Tzu Chi Medical Journal 2022; 34 (4): 456-461

Original Article

Role of folate metabolizing genes and homocysteine in mothers of Down syndrome children

*Amandeep Kaur[a], Anupam Kaur[b]**

[a]Department of Microbiology, Government Medical College, Amritsar, Punjab, India,
[b]Department of Human Genetics, Guru Nanak Dev University, Amritsar, Punjab, India

Submission : 15-Sep-2021
Revision : 18-Oct-2021
Acceptance : 27-Oct-2021
Web Publication : 04-Mar-2022

Abstract

Objectives: Folates are essential nutrients required for the synthesis of DNA/RNA in cell division and segregation. Folates are reduced and methylated in the liver with the help of enzymes such as methylenetetrahydrofolate reductase (MTHFR), MTR MTRR, reduced folate carrier 1, and cystathionine-β-synthase. Variants in the genes encoding these enzymes may lead to hypomethylation, resulting in nondisjunction which in turn increases the risk for Down syndrome (DS). The present study was conducted to genotype these genes and to see their association with homocysteine levels. **Materials and Methods:** A total of 213 mothers having DS children and 220 mothers having normal children were enrolled in the study. Genomic DNA was isolated from lymphocytes followed by polymerase chain reaction/ Restriction Fragment Length Polymorphism for genotyping. Homocysteine levels were checked by chemoassay utilizing coumarin-based fluorescent probe. **Results:** Genotypic frequency of MTHFR 1298 A > C polymorphism was significantly different among cases and controls (χ^2 = 5.83, P = 0.01), presence of C instead of A allele provided protection against DS in mothers (odds ratios = 0.57, 95% confidence interval = 0.35–0.91, P = 0.01). Higher levels of homocysteine were independently associated with the risk of having DS child (P = 0.0001). **Conclusion:** Homocysteine acted as an independent risk factor in the present study and was not associated with folate metabolizing gene variants.

Keywords: *Cystathionine β synthase, Down syndrome, Homocysteine, Methionine synthase reductase*

Introduction

Genetic disorders are the leading cause of infant morbidity and mortality affecting nearly 8% of all conceptions, the common among them is Down syndrome (DS). DS results from the presence of an extra chromosome 21, in most of the cases, nondisjunction leads to trisomy 21 in the mother during meiosis I or II with an incidence of 1/600–1000 live births [1].

Folates are essential nutrients required for the distribution of genetic material during cell division and regulate segregation and other processes. After intestinal absorption, folate metabolism requires reduction and methylation into the liver to form 5-methylenetetrahydrofolate and then released into the blood for cellular uptake, where it can be used for the synthesis of DNA and RNA [2]. The most important enzyme, methylenetetrahydrofolate reductase (MTHFR) regulates the conversion of homocysteine (hcy) to methionine, and the enzyme methionine synthase (MTR) catalyzes the remethylation of homocysteine to methionine and tetrahydrofolate [3]. Methionine synthase reductase (MTRR) catalyzes the regeneration of methylcobalamin, a cofactor of MTR. Thus, MTR activity is maintained by MTRR. Folate transporting proteins are also important in the maintenance of DNA methylation. The reduced folate carrier 1 (RFC I) is the protein located in the intestinal mucosa membrane and plays a role in the folic acid absorption and transportation of 5-MTHFR into the cells. If the folate pathway is impaired, the transsulfuration pathway condenses homocysteine with serine to form cystathionine in the reaction catalyzed by enzyme cystathionine-β-synthase (CBS). Variations in the genes encoding enzymes alter their activity, resulting in hypomethylation which is a possible risk factor for nondisjunction [2]. The present study was conducted to find out the association of SNPs in these genes and homocysteine levels were measured in mothers of DS children (MDS). To

Access this article online

Quick Response Code:

Website: www.tcmjmed.com

DOI: 10.4103/tcmj.tcmj_258_21

*Address for correspondence: Dr. Anupam Kaur, Department of Human Genetics, Guru Nanak Dev University, Amritsar - 143 005, Punjab, India.
E-mail: anupamkaur@yahoo.com

How to cite this article: Kaur A, Kaur A. Role of folate metabolizing genes and homocysteine in mothers of Down syndrome children. Tzu Chi Med J 2022;34(4):456-61.

the best of our knowledge, this is the first study evaluating the role of these polymorphisms from the Punjab region.

Materials and methods

The study enrolled 213 MDS after confirming trisomy 21, and 220 mothers having normal children (without any miscarriage) as controls. Informed consent and approval of the institutional ethical committee were obtained before all the investigations (EC-94/HG dated 9.1.2014.). Detailed family history and pedigree analysis were done. Genomic DNA was isolated from peripheral blood lymphocytes by the phenol extraction method [4] with modifications. Polymerase chain reaction was carried out using specific primers [Table 1] followed by restriction digestion.

Homocysteine measurement

To measure the levels of homocysteine, fasting serum samples were collected from MDS and controls. Samples were then stored at −80°C till further analysis.

Reagents

D,L-Homocysteine was purchased from Sigma Chemicals Co. (St. Louis MO, USA). Sodium borohydride, 10% meta-phosphoric acid, NaOH, EDTA, n-amyl alcohol were obtained from SRL Pvt. Ltd. India.

Deproteinization of serum: To 400 μL serum, 100 μL of freshly prepared 1.43 M sodium borohydride solution (1.5 μM EDTA, 66 mM NaOH and 10 μL n-amyl alcohol) was added. These were mixed and incubated in the water bath at 40°C for 30 min and 250 μL of ice-cold 10% of meta-phosphoric acid was added to precipitate the proteins. After keeping it on ice for 30 min, centrifuged at 21000 g for 15 min to separate the upper phase and filtered it through 0.2 μ filter [5].

Fluorescence measurement of serum samples

The coumarin-based fluorescent probe 1 was synthesized using the described procedure in the literature [6]. Serum (150 μL) from MDS was mixed with an equal amount of cold acetonitrile. The standard calibration curve was prepared by measuring the emission intensity of standard samples containing 10, 20, 50, 100, 150, 200, and 250 μM of homocysteine using fluorescence spectroscopy. The fluorescent intensities of the serum samples were plotted in the standard calibration graph and calculated the homocysteine level of the samples.

Statistical analysis

Genotypic and allelic frequencies were calculated under the assumption of Hardy's Weinberg Equilibrium. Chi-square test was employed to evaluate the relationship between cases and controls, and to estimate the relative risk for DS, odds ratios (OR) at 95% confidence interval (CI) was calculated. To check the difference in the levels of homocysteine between cases and controls, Student's *t*-test and ANOVA were used to analyze gene-environment interaction. All the analyses were performed using SPSS software (Statistical Package for the Social Sciences Inc. 20, Chicago, IL, USA).

Results

Genotype frequencies and Down syndrome risk

In the present study, 213 cases and 220 controls were enrolled and analyzed. Individual analysis of genotype frequencies among MTHFR 677 C > T, MTR 2756 A > G, MTRR 66 A > G, RFC I 80 G > A, and CBS844ins68 showed no difference among cases and controls. Genotype frequency of MTHFR 1298 A > C showed a significant difference between cases and controls (χ^2=5.83, P = 0.01). The allele frequency, OR = 0.53, 95% CI = 0.32–0.87, P = 0.01 indicated that substitution of alanine instead of glutamine provided protection against the occurrence of the disorder [Table 2].

Diplotype analysis

In diplotype analysis, gene-gene interaction showed that presence of MTHFR 677 CT/CBS-/+ and MTR2756 AA/RFC I 80 GA combinations in an individual significantly increases the risk of DS child (OR = 3.89, 95% CI = 1.06–14.24, P = 0.04 and OR = 1.89, 95% CI = 1.02–3.48, P = 0.04). On the other hand, the presence of MTHFR 1298 AC/MTR 2756 AA and MTHFR 1298 AC/CBS-/+ combination in an individual confers protection against the birth of DS child [Table 3].

Haplotype analysis

Haplotype analysis was performed between MTHFR 677 C > T/1298 A > C and the results indicated that both SNPs are not in linkage disequilibrium [Tables 4 and 5].

Table 1: Primer sequences for markers and their respective enzymes

Genes	Primer sequences	Annealing temp (°C)	Enzyme	Fragment size (bp)
MTHFR C677T	F5'TGAAGGAGAAGGTGTCTGCGGGA-3'	62	Hinf I	C - 198
	R5'- AGGACGGTGCGGTGAGAGTG-3'			T - 175,23
MTHFR A1298C	F5'CTTTGGGGAGCTGAAGGACTACTA3'	60	Mbo II	A - 56,31,30,28,18
	R5'CACTTTGTGACCATTCCGGTTTG3'			C - 84,31,30,18
MTR A2756G	F5'CAGTGTTCGCAGCTGTTAGATG3'	60	Hae III	A - 252
	R5'GGGAACTTAAGACACTGAAGGCCTCTG3'			G - 169,83
MTRR A66G	F5'CAGCAGGGACAGGCAAAGGCCATCGCAGAAGACAT3'	58	Nde I	A - 196
	R5'CTGGTGATATCTTACTATACCATATGAACAAACAC3'			G - 230
RFC I G80A	F5'AGTGTCACCTTCGTCCC3'	58	Hha I	G - 125.68,37
	R5'TCCCGCGTGAAGTTCTTG3'			A - 162,68
CBS844ins68	F5'CTGCCTTGAGCCCTGAAGCC3'	60	-	DD - 174
	R5'CTGGACTCGACCTACCGTCCT3'			ID - 242,174

MTHFR: Methylenetetrahydrofolate reductase, MTR: Methionine and enzyme methionine synthase, MTRR: Methionine synthase reductase, RFC 1: Reduced folate carrier 1, CBS: Cystathionine-β-synthase

Table 2: Distribution of genotype and allele frequency of methylenetetrahydrofolate reductase, methionine and enzyme methionine synthase, methionine synthase reductase, reduced folate carrier 1 and cystathionine-β-synthase 844ins68 genes

Genotype	Cases	Controls	χ^2	Alleles	Cases	Controls	OR	*P* (95% CI)
MTHFR C677T								
CC	163 (76.53)	178 (80.91)	3.68	C	372 (87.32)	390 (88.64)	1.32	0.09 (0.95-1.85)
CT	46 (21.59)	34 (15.46)	0.159					
TT	4 (1.88)	8 (3.64)		T	96 (12.68)	76 (11.36)		
MTHFR A1298C								
AA	183 (85.91)	168 (76.36)	5.83	A	396 (92.96)	388 (88.18)	0.57	0.01 (0.35-0.91)
AC	30 (14.09)	52 (23.64)	0.01					
CC	0	0		C	30 (7.04)	52 (11.82)		
MTR A2756G								
AA	101 (47.42)	113 (51.37)	0.69	A	304 (71.36)	324 (73.64)	1.12	0.45 (0.82-1.51)
AG	102 (47.89)	98 (44.55)	0.71					
GG	10 (4.69)	9 (4.09)		G	122 (28.64)	116 (26.36)		
MTRR A66G								
AA	8 (3.76)	8 (3.64)	0.07	A	146 (34.27)	153 (34.77)	1.02	0.88 (0.77-1.35)
AG	130 (61.03)	137 (62.27)	0.97					
GG	75 (35.21)	75 (34.09)		G	280 (65.73)	287 (65.23)		
RFC I G80A								
GG	55 (25.82)	66 (30)	1.09	G	238 (55.87)	254 (57.73)	0.93	0.58 (0.71-1.21)
GA	128 (60.09)	122 (55.46)	0.58					
AA	30 (14.08)	32 (14.55)		A	188 (44.13)	186 (42.27)		
CBS 844ins68								
DD	171 (80.28)	177 (80.46)	0.006	D	384 (90.14)	394 (90.23)	1.00	0.99 (0.64-1.57)
DI	42 (19.72)	43 (19.55)	0.94					
II	0	0		I	42 (9.86)	43 (9.77)		

MTHFR: Methylenetetrahydrofolate reductase, MTR: Methionine and enzyme methionine synthase, MTRR: Methionine synthase reductase, RFC 1: Reduced folate carrier 1, CBS: Cystathionine-β-synthase, OR: Odds ratio, CI: Confidence interval

Table 3: Gene-gene interactions between different genotypes studied

Combinations	Cases	Controls	OR	95% CI	*P*
MTHFR C677T/CBS844ins68					
CTID	11	3	3.89	1.06-14.24	0.04
MTR A2756G/RFC I G80A					
AAGA	69	57	1.89	1.02-3.48	0.04
MTHFR A1298C/MTR A2756G					
ACAA	12	25	0.48	0.23-1.00	0.05
MTHFR A1298C/CBS844ins68					
ACID	2	13	0.15	0.03-0.67	0.01

MTHFR: Methylenetetrahydrofolate reductase, MTR: Methionine and enzyme methionine synthase, OR: Odds ratio, CI: Confidence interval

Homocysteine analysis

Statistically significant difference was observed in levels of homocysteine in cases and controls which suggested that homocysteine acted as an independent risk factor for the birth of DS child [Table 6]. To assess the influence of genetic variations in folate metabolizing genes on homocysteine levels, ANOVA was carried out. There was no significant association between homocysteine levels and SNPs [Table 7].

Discussion

The variations in genes encoding enzymes involved in folate metabolism play an important role in the etiology of birth defects. Gene expressions are regulated by DNA or histone methylation, thus hypomethylation of DNA/histone affects DNA repair, replication, expression, segregation, chromatin conformation, leading to disease conditions. MTHFR is a key enzyme that plays a critical role in DNA/RNA synthesis and methylation pathways. In its homozygous condition, it is responsible for vascular events, NTDs, bad obstetric history, and possibly Down syndrome. Among North Americans, younger mothers with 677 TT were at higher risk of having DS baby [7].

Many studies have suggested that TT genotype could be considered as a risk factor for DS [8,9]. However, Tayeb [10], Kaur and Kaur [11], Coppedè *et al*. [12], and Cretu *et al*. [13] did not observe any association between cases and controls, but the presence of the MTHFR 677T allele slightly increased the risk for DS child in mothers. In the present study, MTHFR 1298 A > C was significantly associated with DS, conferring protection against the disorder, similar to the reports in the literature [14,15]. On the contrary, according to Scala *et al*. [16] presence of 1298A > C allele escalates 2.29 fold risk for the birth of DS. However, Izci *et al*. [8], da Silva *et al*. [17], Santos-Rebouças *et al*. [18], Sukla *et al*. [19] reported that MTHFR 1298 A > C was not associated with the risk of DS child.

The presence of linkage disequilibrium provided evidence that both SNPs (MTHFR 677 C > T and 1298 A > C) have strong interaction with protein stability and activity [16,20]. Scala *et al*. [16] reported that in their population T-C haplotype significantly increased the risk of DS child among mothers. Biselli *et al*. [21] reported the absence of T-C haplotype in

the Brazilian population suggesting negative selection of this haplotype. In this study, the C-C haplotype conferred risk for the birth of DS child [Figure 1].

Another variant in the folate metabolic pathway is MTR 2756 A > G and it has been suggested that the presence of MTR 2756 AG + GG genotype increased the risk of DS child by 3.5 folds [22]. In the present study, no association was observed between DS and this genotype and similar results have been reported [7,12,17,19]. However, Coppedè *et al*. [12] noticed that MTR 2756 G allele frequency among Europian and Brazilian ranged from 18% to 21% whereas Liao *et al*. [23] reported allele frequency of <10% among Asians. Moustafa *et al*. [24] observed that mothers with MTR 2756 G genotype could be considered at risk for DS in Egyptians.

There are limited studies on the variant MTRR 66 A > G but has been found to be associated with DS [1,25]. We did not observe any association between the SNP and the risk of DS and these results are in conformity with other studies [7,21].

Scala *et al*. [16] did not find any association between disease and RFC I 80 G > A genotype but suggested that the presence of RFC I G > A allele increased 2.05 folds risk of DS child. Wang *et al*. [1] and Coppedè *et al*. [26] observed that the presence of RFC I G > A genotype in women increased the risk of delivering DS child. We did not observe any association between the risk of DS child and the presence of RFC I 80 G > A.

CBS enzyme in the transsulfuration pathway converts homocysteine to cystathionine, thus regulating homocysteine levels [27] which otherwise leads to homocysteinemia. More than 17 mutations have been identified in the CBS gene and 68 bp insertion in the coding region of exon 8 is most common. Some studies have reported that CBS844ins68 polymorphism was not associated with the risk of birth of DS child [16,20,28]. Similarly, the nonsignificant association of CBS844in68 polymorphism with the risk of DS child was observed in the present study when analyzed independently.

When studied in combination, MTHFR 677 and MTHFR 1298, MTR 2756, MTRR 66, RFC I 80, and CBS844ins68, these may manifest their effect and result in increased risk of DS offspring. MTR 2756 A > G in amalgamation with other variants (MTHFR 677, 1298, MTRR 66 A > G and CBS844ins68) increased the maternal risk up to 1.2 and 1.7 folds, respectively [17,21,29]. Similarly, Brandalize *et al*. [30] also suggested that the presence of these genotypes confers 4.8–6.9 folds risk for DS. The combination of MTR2756 AA + MTHFR 677 TT genotype elevated the risk by 3.0 folds for DS child [15]. A diplotype analysis showed that MTHFR 677 CC when combined with MTR 2756 (AG or GG), elevated the risk by 6.7 folds and when MTR 2756 AA unites with MTHFR 677 (CT or TT), it confers 4.2 folds risk [23]. In 2006, Coppedè *et al*. [25] observed a slight increase in maternal risk for DS child when mothers carried RFC I 80GG/MTHFR 677TT and reduced risk when carried RFC I 80 (AA or AG)/MTHFR 1298AA. In our study, MTHFR CT genotype when combined with CBS844ins68+/-genotype, the risk for the syndrome increased by 3.89 folds and when MTR 2756 AA amalgamated with RFC I 80 AG, the risk escalated to 1.89 folds. On the other side, MTHFR 1298 AC in alliance with MTR 2756 AA and CBS844ins68 provided protection against DS.

Homocysteine, Vitamin B12, and folate are metabolic and nutritional factors directly related to the folate

Table 4: Haplotype analysis between methylenetetrahydrofolate reductase C677T/A1298C

Haplotype	Frequency	Case ratio	Control ratio	χ^2	*P*
CA	0.794	345/81	342/96	1.041	0.3077
TA	0.112	52/375	45/393	0.702	0.4021
CC	0.085	28/399	46/392	4.583	0.0323

Table 5: Linkage disequilibrium between two markers of single gene

Gene	SNPs	Cases			Controls		
		D'	LOD	R^2	D'	LOD	R^2
MTHFR	C677T/A1298C	0.555	0.12	0.002	0.115	0.01	0.0

MTHFR: Methylenetetrahydrofolate reductase, SNP: Single-nucleotide polymorphism, LOD: Logarithm of the odds

Table 6: Comparison of means (student *t*-test) of homocysteine levels between cases and controls

Cases	Controls	Difference	95% CI	*T*	Df	*P*
104	109	9.05	8.93-9.17	145.17	211	0.0001

CI: Confidence interval

Table 7: Comparison of serum homocysteine levels with different genotypes

Genes	1/1[a]	1/2[b]	2/2[c]	*F*	*P*
MTHFR C677T	15.7±6.3	15.2±4.5	13.25±3.7	0.38	0.69
MTHFR A1298C	15.14±5.7	17.44±5.8	-	2.13	0.15
MTR A2756G	14.39±5.9	16.17±5.3	18.03±8.07	1.7	0.19
MTRR A66G	16.33±4.5	15.20±5.6	15.68±6.15	0.15	0.86
RFC I G80A	17.02±5.4	14.45±6.2	16.19±4.9	2.1	0.13
CBS844ins68	15.56±6.1	15.36±5.2	-	0.01	0.92

MTHFR: Methylenetetrahydrofolate reductase, MTR: Methionine and enzyme methionine synthase, MTRR: Methionine synthase reductase, RFC 1: Reduced folate carrier 1, CBS: Cystathionine-β-synthase, a: Homozygous wild; b: Heterozygous mutant; c: Homozygous mutant

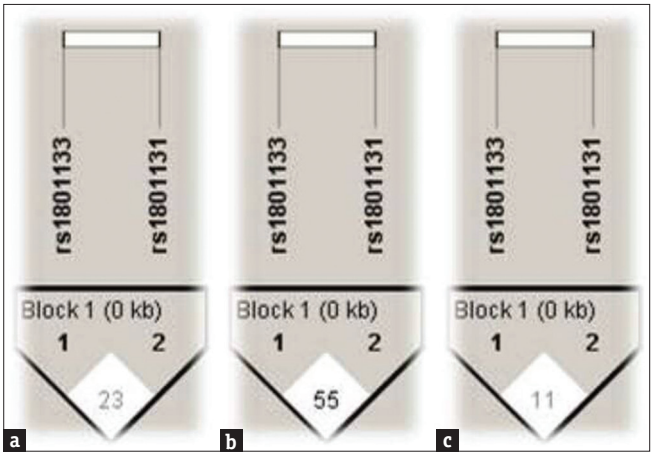

Figure 1: LD plot for methylenetetrahydrofolate reductase gene polymorphisms (a) LD plot for case–control combined (b) LD plot for cases (c) LD plot for controls

pathway and alterations in their concentrations may lead to disturbance in folate metabolism. Various studies have shown that genetic polymorphisms influence the plasma homocysteine concentration either directly or by affecting the folate concentration. Maternal diet before or during conception provides necessary folates to complete the process of meiosis thus, inadequate intake of folic acid or impaired metabolism resulted in abnormal chromosome 21 recombination and malsegregation. A recent study compared the use of folic acid supplements among 702 mothers having DS child due to nondisjunction and 983 mothers having normal children, revealed that lack of folic acid or impaired folate metabolism is associated with risk of birth of DS child [31].

Maintenance of methylation pattern through folate cycle required the action of gene products and micronutrients such as vitamin B12, B6, and methionine obtained from the diet. These elements participate in the conversion of homocysteine to methionine, later the precursor of S-adenosyle methionine (SAM), main methyl donor for DNA, RNA, and protein. SAM, changes into S-adenosylhomocystiene by donating methyl group, whose end product is homocysteine. Thus, the presence of genetic variants alters the enzymatic activity required to convert homocysteine to methionine, predispose to genetic instability, abnormal recombination, and malsegregation. The effect of these variants depends upon the folate status of an individual and high folic acid and other dietary nutrients neutralize the effect of homocysteine and SNPs. Furthermore, variants that reduce the availability of folate/hcy in mothers before or during pregnancy have been observed to be associated with abnormalities such as NTDs, cleft palate, Down syndrome. In our previous study, various risk factors associated with Down syndrome have been studied [32] and maternal age in DS cases was found to be 27.34 ± 5.2 years, while in controls, it was 27.75 ± 4.9 years indicating that the majority of the DS children were born to younger mothers.

Significantly higher levels of homocysteine were observed in the present study which is consistent with the recent report by Kedar and Chadel [33]. However, the association of genotypes and homocysteine levels among cases was not significant in the present study; in contrast to the report suggesting significant levels in the presence of MTHFR 677TT [29]. The elevated levels of hcy are due to insufficient folic acid or reduced absorption of folates in MDS that ultimately affects the Vitamin B12 status. In other words, complex gene-environment interaction that involves maternal diet, lifestyle and genotype could result in risk for DS child [17,19,21,22]. To overcome this food fortification should be made mandatory, which is being recommended by the Indian government to improve nutrition in children, pregnant women, and lactating mothers through various programs.

Limitations of our study

(a) It is restricted only to Punjab; (b) analysis of gene-environment interaction was not done which would otherwise increase the power of the study.

Conclusion

This is the first report from Punjab analyzing six SNPs and their association with homocysteine levels. The alliance of SNPs was significantly associated either with the risk or protection of having DS child and homocysteine came out to be independent risk factor among women. However, individual SNPs and their association with hcy did not provide risk. A larger sample size is required to see gene-gene and gene-nutrient interaction and will help in establishing the link between disease and genotype and awareness about intake of folic acid either orally or in fortified food products, thus providing opportunities to create new strategies to improve public health and prevent the birth of DS.

Acknowledgment

A special thanks to Dr. Vandana Bhalla (Professor) and Dr. Shahi Imam Reja, Department of Chemistry, for their help and guidance.

Financial support and sponsorship

We gratefully acknowledge the support from (DST) grant and fellowship (SR/WOS-A/LS-348/2013) awarded to Amandeep Kaur.

Conflicts of interest

There are no conflicts of interest.

References

1. Wang SS, Wang C, Qiao FY, Lv JJ, Feng L. Polymorphisms in genes RFC-1/CBS as maternal risk factors for Down syndrome in China. Arch Gynecol Obstet 2013;288:273-7.
2. Nazki FH, Sameer AS, Ganaie BA. Folate: Metabolism, genes, polymorphisms and the associated diseases. Gene 2014;533:11-20.
3. Coppedè F, Grossi E, Migheli F, Migliore L. Polymorphisms in folate-metabolizing genes, chromosome damage, and risk of Down syndrome in Italian women: Identification of key factors using artificial neural networks. BMC Med Genomics 2010;3:42.
4. Adeli K, Ogbonna G. Rapid purification of human DNA from whole blood for potential application in clinical chemistry laboratories. Clin Chem 1990;36:261-4.
5. Melnyk S, Pogribna M, Pogribny I, Hine RJ, James SJ. A new HPLC method for the simultaneous determination of oxidized and reduced plasma aminothiols using coulometric electrochemical detection. J Nutr Biochem 1999;10:490-7.
6. Yap AC, Mahamad UA, Lim SY, Kim HJ, Choo YM. A coumarin-based fluorescent probe as a central nervous system disease biomarker. Sensors (Basel) 2014;14:21140-50.
7. James SJ, Pogribna M, Pogribny IP, Melnyk S, Hine RJ, Gibson JB, et al. Abnormal folate metabolism and mutation in the methylenetetrahydrofolate reductase gene may be maternal risk factors for Down syndrome. Am J Clin Nutr 1999;70:495-501.
8. Izci Ay O, Ay ME, Erdal ME, Cayan F, Tekin S, Soylemez F, et al. Folate metabolism gene polymorphisms and risk for Down syndrome offspring in Turkish women. Genet Test Mol Biomarkers 2015;19:191-7.
9. Jaiswal SK, Sukla KK, Mishra SK, Lakhotia AR, Kumar A, Rai AK. Association of genetic polymorphisms in genes involved at the branch point of nucleotide biosynthesis and remythylation with Down syndrome birth risk – A case-control study. J Mol Genet Med 2016;10:207.
10. Tayeb MT. The methylenetetrahydrofolate reductase gene variant (677 C-T) in risk mothers with Down syndrome among Saudi population. Egypt J Med Hum Genet 2012;13:263-8.
11. Kaur A, Kaur A. Prevalence of methylenetetrahydrofolate reductase 677

C-T polymorphism among mothers of Down syndrome children. Indian J Hum Genet 2013;19:412-4.

12. Coppedè F, Bosco P, Lorenzoni V, Migheli F, Barone C, Antonucci I, et al. The MTR 2756A>G polymorphism and maternal risk of birth of a child with Down syndrome: A case-control study and a meta-analysis. Mol Biol Rep 2013;40:6913-25.
13. Cretu R, Neagos D, Radoi VE. Clinical study regarding the link between cystathionine β synthase 844ins68 polymorphism and maternal risk for Down syndrome. GINECO.eu 2013;9:15-8.
14. Meguid NA, Dardir AA, Khass M, Hossieny LE, Ezzat A, El Awady MK. MTHFR genetic polymorphism as a risk factor in Egyptian mothers with Down syndrome children. Dis Markers 2008;24:19-26.
15. Coppedè F. The complex relationship between folate/homocysteine metabolism and risk of Down syndrome. Mutat Res 2009;682:54-70.
16. Scala I, Granese B, Sellitto M, Salomè S, Sammartino A, Pepe A, et al. Analysis of seven maternal polymorphisms of genes involved in homocysteine/folate metabolism and risk of Down syndrome offspring. Genet Med 2006;8:409-16.
17. da Silva LR, Vergani N, Galdieri Lde C, Ribeiro Porto MP, Longhitano SB, Brunoni D, et al. Relationship between polymorphisms in genes involved in homocysteine metabolism and maternal risk for Down syndrome in Brazil. Am J Med Genet A 2005;135:263-7.
18. Santos-Rebouças CB, Corrêa JC, Bonomo A, Fintelman-Rodrigues N, Moura KC, Rodrigues CS, et al. The impact of folate pathway polymorphisms combined to nutritional deficiency as a maternal predisposition factor for Down syndrome. Dis Markers 2008;25:149-57.
19. Sukla KK, Jaiswal SK, Rai AK, Mishra OP, Gupta V, Kumar A, et al. Role of folate-homocysteine pathway gene polymorphisms and nutritional cofactors in Down syndrome: A triad study. Hum Reprod 2015;30:1982-93.
20. Zampieri BL, Biselli JM, Goloni-Bertollo EM, Vannucchi H, Carvalho VM, Cordeiro JA, et al. Maternal risk for Down syndrome is modulated by genes involved in folate metabolism. Dis Markers 2012;32:73-81.
21. Biselli JM, Goloni-Bertollo EM, Zampieri BL, Haddad R, Eberlin MN, Pavarino-Bertelli EC. Genetic polymorphisms involved in folate metabolism and elevated plasma concentrations of homocysteine: Maternal risk factors for Down syndrome in Brazil. Genet Mol Res 2008;7:33-42.
22. Bosco P, Guéant-Rodriguez RM, Anello G, Barone C, Namour F, Caraci F, et al. Methionine synthase (MTR) 2756 (A -> G) polymorphism, double heterozygosity methionine synthase 2756 AG/methionine synthase reductase (MTRR) 66 AG, and elevated homocysteinemia are three risk factors for having a child with Down syndrome. Am J Med Genet A 2003;121A: 219-24.
23. Liao YP, Bao MS, Liu CQ, Liu H, Zhang D. Folate gene polymorphism and the risk of Down syndrome pregnancies in young Chinese women. Yi Chuan 2010;32:461-6.
24. Moustafa M, Gaber E, Fath GA. Methionine synthase A2756G and reduces folate carrier 1 A80G gene polymorphisms as maternal risk factor for Down syndrome in Egypt. Egypt J Med Hum Genet 2016;17:217-21.
25. Coppedè F, Bosco P, Lorenzoni V, Denaro M, Anello G, Antonucci I, et al. The MTRR 66A>G polymorphism and maternal risk of birth of a child with Down syndrome in Caucasian women: A case-control study and a meta-analysis. Mol Biol Rep 2014;41:5571-83.
26. Coppedè F, Lorenzoni V, Migliore L. The reduced folate carrier (RFC-1) 80A>G polymorphism and maternal risk of having a child with Down syndrome: A meta-analysis. Nutrients 2013;5:2551-63.
27. Zhang J, Handy DE, Wang Y, Bouchard G, Selhub J, Loscalzo J, et al. Hyperhomocysteinemia from trimethylation of hepatic phosphatidylethanolamine during cholesterol cholelithogenesis in inbred mice. Hepatology 2011;54:697-706.
28. Yang M, Gong T, Lin X, Qi L, Guo Y, Cao Z, et al. Maternal gene polymorphisms involved in folate metabolism and the risk of having a Down syndrome offspring: A meta-analysis. Mutagenesis 2013;28:661-71.
29. Jiajin L, Shuyan C, Ying W, Junxiao C, Xiudi W. Genetic polymorphisms in folate metabolism as risk for Down syndrome in the southern China. J Matern Fetal Neonatal Med 2019;32:2030-5.
30. Brandalize AP, Bandinelli E, Dos Santos PA, Schüler-Faccini L. Maternal gene polymorphisms involved in folate metabolism as risk factors for Down syndrome offspring in Southern Brazil. Dis Markers 2010;29:95-101.
31. Hollis ND, Allen EG, Oliver TR, Tinker SW, Druschel C, Hobbs CA, et al. Preconception folic acid supplementation and risk for chromosome 21 nondisjunction: A report from the National Down Syndrome Project. Am J Med Genet A 2013;161A: 438-44.
32. Kaur A, Kaur A. Assessment of risk factor associated with Down syndrome. J Pediatr Assoc India 2020;9:24-30.
33. Kedar R, Chandel D. MTHFR gene polymorphism and associated nutritional deficiency in the etiology and pathogenesis of Down syndrome. Egypt J Med Hum Genet 2019;20:12.