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Original Research Article

Folate Metabolizing Genes Polymorphism in Mentally Retarded people of North Coastal Andhra Pradesh

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Abstract

Background: Mental Retardation (MR) is a genetic disorder manifested in childhood and is significantly characterized by decreased intelligence and adaptive skills. It is also the most common developmental disorder with overall below average intellectual functioning and deficits in adaptive behavior. MR in young children is often missed by clinicians. The condition is present in 2 to 3 percent of the population, either as an isolated finding or as part of a syndrome or broader disorder. ***Aim:*** The aim of the present study was to investigate the association of folate metabolizing gene variants MTHFR(C677T) & (A1298C); MTR(A2756G); MTRR(A66G); MTHFD1(G1958A) and RFC(A80G) in mental retarded (MR) cases and controls in North Coastal Andhra Pradesh. ***Methods:*** A total of 200 samples (100 MR cases and 100 controls) were included in the present study and genotyping was accomplished by using PCR - RFLP technique. Data was analyzed by SPSS software. ***Results:*** The odds ratio p value of the variant MTHFR C677T genotype CC and CT were statistically insignificant whereas TT genotype was found to be statistically significant. The odds ratio p value of the variant (A1298C) of MTHFR, MTR (A2756G), MTRR (A66G), and RFC1 (A80G) genes were not statistically significant with MR cases. The odds ratio p value of the variant MTHFD1 (G1958A) genotype AG was found to be showing small risk compared to AA and GG genotypes. ***Conclusions:*** The present study concludes that the MTHFR 677 C>T and MTHFDI genes shows association with MR and MTHFR 1298 A>C, MTRR A66G , MTR A2756G and RFC 1A80G genes does not shows any association with MR.

Keywords: MR, MTHFR, MTR, MTRR, MTHFD1, RFC1, Gene Polymorphism, Polymerase Chain Reaction, Restriction Fragment Length Polymorphism, Statistical Package of Social Sciences Software program.

INTRODUCTION

Mental Retardation (MR) is a genetic disorder manifested in childhood and is significantly characterized by decreased intelligence and adaptive skills. It is also the most common developmental disorder [1] with overall below average intellectual functioning and deficits in adaptive behavior. MR in young children is often missed by clinicians. The condition is present in 2 to 3 percent of the population, either as an isolated finding or as part of a syndrome or broader disorder [2]. In addition, many studies are based on broad categories of either severity (using labels such as mild, moderate, severe and profound MR) or etiology (utilizing the terms cultural or familial and organic MR).

The American Association on Intellectual and Developmental Disabilities (3) has defined MR as significant limitations both in intellectual functioning and in adaptive behavior, which covers many everyday social and practical skills. Intellectual limitations refer to an Intelligence Quotient (IQ) which falls two standard deviations below the population mean of 100 (<70), and adaptive functioning limitations refer to impairments in at least two out of ten skill areas [4].

According to World Health Organization [5], the true prevalence rate of total MR in industrialized countries comes close to 3% [6, 7], but in the United States controversy exists over whether the rate is 1 versus 3% [8, 9, 10 ,11] whereas the Scandinavian countries claim that the 1 % Figure is their true prevalence [12, 13]. An average of 2.5% of all children is mild and moderately retarded, and 0.5% are severely retarded. A number of environmental, genetic or multiple factors can cause Mental retardation.

Unfortunately, in approximately 30 to 50 percent of cases, the etiology is not identified even after thorough diagnostic evaluation [14, 15].

Down Syndrome (DS), also known as trisomy 21, is a genetic disorder occurring in approximately 1 in 650 to 1000 live births [16]. It is the most common genetic cause of Mental retardation accounting for 25-30% worldwide [17].

Since [18] hypothesised that abnormal folate metabolism, due to a polymorphism involved in folate metabolism gene, might increase the risk for chromosome 21 non-disjunction in young mothers, the relationship between maternal gene polymorphisms involved in folate metabolism and the risk of having a DS offspring has aroused people's attention in recent years. But the results remain conflicting, making it difficult to clarify the nature of the maternal SNPs' contribution to the risk of having a DS offspring.

The folate metabolism begins with the intake of folic acid through diet. It is rapidly reduced to its active form, tetrahydrofolate, which converts to 5,10 methylenetetrahydrofolate by the enzyme methylenetetrahydrofolate reductase, encoded by the MTHFR gene [19] . This substrate is vital for nucleic acids metabolism, including those necessary for synthesis of nucleotides and consequent cell division [20]. The product of this reaction is methyl groups used for the synthesis of methionine, necessary for DNA methylation. Subsequently, the enzyme methioninesynthase, encoded by the MTR gene, catalyzes the remethylation of homocysteine to methionine, necessary for the production of S-adenosylmethionine, the universal methyl donor. Vitamin B12 acts as a cofactor for methylation [21]. It becomes oxidized over time and the enzyme methionine synthase is inactivated. Functional regeneration of this methionine synthase requires the participation of another enzyme, methioninesynthase reductase, which is encoded by the MTRR gene [22]. In the folate metabolic pathway, the methylenetetrahydrofolate dehydrogenase (MTHFD) gene encodes a trifunctional enzyme that catalyzes the conversion of tetrahydrofolate to 10-formyl, 5,10-methenyl, and 5,10-methylenetetrahydrofolate. Another important protein involved in this metabolism is folate-transporting protein (RFC 1), transporting 5-methyltetrahydrofolate into the cells.

The impact of folic acid intake on pregnancy outcome, however, is modified by polymorphisms in both the maternal and fetal genes that code for enzymes involved in folate metabolism [23]. Data on the genotype and allele frequencies of MTHFR C677T polymorphism in DS mothers compared to non‐DS mothers are numerous and show significant differences, but reports regarding distribution of MTHFR mutant allele and genotype frequencies in DS individuals are very few [24, 25, 26].

From the literature it is revealed that Down Syndrome is the most common genetic cause of mental retardation occurring for 25% to 30% worldwide. After cytogenetic analysis of the present study out of 100MR patients 24(24%) Down syndrome cases were observed. So the present study was carried out to evaluate the folate metabolising genes polymorphisms as a risk factor for the occurrence of DS cases. Several clinical and experimental studies have hypothesized/reported that patients with DS have disturbed one‐carbon metabolism [18, 27]. t‐Hcy, vitamin B‐12and folate are metabonutritional factors directly related to this metabolism. These factors along with the associated genetic polymorphisms might aggravate the age related MR in Down syndrome.

MATERIALS AND METHODS

The present study was carried out with100 MR (55 males and 45 females) from Labenshiff Mentally Handicapped, Visakapatnam; Asramdham Manovikas Kendram, Anakapalli; Behara Manovikas Kendram, Srikakulum and 100 age and sex matched controls (63 males and 37 females) above 25 years from North Coastal Andhra Pradesh during the period 2011-2013. The study was approved by the institutional ethical committee for blood sample collection. The informed consent was obtained from each and every participant for taking blood sample for the evaluation of the folate metabolizing gene polymorphisms of MTFR C677T; MTHFR A1298C; MTRA 2756G; MTRR A66G; MTHFD G1958A and RFC-1.

The DNA was isolated by salting out method for the analysis of folate metabolizing gene variants MTHFR(C677T) & (A1298C); MTR(A2756G); MTRR (A66G); MTHFD1 (G1958A) and RFC (A80G) by the PCR-RFLP technique. The data was analysed by using SPSS software for calculating genotype and allele frequencies.

RESULTS

Table 1: Genotype and allele frequencie s of MTHFR (677C>T) MTHFR (A1298C) in MR cases and Controls

Genes	Genotypes and alleles	MR cases		Controls		ODDS RATIO (CI 95%)	p value
		n (100)	%	n (100)	%		
MTHFR C677T	CC	46	44.89 %	56	58.52 %	0.669 (0.38-1.169)	0.078^{NS}
	CT	42	44.22 %	41	35.96 %	1.042 (0.5937-1.829)	0.442^{NS}
	TT	12	10.89 %	3	5.52 %	4.409 (1.205-16.14)	0.007^{**}
	C	134	67 %	153	76.5 %		
	T	66	33 %	47	23.5 %	-	0.034^{*}
MTHFR A1298C	AA	36	38.44 %	42	43.56 %	0.776 (0.4395 -1.373)	0.192^{NS}
	AC	52	47.12 %	48	44.88 %	1.174 (0.6739 - 2.044)	0.285^{NS}
	CC	12	14.44 %	10	11.56 %	1.227 (.5044 - 2.986)	0.325^{NS}
	C	124	62%	132	66%	-	0.202^{NS}
	A	76	38%	68	34 %		

Table 1 describes the genotypic frequencies of MTHFR (677 C>T) in MR cases and controls. The genotypic frequencies of CC was higher in controls (58.52%) than MR cases (44.89%) whereas the frequencies of genotype CT (44.22%) and TT (10.89%) were higher in MR cases than frequencies of genotypes CT (35.96%) and TT (5.52%) in controls. The odds ratio for TT genotype conferred 4.409 times higher risk for MR cases compared to CC and CT genotypes. The odds ratio p value of genotype CC and CT were statistically insignificant whereas TT genotype was found to be statistically significant.

The frequency of C allele was 67% in MR cases and 76.5% in controls. The T allele frequencies in MR cases and controls was 33% and 23.5% respectively. The Chi square p value reveal that MTHFR 677 C>T gene shows association with MR.

Table 2: Genotype and allele frequencies of MTRR A66G and MTR A2756G in MR cases and Controls.

Genes	Genotypes and alleles	MR cases		Controls		ODDS RATIO (IC 95%)	p value
		n (100)	Frequency	n(100)	Frequency		
MTRR A66G	AA	42	42.25 %	52	51.13 %	0.668 (0.3825 - 1.168)	0.078^{NS}
	AG	46	45.5 %	39	40.75 %	1.332 (0.7594 - 2.338)	0.159^{NS}
	GG	12	12.25 %	09	8.12 %	1.379 (0.5536 - 3.434)	0.244^{NS}
	G	130	65 %	143	71.5 %		
	A	70	35 %	57	28.5 %	-	0.081^{NS}
MTR A275G	AA	39	43.56 %	35	37.21 %	1.187 (0.6683 - 2.109)	0.279^{NS}
	AG	54	44.88 %	52	47.58 %	1.084 (0.6218 - 1.888)	0.388^{NS}
	GG	07	11.56 %	13	15.21 %	0.5037 (0.1921 - 1.321)	0.078^{NS}
	G	132	66 %	122	61 %		
	A	68	34 %	78	39 %	-	0.150^{NS}

The genotypic frequencies of MTHFR A1298C in MR cases and controls .The genotypic frequency of AA was higher in controls (43.56%) than MR cases (38.44%).The frequencies of genotypes AC (47.12%) and CC(14.44%) were higher in MR cases than frequencies of genotypes AC (44.88%) and CC (11.56%) in controls. The odds ratio for AC and CC genotypes were showing small risk compared to AA genotype. The odds ratio p value of the variant A1298C of MTHFR gene was not statistically significant with MR cases.

The frequency of C allele was 62% in MR cases and 66% in controls. The A allele frequencies MR cases and controls was 38% and 34% respectively. The Chi square p value reveal that MTHFR 1298 A>C gene does not seen to exhibit significant association with MR.

Table 2 shows genotypic frequencies of MTRR A66G in MR cases and controls. The genotypic frequency of AA was higher in controls (51.13%) than MR cases (42.25%) whereas the frequencies of AG (45.5%) and GG (2.25%) was higher in MR cases than the frequencies of genotypes AG (40.75%) and GG (8.12%) in controls. The odds ratio for AG and GG genotypes were found to be showing small risk compared to AA genotype. The odds ratio p value of the variant MTRR A66G was statistically insignificant with MR cases.

The frequency of G allele was 65% in MR cases and 71.5% in controls. The A allele frequencies in MR cases and controls was 35% and 28.5 % respectively. The Chi square p value reveals that MTRR A66G gene does not shows association with MR.

The genotypic frequencies of MTR A2756G in MR cases and controls. The genotypic frequency of AA was higher in MR cases (43.56%) than controls (37.21%) whereas the frequencies of genotypes AG (47.58%) and GG (15.21%) were higher in controls than frequencies of genotypes AG (44.88%) and GG (11.56%) in MR cases. The odds ratio for AG and GG genotypes were found to be showing small risk when compared to AA genotype .The odds ratio p value of the variant MTR A2756G gene was not statistically significant with MR cases.

The frequencies of G allele was 66% in MR and 61% in controls .The A allele frequencies in MR cases and controls was 34% and 39% respectively. The Chi square p value of MTR A2756G gene does not shows association with MR.

Table-3: Genotypic frequencies of MTHFD1 G1958A in MR cases and Controls.

Genes	Genotypes and alleles	MR cases		Controls		ODDS RATIO (IC 95%)	p value
		n (100)	%	n (100)	%		
MTHFD1 G1958A	GG	37	33.64%	51	44.22%	0.5643 (0.3209 - 0.9921)	0.023^{NS}
	AG	42	48.72%	31	44.56%	1.612 (0.9018 - 2.881)	0.053^{NS}
	AA	21	17.64%	18	11.22%	1.211 (0.6006 - 2.442)	0.296^{NS}
	G	116	58%	133	66.5%	-	0.039^{*}
	A	84	42%	67	33.5%		
RFC1 A80G	AA	18	18.92%	21	19.8%	0.825 (0.4096 - 1.665)	0.296^{NS}
	AG	51	49.15%	47	49.4%	1.174 (0.6739 - 2.044)	0.285^{NS}
	GG	31	31.92%	32	30.8%	0.954 (0.5257 - 1.734)	0.439^{NS}
	G	87	(43.5%)	89	(44.5%)	-	0.42^{S}
	A	113	(56.5**%**)	111	(55.5%)		

Table 3 represents the genotypic frequencies of MTHFD G1958A in MR cases and controls. The genotypic frequency of GG (44.22%) was higher in controls then MR cases (33.64%) whereas the frequencies of genotypes AG (48.72%) and AA (17.64%) were higher in MR cases than frequencies of genotypes AG (44.56%) and AA (11.22%) in controls .The odds ratio for AG and `genotypes were found to be showing small risk compared to GG genotype. The odds ratio p value of genotypes GG and AG were statically significant whereas AA genotype was found to be statically insignificant.

The frequency of G allele was 58% in MR cases and 66.5% in controls. The A allele frequencies in MR cases and controls were 42% and 33.5% respectively. The chi-square p value reveal that MTHFDI gene seem to exhibit significant association with MR.

The genotypic frequencies of RFC1 A80G in MR cases and controls. The genotype frequency of AA was higher in controls (19.8%) than MR cases (18.92%).The genotype frequency of MR cases (49.15%) shows slight variation with the frequency of controls (49.4%) whereas the genotype frequency of GG was higher in MR cases (31.92%) than controls (30.8%) .The odds ratio for AG genotype was found to be showing small risk compared to AA and GG genotypes. The odds ratio p value of the variant A80Gof RFC gene was not statistically with MR cases.

The frequency of G allele was 43.5% in MR cases and 44.5% in controls. The A allele frequencies in MR cases and controls was 56.5% and 55.5% respectively .The Chi square p value reveals that RFC 1A80G gene does not seem to exhibit significant association with MR.

Table 4: MTHFR (C677T) polymorphism and its interaction with othe polymorphisms of MTHFR 1298, MTRR, MTR, MTHFD1 and RFC 1 genes in MR cases and Controls.

MTHFR C677T CC v/s				
	MR	**Controls**	**OR**	**p-value**
	n=100	**n=100**	**(95% CI)**	
MTHFR A1298C				
AA	24	34	0.613 (0.3305-1.137)	0.05*
AC	30	40	0.6429 (0.3579, 1.155)	0.06
CC	08	09	0.8792 (0.3249, 2.379)	0.39
MTRR A66G				
AA	18	20	0.878 (0.4328, 1.781)	0.359
AG	28	30	0.9074 (0.4925, 1.672)	0.37
GG	09	07	1.314 (0.4695, 3.677)	0.30
MTR A2756G				
AA	22	28	0.7253 (0.381, 1.381)	0.16
AG	42	39	1.133 (0.6438, 1.993)	0.33
GG	05	10	0.4737 (0.1559, 1.439)	0.08
MTHFD1 G1958A				
GG	22	32	0.5994 (0.3183, 1.128)	0.05*
GA	26	09	3.553 (1.568, 8.047)	0.0007**
AA	12	07	1.812 (0.6823, 4.811)	0.11
RFC1 A80G				
GG	05	12	0.386 (0.1307, 1.14)	0.03*
GA	25	39	0.5214 (0.2846, 0.955)	0.01*
AA	22	28	0 .72 (0.381, 1.381)	0.16

MTHFR C677T CT+TT v/s

MTHF R A1298C				
AA	12	08	1.568 (0.6119, 4.019)	0.17
AC	22	08	3.244 (1.368, 7.692)	0.002**
CC	04	01	4.125 (0.4529, 37.57)	0.08
MTRR A66G				
AA	24	32	0.6711 (0.3602, 1.25)	0.10
AG	18	09	2.22 (0.9449, 5.213)	0.03*
GG	03	02	1.515 (0.2478, 9.269)	0.32
MTR A2756G				
AA	17	07	2.721 (1.075, 6.887)	0.01*
AG	12	13	0.9126 (0.3945, 2.111)	0.41
GG	02	03	0.6599 (0.1079, 4.036)	0.32
MTHFD1 G1958A				
GG	15	19	0.7523 (0.3582, 1.58)	0.22
GA	16	22	0.6753 (0.3307, 1.379)	0.14
AA	09	11	0.8002 (0.3163, 2.024)	0.31
RFC1A80G				
GG	13	09	1.511 (0.6147,3.713)	0.18
GA	26	08	4.041 (1.728, 9.448)	0.0003**
AA	09	04	2.374 (0.7063, 7.977)	0.07

n = number of alleles, p value = probability value of the statistical test,
**p< 0.01 - Highly significant, p<0.05 - Significant, NS - Not Significant

Table 4 explains the MTHFR C677T polymorphism and its interaction with other polymorphisms of MTHFR A1298C, MTRR A66G,MTR A2756G, MTHFD1G1958A and RFC1 A80G genes in Mental retardation cases and controls. From the above table, it is evident that the CC genotype of MTHFR C677T gene polymorphism was highly significant with GA genotype of MTHFD1 G1958A gene. The CC genotype of MTHFR C677T gene polymorphism was statistically significant with AA genotype of MTHFR A1298CGG genotype of MTHFD1 G1958A, GG and GA genotypes of RFC 1A80G genes, whereas AC and CC genotypes of MTHFR A1298C, AA,AG and GG genotypes of MTRR A66G,AA,AG and GG genotypes of MTR A2756G, AA genotype of MTHFD1 G1958A, AA

genotype of RFC 1A80G genes were statistically insignificant with CC genotype of MTHFR C677T gene polymorphism.

The CT and TT genotypes of MTHFR C677T gene polymorphism was highly significant with AC genotype of MTHFR A1298C and GA genotype of RFC1 A80G genes. The CT and TT genotypes of MTHFR C677T gene polymorphism was statistically significant with AG genotype of MTRR A66G and AA genotype of MTR A2756G genes, whereas AA and CC genotypes of MTHFR A1298C,AA and GG genotypes of MTRR A66G, AG and GG genotypes of MTR A2756G,GG,GA and AA genotypes of MTHFD1 G1958A,GG and AA genotypes of RFC1 A80G genes were statistically insignificant with CT and TT genotypes of MTHFR C677T gene polymorphism.

Table-5: MTHFR A1298C polymorphism and its interaction with other polymorphisms of MTHFR677, MTRR, MTR, MTHFD1 and RFC 1genes in mental retardation cases and controls.

	MR	**Controls**	**OR**	**p-value**
	n=100	**n=100**	**(95% CI)**	
MTHFR A1298C AA v/s				
MTHFR C677T				
CC	18	34	0.4261 (0.2209, 0.8218)	0.004**
CT	20	22	0.8864 (0.4486, 1.751)	0.36
TT	08	02	4.261 (0.8818, 20.59)	0.02*
MTRR A66G				
AA	04	20	0.1667 (0.05472, 0.5076)	0.0002**
AG	04	10	0.375 (0.1136, 1.238)	0.04*
GG	06	02	3.128 (0.6159, 15.88)	0.07
MTR A2756G				
AA	08	16	0.4565 (0.1858, 1.121)	0.04*
AG	05	20	0.2105 (0.07561, 0.5862)	0.0006**
GG	02	11	0.1651 (0.03562, 0.7653)	0.004**
MTHFD1 G1958A				
GG	05	30	0.1228 (0.04537, 0.3324)	0.000001**
GA	05	15	0.2982 (0.104, 0.8553)	0.009**
AA	02	04	0.4898 (0.08767, 2.737)	0.20
RFC1 A80G				
GG	02	07	0.2711 (0.05492, 1.339)	0.04*
GA	04	20	0.1667 (0.05472, 0.5076)	0.0002**
AA	02	13	0.1366 (0.02998, 0.6222)	0.001**
MTHFR C677T CT+TT v/s				
MTHF R A1298C				
AA	12	08	1.568 (0.6119, 4.019)	0.17
AC	22	08	3.244(1.368, 7.692)	0.002**
CC	04	01	4.125(0.4529, 37.57)	0.08
MTRR A66G				
AA	24	32	0.6711(0.3602, 1.25)	0.10
AG	18	09	2.22(0.9449, 5.213)	0.03*
GG	03	02	1.515(0.2478, 9.269)	0.32
MTR A2756G				
AA	17	07	2.721(1.075, 6.887)	0.01*
AG	12	13	0.9126(0.3945, 2.111)	0.41
GG	02	03	0.6599(0.1079, 4.036)	0.32
MTHFD1 G1958A				
GG	15	19	0.7523(0.3582, 1.58)	0.22
GA	16	22	0.6753(0.3307, 1.379)	0.14
AA	09	11	0.8002(0.3163, 2.024)	0.31
RFC1 A80G				
GG	13	09	1.511(0.6147, 3.713)	0.18
GA	26	08	4.041 (1.728, 9.448)	0.0003**
AA	09	04	2.374(0.7063, 7.977)	0.07

n = number of alleles, p value = probability value of the statistical test,
**p< 0.01 - Highly significant, p<0.05 - Significant, NS - Not Significant

Table 5 illustrates the MTHFR A1298C polymorphism and its interaction with other polymorphisms of MTHFR C677T, MTRR A66G, MTR A2756G, MTHFD1 G1958A and RFC1 A80G genes in Mental retardation cases and controls. From the above table, it is clear that AA genotype of MTHFR A1298C gene polymorphism was highly significant with CC genotype of MTHFR C677T, AA genotype of MTRR A66G,AG and GG genotypes of MTR A2756G,GG and GA genotypes of MTHFD1 G1958A,GA and AA genotypes of RFC1 A80G genes. The AA genotype of MTHFR A1298C gene polymorphism was statistically significant with TT genotype of MTHFR C677T, AG genotype of MTRR A66G,AA genotype of MTR A2756G, GG genotype of RFC1 A80G genes, whereas CT genotype of MTHFR C677T,GG genotype of MTRR A66G,AA genotype of MTHFD1 genes were statistically insignificant with AA genotype of MTHFR A1298C gene polymorphism.The AC and CC genotypes of MTHFR A1298C was highly significant with AG genotype of MTR A2756G, GA genotype of MTHFD1 G1958A, GA genotype of RFC1 A80G genes. The AC and CC genotypes of MTHFR A1298C was statistically significant with AG genotype of MTRR A66G, AA genotype of MTR A2756G, GG genotype of MTHFD1 G1958A,AA genotype of RFC1A80G genes, whereas AA,AC and CC genotypes of MTHFR C677T, AA,GG genotypes of MTRR A66G, GG genotype of MTR A2756G,AA genotype of MTHFD1 G1958A, GG genotype of RFC1 A80G were statistically insignificant with AC,CC genotypes of MTHFR A1298C gene polymorphism.

DISCUSSION

In the last few years, a number of studies have evaluated a possible link between polymorphism in maternal folate metabolising genes and Downs syndrome [28, 29].

In 1999, on the basis of evidence that abnormal folate and methyl metabolism can lead to DNA hypomethylation and abnormal chromosomal segregation, [18] hypothesised that the MTHFR 677C>T polymorphism might be a risk factor for maternal meiotic chromosome 21 non-disjunction and DS risk in the offspring. The MTHFR enzyme is constituted by dimers in humans, each of which has the catalytic domain harbouring position 222 and the regulatory domain harbouring position 429, and two common polymorphisms changing the amino acids at those regulatory positions are known to reduce MTHFR activity: MTHFR 677C>T (Ala222Val) and MTHFR 1298A>C (Glu429Ala).

In the present study, the C allele frequency of MTHFR C677T variant in MR cases and controls was 67% and 76.5% respectively. The T allele frequency in MR cases and controls was 33% and 23.5% respectively. The Chi square p value reveal that MTHFR 677 C>T gene shows association with MR. In the study of [26], the C allele frequency of MTHFR C677T variant in MR cases and controls was 91% and 90% respectively. The T allele frequency in MR cases and controls was 9% and 10% respectively. The Chi square p value of MTHFR 677 C>T gene does not shows association with MR. In the study of (30), the C allele frequency of MTHFR C677T variant in MR cases and controls was 59.8% and 52.8% respectively. The T allele frequency in MR cases and controls was 40.2% and 47.2% respectively. The Chi square p value of MTHFR 677 C>T gene does not shows association with MR. In the study of [31], Chi square p value of MTHFR 677 C>T gene does not shows association with MR.

In the present study, the C allele frequency of MTHFR A1298C variant in MR cases and controls was 62% and 66% respectively. The A allele frequency of MR cases and controls was 38% and 34% respectively. The chi square p value reveal that MTHFR 1298 A>C gene does not seem to exhibit significant association with MR.

In the study of [30], the C allele frequency of MTHFR A1298C variant in MR cases and controls was 36.1% and 23.6% respectively. The A allele frequency of MR cases and controls was 63.9% and 76.4% respectively. The chi square p value reveal that MTHFR 1298 A>C gene seems to exhibit significant association with MR.

In the study of [31], the chi square p value reveal that MTHFR 1298 A>C gene seems to exhibit significant association with MR.

In the present study, the G allele frequency of MTRR A66G variant in MR cases and controls was 65% and 71.5% respectively. The A allele frequency in MR cases and controls was 35% and 28.5 % respectively. The chi square p value reveals that MTRR 66 A>G gene does not shows association with MR. In the study of [30], the G allele frequency of MTRR A66G variant in MR cases and controls was 48.3% and 53.1% respectively. The A allele frequency in MR cases and controls was 51.6% and 46.9 % respectively. The chi square p value reveals that MTRR 66 A>G gene does not shows association with MR

In the present study, the G allele frequency of MTR A2756G variant of MR cases and controls was 66% and 61% respectively. The A allele frequency in MR cases and controls was 34% and 39% respectively. The chi square p value of MTR A2756G gene does not shows association with MR. In the study of [30], the G allele frequency of MTR A2756G variant in MR cases and controls was 18.9% and 10.2% respectively. The A allele frequency in MR cases and controls was 81.1% and 89.8 % respectively. The chi square p value reveals that MTRR 66 A>G gene does not shows association with MR. In the study of [31], The chi square p value

reveals that MTR A2756G gene does not shows association with MR.

Polymorphism in the methylenetetrahydrofolate dehydrogenase gene, which is involved in folate metabolism, affect maternal risk for Down syndrome. There is evidence that some mothers of infants with DS have abnormal folate and methyl metabolism, as well as mutations in folate genes, which are features that are also seen in neural-tube defects [18, 23, 28].

In the present study, the G allele frequency of MTHFD1 G1958A variant in MR cases and controls was 58% and 66.5% respectively. The A allele frequency in MR cases and controls was 42% and 33.5% respectively. The chi-square p value reveals that MTHFDI gene shows significant association with MR.

Common polymorphism in RFC 1 gene is A80G substitution leading to change of an adenine to guanine. The neural tube defects and Down syndrome are influenced by the same genetic determinants of folate metabolism [32].

In the present study, the G allele frequency of RFC 1 A80G variant in MR cases and controls was 43.5% and 44.5% respectively. The A allele frequency in MR cases and controls was 56.5% and 55.5% respectively .The chi square p value reveals that RFC 1 A80G gene does not seems to exhibit significant association with MR.

The studies of [30, 31, 26] failed to reveal any association between MTHFR C677T polymorphism and risk of being DS child unlike the present study. The present study shows association with MTHFR C677T polymorphism, which correlates with the study of [33].The present study does not shows association with MTHFR A1298C, contradictory to the studies of [30, 31, 33] identified significant association with MTRR A66G unlike the present study and studies of [33].The present study and studies of [30, 31] failed to found any association with the variant MTR A2756G.

In the present study for the variant MTHFR C677T, the genotypic frequency of CC was higher in controls (58.52%) than MR cases (44.89%) whereas the frequencies of genotype CT (44.22%) and TT (10.89%) were higher in MR cases than frequencies of genotypes CT (35.96%) and TT (5.52%) in controls. The odds ratio for TT genotype conferred 4.409 times higher risk for MR cases compared to CC and CT genotypes. The odds ratio p value of genotypes CC and CT were statistically insignificant whereas TT genotype was found to be statistically significant for the variant MTHFR C677T.In the study of [26] the genotypic frequencies of CC in controls was (81.25%) and in MR cases (81.25%) whereas the frequencies of genotype CT in MR cases and controls were 18.75% and 17.19% and the frequencies of genotype TT were (0%) in MR and 1% in controls. The odds ratio p value was statistically insignificant for the variant MTHFR C677T. In the study of [31] the genotypic frequencies of CC,CT and TT in MR cases were 41.07%, 42.86% and 16.07% respectively for the variant MTHFR C677T .

In the present study, for the variant MTHFR A1298C the genotypic frequency of AA was higher in controls (43.56%) than MR cases (38.44%).The frequencies of genotypes AC (47.12%) and CC(14.44%) were higher in MR cases than frequencies of genotypes AC (44.88%) and CC (11.56%) in controls. The odds ratio for AC and CC genotypes were showing small risk compared to AA genotype. The odds ratio p value of the variant 1298A>C of MTHFR gene was not statistically significant with MR cases. In the study of [31] the genotypic frequencies of AA,AC and CC in MR cases were 66.07%, 25% and 8.93% respectively for the variant 1298A>C.

In the present study, for the variant MTRR A66G the genotypic frequency of AA was higher in controls (51.13%) than MR cases (42.25%) wheras the frequencies of AG (45.5%) and GG (2.25%) were higher in MR cases than the frequencies of genotypes AG (40.75%) and GG (8.12%) in controls. The odds ratio for AG and GG genotypes were found to be showing small risk compared to AA genotype. The odds ratio p value of the variant MTRR 66A>G was statistically insignificant with MR cases

In the present study, for the variant MTR A2756G the genotypic frequency of AA was higher in MR cases (43.56%) than controls (37.21%) wheras the frequencies of genotypes AG (47.58%) and GG (15.21%) were higher in controls than frequencies of genotypes AG (44.88%) and GG (11.56%) in MR cases. The odds ratio for AG and GG genotypes were found to be showing small risk when compared to AA genotype .The odds ratio p value of the variant MTR A2756G gene was not statistically significant with MR cases. In the study of [31], the genotypic frequencies of AA, AG and GG in MR cases were 69.64%, 25% and 5.36% respectively for the variant MTR A2756G.

In the present study, for the variant MTHFD1 G1958A the genotypic frequency of GG (44.22%) was higher in controls then MR cases (33.64%) wheras the frequencies of genotypes AG (48.72%) and AA (17.64%) were higher in MR cases than frequencies of genotypes AG (44.56%) and AA (11.22%) in controls. The odds ratio for AG and AA` genotypes were found to be showing small risk compared to GG genotype. The odds ratio p value of genotypes GG and AG were statically significant wheras AA genotype was found to be statically insignificant for the variant MTHFD 1G1958A

In the present study, for the variant RFC 1A80G the genotype frequency of AA was higher in controls (19.8%) than MR cases (18.92%).The AG genotype frequency of MR cases(49.15%) shows slight variation with the frequency of controls (49.4%) wheras the genotype frequency of GG was higher in MR cases (31.92%) than controls (30.8%) .The odds ratio for AG genotype was found to be showing small risk compared to AA and GG genotypes .The odds ratio p value of the variant RFC1 A80G gene was not statistically significant with MR cases. In the study of [31], the genotypic frequencies of AA, AG and GG in MR cases were 18.2%, 69.1% and 12.7% respectively for the variant RFC1 A80G gene.

Differences in allele distribution and in gene nutrition and gene gene interaction in the different series already published suggest that the impact of these polymorphisms varies in function of the geographical area and that other determinants affecting the methylation pathway and the homocysteine metabolism remains to be identified.

In summary, the accumilated evidence has indicated association between MTHFR 677 C>T and MTHFD G1958A gene polymorphisms and Mental retardation and lack of association found between MTHFR 1298A>C, MTRR 66A>G, MTR A2756G and RFC-1 A80G gene polymorphisms and MR. In the present study, the combination of one or more of these polymorphisms, however results in an increased risk in DS, suggesting the existence of a synergistic relationship among them in a multifactorial way. Interaction of MTHFRC677T polymorphism with other polymorphisms of MTHFR A1298C, MTRR A66G,MTR A2756G, MTHFD 1G1958A, RFC 1A80G and the interaction of MTHFR A1298C polymorphism with other polymorphisms of MTHFR C677T, MTRR A66G, MTR A2756G, MTHFD 1G1958A, RFC 1A80G showed the risk in DS child in the present study.

In conclusion, the description of folate metabolic polymorphism as a risk factor provides an additional argument for suggesting that genetic polymorphisms that affect the one-carbon metabolism may be contributing risk factors for mothers having a DS child and DS cases. Differences in allele distribution and in gene-nutrition and gene-gene interaction in the different series already published suggest that the impact of these polymorphisms varies in function of the geographical area and that other determinants affecting the methylation pathway and the homocysteine metabolism remains to be identified.

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