE ARAŞTIRMA HASTANESİ'NDE 5 YILLIK BİR ÇALIŞMA

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Abstract

Objective: To investigate the relationship between 5,10-methylenetetrahydrofolate reductase enzyme (MTHFR) C677T polymorphism and the development of neural tube defect (NTD) in our center.

Material and Methods: This case-control study performed in Aegean Obstetrics and Gynecology Training and Research Hospital between 2001 and 2005 was designed to compare 36 women with histories of spina bifida (SB) (n= 18), anencephaly (n= 11) or occipital encephalocele (n= 7) in prior pregnancies (NTD group) with 40 women without histories of NTD birth (control group) for the presence of MTHFR C677T gene polymorphism. DNA analyses were performed using Polymerase Chain Reaction (PCR).

Results: The overall maternal MTHFR mutant (T) allele frequency was 28.3% (21.5/76). MTHFR C677T polymorphism prevalence was 41.7% (15/36) for NTD group and 42.5% (17/40) for control group (p= 0.48). This rate was found to be 50% (9/18), 36.4% (4/11), and 28.6% (2/7) for SB, anencephaly, and occipital encephalocele sub-groups; respectively (p= 0.04).

Conclusion: Our findings showed no statistically significant relation between maternal MTHFR C677T polymorphism and development of fetal NTD. However, relatively high prevalence of MTHFR C677T polymorphism in SB sub-group compared with other NTDs, has suggested that this polymorphism might cause a specific defect in neural tube closure.

Key Words: Neural tube defects; 5,10-methylenetetrahydrofolate reductase (FADH2); polymorphism, genetic

Özet

Amaç: Merkezimizde 5,10-metilentetrahidrofolat redüktaz (MTHFR) C677T polimorfizmi ile nöral tüp defekti (NTD) gelişmesi arasındaki ilişkiyi araştırmak.

Gereç ve Yöntemler: Ege Doğumevi ve Kadın Hastalıkları Eğitim ve Araştırma Hastanesi'nde 2001-2005 yılları arasında gerçekleştirilen bu vaka-kontrol çalışması, önceki gebeliğinde spina bifida (SB) (n=18), anensefali (n=11) veya ensefalosel (n=7)'li fetus öyküsü olan 36 kadın (NTD grubu) ve NTD'li gebelik öyküsü olmayan 40 kadın (kontrol grubu)'ı MTHFR C677T gen polimorfiziminin varlığı açısından karşılaştıracak şekilde dizayn edildi. DNA analizleri Polimeraz Zincir Reaksiyonu (PCR) kullanarak gerçekleştirildi.

Bulgular: Genel olarak maternal MTHFR mutant (T) allel sıklığı %28.3 (21.5/76) idi. MTHFR C677T polimorfizm sıklığı NTD grubu için %41.7 (15/36), kontrol grubu için ise %42.5 (17/40) idi (p= 0.48). Bu oran sırasıyla SB, anensefali ve oksipital ensefalosel için %50 (9/18), %36.4 (4/11) ve %28.6 (2/7) olarak bulundu (p= 0.04).

Sonuç: Bulgularımız maternal MTHFR C677T polimorfizmi ile fetusta NTD gelişmesi arasında istatistiki olarak anlamlı bir fark göstermedi. Bununla birlikte, diğer NTD grupları ile karşılaştırıldığında SB alt-grubunda MTHFR C677T polimorfizminin göreceli olarak daha yüksek frekansı bu polimorfizmin nöral tüpün kapanmasında spesifik bir defekte neden olabileceğini düşündürmektedir.

Anahtar Kelimeler: Nöral tüp defektleri; 5,10-metilentetrahidrofolat redüktaz; genetik, polimorfizm

Turkiye Klinikleri J Gynecol Obst 2007, 17:337-341

Geliş Tarihi/*Received:* 04.01.2007 **Kabul Tarihi/*Accepted:*** 22.05.2007

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Neural tube defect (NTD) prevalence is different in populations with different geographical and socioeconomic characteristics.[1,2] It is one of the most common congenital structural defects worldwide. NTD is also a com-

mon congenital anomaly in Turkey with a prevalence of 30/10 000.[3]

Cerebral hemispheres appear as a midline structure and closure of the neural tube is completed 28 days after conception.[4] Neural tube formation is a multifactorial process determined by both extrinsic and intrinsic factors. Therefore, both genetic and environmental factors attribute to pathogenesis of NTD. British Medical Research Council (MRC) Vitamin Study Research Group reported that NTD risk might be decreased with periconceptional folate intake.[5] Maternal low folate and raised homocysteine (Hcy) levels in early pregnancy are risk factors for the development of NTDs in fetuses.[6,7]

The 5,10-methylentetrahydrofolate reductase (MTHFR) C677T polymorphism has been associated with elevated Hcy, especially under condition of low folate status.[8,9] Studies revealed that MTHFR C677T polymorphism was observed significantly more in children with NTD and their mothers when compared with control groups.[10-14] Both the homozygous (TT) and heterozygous (CT) MTHFR C677T polymorphisms are associated with decreased MTHFR activity and increased plasma Hcy concentrations.[14] However, the relevance of the maternal C677T polymorphism for the development of NTD in fetuses has recently been questioned by some authors.[15,16]

The aim of this study was to investigate the relationship between MTHFR C677T polymorphism and the development of NTDs in their fetuses.

Material and Methods

Study Population

A total of 36 pregnant women with the history of a prenatal diagnosis of spina bifida (SB) at different levels (n: 18), anencephaly (n: 11) and occipital encephalocele (n: 7) were enrolled into this case-control study in Aegean Obstetrics and Gynecology Training and Research Hospital between January 2001 and December 2005. Control group was constituted from randomly selected 40 women who delivered babies without any congenital anomaly in the same period.

The study was approved by Institutional Review Board (IRB) of our hospital. Informed consent was obtained from each patient after the purpose and nature of the study had been fully explained. The work was also conducted following the principles of 'Helsinki declaration' on human experimentation.

NTDs of the fetuses were diagnosed with second trimester MSAFP and ultrasonography. All pregnancies with NTDs were evaluated by "Perinatology Council" formed by obstetrician, neonatologist, pediatric surgeon, genetic diseases specialist and pathologist, and termination decision for 30 fetuses with different NTD was declared. Pregnancies with decision of "continuation" were 6 cases, and two out of these 6 cases were diagnosed in the second trimester. One of these two cases with thoraco-lumbar SB was delivered with cesarean section at 39th week of gestation and transferred to pediatric surgery department, but the other was aborted in 22nd week of gestation.

DNA isolation and diagnosis of MTHFR C677T polymorphism

Peripheral blood samples of 2.5 cc were drawn from the cases and put into the standard K3 EDTA tubes. The samples were studied in Tissue Typing and Molecular Diagnosis Laboratory of Tepecik Training and Research Hospital. Nucleospin® (MN Macherey-Nagel GmbH, Germany) nucleic acid isolation kits were used in order to get genomic DNA from peripheral blood samples. After confirmation of presence of isolated DNA by adding to the Agarose gel, amplification was applied. FV-PTH strip assay kits (Vienna Lab. Labordiagnostika GmBH, Austria) were used for MTHFR gene analysis to be performed under principle of reverse hybridization. "Taq polymerase" was used in order to amplify mutation loci with multiplex Polymerase Chain Reaction (PCR) in Thermal Cycler. Gene analysis was applied to the PCR product obtained from the Thermal Cycler. Specimens were studied and evaluated according to market-kit procedure with Inna Genetics Auto Lipa mutation device.

Statistical Evaluation

Statistical analyses were carried out using the pocket program of Statistical Program for Social Sciences, version 11.0 for Windows (SPSS Inc., Chicago, IL, USA). Fischer's exact test and Student's t test were used in statistical estimations and two tailed P<0.05 was accepted as statistical significance.

Results

Mean age of women in NTD and control groups was 26.3±4.5 and 25.9±4.1 years, (p: 0.78), respectively.

The overall maternal MTHFR mutant (T) allele frequency was found to be 28.3% (21.5/76); 27.8% (10/36) for NTD group and 28.8% (11.5/40) for control group (p: 0.65). The maternal T allele frequency for SB, anencephaly, and occipital encephalocele was found to be 33.3% (6/18), 22.7% (2.5/11) and 21.4% (1.5/7), respectively, when evaluated according to the type of the NTD (p: 0.04).

The maternal MTHFR C677T polymorphism was observed in 15 women from NTD group (41.7%) (10 CT, 5TT), and in 17 women from control group (42.5%) (11 CT, 6 TT) (p: 0.48). Presence of this polymorphism both as CT (p: 0.76) and TT (p: 0.17) forms in between both groups revealed no statistically significant different. Additionally, the maternal MTHFR C677T polymorphism prevalence for SB, anencephaly, and occipital encephalocele was found to be 50% (9/18), 36.4% (4/11) and 28.6% (2/7), respectively; when evaluated according to the type of the NTD (p: 0.04). Table 1 and Table 2 show the maternal MTHFR C677T gene status of the groups.

Table 1. Comparison of both groups according to MTHFR C677T gene polymorphism.

	NTD Group (n=36)	Control Group (n=40)	Significance
MTHFR 677CC	21 (58.3%)	23 (57.5%)	P=0.98
MTHFR 677CT	10 (27.8%)	11 (27.5%)	P=0.76
MTHFR 677TT	5 (13.9%)	6 (15.0%)	P=0.07

CC: Normal, CT: Heterozygous, TT: Homozygous

Table 2. Distribution of MTHFR C677T gene polymorphism according to the type of the defects in NTD group.

	Spina bifida (n=18)	Anencephaly (n=11)	Encephalocele (n=7)
MTHFR 677CC	9 (50%)	7 (63.6%)	5 (71.4%)
MTHFR 677CT	6 (33.3%)	3 (27.3%)	1 (14.3%)
MTHFR 677TT	3 (16.7%)	1 (9.1%)	1 (14.3%)

CC: Normal, CT: Heterozygous, TT: Homozygous

Discussion

Hcy play a critical role in methionine metabolism. With the effect of mutation of several genes in methionine pathway (i.e. MTHFR), circulating Hcy levels may increase (hyperhomocysteinemia). B12 and folate deficiencies can also augment this increase.[6-9] There are some clinical suspicious about the possible relationships between hyperhomocysteinemia and adverse obstetric and perinatal outcomes such as pregnancy-induced hypertension, abruptio placentae, and intrauterine growth restriction.[17] However, these issues need further studies.

There exists many data about genetic basis of NTDs. A relationship in between SB and HLA-B5 tissue type was reported.[18] NTDs can be associated with autosomal trisomy and triploidy.[19] Mendelian inheritance model had also been reported.[20] MTHFR C677T polymorphism is the first genetic polymorphism being established in etiology of NTD. Women carrying homozygote MTHFR C677T polymorphism have 1.8 times increased risk of having a fetus with NTD, according to the results of a very large review of many studies from Europe, USA, Asia and Australia.[21]

MTHFR C677T mutant allele frequency differs in between 6-59% with rates in different populations.[21,22] It was reported as 28.5% for Turkey in a previously published report.[23] Data obtained from this present study (28.3%) are accordance with the literature.

This study could not demonstrate a relationship in between the presence of maternal MTHFR C677T polymorphism and NTD presentation in fetuses. Similarly, a study by Nitsche et al comparing 58 blood samples from mothers who had a child diagnosed with SB and 184 healthy mothers with no NTD offspring concluded that there were no differences in the C677T polymorphism of the MTHFR observed between two groups.[15] It is possible that low folate intake combined with other genetic factors besides MTHFR C677T polymorphism plays a more important role in the cause of this disease. A case-control study by Relton et al reported that both independent genetic effects of polymorphisms in different genes coding for proteins in the folate-dependent Hcy pathway and gene-gene interaction were observed in relation to NTD risk.[24] More comprehensive studies are essential in order to investigate other possible etiologic genetic factors.

Mothers with the history of a SB had significantly higher rate of MTHFR C677T polymorphism than those had anencephaly or occipital encephalocele. It is known that spinal closure begins synchronously at five different points independently and continues as a zip closure (multi-site closure model). These points are, face area, merocronium (anencephaly is observed due to closure defect), occiput (occipital encephalocele is observed due to closure defect), cervicolumbar area and sacral area (spinal defects at different levels are observed due to closure defects). Recent scientific literature has claimed that there is an association between the presence of Hcy pathway gene polymorphisms and the levels of defect in children with NTD.[25-27] Wenstrom et al have suggested that MTHFR C677T polymorphism and elevated Hcy levels appear to be disproportionately associated with defects spanning the cervical-lumbar spine, lumbosacral spine, and occipital encephalocele while anencephaly, exencephaly, and defects confined to the sacrum may not be related to altered Hcy metabolism.[27] A report of genetic analysis of 11 affected families, by Sadewa et al, suggested that the maternal MTHFR C677T polymorphism did not associated with the development of frontoethmoidal encephalocele which is the most common form of NTD in East Java, Indonesia.[25]

Recently, Dodelson de Kremer and Grosso have researched the relationship between hypoxic-ischemic encephalopathy of the newborn (HIEN) and the MTHFR C677T polymorphism as a genetic risk for this condition. The prevalence of the MTHFR C677T allele was studied in 11 children with HIEN, their respective mothers, and 85 healthy individuals. The authors concluded that the MTHFR C677T mutation in mothers, either in a homozygous or heterozygous state, together with poor nutritional status (probable folate deficiency) may represent a risk factor for irreversible HIEN in the offspring.[28] Another recent study by O'Leary et al has showed that there is a possible association between NTD cases and new MTHFR polymorphisms such as MTHFR C116T.[29]

Major limitations of this study were, to include relatively a small number of cases, inability to investigate MTHFR C677T polymorphism in fetuses, no data about serum Hcy and folate levels, no data about folate intake and MTHFR C677T polymorphism relation with pathophysiologic mechanisms leading to NTD development, and inability to investigate other possible gene polymorphisms (ie; MTHFR A1298C, etc). Besides, data suggest no relationship in between maternal MTHFR C677T polymorphism and fetal NTD in our study population. However, these findings have suggested that this polymorphism might cause a specific defect in the closure of neural tube. It is needed further studies to explain this subject. Additionally, future researches should focus on possible relationships between MTHFR C677T polymorphism and hypoxic-ischemic encephalopathy of the newborn and between new MTHFR polymorphisms and NTD cases.

REFERENCES

1. Greenberg F, James LM, Oakley GP. Estimates of birth prevalence rates of spina bifida in United States from computer generated maps. Am J Obstet Gynecol 1983; 145:570-3.
2. Hall JG, Friedman JM, Kenna BA, Popkin J, Jawanda M, Arnold W. Clinical genetic and epidemiological factors in neural tube defects. Am J Hum Genet 1988;43:827-37.

3. Tuncbilek E, Alikasifoglu M, Akadlı B, Boduroglu K. The frequency, distrubution, and risk factors of congenital abnormalities in Turkey. Ankara: TUBITAK Matbaasi 1996; 94-5.
4. Sadler TW. Mechanism of neural tube closure and defects. Ment Retard Dev Disabil Res Rev 1998;4:247-53.
5. MRC Vitamin Study Research Group. Prevention of neural tube defects: Results of the Medical Research Council vitamin study. Lancet 1991;338:131-7.
6. Mills JL, McPartlin JM, Kirke PN, et al. Homocysteine metabolism in pregnancies complicated by neural-tube defects. Lancet 1995;345:149-51.
7. Steegers-Theunissen RP, Boers GH, Bloom HJ, et al. Neural tube defects and elevated homocysteine levels in amniotic fluid. Am J Obstet Gynecol 1995;172:1436-41.
8. Guttormsen AB, Ueland PM, Nesthus I, et al. Determinants of intermediate hyperhomocysteinemia (>40 µmol/l). The Hordal and homocysteine study. J Clin Invest 1996;98:2174-83.
9. Kluijtmans LAJ, van den Heuvel LPWJ, Boers GHJ, et al. Molecular genetic analysis in mild hyperhomocysteinemia: A common mutation in the methylenetetrahydrofolate reductase gene is a genetic risk factor for cardiovascular disease. Am J Hum Genet 1996;58:35-41.
10. Goyette P, Frosst P, Rosenblatt DS, Rozen R. Seven novel mutations in the methylenetetrahydrofolate reductase gene and genotype/phenotype correlations in severe methylenetetrahydrofolate reductase deficiency. Am J Hum Genet 1995;56:1052-9.
11. Grandone F, Corrao AM, Colaizzo D, et al. Homocysteine metabolism in families from southern Italy with neural tube defects: Role of genetic and nutrional determinants. Prenat Diagn 2006;26:1-5.
12. Martinez de Villarreal LE, Delgado-Enciso I, Valdez-Leal R, et al. Folate levels and N(5), N(10)-methylenetetrahydrofolate reductase genotype (MTHFR) in mothers of offspring with neural tube defects: A case-control study. Arch Med Res 2001;32:277-82.
13. Van der Put NMJ, Eskes TKAB, Blom HJ. Is the common C677T mutation in the methylenetetrahydrofolate reductase gene a risk factor for neural tube defects? A meta-analysis. Q J Med 1997;90:111-5.
14. Van der Put NMJ, Steegers-Theunissen RPM, Frosst P, et al. Mutated methylenetetrahydrofolate reductase as a risk factor for spina bifida. Lancet 1995;346:1070-1.
15. Nitsche F, Alliende MA, Santos JL, et al. Frequency of C677T polymorphism of 5, 10-methylenetetrahydrofolate reductase (MTHFR) in Chilean mothers of spina bifida cases and controls. Rev Med Chil 2003;131:1399-404.
16. Perez AB, D'Almeida V, Vergani N, de Oliveira AC, de Lima FT, Brunoni D. Methylenetetrahydrofolate reductase (MTHFR): Incidence of mutations C677T and A1298C in Brazilian population and its correlation with plasma homocysteine levels in spina bifida. Am J Med Genet 2003; 119:20-5.
17. Steegers-Theunissen RP, Van Iersel CA, Peer PG, Nelen WL, Steegers EA. Hyperhomocysteinemia, pregnancy complications, and the timing of investigation. Obstet Gynecol 2004;104:336-43.
18. Vannier JP, Lefort J, Cavalier B, Ledosseur P, Assailly C, Feingold J. Spina bifida cystica families, X-ray examination and HLA typing. Pediatr Res 1981;15:326-9.
19. Coerdt W, Miller K, Holzgreve W, Rauskolb R, Schwinger E, Rehder H. Neural tube defects in chromosomally normal and abnormal human embryos. Ultrasound Obstet Gynecol 1997;10:410-5.
20. Jensson O, Arnosan A, Gunnarsdottir H, Petursdottir I, Fossdal R, Hreidarsson S. A family showing apperent X-linked inheritance of both anencephaly and spina bifida. J Med Genet 1988;25:227-9.
21. Botto LD, Yang Q. 5, 10-Methylenetetrahydrofolate reductase gene variants and congenital anomalies: A HuGE Review. Am J Epidemiol 2000;151:862-77.
22. A EUROCAT Working Group Report 7. 15 years of surveillance of congenital anomalies in Europe 1980-1994. Brussels: Scientific Institute of Public Health-Louis Pasteur 1997;50-79.
23. Boduroglu K, Alikasifoglu M, Anar B, Tuncbilek E. The 677C→T mutation of the methylenetetrahydrofolate reductase gene is not a risk factor for neural tube defects in Turkish population. Arch Dis Child Fetal Neonat 1998; 78:235.
24. Relton CL, Wilding CS, Pearce MS, 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.
25. Sadewa AH, Sutomo R, Istiadjid M, et al. C677T mutation in the MTHFR gene was not found in patients with frontoethmoidal encephalocele in East Java, Indonesia. Pediatr Int 2004;46:409-14.
26. Volcik KA, Shaw GM, Lammer EJ, Zhu H, Finnell RH. Evaluation of infant methylenetetrahydrofolate reductase genotype, maternal vitamin use, and risk of high versus low level spina bifida defects. Birth Defects Res A Clin Mol Teratol 2003;67:154-7.
27. Wenstrom KD, Johanning GL, Owen J, et al. Amniotic fluid homocysteine levels, 5,10-methylenetetrahydrafolate reductase genotypes, and neural tube closure sites. Am J Med Genet 2000;90:6-11.
28. Dodelson de Kremer R, Grosso C. Maternal mutation 677C>T in the methylenetetrahydrofolate reductase gene associated with severe brain injury in offspring. Clin Genet 2005;67:69-80.
29. O'Leary VB, Mills JL, Parle-McDermott A, et al. Screening for new MTHFR polymorphisms and NTD risk. Am J Med Genet A 2005;138:99-106.