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MTHFR gene polymorphism and associated nutritional deficiency in the etiology and pathogenesis of Down syndrome

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Abstract

Background: Our aim was to evaluate the influence of *methylenetetrahydrofolate reductase* (MTHFR) gene polymorphism on maternal risk for Down syndrome (DS) and observe the impact of this polymorphism on folate, homocysteine, and vitamin B_{12} concentrations and their association with pregnancy outcome in addition to malformations in DS offspring.

Results: The prevalence of MTHFR gene polymorphism at 677 positions in mothers of DS children (DSM) ($n = 118$) was compared with control mothers (CM) who were age matched with normal children and no history of spontaneous abortion (SA) ($n = 118$). The MTHFR gene polymorphism was detected using the PCR-RFLP method. MN frequency was measured by CBMN assay and folate; homocysteine and vitamin B_{12} were measured using the biochemical analyzer. All statistical analyses were carried out using the chi-square test and *t* test by using GraphPad Prism 7.0 software.
Heterozygous (C/T) genotype was highly significant ($p < 0.001$) in DSM occurring at 64.4 %, while only 33% CM showed C/T genotype, with an odds ratio of 4.1. Significantly lower levels of folate ($p < 0.01$), vitamin B_{12} ($p < 0.001$), and higher levels of homocysteine ($p < 0.01$) were found in DSM compared to CM. The MN frequency was highly significant ($p < 0.001$) in DSM with C/T genotype when compared to CM. Within DSM, significantly higher ($p < 0.001$) MN frequencies were observed in DSM with C/T genotype than DSM with C/C genotype. This shows the susceptibility of chromosome malsegregation leading to DS in these women. In addition, the frequency of SA in DSM with C/T genotype was significantly higher ($p < 0.01$). The DS children showed significantly higher rates of congenital heart defect, preterm birth and low birth weight when mother had C/T genotype.

Conclusion: The present study supports the association of MTHFR C677T with DS risk and the above mentioned associated abnormalities in the child. We suggest that identification of MTHFR genotype and adequate folate and vitamin B_{12} intake during the preconception and pregnancy period could help protect against congenital malformations and improving pregnancy outcome.

Keywords: Down syndrome, Nondisjunction, MTHFR gene polymorphism

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Background

Down syndrome (DS) is the most common chromosomal abnormality, associated with mild to moderate mental retardation with an incidence of 1 in 700 live-births. In most cases of DS, the extra chromosome is present as a result of the failure of normal chromosome segregation during meiosis [1]. The nondisjunction (ND) event is maternal in 95% of cases, occurring primarily during meiosis I in the maturing oocyte before conception [1]. The etiological factors for ND leading to free trisomy 21 are still unknown, although many factors have been identified. The relationship between chromosomal nondisjunction and folate metabolism has drawn attention in recent years and research in this field has accelerated.

Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in folate and homocysteine metabolism. MTHFR catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which provides the methyl group for the remethylation of homocysteine to methionine. Reduced MTHFR activity results in an increased requirement for folic acid to maintain normal homocysteine remethylation to methionine. In the absence of sufficient folic acid, intracellular homocysteine accumulates, methionine resynthesis is reduced, and remethylation reactions are interrupted. Folate is also essential for various cellular processes such as synthesis of DNA, RNA, methylation, and embryonic developmental processes including the cardiovascular system [2]. Congenital heart defects (CHD) account for a major portion of life-threatening birth defects including atrioventricular septal defect (AVSD) and ventricular septal defects (VSD) which are common cardiac malformations in DS cases [3]. Several studies were performed on human cell cultures; in vivo studies in humans and studies involving animal models have demonstrated that folate depletion from the media, or inadequate folate dietary intake, results in DNA hypomethylation, chromosome breakage, increased frequency of micronuclei (MN), and aneuploidy [4]. The MTHFR gene C677T polymorphism is commonly associated with defects in folate-dependent homocysteine metabolism and has been implicated as risk factors for recurrent embryo loss in early pregnancy [5] and known to be a key player in the development of unexplained recurrent spontaneous abortion [6].

The present study was carried out to determine whether the MTHFR polymorphism is associated with increased risk of having a child with Down syndrome and the role of this polymorphism on fetal development. Hence, we examined the prevalence of MTHFR genotypes in mothers who had given birth to a child with Down syndrome (DSM) and correlated it with the micronucleus frequency and compared the results with control mothers (CM). In addition, the association of MTHFR gene polymorphism with nutrition factors and its relation with different conditions in DSM and DS children were also studied.

Subjects and methodology

This study was conducted at the Zoology Department, Gujarat University, Ahmedabad, India, from 2015 to 2018. The study was approved by the Institutional Ethics Committee (Reference Number: GUZOOLDHEC_16_2015). Women who had given birth to a DS child confirmed to have trisomy 21 by karyotyping were included in the study as DSM, and women whose children were not affected by trisomy 21 and who had never suffered a miscarriage were enrolled as control mother (CM). The study followed the Helsinki declaration, and a written informed consent was taken before enrolling the individuals in the study. Maternal age was calculated considering the age of the mother at the birth of the DS child. The study was divided into three components: cytogenetic, molecular, and biochemical. Blood samples (10 ml) were collected from DSM and age-matched control mothers and were used for DNA isolation, cytokinesis blocked micronucleus assay (CBMN) assay, and serum analysis.

Genotype analysis

The MTHFR 677C/T mutations were analyzed by polymerase chain reaction by using forward primer 5′TGA AGG AGA AGG TGT CTG CGG GA 3′ and reverse primer 5′AGG ACG GTG CGG TGA GAG TG 3′ [7] followed by allele-specific restriction digestion with Hinf I which was then analyzed by electrophoresis in 3% agarose gel. The enzyme digested product was checked against the standard 100 bp DNA Ladder. The MTHFR C/C and C/T genotype bands were observed in DSM and CM and gel photos were recorded (Fig. 1).

Cytokinesis blocked micronucleus assay (CBMN)

Micronucleus assay was done following the method of Fenech [8] with slight modifications. The peripheral blood lymphocyte cultures (PBLC) were set up according to the standard protocol and 7 μg/ml Cytochalasin B was added after 68 h of incubation, at 96 h the cultures were centrifuged, and the media was pipetted off and hypotonic treatment given by prewarmed (37 °C) 0.075 M KCl. The cells were then fixed in fresh chilled fixative, and the slides were prepared within 2 h of harvesting initiation after another wash with fixative and stained in 2% Giemsa. One thousand binucleates within one cytoplasm were scanned for the presence of micronucleus under high power (× 40) objective for each individual.

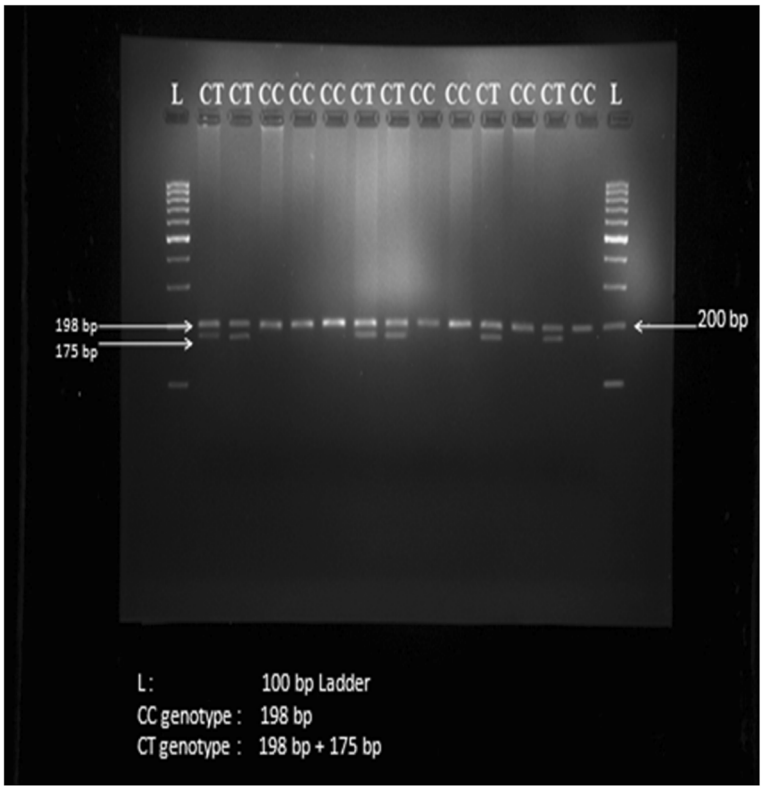

Fig. 1 Gel picture showing MTHFR C/C and C/T polymorphism

Serum analysis

Folate and vitamin B_{12} were measured by using electrochemiluminescence immunoassay (ECLIA) in Cobas e 411 analyzer by ROCHE kits. Homocysteine was measured by using homocysteine enzymatic assay in COBAS INTEGRA® 400 plus.

Statistical analysis

The statistical analysis of the data was carried out by GraphPad Prism 7.0 software. The biochemical parameters were analyzed by student t-test, while the molecular analysis by chi-square tests in the software. A value of $p < 0.05$ is considered significant. Odds ratio and 95% confidence interval (95%CI) were calculated to estimate the risk of different genotypes.

Result

A total of 236 individuals, DSM (118) and CM (118), were studied. MTHFR gene polymorphism study showed that the prevalence of MTHFR C/C genotype frequency among mothers of children with Down syndrome was 42.5% compared to 67% in control mothers; 64.4% C/T genotype frequency ($p < 0.001$) in DSM and 33% in CM, while homozygous for T allele was not observed in both groups (Table 1). The "C" allele frequency was found to be 0.84 in CM and 0.67 in DSM. The "T" allele frequency was found to be 0.65 and 0.82 among CM and DSM respectively.

CBMN in DSM and CM

The MN frequency was non-significant (3.436 ± 0.404 vs 3.078 ± 0.151) for both the CM and DSM with C/C genotype, while it was significantly higher ($p < 0.001$) in DSM as compared to the CM for the C/T genotype (4.309 ± 0.462 vs 2.44 ± 0.252). Also, significantly higher ($p < 0.01$) MN frequency was observed in C/T genotype than the C/C genotype in CM. Within the DSM, MN frequencies were found significantly higher ($p < 0.001$) in DSM with C/T genotype than DSM with C/C genotype (4.309 ± 0.462 vs 3.078 ± 0.151) (Table 2) (Figs. 2 and 3).

Table 1 MTHFR genotype frequency distribution in DSM and CM

Genotype	Tested group			
	CM *n*, *f*(%)	DSM *n*, *f*(%)	OR (CI)	*p* value
CC	82 (67)	42 (42.5)	Reference	Reference
CT	36 (33)	76 (64.4)	4.122 (2.3 to 7.1)	$p < 0.001$***

Note: No TT genotype was observed in both cases
n number of genotype, *f* frequency, OR odds ratio, CI 95% confidence interval
***$p < 0.001$

Table 2 Micronucleus (MN) frequency and MTHFR genotype in DSM and CM

Genotype	MN frequency	
	CM	DSM
CC	3.436 ± 0.404	3.078 ± 0.151^{NS}
CT	2.44 ± 0.252**	$4.309 \pm 0.462^{***\#\#}$

NS not significant
CM vs DSM (CC/CC): NS; CM (CC/CT): $^{**}p < 0.01$,
CM vs DSM (CT/CT): $^{***}p < 0.001$; DSM (CC/CT): $^{\#\#}p < 0.01$

MTHFR and spontaneous abortion (SA) in DSM

Among the total 118 DSM, 68 (57.6%) DSM suffered at least one instance of spontaneous abortion and 50 (42.4%) DSM did not report a SA. Within the DSM, 26.7% with C/C genotype and 31.3% with C/T genotype did not report a SA while 33% DSM with C/T genotype reported a SA which was significantly higher ($p < 0.01$) than the C/C genotype (9.3%) (Table 3).

MTHFR and Congenital heart disease (CHD) in DS child

MTHFR polymorphism in all DSM was checked for an association of CHD in their DS child: DS child who showed CHD were 73 and 42 did not show CHD, while 3 DSM were not aware about the heart condition of the child. CHD in the DS child was reported by 10.43% DSM with C/C genotype and 53% DSM with C/T genotype which is statistically highly significant ($p < 0.001$) as shown in Table 4.

MTHFR and neonatal jaundice in DS child

In all the DS children, only 42 had neonatal jaundice while 76 children did not show neonatal jaundice. Among the DSM, 26.2% with the C/T genotype and only 9.3% DSM with the C/C genotype reported neonatal jaundice in the DS child which was statistically not significant as shown in Table 5.

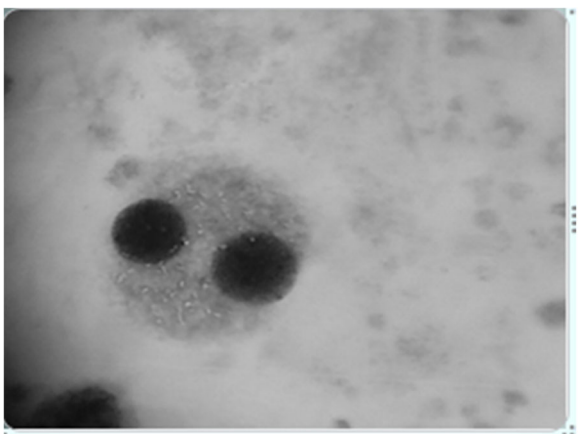

Fig. 2 Binucleated cell

MTHFR and CHD with neonatal jaundice in DS child

Both the CHD and neonatal jaundice in DS child was reported by 7.6% DSM with CC genotype and 12.7% DSM with CT genotype which is statistically highly significant ($p < 0.001$) (Table 6).

MTHFR and low birth weight (LBW) in DS child

In the DSM, 44.9% with C/T genotype and 11.8% with C/C genotype reported low birth weight in their DS child which was highly significant ($p < 0.001$) as shown in Table 7.

MTHFR and Delivery status of DS child

Premature birth was observed in 35 DS children, 24 children had postmature birth and 42 children had full-term birth and delivery status was unknown for 17 DS children. Among DSM 9.9% with C/C genotype and 24.7% with C/T genotype reported premature delivery which was statistically significant ($p < 0.01$), also 9.9% DSM with C/C genotype and 13.8% DSM with C/T genotype reported post mature delivery which was statistically not significant. The full-term delivery was observed in 25.7 % DSM with C/C genotype and 15.8% DSM with C/T genotype as shown in Table 8.

Serum analysis in DSM and CM

The biochemical analysis in this study involved examining cases (DSM) and controls (CM) for the effect of serum folate, vitamin B_{12} and homocysteine status (Table 9). Levels of vitamin B_{12} (176.9 ± 19.77 pg/ml vs 379.7 ± 31.18 pg/ml vs., $p < 0.001$) were significantly lower in all mothers of DS children than the CM respectively. DSM with C/T genotype showed lower levels of Vitamin B_{12} as compared with C/T genotype of CM which was highly statistically significant (121.4 ± 10.11 vs. 375.5 ± 41.14 pg/ml, $p < 0.001$, respectively) (Table 10). Also, levels of folic acid (2.811 ± 0.2746 ng/ml vs 6.09 ± 1.120 ng/ml, $p < 0.01$), were significantly lower in mothers of DS children than the CM. DSM with C/T genotype showed lower levels of folate as compared with C/T genotype of CM which was significant (2.044 ± 0.2161 ng/ml vs 4.117 ± 0.6469 ng/ml, $p < 0.05$, respectively) (Table 10). Serum homocysteine levels also were significantly higher in all mothers of DS children compared with the Control mothers (15.58 ± 1.583 μmol/l vs. 26.45 ± 2.643 μmol/l, $p < 0.01$). Further, levels of homocysteine in DSM group with C/T genotype (32.53 ± 3.878 μmol/l) showed a significant increase ($p < 0.01$) when compared to C/T genotype of CM group (17.05 ± 2.979 μmol/l) (Table 10).

Discussion

Nondisjunction (ND) is the failure of one or more pairs of homologous chromosomes or sister chromatids to

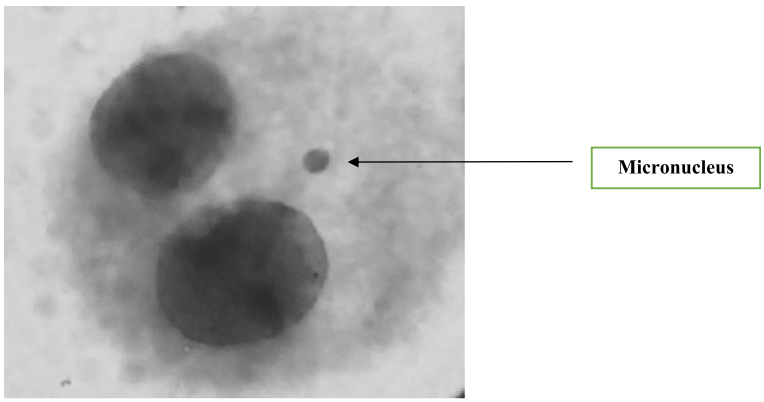

Fig. 3 Binucleated cell with micronucleus (MN)

separate normally during nuclear division which can result in an abnormal distribution of chromosomes in the daughter nuclei. The etiological factors for ND leading to DS are still unknown after decades of research. Maternal ND has been identified in more than 95% cases of DS [1]. One of the major limits for the study of the molecular mechanisms leading to chromosome 21 malsegregation is the unavailability of human egg cells from DSM, so that most of the studies performed so far have been in peripheral lymphocytes from them. MTHFR is one of the major enzymes of the folate metabolic pathway, whose genetic polymorphisms have been associated with chromosome damage and maternal risk of birth of a child with DS [9]. James et al. [10] first examined the distribution of the MTHFR genotypes in the mothers of Down syndrome individuals (DSM) and mothers of normal children (CM) and observed a significantly higher frequency of MTHFR gene polymorphism in mothers of children with DS than in control women. Afterwards, numbers of studies were conducted in different regions of the world with a lot of variation in the results [9, 11]. Some studies on MTHFR polymorphism have been conducted in India also and have shown variation in results which could be due to differences in geographical regions [12, 13]. The present study showed that the distribution of MTHFR C677T polymorphisms was significantly higher in the DSM group when compared to the CM group. The T allele was observed to be higher in DSM and lower in CM and no individual showed T/T genotype in both the groups. Some other Indian studies of different regions also did not observe T/T genotype in DSM [7, 12], while few studies found T/T genotype in DSM in very low frequency [14, 15]. These results suggest that the distribution of T/T genotype is lower in the Indian population. In addition, we also observed a significantly increased MN frequency and spontaneous abortion (SA) in the DSM group. The DSM with C/T genotype of MTHFR gene reported a significantly higher frequency of CHD, PTB, and LBW in their children.

There are reports which have found the association of nondisjunction with increased maternal age [16], but in the present study, we observed significantly higher MN frequency in young mothers with a DS child, suggesting that these women have a tendency for chromosome malsegregation events. Also, genetic factors may be involved in the chromosome nondisjunction causing aneuploidy, since young parents can have DS progeny, a fact indicating susceptibility to abnormal chromosome segregation in these cases. The increased MN frequency is a sign of cytogenetic damage, probably associated with spindle disruption leading to nondisjunction. There are several possible molecular mechanisms causing abnormal chromosome segregation that result in MN formation. One of these mechanisms is related to the hypomethylation of cytosine in centromeric and pericentromeric repetitive sequences [17]. In the present

Table 3 Genotype distribution of MTHFR and spontaneous abortion (SA) in DSM

Genotype	DSM group			
	No SA *n*, *f*(%)	SA *n*, *f*(%)	OR (CI)	*p* value
CC	31 (26.7)	11 (9.3)	Reference	Reference
CT	37 (31.3)	39 (33)	2.971 (1.3 to 6.7)	$p = 0.0082$**

n number of genotype, *f* frequency, OR odds ratio, CI 95% confidence interval
**$p < 0.01$

Table 4 Genotype distribution of MTHFR in DSM and Congenital heart disease (CHD) in the DS child

Genotype	DSM group			
	No CHD *n*, *f*(%)	CHD *n*, *f*(%)	OR (CI)	*p* value
CC	29 (25.2)	12 (10.43)	Reference	Reference
CT	13 (11.3)	61 (53.0)	11.34 (4.6 to27.9)	$p < 0.001$***

n number of genotype, *f* frequency, OR odds ratio, CI 95% confidence interval
***$p < 0.001$

Table 5 Genotype distribution of MTHFR in DSM and neonatal jaundice in the DS child

Genotype	DSM group			
	No neonatal jaundice *n*, *f*(%)	Neonatal jaundice *n*, *f*(%)	OR (CI)	*p* value
CC	31 (26.2)	11 (9.3)	Reference	Reference
CT	45 (38.1)	31 (26.2)	1.9 (0.8 to 4.4)	$p = 0.1128^{NS}$

n number of genotype, *f* frequency, OR odds ratio, CI 95% confidence interval, NS not significant

study, we have observed a correlation between the MTHFR Polymorphism and the baseline frequency of binucleate with micronucleus (BNMN) in lymphocytes and observed significantly higher MN frequency in DSM who had C/T genotype with low folate levels, suggesting a direct link between the amount of chromosome damage observed and a variant of a gene whose product is involved in DNA methylation and homocysteine metabolism. Folate deficiency can lead to heterochromatin demethylation causing defects in the structure of the centromere, which could induce an abnormal distribution of replicated chromosomes during nuclear division [4]. Considering that MN is a biomarker of chromosome breakage and/or whole chromosome loss, an increased frequency of MN in these mothers of a DS child suggests a higher predisposition to aneuploidy. In chromosome segregation, a significant role should be attributed to the microtubular system. Ford [18] has proposed that a mechanism controlling microtubular polymerization and/or alteration in the microtubular structures is responsible for the formation of trisomic cells during mitosis and meiosis. Beetstra et al. [19] observed that folate deficiency or inherited defects in folate metabolism may lead to increased chromosome 21 mosaicism *in vivo* during the fetal stage. The reduction of folate intake by mother and maternal grandmothers seems to be relevant in chromosome nondisjunction and consequently could be associated with a higher risk of DS progeny [20]. In the current study, C/T genotype of MTHFR gene with low folate levels in DSM might hinder the microtubule formation and lead to ND.

Studies have shown that folate deficiency is associated with genomic damage and formation of MN and other nuclear abnormalities in human lymphocytes [21]. Furthermore, folate supplementation led to a pronounced reduction in DNA damage and MN formation in another study [22]. Folate is also essential for normal body growth and in DNA methylation mechanism. Methylation is an important epigenetic characteristic that plays an important role in DNA repair and the stability of the genome. These data provide additional support to the epigenetic mechanisms of MN formation. Based on all the abovementioned facts and lines of evidence, we may conclude that one mechanism of MN formation is induced epigenetically mainly through the loss of DNA methylation due to MTHFR gene polymorphism. Abnormal folate levels directly have an effect on the homocysteine levels. These two atypical levels together make more difficulties in metabolic pathways and lead to different anomalous conditions. Hence, the results of the present study recommend that maternal aging is not involved in Down syndrome, but biochemical pathways could promote maternal meiotic nondisjunction and the risk of having a DS child.

SA and DSM

In the present study, we investigated the possible association of SA in DSM with MTHFR polymorphism and found significantly higher spontaneous abortion in DSM who had C/T genotype than the DSM with C/C genotype. Observation of higher frequency of C/T genotype indicates the importance of this gene which may be affecting the growth of developing embryo and/or increasing the risk for ND as mentioned earlier. It has been reported that more than 50% of early pregnancy terminations are due to chromosomal aneuploidy and more than 80% of DS conceptuses abort, and MTHFR 677 T and 1298C *SNPs* are risk factors in mothers with recurrent pregnancy failure [23]. The mechanism by

Table 6 Genotype distribution of MTHFR in DSM and congenital heart disease (CHD) and neonatal jaundice in DS child

Genotype	DSM group			
	No CHD *n*, *f*(%)	CHD and neonatal jaundice *n*, *f*(%)	No neonatal jaundice *n*, *f*(%)	*p* value
CC	29 (25.2)	2 (7.6)	31 (26.2)	Reference
CT	13 (11.3)	24 (12.7)***	45 (38.1)	$p < 0.001$***

n number of genotype, *f* frequency
***$p < 0.001$

Table 7 Genotype distribution of MTHFR in DSM and birth weight in the DS child

Genotype	DSM group			
	Normal *n, f*(%)	LBW *n, f*(%)	OR (CI)	*p* value
CC	28 (23.7)	14 (11.8)	Reference	Reference
CT	23 (19.4)	53 (44.9)	4.609 (2.0 to10.3)	$p < 0.001$***

n number of genotype, *f* frequency, OR odds ratio, CI 95% confidence interval
***$p < 0.001$

which the MTHFR polymorphisms increase the risk of SA is not known. A few studies have addressed the issue of folate deficiency and levels of homocysteine in relation to spontaneous abortion, recurrent pregnancy loss, and stillbirth [24, 25]. In vitro studies on human cells show that low levels of serum folate are associated with misincorporation of uracil into DNA and DNA damage, as well as with aneuploidy of chromosomes involved in tumors and other diseases associated with folate deficiency [19, 26]. In our study, the low folate levels and high homocysteine levels were observed in DSM who had at least one SA when compared with DSM who did not report SA which further supports the observation mentioned earlier that folate and homocysteine levels are very essential for normal chromosomal segregation, structural aberration, and aneuploidy.

Study of Puri et al. [13] in north Indian population observed that hyperhomocysteinemia was significantly associated with recurrent pregnancy loss. They also found T allele to increase homocysteine levels among cases. In the present study, the abnormal levels of these micronutrients show that it might be one of the risk factors for SA in DSM. Previously, authors have hypothesized that the increased risk may be due to an increase in homocysteine levels [27] which is consistent with the higher levels of homocysteine that we detected in our cases within the DSM. Hyperhomocysteinemia reduces methyl availability [28]. Hence, such harmful changes in the genes can increase the risk of fetal loss and/or nondisjunction (ND).

Table 8 Genotype distribution of MTHFR in DSM and delivery status of the DS child

Genotype	DSM group				
	Full term *n, f*(%)	Postmature *n, f*(%)	Premature *n, f*(%)	Unknown *n, f*(%)	OR (CI)
CC	26 (25.7)	10 (9.9)	10 (9.9)	6 (5)	Reference
CT	16 (15.8)	14 (13.8)^{NS}	25 (24.7)**	11 (9.3)	3.43 (1.2 to 9.1)

n number of genotype, *f* frequency, OR odds ratio, CI 95% confidence interval, NS not significant
**$p < 0.01$

Table 9 Serum levels of vitamin B_{12}, folate, and homocysteine in CM and DSM

Serum	CM	DSM	Normal range
Vitamin B_{12}	379.7 ± 31.18	176.9 ± 19.77***	250–1100 pg/ml
Folic acid	6.09 ± 1.120	2.811 ± 0.2746**	3.1–20.5 ng/ml
Homocysteine	15.58 ± 1.583	26.45 ± 2.643**	< 15.00 μmol/l

**$p < 0.01$, ***$p < 0.001$

CHD and DS

Brandalize et al. [28] conducted the first study on the presence of maternal MTHFR polymorphisms C677T and A1298C as a risk factor for CHD in DS children. Folate and homocysteine levels in pregnancy may affect the development of the heart in the fetus. We attempted to check the association of the MTHFR gene polymorphism within the DSM group for the presence or absence of CHD conditions in their DS children. We observed that C/T genotype was significantly higher in mothers of the DS children who suffered from CHD condition than the C/C genotype. In addition, low folate levels and high levels of homocysteine were also found abnormal in DSM whose DS children suffered from CHD condition. This evidence suggests a role for folic acid and homocysteine in the development of CHD which leads to the hypothesis that mutations in folate metabolism could alter susceptibility to CHD.

In India, approximately 21,000 babies are born with DS every year [29]. Approximately, CHD accounts for 28% of the major congenital anomalies [30] in neonates. Studies show a widely varying prevalence of maternal folate deficiency during pregnancy, specifically in the Indian population (0.2–26.3%) [31, 32]. Botto and his study group [33] suggested that approximately one in four major cardiac defects could be prevented by periconceptional multivitamins use. Investigators from Canada reported a 6% decrease per year in the rates of severe CHD after folic acid fortification of grain products [34]. A case-control study from Spain reported that the absence of maternal folic acid supplementation was more frequent in DS with CHD compared to DS without CHD [35]. Thus, maternal supplementation with folic acid is likely to be associated with reduced risk of CHD in DS. The data obtained from these studies including the present one suggests that folic acid is essential for normal fetal cardiac development during early embryogenesis. The abnormal levels of folate and homocysteine due to this polymorphism might be one of the risk factors which is responsible for CHD condition in DS children. We also observed a significantly low level of vitamin B_{12} in DSM with C/T genotype than the C/C genotype which also directly affects the homocysteine levels and increased the risk for CHD in their children.

Table 10 Genotype distribution of MTHFR and serum levels of vitamin B_{12}, folate, and homocysteine in DSM and CM

Serum	CM		DSM	
	CC	CT	CC	CT
Vitamin B_{12}	383.9 ± 49.35	375.5 ± 41.14^{NS}	$232.3 \pm 28.11^{\#}$	$121.4 \pm 10.11^{***+++}$
Folic acid	6.444 ± 0.6534	4.117 ± 0.6469^{NS}	$3.578 \pm 0.3562^{\#\#}$	$2.044 \pm 0.2161^{*+++}$
Homocysteine	4.11 ± 1.116	17.05 ± 2.979^{NS}	$20.38 \pm 2.329^{\#\#\#}$	$32.53 \pm 3.878^{**+++}$

$^{\#}$CM vs DSM (CC/CC), $^{\#}p < 0.05$), $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$
*CM Vs DSM (CT/CT), $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$
$^{+}$DSM (CC Vs CT), $^{+}p < 0.05$), $^{++}p < 0.01$, $^{+++}p < 0.001$, NS CM (CC/CT), not significant

We also checked the MTHFR gene polymorphism association with neonatal jaundice. There is no data available concerning the MTHFR correlation with neonatal jaundice in DS children born at birth. In the study of Kaplan et al. [36], more than 55% incidence of neonatal jaundice was observed in Down syndrome cohort and they concluded Down syndrome neonates had a greater risk of hyperbilirubinemia. The C/T genotype in DSM was non-significantly higher as compared to the C/C genotype for neonatal jaundice in the child. In addition, DSM with C/T genotype showed a highly significant number of affected DS child for both conditions (CHD and neonatal jaundice). Hence, this further suggests that the MTHFR polymorphism, folate, and homocysteine levels are liable for congenital malformation in the DS child.

PTB and LBW

Studies concerning a potential role of the MTHFR C677T in PTB and LBW susceptibility have been conducted by researchers but with inconsistent results, which might be mainly caused by insufficient population size of one single study [37]. In our study, the observation of significant C/T genotype and low folate levels in DSM with low birth weight children suggests that this polymorphism in DSM reduced the MTHFR enzyme activity and increased adverse pregnancy outcome. There are some hypotheses that could link reduced folate levels and premature birth. Firstly, periconceptional folate supplementation may influence early placentation processes [38]. In fact, folate is potentially important in a number of crucial early stages of placental development, including extravillous trophoblast invasion, angiogenesis, and secretion of matrix metalloproteinases [39]. Secondly, micronutrient status at the time of implantation could have a role in inflammation, and early PTB is often caused by intrauterine infection and folate is reduced in PTB [40]. DSM also showed higher levels of homocysteine in our study. Increased homocysteine levels induce cytotoxic and oxidative stress on placental vascular and endothelial functions [41] and exposure of trophoblast cells to homocysteine may increase apoptosis [42].

Some studies found evidence that the MTHFR C677T polymorphism was associated with an increased risk of placental abruption and perhaps intra-uterine growth restriction which may be a factor responsible for low birth weight [43]. It is well established that folate requirements increase throughout pregnancy in order to facilitate the rapid cellular division and growth of the mother and fetus [44]. Rogne et al. [45] also observed that lower maternal vitamin B_{12} levels are associated with increased risk of preterm birth and that the risk of preterm birth was particularly high in the presence of B_{12}-deficiency during pregnancy. Low birth weight is a result of preterm birth, of being born small at term, or a combination of the two. Additionally, Indian newborns are among the smallest in the world [46]. Indian women generally have a lower dietary intake of B_{12}, due to a mainly vegetarian diet, making them susceptible to B_{12}-deficiency [32]. Study of Rogne et al. [45] suggest that pregnancies already at greatest risk of giving birth to small newborns were the ones most vulnerable to low levels of B_{12}, which supports the low vitamin B_{12} levels observed in DSM of our study; also the C/T genotype might be affecting the folate deficiency.

Conclusion

We observed a significantly higher MTHFR C/T genotype in the DSM and suggest that this polymorphism might be an etiological factor for DS risk. We also observed low levels of folate, vitamin B_{12} and high homocysteine in DSM compared to the CM which add to the evidence that supports the association of the folate-Homocysteine pathway genes and micronutrient deficiency as risk factors for ND. Further, we also observed an association of this polymorphism and folate-homocysteine pathway with CHD, neonatal jaundice, LBW, and PTB in DS children. The results from our study support the hypothesis that folate deficiency may increase the rate of aneuploidy and other malformations and pregnancy complications including spontaneous abortion. Hence, it is advisable to provide micronutrient supplementation (including folate and vitamin B_{12}) to women prior to conception onwards to reduce the risk of maternal ND and during

pregnancy to avoid malformations in the developing fetus. Still large intervention trials as well as prospective studies on identification of MTHFR genotype and measuring homocysteine, folate status and vitamin B_{12} during pregnancy are needed to establish the role of these nutrients and related factors as predictors or etiologic factors for these abnormalities.

Abbreviations

DS: Down Syndrome; DSM: Mother of Down syndrome; CM: Control mother; ND: Nondisjunction; MI: Meiosis I; CHD: Congenital heart diseases; PTB: Preterm birth; MTHFR: Methylene tetrahydro folate reductase; Hcy: Homocysteine; SNP: Single nucleotide polymorphism; SA: Spontaneous abortion; DNA: Deoxyribonucleic acid; LBW: Low birth weight; MN: Micronucleus; WHO: World Health Organization; ROS: Reactive oxygen species; CBMN: Cytokinesis blocked micronucleus assay

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Authors' contributions

Both authors read and approved the final manuscript. RK carried out cytogenetics, molecular genetic work, and drafted the manuscript. DC conceived the study, carried out karyotyping, and helped to draft the manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study was approved by the Institutional Ethics Committee, Gujarat University, India (Reference Number: GUZOOLDHEC_16_2015), and an informed written consent has been taken from all the women while enrolling them for this study.

Consent for publication

The consent to publish has been taken from each participant at the start of this work.

Competing interests

The authors declare that they have no competing interests.

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