Journal of Cell and Molecular Research (2014) 6 (2), 103-104

Letter to Editor

Impact of MTHFR and RFC-1 gene in the development of neural tube defect

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Received 03 Jun 2014 Accepted 07 Jul 2014

Summary

Neural tube defects (NTDs) are complex problem of central nervous system including brain and spinal cord. Anencephaly and myelomeningocele are the two most common forms of NTDs. Epidemiological studies reveal that genetic and environmental factors are responsible for the development of NTDs. During embryogenesis large numbers of extrinsic and intrinsic factors are responsible for the closure of neural tube which is responsible to maintain the three germ layers including neural ectoderm. The role of MTHFR and RFC-1 gene in etiopathology of NTDs has not been clearly defined in Indian population. Hence, the curiosity has been developed with the aim to evaluate folate metabolism and folate regulatory gene in clinically diagnosed NTDs by using PCR based DNA analysis with selected specific forward/reverse primers. Interestingly, the highest frequency (12.5%) of CT has been appeared of MTHFR C677T gene noticed in NTDs mother. We also observed the similar frequency of heterozygous AG genotype in NTDs of A80G RFC-1 gene. Therefore, C and A allele have high prevalence among than other genotype. However, the mutation in MTHFR partially have protective effect of embryo and these selected candidate folate markers are responsible to influence the cells of neural crest confirming the folding of neural tube associated with severity of disease in NTDs.

***Keywords*:** NTDs, MTHFR, RFC-1, Folate, Homocystine

Dear Editor…

Low folate intakes and impaired folate metabolism play major role in the etiology of neural tube defects (NTDs), which is complex problem of central nervous system including brain and spinal cord. Anencephaly and myelomeningocele are the two most common forms of NTDs. Epidemiological studies reveal that genetic and environmental factors are responsible for the development of NTDs1. Periconceptional folic acid supplementation diminishes the risk of NTDs in new born baby. Mutation in 5, 10 methylenetetrahydrofolatereductase (MTHFR; 677→CT) gene, reduced enzymatic activity and results in increased plasma homocysteine levels. Such defects can be lowered by supplementing folic acid in the diet. Another gene, reduced-folate carrier-1 (RFC-1), has a critical role in anti-folate transport with resistance and higher affinity to reduce the folate, including the physiological substrate 5-methyltetrahydrofolate and oxidized folic acid 2-3. Still it is not absolutely clear how genetic and epigenetic factors regulate neural tube folding during neurogenesis. We studied the genotypic distributions and allele frequencies of MTHFR C677T and RFC-1 A80G with level of folic acid, RBC and homocysteine in the clinically diagnosed NTDs and in their mothers.

Blood for mutation analysis was obtained after written informed consent from study subjects NTDs and their mothers. The study was approved by ethical committee of the Institute medical sciences, Varanasi, India. The study population comprised of 50 subjects with NTDs, and their mothers (n=50). The control group consisted of 59 healthy infants with their mothers (n=59). Genotype analysis of the blood samples were done using polymerase chain reaction based DNA analysis with selected specific forward/reverse primers and allele specific restriction digestion(Hinf-I and HhaI; MTHFRC677T andRFC-1 A80G respectively), according to the method described by Frosst et al4 and Chang et al 20005. The total homocysteine

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(tHcy) and folic acid in the NTDs cases from the plasma was determined by HPLC and fluorescence detector 6 and RBC investigated from central collection investigation laboratory, S.S. Hospital Varanasi.

Our data showed evidence for an association between the 677→C and A80G-RFC-1, with the occurrence of NTDs (table 1). The average concentration of plasma homocysteine are in higher (8.87 µmol/L) in the NTDs case as compared to the controls (5.65 µmol/L) whereas the level of folic acid and RBC are higher in controls than the cases. Interestingly, the highest frequency (12.5%) of CT has been appeared of MTHFR C677T gene noticed in NTDs mother and the similar frequency of heterozygous AG genotype in NTDs of A80G RFC-1 gene. Therefore, C and A alleles have high prevalence than other genotype. On the other hand, the mutations in MTHFR have partial protective effect on embryo and these selected candidates. Folate markers are responsible to influence the cells of neural crest, confirming the folding of neural tube associated with severity of disease in NTDs. Therefore, NTDs have multifactor origin of CNS disorders with common variant in more than one gene involved in folate and tHcy could interact to increase in infant's NTDs risk.

Table 1. Genotype and allele frequencies of MTHFR 677CT and RFC-1 in Indian subjects

Gene	**Genotype % frequency**			**Allele frequency**	
MTHFR C677T	***CC***	***CT***	***TT***	***C***	***T***
NTDs Cases	10	9.0	6.0	0.58	0.42
NTDs Mother	7.5	12.5	5.0	0.55	0.45
Control Child	24.7	7.0	2.9	0.81	0.18
Control Mother	27.1	6.4	1.1	0.87	0.12
RFC-1	***AA***	***AG***	***GG***	***A***	***T***
NTDs Cases	9	12.5	5.0	0.61	0.45
NTDs Mother	9	9.5	6.5	0.45	0.55
Control Child	7.0	6.4	1.1	0.29	0.06
Control Mother	25.3	7.0	2.3	0.83	0.16

Earlier studies have implicated susceptibility to NTDs due to the MTHFR and RFC-1 gene polymorphism. Folate supplement has potential effect in prevention and management of NTD and reduces the risk of the RFC-1 carrying variant. This study has important implications in the assessment of potential "risk factor" either due to folate deficient diet (nutritional factor) or unknown environmental factor responsible for NTDs and has been proven that mutant genotype has impact on the pregnancy outcome with possible maternal-foetal interaction. The results of this study indicate that 677→CT and RFC-1 mutation are responsible for NTDs in Indian patients.

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