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Original Article

Genetic analysis of *MTR* and *MTRR* gene polymorphisms in healthy mothers from Eastern part of India

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ABSTRACT: The polymorphic variation of MTR and MTRR genes can influence the folate metabolism and homeocysteine remethylation processes and consequently has strong impact on Neural Tube Defects (NTD). The SNP of MTR and MTRR genes has been reported in some populations, but in the Indian population has never been explored. This study is focused on the frequency distributions of rs1805087 (D919G/A2756G) and rs1801394 (I22M/A66G) of these genes in the West Bengal population, India for the first time. The G allele frequencies of rs1085087 and rs1801394 were 8% and 47% respectively. The G allele frequency of MTR rs1085087 in Indian population is less than the other major population; such as, in European (17%), American (18%), African (28%) and South Asian (32%) population. The G allele frequency of MTRR rs1801394 is quite like the European and south Asian population (G=52%) but greatly differs from African (25%), East Asian (26%) and mixed American (28%) population. This observation might help us to understand pattern of genetic alteration and development of single screening protocol for early detection and better prognosis in future.

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INTRODUCTION

The second most common complicated and multifactorial congenital malformation of central nervous system is Neural Tube Defects (NTD) [25, 28]. According to 2012 WHO report, three lakh babies were affected per year worldwide by NTD. The acquired or inherited pattern of NTD is strongly guided by both genetic and environmental factors which acted throughout the period of development of the foetus [17, 19].

Most of the studies reported that 1-28 days of gestational period is the crucial time for development of NTD during pregnancy. The incident rate of NTD is 1-2 per 1000 live birth worldwide [21,24] and in India is 2.41-4.1per 1000 live birth [2, 13]. In India the prevalence is higher in northern India (11 per 1000 birth) compare to southern India (5 per 1000 birth) [8].

Folic acid deficiency and defected methionine metabolism are two most reported cause for NTD development, which can be determined by high homocysteine levels during pregnancy [4]. Although peri-conceptional uptake of folic acid reduces the risk of NTDs by 50–70% [26]. The folate –homoecysteine metabolic pathway is controlled by several enzymes like MTHFR, DHFR, MTRR, MTR etc [10].

The MTR and MTRR gene is responsible for the conversion of homocysteine to methionine infolate-homeocystine metabolism pathway [4, 14]. The circulating form of folate in blood is 5-Methyltetrahydrofolate (5-methylTHF), which donate its methyl group to homocysteine, via the cofactor cob(I)alamin by the help of methionine synthase (MTR) enzyme. This Cob (I) alamin oxidized to cob(II)alamin which inactivates the cobalamin–MTR–enzyme complex.

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Methionine synthase reductase (MTRR) induced MTR activity by reductive methylation cob (II)alamin. Methionine amino acid plays an important role in protein synthesis and one-carbon metabolism and the synthesis of Methionine guided by enzyme methionine synthase. Reductive methylation of cobalamin by the enzyme MTR reductase (MTRR, EC 2.1.1.135) regenerates inactive MTR [5]. Normal functioning of MTR adversely affected by deficiency MTRR result in increased homocysteine levels and also methylation processes. Vitamin B12-dependent enzyme MTR (EC 2.1.1.13) controls the homocysteine levels and folate pools within a cell by the remethylation of homocysteine to methionine. Chromosomal location of human MTR gene 1q43 produces 1265 amino acid long polypeptide chain whose molecular weight is approximately 140.5 kDa. The nucleotide changes of MTR gene (MTR A2756G; rs1805087) have a great impact on the amino acid aspartic acid that changes into glycine (D919G). Most of the studies have reported that this A2756G polymorphism works as a risk factor for the development of NTD [1, 6, 7, 15, 18, 27, 29].

MTRR classified as the member of the ferredoxin NADP (+) reductase (FNR) family of electron transferases. Chromosomal location of human MTRR gene is 5p15.2-15.3.the nucleotide change of MTRR gene A66G (rs1801394) have an impact on the amino acid change isoleucine to methionine (I22M). Most of the studies have been reported that this polymorphism increases the risk for NTDs [18, 20, 22, 26, 29].

As stated above, rs1805087 and rs1801394 play significant role in NTDs. Aside, NTDs, we want to observe the normal distribution of these SNPs in healthy mothers, so later we can draw a rational correlation with the case population. In West Bengal, there is no report of allelic spectrum of these particular rs1805087 of MTR gene and rs1801394 of MTRR gene. Our study first time aimed to identify the frequency distributions of Genotypic as well as allelic of rs1805087 and rs1801394 in healthy Bengali mothers of residence of West Bengal, India.

METHODOLOGY

Collection of samples

In the present study 50 unrelated healthy mothers were identified and enrolled who were visiting the Department of Neonatology, Institute of Post Graduate Medical Education and Research (IPGME&R), SSKM Kolkata. They were selected by checking several parameters like socio-economic status, occupation, previous history of diabetes, neural disease or any other birth defects, age, body mass index (BMI), smoking habits, alcohol drinking by physical examination and personal interrogation for each of the individuals. The study has been started after achieving the ethical clearance from Ethical Committee of IPGME&R, SSKM Kolkata. Written informed consent was obtained from all adult participants included in this study [Memo No. Inst/IEC/2015/43]. Samples were then analyzed by isolating the Genomic DNA using QIAamp Blood Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. After the isolation, the concentrations and purity of DNA was measured in each sample using Nano-photometer light absorbance at 260 and 280 nm.

Subject exclusion criteria:

1. Babies with meningomyelocele, meningocele, encephalocele, anencephaly disorder.
2. Pregnant women with low folate and Hcy level.
3. Mothers with pre-existing medical disease (diabetes) [24]

Subject inclusion criteria:

1. All normal pregnant women.

Genotyping

Polymerase chain reactions (PCR) was done by using following PCR mixture and cycling program in Thermocyclerfor standardizing specific exon containing mentioned SNPs. The 25 μLofreaction mixture contained 40–100 ng of genomic DNA, 1.5mM $MgCl_2$, 100μM of each dNTP, 0.4μM of each primer, and 0.5 unit of Taq DNA polymerase (Applied Biosystems). The cycling program is maintained by denaturation at 95°C for 30 seconds, annealing at 58°C (for MTR) and 60°C (for MTRR) for 30 seconds, and extension at 72°C for 30 seconds were performed and these whole steps were repeated for 42 cycles. The conformation of the PCR product size was done by preparing 1.5% agarose gel followed by electrophoresis. PCR fragments would be checked and purified from agarose gel using QIAEX II (Qiagen, Hilden, Germany). Finally, the PCR products were subjected to direct sequencing to identify SNPs. Direct sequencing would be performed using Taq Dye Deoxy Terminator sequencing kit Applied Biosystems, City, USA with an ABI Prism 377 DNA Sequencer (PE Applied Biosystems). Pair wise sequence alignments were performed to find the best-matching piecewise (local) or global alignments of two query sequences using clustalW program both in database and controls. These following primers set were used during the PCR reaction (Table 1).

Table 1: Set of Primers for MTR and MTRR

rs1805087	
MTR F	5'CAGTGTTCCCAGCTGTTAGAT3'
MTR R	5'AGACACTGAAGACCTCTGATTTG3'
rs1801394	
MTRR F	5'CAAGTAGTTTCGAGCCGATCAT3'
MTRR R	5'GCTGGTGATATCTTACTATACCATTGA3'

Statistical analysis

The genotype and allele frequencies were determined by using GraphpadInstat 3 software and the genotype data analyzed by using Chi-square test. The comparison between observedand expected frequencies was done and tested the population for Hardy-Weinberg equilibrium. Furthermore, allele frequencies differences between West Bengal and other ethnic populations has been measured by using 1000 genome project. Haploview software (version 4.1. A) has-been used for the calculation of Linkage disequilibrium (LD) where *P*-value <0.05 was considered as statistically significant level.

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RESULT

In this study, most of the mothers showed rural inhabitation and few of them are devoted for cultivation purpose where as other are housewives and most of them are aged bellow 30. The details of the study population were mentioned in the Table 2.

The genetic study revealed that, 8 individuals (16.0%) were found heterozygous (A/G) carriers and 42 individuals (84.0%) with homozygous wild type (A/A) allele for rs1805087. We did not find any homozygous G/G genotype in the studied population regarding this polymorphism. The allele frequency for A is 92% and G is 8% for the rs1805087 (Table 3). The G allele frequency in West Bengal population (8%) shows a similar pattern with East Asia population (11%) but greatly differs from the other major populations; such as, European (17%), American (18%), African (28%) and South Asian (32%) populations. The genotype frequencies of rs1801394 homozygote wild type (A/A), heterozygote (A/G) and homozygote variant (G/G) were 30.0%, 46.0% and 24.0% respectively for MTRR gene (Table 3). The allele frequency for the A allele is 53% and 47% for the G allele, which is quite similar to the European and south Asian population (G=52%) but greatly differs from African (25%) East Asian (26%) and mixed American (28%) population. The studied polymorphisms were in Hardy-Weinberg equilibrium.

Table 2: Epidemiological Data: Socio-economic characters of study participants (mothers) [n=total no of healthy mother]

Epidemiological parameter	Number-50 (n %)
Location	
Rural	32(64)
Urban	18(36)
Age	
Bellow 30	43(86)
Above30	7(14)
Education	
Bellow 8th Standard	8(16)
8th pass	14(28)
10th pass	17(34)
12th pass	8(16)
Graduation	3(6)
Smoking habit	0(0)
Drinking habit	0(0)
Oral tobacco	0(0)
Occupation	
Working	2(4)
Housewife	48(96)

Table 3: Genotype and allele frequencies of MTR and MTRR genes obtained in the study population

Polymorphism	Genotype frequency %			Allele frequency %	
MTR (n=50)	**AA**	**AG**	**GG**	**A**	**G**
rs1805087 (2756 A> G)	84	16	0	92.0	8.0
MTRR(n=50)					
rs1801394 (66 A >G)	30	46	24	53.0	47.0

DISCUSSION

Polymorphism of genes leading to inter-individual genetic difference which can be translated in to abnormal folate metabolism considered to be an important cause for development of NTD. The polymorphisms in folate metabolism pathway genes and folate carrier genes also play crucial role in methylation process and several human congenital defects of central nervous system. Thus, we carried out a screening program in the population of West Bengal, India to find out the distribution of some specific SNPs that was reported earlier as a probable cause of NTD.

It is reported that A2756G (rs1805087) polymorphism of MTR gene is considered as a risk factor for spina bifida in the American population [7] and in some other population as well [9, 11]. In the Jordan population, association of AG genotype is frequently observed in mothers with NTD-affected children compared to AA genotype [1]. But in our study, AG genotype is present in 16% of control mother, while most of the mothers show the AA genotype (84%). European and Hispanic–American populations strongly claimed that A2756G polymorphism induces the risk for NTDs [6, 7, 10, 12, 18, 29]. On the other hand, UK and Netherlands populations did not show any association between the polymorphism and risk of NTD [27, 16].

Our result showed that the MAF for MTR; rs1805087 (A2756G) is 8 % where as in other population like white European, Irish, American Caucasians and African Americans shows that MAF is 21%,20%,17%,22% respectively, which are quite higher frequencies than our study [18, 22] (Table 4). In contrast the data obtained from the 1000 genome project shows that African, Mixed American European and South Asian have the MAF 28%, 18% 17%, 32%, respectively. (Table 5) This difference reflects a change in population origin. The individuals included in our study population belong to mixed ethnic background-Indo European and Austro Asiatic [3]. This might be the probable reason for the altered frequency of MTR; rs1805087 (A2756G) polymorphism in our study.

Several studies indicated that MTRR A66G polymorphism is strongly associated with NTD, whereas few studies from different population have failed to establish this association between A2756G polymorphism with NTD. Van der Linden, 2006, have found the association of NTDs with MTRR-A66G polymorphism in Caucasians population while Wilson *et al*, 1999 reported that Low vitamin B12 levels with MTRR-A66G polymorphism increases NTDs risk.

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Some studies reported that 1.9-fold to 3.1-fold increased risk for spina bifida conducted by A66G homozygous genotype [20, 26, 29]. On the contrary, in Irish and Indian populations, the A66G polymorphism was not associated with susceptible risk for NTDs [15].

Our results basically show the distribution of these SNPs in healthy mothers of West Bengal.In our control population, we got 46% bearing the AG allele, whereas 24% bear GG allele for A66G polymorphism.The A66G polymorphism (rs1801394) of MTRR in our healthy mother group shows the MAF 47%, which lies in between American Caucasians population and European (45%and 51%),Whereas moderately differ with white European (40%) and also greatly differ from African American (67%) population [25,18] (Table 4).Similarly when our study group compare with the others world population's data obtained from 1000 genome projects it has been found that MAF greatly differ with African, Mixed American, East Asian(25%, 28%, 26%) population but shows a pretty similar with European (52%) and South Asian (52%) (Table 5). This deviation in MAF with other populations to our studied group may be due to their different genetic hierarchy and ancestral pattern with different genetic composition as well as different ethnicity.

Table 4: Genotype and allele frequencies of MTRR and MTR polymorphisms in white European, Irish controls, American-Caucasians, and African-Americans [18, 20]

Population Name	n	Genotype Frequency (%)			Allele Frequency (%)	
		AA	AG	GG	A	G
MTR (A2756G) rs1805087						
Present study	50	**84**	**16**	**0**	**92.0**	**8.0**
White European	188	63	32	5	79	21
Irish controls	487	64	32	04	80	20
American-Caucasians	89	70	27	03	83	17
African-Americans	95	60	37	3	78	22
MTRR (A66G) rs1801394						
Present study		**30**	**46**	**24**	**53.0**	**47.0**
White European	184	22	54	24	49	51
Irish controls	476	37	47	16	60	40
American-Caucasians	91	32	47	21	55	45
African-Americans	96	11	44	45	33	67

According to Zhu *et al*. 2003, MTR A2756G polymorphism alone has no serious impact in formation of NTD but combined with MTRR A66G, it yielded NTD strongly as MTRR is the only responsible enzyme for the activation of MTR. In a line, van der Linden *et al* reported that, mothers with both the MTRR 66 GG and MTR 2756GG/AG genotypes had a 3 fold increased risk of spina bifida child, compared to 66AA/AG and 2756AA genotype [26]. However, our results revealed that, only 1 mother carrying MTRR 66GG + MTR 2756AG but not a single mother was found to carryMTRR 66 GG + MTR 2756GG genotype. This is probably; we included only normal individuals in our study.

Table 5: Allele frequencies of MTRR and MTR polymorphisms in other major population [30]

Population Name	n	Allele Frequency (%)	
		A	G
MTR (rs1805087)			
Present study	**50**	**92**	**8**
African	661	72	28
Mixed American	347	82	18
East Asian	504	89	11
European	503	83	17
South Asian	489	68	32
Bengali in Bangladesh	86	67	33
MTRR (rs1801394)			
Present study	50	**53**	**47**
African	661	75	25
Mixed American	347	72	28
East Asian	504	74	26
European	503	48	52
South Asian	489	48	52
Bengali In Bangladesh	86	47	53

To the best of our knowledge, this is the first study reported from eastern part of India that mapped the allele and genotype frequency distribution ofrs1805087 (D919G/A2756G) and rs1801394 (I22M/A66G). Hence, our study can function as a platform for future studies to evaluate the association of NTD with these variants in MTR and MTRR genes in ethnically distinct East Indian population.

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Conflicts of interest: None

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