TÜBİTAK

ORIGINAL ARTICLE

Turk J Med Sci
2008; 38 (5): 383-386
© TÜBİTAK
E-mail: medsci@tubitak.gov.tr

Neural Tube Defects and 19 bp Deletion Within Intron-1 of Dihydrofolate Reductase Gene

Nejat AKAR
Ece AKAR
Yonca EĞİN
Gülhis DEDA
Saadet ARSAN
Mesiha EKİM

Department of Pediatrics,
Faculty of Medicine,
Ankara University,
Ankara - TURKEY

Aims: Dihydrofolate reductase (DHFR) is necessary for the reduction of the ingested folates before they are used in the body metabolism. Thus, the DHFR enzyme has an important role in folate supplementation and the DHFR gene is a strong candidate for a teratogenic locus for neural tube defects (NTDs). There is a 19 bp deletion within the first intron of the DHFR gene, which may have an effect on folate reduction. We thus studied this mutation in Turkish spina bifida patients and their mothers to determine whether there is an association with the occurrence of NTD.

Materials and Methods: The case-control study included 69 meningomyelocele (MMC) patients and 104 mothers who gave birth to NTD babies. One hundred sixty eight women, consecutively selected, who admitted to the laboratory, were included as controls. One hundred thirty-six newborns without MMC were also included.

Results: DHFR gene 19 bp deletion in homozygous state was significantly higher in the MMC group compared to control newborns [Odds Ratio 2.4 (CI 95% 0.95-6.08)]. It also brought 2.4- fold risk [Odds Ratio 2.4 (CI 95% 1.04-5.6)] in NTD mothers compared to controls.

Conclusions: Our data revealed that DHFR gene 19 bp deletion homozygosity and other possible mutations in the DHFR gene should be studied further especially in folate-supplemented mothers with NTD recurrence.

Key Words: Neural tube defects, spina bifida, dihydrofolate reductase

Neural Tüp Defektleri ve Dihidrofolat Redüktaz Geni İntron-1'de Yer Alan 19 bp'lik Delesyon

Amaç: Dihidrofolat redüktaz folatın indirgenmesinde rol alan bir enzimdir. Bu nedenle folat suplementasyonu yapılması halinde önem kazanmaktadır. Özellikle neural tüp defektlerinde önemi olan folatın, annelere supplementasyonunda önemli rol oynayabilir. DHFR geninin birinci intronunda 19 bp'lik bir delesyon bulunmaktadır. Bu delesyon folat indirgenmesinde rolü olabilecek bir gen değişimidir.

Yöntem ve Gereç: Vaka-kontrol şeklinde tasarımlanan çalışmamızda, 69 meningomyleoselli olgu ile 104 NTD'li bebek sahibi olmuş anneler ile 168 sağlıklı kadın ile 136 yenidoğan çalışmaya alınmışlardır. Çalışmaya alınan bireylerde ve/veya ebeveynlerinden yazılı onay alınmıştır.

Bulgular: DHFR geni 19 bp delesyonunu homozigot olarak taşıma oranı MMS'li olgularda yenidoğan sağlıklı bebeklere oranla daha yüksek bulunmuştur [Odds Ratio 2.4 (CI 95 % 0.95 -6.08)]. Anneler içinde 2.4 kat risk getirmektedir [OR 2.4 (CI 95 % 1.04-5.6)].

Sonuç: Verilerimiz, folat supplemenatsayonundan yarar görmeyen NTD'li bebek doğurmuş annelerde önemli bir gen olarak görülmüş ve ileri araştırmalar için iyi bir aday gen olabileceği düşüncesine ulaşılmıştır.

Anahtar Sözcükler: Neural tüp defekti, Spina bifida, Dihidrofolat redüktaz

Abbreviations: NTD: Neural Tube Defects, DHFR: Dihydrofolate Reductase

Received: January 25, 2008
Accepted: June 18, 2008

Correspondence
Nejat AKAR
Koru Mah. 606. Sokak
Uyum Sitesi 19
Çayyolu/Ankara - TURKEY

akar@medicine.ankara.edu.tr

Introduction

Meningomyelocele (MMC), encephalocele and anencephaly are the most common severe congenital malformations of the central nervous system (CNS). These malformations arise due to failure of closure of the neural tube and are referred to as neural tube defects (NTDs), with high incidence in some parts of the world, including Turkey (incidence is 4 in 1000 live births). NTD causation is believed to be multifactorial, with genetic and environmental components (1,2). The relationship between periconceptional dietary folate supplementation and the prevention of up to

approximately 70% of NTDs is well recognized, although the mechanisms underlying the beneficial effect of folate are only partly understood (3).

There is a growing interest in the relationship between variation in genes that are involved in the folate-homocysteine metabolic pathway and the risk of spina bifida. The evaluation of this relationship is, however, complicated by the potential involvement of both the maternal and the embryonic genotype in determination of disease risk.

Our group and others have published data on homocysteine metabolism-related polymorphisms in patients with spina bifida and their mothers in different populations. Although the data varies among study groups, our data revealed that none of the common risk mutations in three genes (methylenetetrahydrofolate reductase (MTHFR) 677C-T and 1298 A-C, methionine synthase (MS) 2756 A-G, and cystathionine B-synthase (CBS) Exon 8, 844 Ins 68), either alone or in combination, increased the risk for the occurrence of spina bifida in our population (4,5).

Homocysteine (Hcy) accumulation can be due to dietary (e.g. low folate), environmental or genetic factors and is mildly elevated in some pregnant women who subsequently give birth to infants with NTDs (6). Partial prevention of recurrence of NTDs including spina bifida was found for mothers taking multivitamins containing folic acid during pregnancy (7,8).

It is logical that genes that mediate absorption of ingested folate and genes that metabolize folate to forms required for cellular metabolism are candidates for teratogenic loci.

Dihydrofolate reductase (DHFR) has a key role in folate biosynthesis and is responsible for the generation of the DNA base, deoxythymidine monophosphate. DHFR is also involved in the biosynthesis of purine nucleotides and the amino acids histidine and methionine. Humans do not have the ability to synthesize folates de novo and instead use a membrane-bound reduced folate carrier to bring dietary folic acid, which is then reduced to tetrahydrofolate by DHFR into the cell (9).

Johnson et al. (10) (2004) proposed the DHFR gene as a strong candidate for a teratogenic locus because ingested folates must be fully reduced by the enzyme before being used in the body metabolism. All of the folate from vitamin supplements and food fortification is folic acid, a form of folate that is unreduced and requires the action of DHFR before it can participate in cellular reactions. The DHFR gene, synthesizing the DHFR enzyme, is a genetic factor that is well placed to interact with the environmental component of the folate risk factor for adverse pregnancy outcome, i.e., dietary folates and folic acid supplements. Human cells also depend on DHFR for DNA replication, a crucial feature of gestation and development. Johnson et al. also reported a polymorphic 19 bp deletion within intron-1 of DHFR, hypothesizing that this polymorphism might function as a teratogenic allele in spina bifida mothers, acting to decrease the supply of reduced folate to the fetus. Their data showed a significantly increased fraction of spina bifida mothers compared to controls were DHFR 19 bp deletion homozygotes, a finding not seen in individuals with spina bifida or their fathers. This supported the hypothesis that the DHFR deletion allele acts as a teratogenic allele, i.e., it acts in the mother during pregnancy to alter the development of the fetus, contributing to the spina bifida phenotype (10).

However, two recent studies found the contrary, and one even showed a protective effect to the mothers (11,12)

We thus studied this mutation in Turkish spina bifida patients and their mothers to determine whether there is an association with the occurrence of NTD.

Materials and Methods

Patients

This case-control study included 69 MMC patients and 104 mothers who gave birth to NTD babies. These individuals were simplex non-syndromic families and none of the mothers had folate supplementation. These were included consecutively on admission to the hospital. All the MMC cases had lesions at the lumbosacral area. One hundred sixty eight women, consecutively selected, who admitted to the laboratory, were included as controls. One hundred thirty-six newborns without MMC were also included. All subjects were from Ankara, Turkey without personal or family history of NTD.

Methods

Blood for mutation analysis was obtained after provision of written informed consent by the parents and DNA was extracted by conventional methods. Genotyping

of the DHFR 19 bp deletion was analyzed by polymerase chain reaction (PCR) amplification using three allele specific primers (5'-CCACGGTCGGGGTACCTG-3'; (Forward-2) 5'-ACGGTCGGGGTGGCCGACTC-3' and (Reverse) 5'–AAAAGGGGAATCCAG TCGG-3') as described previously. 113 bp product was separated on 10% polyacrylamide gel (10).

Results

Genotype distribution of DHFR gene 19 bp deletion of intron-1 is given in Table. Case mothers and the patient group demonstrated the distribution expected under Hardy-Weinberg.

DHFR gene 19 bp deletion in homozygous state was higher in the MMC group compared to control newborns [(Odds Ratio 2.4 (CI 95% 0.95-6.08)]. It brought a 2.4-fold risk [Odds Ratio 2.4 (CI 95% 1.04-5.6)] in NTD mothers compared to controls.

Discussion

Previous data revealed that MTHFR 677 C-T alteration did not increase the risk for the occurrence of spina bifida in our population (4,5). The combination of homozygous MTHFR 677T and homozygous DHFR gene 19 bp deletion was found only in one individual in controls and mothers, but in none in the MMC group (data not shown).

The C677T allele of the MTHFR gene and some other functional polymorphisms are reported as risk factors for spina bifida in some populations. However, despite extensive study, the genetic risk factors for spina bifida are incompletely understood. Polymorphic alleles that diminish bioavailability of reduced folate in the mother during pregnancy could contribute to spina bifida in her fetus, acting in the mother as teratogenic alleles. Johnson et al. (10) (2004) reported a polymorphic 19 bp deletion within intron-1 of DHFR, hypothesizing that DHFR deletion allele might decrease DHFR expression, revealing a risk for the spina bifida mothers who were found to carry intron-1 19 bp homozygous deletion allele compared to controls. However, two recent studies found the contrary, and one even showed a protective effect to the mothers (11,12). Our data is in concordance with Johnson et al.'s hypothesis showing that NTD mothers homozygous for 19 bp del have 2.4-fold greater risk compared to the controls in our population.

DHFR gene 19 bp deletion homozygosity and other possible mutations in the DHFR gene should be studied further, especially in folate-supplemented mothers with NTD recurrence. Until then, since about 75% of the NTDs are preventable with folate supplementation in the mother before conception, it is appropriate that folate supplementation should be undertaken irrespective of their genotypes.

Table. Distribution of DHFR -19 bp del in Turkish NTD mothers and patients.

	n	DHFR -19 bp del						
		W/W		W / 19 bp del		Homozygous 19 bp del		Frequency of 19 bp del
		n	%	n	%	n	%	
Meningomyelocele	69	28	40.57	30	43.5	11	15.94	37.6
Controls (newborn)	136	61	44.8	66	48.5	9	6.6	30.8
Mothers	104	40	38.46	49	47.1	15	14.42	37.9
Controls (mothers)	168	81	48.2	77	45.8	10	5.9	28.8

References

1. Campbell LR, Dayton DH, Sohal GS. Neural tube defects: a review of human and animal studies on the etiology of neural tube defects. Teratology 1986; 34: 171-8.

2. Akar N, Çavdar AO, Arcasoy A. High incidence of neural tube defect in Bursa, Turkey. Paediatric Perinat Epid 1988; 2: 89-92.

3. MRC Vitamin Study Research Group. Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. Lancet 1991; 338: 131-7.

4. Akar N, Akar E, Deda G, Arsan S. Spina bifida and common mutations at the homocysteine metabolism pathway. Clin Genet 2000; 57: 230-1.

5. Boduroglu K, Alikasifoglu M, Anar B, Tuncbilek E. 677 CT mutation on the methylenetetrahydrofolate reductase gene is not a risk factor for neural tube defects in Turkey. Arch Dis Child 1998; 78: 235.

6. Steegers-Theunissen RPM, Boers GHJ, Trijbels FJJM, Finkelstein JJD, Blom HJ, Thomas CMG. Maternal hyperhomocysteinemia: a risk factor for neural-tube defects? Metabolism 1994; 43: 1475-80.

7. Smithells RW, Sheppard S. Possible prevention of neural tube defects by periconceptional vitamin supplementation. Lancet 1980; 1: 647.

8. Wild J, Read AP, Sheppard S, Seller MJ, Smithells RW, Nevin NC. Recurrent neural tube defects, risk factors and vitamins. Archs Dis Child 1986; 61: 440-4.

9. Anderson AC. Targeting DHFR in parasitic protozoa. Drug Discovery Today 2005; 10(2): 121-8.

10. 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; 124A(4): 339-45.

11. Van der Linden IJ, Nguyen U, Heil SG, Franke B, Vloet S, Gellekink H et al. Variation and expression of dihydrofolate reductase in relation to spina bifida. Mol Genet Metab 2007; 91(1): 98-103.

12. Parle-McDermott A, Pangilinan F, Mills JL, Kirke PN, Gibney ER, Troendle J. The 19-bp deletion polymorphism in intron-1 of dihydrofolate reductase may decrease rather than increase risk for spina bifida in the Irish population. Am J Med Genet A 2007; 143(11): 1174-80.