Research Journal of Medical Sciences 12 (6): 84-89, 2018
ISSN: 1815-9346
© Medwell Journals, 2018

Maternal MTHFR A1298C not C677T Polymorphism as the Risk Factor in Children with Down Syndrome

[1]Tiar M. Pratamawati, [2]Tri Indah Winarni, [3]Hardian and [2]Sultana M.H. Faradz
[1]Department of Genetics, Faculty of Medicine, University Swadaya Gunung Jati, Cirebon, Indonesia
[2]Center for Biomedical Research (CEBIOR),
[3]Department of Medical Physiology, Faculty of Medicine, Diponegoro University, Semarang, Indonesia

Abstract: Down Syndrome (DS) is the most common genetic cause of intellectual disability. The incidence is increased in advanced maternal age of more than 35 years and mostly caused by meiotic non disjunction. MTHFR gene polymorphism has been identified as a genetic contributor to human meiotic non disjunction. This research aimed to analyze MTHFR C677T and A1298C polymorphisms as a risk factor for mothers to have children with classical DS. This study was an observational analytical study with case-control design. Blood samples were obtained from 30 mothers with cytogenetically confirmed children with classical DS (47, XX, +21 or 47, XY,+21) and 30 mothers of children without DS as controls. The DNA was extracted using salting out method, followed by PCR-RFLP analysis for MTHFR C677T and A1298C gene polymorphisms. The genotype frequency for MTHFR C677T was 56.7% homozygous CC (wild type) and 43.4% heterozygous CT in the cases and 63.4% homozygous CC (wild type), 33.3% heterozygous CT (heterozygote) and 3.3% homozygous TT (mutant type) in the controls. The genotype frequency for MTHFR A1298C was 50% homozygous AA (wild type), 43.3% heterozygous AC and 6.7% homozygous CC (mutant type) in the cases and 76.7% homozygous AA (wild type), 20% heterozygous AC and 3.3% homozygous CC (mutant type) in the controls. MTHFR C677T was inconclusive as risk factor for having children with DS ($p = 0.598$ or = 1.321, CI 95% = 0.46-3.72) whereas MTHFR A1289C was a risk factor for having children with DS ($p = 0.014$ or = 4.62, CI 95% = 1.36-15.65) in 73.3% young maternal age (<35 years). An MTHFR 1298C allele is a risk factor for mothers to have children with DS with a risk of 4.62 times higher compared with mother who has 1298A allele.

Key words: MTHFR gene, polymorphism, down syndrome, polymorphism, cytogenetically, analyze

INTRODUCTION

Down Syndrome (DS) is a numerical chromosomal abnormality with 47 chromosomes instead of the normal 46 chromosomes due to the presence of additional chromosome 21 (trisomy 21) Vision and Health, DS incidence prevalence is estimated in 1:700-1000 live births with a predicted four million cases worldwide (Parker *et al*., 2010). DS is the most common cause of non-inherited genetic cause of intellectual disabilities and more than 90% of DS is caused by maternal meiotic nondisjunction (Shalaby, 2011). Meiosis is a tightly regulated process with various checkpoints to ensure that cell division happens correctly. Nondisjunction is a mistake during chromosomal segregation involving spindle fibers. Spindle check point was utilized to ensure that the spindle fibers are attached correctly to chromosomes and a failure during this check point may cause chromosomal nondisjunction (Bernasconi, 2018). Advanced maternal age remains the main risk factor and may be the cause of degenerating oocyte pool in mothers (Christianson *et al*., 2004). Methylenetetrahydrofolate reductase is an enzyme in the methyl cycle expressed by the MTHFR gene. This gene is located at chromosome 1p36.3 at base pair of 11 785, 730-11 806 103. Methylenetetrahydrofolate reductase catalyzes 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate a co-substrate for homocysteine re-methylation to methionine. Methionine is crucial for cell division and MTHFR gene polymorphisms have been suggested to play an important role in cellular division failure that caused nondisjunction, leading to DS.

In addition to the fact that folic acid intake/supplementation and folate metabolism is strongly associated with meiosis non disjunction, high prevalence of folat-sensitive Neural Tube Defect (NTD) was reported

Corresponding Author: Tiar M. Pratamawati, Magister of Biomedical Science Majoring Genetic Counseling, Faculty of Medicine, Diponegoro University, Semarang, Indonesia

in lower-middle income countries such as Algeria, Nigeria, Iran, Jordania, Pakistan. It may associated with insufficient intake of folic acid in female reproductive age. Zaganjor *et al*. Describing the Prevalence of Neural Tube Defects Worldwide: A systematic literature review. PLoS ONE 11(4): e0151586) As far as we are concerned, there is no previous study was conducted in Indonesia regarding MTHFR gene polymorphism in mothers who had children with DS. This study aimed to analyze C677T and A1298C polymorphisms as a risk factor of mothers who had classical DS children.

MATERIALS AND METHODS

This research was a case-control study with 30 cases and 30 controls. The ethical clearance was obtained from Ethical Committee on Medical and Health Research, Faculty of Medicine Diponegoro University/Dr. Kariadi Hospital. Case group participants were mothers with cytogenetically confirmed children with classical DS who had undergone cytogenetic analysis at the Center for Biomedical Research, Faculty of Medicine, Diponegoro University, Semarang, Central Java, Indonesia. Control group participants were mothers with normal children. DNA from the peripheral blood in EDTA was extracted, followed by PCR amplification by using the following forward and reverse primers. For C677T polymorphisms, the primers were 5'TGAAGGAGAAGGTGTCTGCGGGA3 and 5‘AGGACGGTGCGGTGAGAGTG3’. PCR was conducted using the following settings: initial denaturation at 95°C for 5 min, 35 cycles of denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec, extension at 72°C for 30 sec and final extension at 72°C for 5 min. For A1298C, 5‘CAAGGAGGAGCTGCTGAAGA3 and 5'CCACTCCAGCATCACTCACT3 were used with the following PCR settings: initial denaturation at 94°C for 4 min, 30 cycles of denaturation at 94°C for 60 sec, annealing at 60°C for 60 sec, extension at 72°C for 60 sec and final extension at 72°C for 10 min. The C677T PCR product was then digested using HinfI and visualized with 1% agarose gel. The A1298C PCR product was subsequently digested using MboII and visualized with 1% agarose gel.

Statistical analysis was done using chi square, Odds Ratio (OR) and logistic regression. Statistical significance was set at a probability of 0.05. Normality and homogeneity of the group were performed using Kolmogorov-Smirnov test (0.071) with skewness (0.421) and kurtosis (0.521). Both groups showed normal and homogeny distribution.

RESULTS AND DISCUSSION

The average age of the case group was 40.80 years old (±8.779) whereas that in the control group was 40.83 years old (±8.816). The average of maternal age in cases was 34.03 years old (±5.623) and in controls was 29.83 years old (±5.180). The case group had 13 (43.4%) maternal age above 35 years old with only 5 in the control group (16.7%) (Table 1-4).

MTHFR C677T genotype distribution in the case group was 17 (56.7%) of CC and 12 (43.4%) of CT whereas genotype distribution in the control group was 19 (63.4%) of CC, 10 (33.3%) of CT and 1 (3.3%) of CC. The allelic distribution for C was 47 (78.3%) in the case group and 48 (80%) in the control. Meanwhile, the distribution for T was 13 (21.7%) in the case group and 12 (20%) in the control (Table 4).

MTHFR A1298C genotype distribution in the case group was 15 (50%) of AA, 13 (43.3%) of AC and 2 (6.7%) of CC whereas in the control group, the genotype distribution was 23 (76.7%) of AA, 6 (20%) of AC and 1 (3.3%) of CC. The allelic distribution of A was 43 (71.7%) in the case group and 52 (86.7%) in the control. Meanwhile, the distribution for C was 17 (28.3%) in the case group and 8 (13.3%) in the control.

Statistical analysis showed no significant value between MTHFR C677T and having children with DS with OR of 1.321 (95% confidence interval [CI] 0.46-3.72, p = 0.598). However, a significant value was observed in MTHFR A1298C and having children with DS with OR of 3.286 (95% CI 1.09-9.95, p = 0.032). Maternal age showed OR of 3.824 (95% CI 1.15-12.71) (Table 2). Multivariate analysis for maternal age and MTHFR A1298C showed OR of 5.41 (95% CI 1.46-20.07, p = 0.012) and of 4.62 (95% CI 1.36–15.65, p = 0.014) (Table 3).

Interest in evaluating polymorphisms of the maternal genes involved in folate metabolism in relation to the risk of trisomy 21 has increased in the last decade. Various enzymes involved in metabolic pathways of the folates were studied and different groups obtained contradictory results. The explanation can be rooted in variations of genetic structure in different populations and ethnic groups in which different epigenetic and environmental factors are involved (Bucerzan *et al*., 2017).

Table 1: Demographic data of participants

Characteristic	Case (n = 30)	Control (n = 30)	p-values
Age			
At collection	40.80±8.779	40.83±8.816	0.071
Maternal age in pregnancy maternal age group in pregnancy	34.03±5.623	29.83±5.180	
>35 years	13 (43.3%)	5 (16.7%)	
≤35 years	17 (56.7%)	25 (83.3%)	

Table 2: Risk factors calculation between MTHFR polymorphism and maternal age with mothers having DS child

Variables	Down syndrome (+)	Down syndrome (-)	OR (95% CI)	p-values
C677T				
Polymorphism	13 (43.3%)	11 (36.7%)	1.321 (0.46-3.72)	0.598
Wild Type	17 (56.7%)	19 (63.3%)	1	
A1298C				
Polymorphism	15 (50,0%)	7 (23,3%)	3,286 (1,09-9,95)	0,032
Wild type	15 (50,0%)	23 (76,7%)	1	
Maternal age (Years)				
>35	13 (43,3%)	5 (16,7%)	3,824 (1,15-12,71)	0,024
≤35	17 (56,7%)	25 (83,3%)	1	

p-value obtained with chi-square

Tabel 3: Risk factors estimations of variable that affect mothers having DS child

Variable	OR (95% CI)	p-values
Maternal age positive	5.41 (1.46-20.07)	0.012
Polimorfisme A1298C positive	4.62 (1.36-15.65)	0.014
Constant	0.350	0.017

Tabel 4: Genotype frequency distribution of MTHFR polymorphism in case and control group

Polymorphism	Genotype	Case (n = 30) (%)	Control (n = 30) (%)
	CC	17 (56.7)	19 (63.4)
C677T	CT	13 (43.3)	10 (33.3)
	TT	0 (0)	1 (3.3)
A1298C	AA	15 (50.0)	23 (76.7)
	AC	13 (43.3)	6 (20)
	CC	2 (6.7)	1 (3.3)

Genes known for folate regulation includes MTHFR which codes for methylenetetrahydrofolate reductase; MTRR which codes for methionine synthase reductase; MTR which codes for methionine synthase; CBS which indicates cystathionine-beta-synthase; RFC1 which codes for reduced folate carrier 1 and MTHFD which signifies methylenetetrahydrofolate dehydrogenase (Scala *et al*., 2006).

Several MTHFR gene polymorphisms have been reported and documented but only C677T and A1298C have been successfully expressed and confirmed to have altered enzyme activity. This study found no association of MTHFR C677T with the maternal risk of having children with classical DS. This result was similar with the recent findings from a meta-analysis conducted by Rai and Kumar (2018) from five studies in India and France and in 401 cases and 529 controls, showing no association between MTHFR C677T polymorphism and the risk of having children with DS (Rai and Kumar, 2018). However, Liao *et al*. (2010) reported an increased risk (OR = 3.51, CI = 1.3-9.4) of having DS in mothers with MTHFR C677T. This study found that having MTHFR A1298C and not C677T, increased the risk of having children with DS by 4.62 times (OR = 4.62, CI = 1.36-15.65) in Asian population.

MTHFR indirectly affects meiotic nondisjunction as the most common etiology in DS cases (Patterson, 2008). Folate is a component in the formation of S-Adenosyl Methionine (SAM), a crucial methyl donor for DNA methylation in epigenetic processes and gene expression regulation and maintains centromeric chromosomal integrity. Folate deficiency decreased SAM, resulting in DNA hypomethylation. Pericentromeric hypomethylation may cause defect in heterochromatin formation and kinetochore assembly, leading to chromosomal nondisjunction. Chromatin with stable centromere is influenced by epigenetic inheritance of centromere-specific methylation pattern and centromeric chromatin binds to methyl-sensitive specific proteins, maintaining the chromosomal structure needed for kinetochore assembly (Patterson, 2008). We studied MTHFR C677T and A1298C polymorphisms in 93 Romanian mothers with children with DS and 202 controls and only A1298C polymorphism significantly increased the risk with OR 2.57 (Bucerzan *et al*., 2017). A risk study between MTHFR C677T and A1298C polymorphisms and cleft lips/palate in Indonesian Sasak Tribe population by Rochmah *et al*. (2018) showed MTHFR A1298C polymorphism as a risk factor with OR of 2.7 (CI 1.1-7.0) but not C677T (Rochmah *et al*., 2018). Similarly, our study revealed that MTHFR A1298C polymorphism is a risk factor of having DS children (OR = 4.62 [95% CI = 1.36-15.65], p = 0.014).

Maternal age remains the strongest risk factor for DS and the multivariate analysis result showed that, combined with A1298C polymorphism, increases the risk of having children with DS by up to four times. Additionally, most cases (73.3%) occur in the age group below 35 years old despite the high risk maternal age factor above 35 years old. This finding shows that MTHFR A1298C has an increased risk of children with DS (OR 3.286) even if the pregnancy occured below aged 35 years and the risk is almost doubled when combined with advanced maternal age (OR 5.41). As far as the researcher is concerned, this research is the first study conducted on MTHFR polymorphism as risk of having children with DS in Indonesian population.

The increased risk of having children with DS with the A1298C polymorphism screening can be used as an indication of further prenatal screening such as the

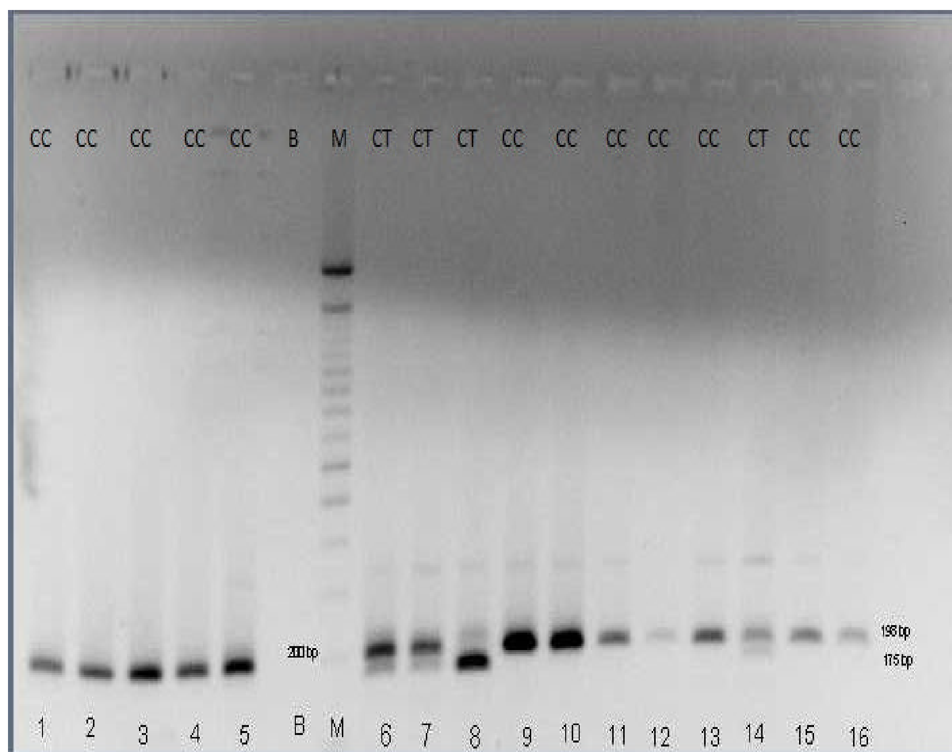

Fig. 1: Agarose gel of MTHFR C677T polymorhism PCR-RFLP product, row 1-5; 8-18: digestion result (CC: 198 bp; CT: 198 bp, 175 bp; TT: 175 bp), B: Blank, M: Marker

non-invasive combined First Trimester Screening (cFTS) and Non-Invasive Prenatal Testing (NIPT) or the invasive amniocentesis and chorionic villus sampling, especially in high risk women at the age of above 36 years old or having previous history of trisomic pregnancy. cFTS utilizes biochemical markers such as pregnancy-associated plasma protein-A, free a-human chorionic gonadotropin and ultrasound markers such as nuchal translucency (Vogel and Petersen, 2018). NIPT is an advanced method of detection of fetal chromosomal aneuploidies by using cell-free fetal DNA and because of its non-invasive nature, it has quickly spread across the globe after being introduced in the United States and Hong Kong in 2011 (Allyse *et al*., 2015). This result may also, a premature explanation of the cause of increase DS baby number in young mother. Mary *et al*, Increased Frequency of Down Syndrome in Young Mothers of Rural Population, Journal of Ecobiotechnology 2/3: 40-44.

A prenatal screening study in the United States between 2009 and 2012 showed that among the screen-positive women, 83.1% were predicted to be detectable with current non-invasive testing methods and 16.9% were considered not currently detectable with trisomy 21 accounting for 53.2% of the abnormal result (Norton *et al*., 2014). The Netherlands has been implementing prenatal screening for trisomy 21, since, 2007 and manages to keep a stable trisomy 21 live birth prevalence of 13.6 per 10 000 live births despite the increasing number of mothers aged = 36 years and increasing proportion of fetus diagnosed with trisomy 21 (De Groot-Van *et al*., 2018).

Traditionally, only women with the age above 36 years old and with previous history of chromosomal abnormality are included in prenatal screening, however, in developed countries such as the United States, Australia and many European Countries, NIPT recently become part of healthcare program and offered also, to young women who desire to do NIPT with their own budget. However, in recent years, developing countries, such as Algeria, Brazil, Malaysia, India, Iran, Mexico and several countries, have steadily adopted prenatal screening and now of fers NIPT. There is no report available regarding prenatal screening program in Indonesia. Thus, Indonesia has not yet incorporate prenatal screening program based on specific indication such as advanced maternal and having A1298C allele can be used to consider prenatal screening (Fig. 1 and 2).

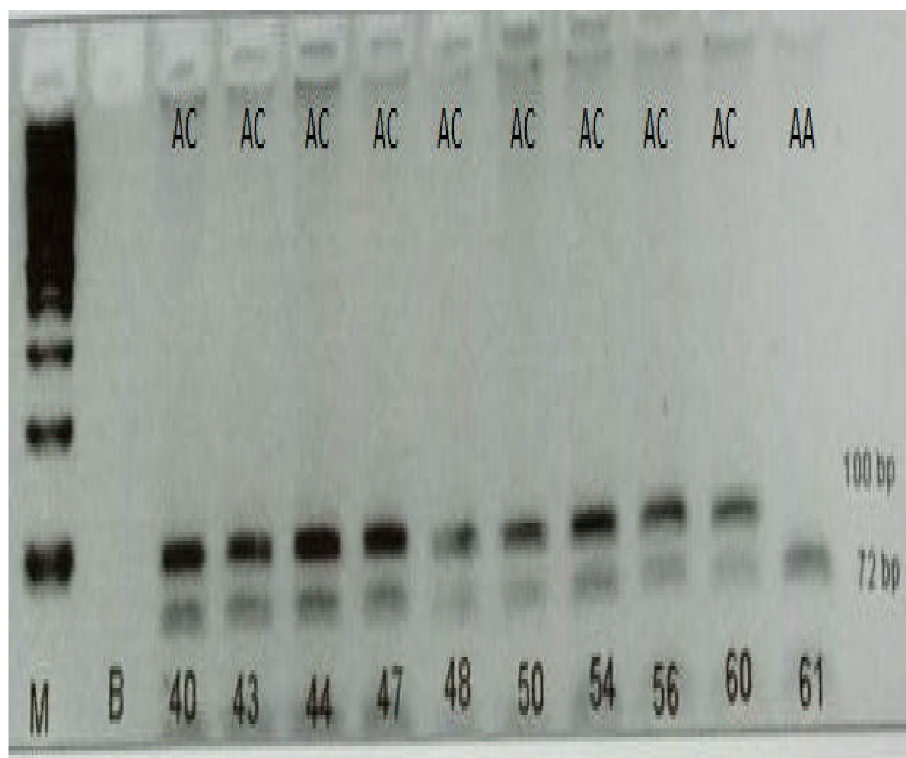

Fig. 2: A1298C RFLP result, M: Mark, B:Blank, row 3-12: digestion result (AA: 72 bp; AC: 100 bp and 72 bp; CC: 100 bp)

ACKNOWLEDGEMENTS

This research was funded by Faculty of Medicine Swadaya Gunung Djati University internal research fund. Cytogenetic results for DS children were provided by Center for Biomedical Research (CEBIOR) Faculty of Medicine Diponegoro University, Semarang, Central-Java, Indonesia.

REFERENCES

Allyse, M., M.A. Minear, E. Berson, S. Sridhar and M. Rote *et al*., 2015. Non-invasive prenatal testing: A review of international implementation and challenges. Intl. J. Womens Health, 7: 113-126.

Bernasconi, A., 2018. Nondisjunction-the causes of nondisjunction and its frequency in humans. Net Industries Inc., Indianapolis, Indiana. http://medicine.jrank.org/pages/2605/Nondisjunction-Causes-Nondisjunction-Its-Frequency-in-Humans.html

Bucerzan, S., R.A. Popp, R.M. Vlad, C. Lazea and R. Nicolaescu *et al*., 2017. Evaluation of c677t and a1298c polymorphism of the methylenetetrahydrofolate reductase gene as a maternal risk factor for trisomy 21 (A Monocentric Study). Rom. J. Lab. Med., 25: 27-35.

Christianson, R.E., S.L. Sherman and C.P. Torfs, 2004. Maternal meiosis II Nondisjunction in Trisomy 21 is associated with maternal low socioeconomic status. Genet. Med., 6: 487-494.

De Groot-Van Der Mooren, M.D., S. Tamminga, D. Oepkes, M.E. Weijerman and M.C. Martina, 2018. Older mothers and increased impact of prenatal screening: Stable livebirth prevalence of Trisomy 21 in the Netherlands for the period 2000-2013. Eur. J. Hum. Genet., 26: 157-165.

Liao, Y.P.1, M.S. Bao, C.Q. Liu and H.Z.D. Liu, 2010. No title. Yi Chuan, 32: 461-466.

Norton, M.E., L.L. Jelliffe-Pawlowski and R.J. Currier, 2014. Chromosome abnormalities detected by current prenatal screening and noninvasive prenatal testing. Obstetrics Gynecology, 124: 979-986.

Parker, S.E., C.T. Mai, M.A. Canfield, R. Rickard and Y. Wang *et al*., 2010. Updated national birth prevalence estimates for selected birth defects in the United States, 2004-2006. Birth Defects Res., 88: 1008-1016.

Patterson, D., 2008. Folate metabolism and the risk of down syndrome. Down Syndrome Res. Pract., 12: 93-97.

Rai, V. and P. Kumar, 2018. Fetal MTHFR C677T polymorphism confers no susceptibility to Down syndrome: Evidence from meta-analysis. Egyptian J. Med. Hum. Genet., 19: 53-58.

Rochmah, Y.S., L. Suwarsi, S. Harumsari, A. Sosiawan and S. Fatimah-Muis *et al*., 2018. Maternal polymorphism MTHFR A1298C not C677T and MSX1 as the risk factors of non-syndrome cleft Lips/Palate in Sasak Tribe Indonesia. J. Intl. Dental Med. Res., 11: 120-123.

Scala, I., B. Granese, M. Sellitto, S. Salome and A. Sammartino *et al*., 2006. Analysis of seven maternal polymorphisms of genes involved in homocysteine/folate metabolism and risk of Down syndrome offspring. Genet. Med., 8: 409-416.

Shalaby, H.M.A., 2011. A study of new potential risk factors for Down syndrome in Upper Egypt. Egypt. J. Med. Hum. Genet., 12: 15-19.

Vogel, I. and O.B. Petersen, 2018. Prenatal screening for atypical chromosomal abnormalities: Past or future?. Ultrasound Obstetrics Gynecology, 51: 434-435.