Medical
Communication

Biosci. Biotech. Res. Comm. 12(3): 577-583 (2019)

BBRC
Bioscience Biotechnology
Research Communications

Genetic Polymorphism Studies in MTHFR Gene with Acute Myeloid Leukemia in the Saudi Population

Abdullah Farasani[1,2*]

[1]*Medical Research Center, Jazan University, Jazan, Saudi Arabia*
[2]*Department of Medical Laboratory Technology, Faculty of Applied Medical Sciences, Jazan University, Jazan, Saudi Arabia*

ABSTRACT

Acute myeloid leukemia (AML) is connected with the leukemia cells, highly malignant, which invades the bone marrow and results in normal hematopoiesis. AML is the most commonly seen in adults as acute leukemia. The current study aims to investigate the possible association between C677T and A1298C polymorphisms in the *MTHFR* gene in AML patients in the Saudi population. In this case-control study, 100 AML cases and 100 normal healthy controls were adopted based on the inclusion and exclusion criteria of the subjects. For each patient, 2 mL of the peripheral blood was collected in an EDTA vacutainer and genomic DNA was extracted using the specialized kits. Polymerase chain reaction was performed for the C677T and A1298C variants using the specific primers in both AML cases and controls. The risk of AML through molecular analysis of cases and controls were analyzed through statistical analysis. The significant difference was found with the age in AML cases and controls (p=.02), but not with the gender (p>.05). No significant association was occurred either with allele or genotype frequencies in C677T (T vs C: OR-0.75 (95% CI:0.38-1.46); p=.39); CT vs CC: OR-0.72 (95% CI:0.35-1.46); p=.37) and A1298C polymorphisms (C vs A: OR-1.03 (95% CI:0.60-1.76); p=.89); AC vs AA: OR-1.04 (95% CI:0.57-1.89); p=.88). The results of this case-control study suggested for the first time in the Saudi population that the C677T and A1298C polymorphisms were not associated and may not constitute a shared genetic risk factor for AML patients in the Saudis.

KEY WORDS: ACUTE MYELOID LEUKEMIA, MTHFR, C677T AND A1298C

Article Information:*Corresponding Author: aofarasani@jazanu.edu.sa
Received 12/07/2019 Accepted after revision 23/09/2019
Published: 30th Sep 2019 Pp-577-583

Published by Society for Science & Nature, Bhopal India.
Available at: https://bbrc.in/
Article DOI: http://dx.doi.org/10.21786/bbrc/12.3/4

INTRODUCTION

Leukemias are defined as group of heterogeneous malignancies which are normally categorized through acquiring somatic mutations such as chromosomal translocations, inversions and deletions, (Liu et al., 2016). Acute myeloid leukemia (AML) is one type of leukemia, which remains as one of the leading causes of global disease in both children and adolescents, (Zampini et al., 2018). AML, one of the common blood cancers, which is highly prevalent, is organized as an aberrant developmental hierarchy maintained through functionally distinct in leukemia stem cells, (Fujita et al., 2018, Wingelhofer et al., 2018). AML is known to be a heterogeneous group of disease characterized by high degree of heterogeneity with respect to chromosomal abnormalities and genetic/non-genetic variants, which translate to marked alterations in treatment response and survival, (Park et al., 2018).

Cytogenetic analysis is closely associated with specific cyto morphological subtypes well-defined by French-American-British criteria, consists of translocations between t (8; 21), t (15; 17), and t (16; 16) /inv (16), (Rose et al., 2017). As per the classification of world health organization (WHO), there were about more than 20% of AML blasts, (Bosshard et al., 2018). Almost 80% of AML patient receives complete remission and among them 50% of them relapse at a later time. The cytogenetic and molecular genetic changes play a major role in the pathogenesis of AML and also have an impact on prognosis of AML patients, (Both et al., 2017).

The precise molecular mechanisms of AML are unknown. Apart from this both genetic and environmental factor plays a major role in the development of AML. (Rashed et al., 2018) Genetic polymorphism has been identified with AML disease and role of polymorphism can affect the protein function, promoter activity, and mRNA stability and splice variants.(Seedhouse et al., 2004) Case-control, epidemiological and meta-analysis studies have connection with AML and methyltetrahydrofolate reductase (*MTHFR*) gene. (Qin et al., 2014) (Lien et al., 2017) MTHFR is a key enzyme in the encoded protein in folate metabolism converts 5,10-methylenetetrahydrofolate to 5,10-methylenetetrahydrofolate, a co-substrate for homocysteine methylation to methionine, (Smolkin and Perrotta, 2016).

Modifications in quantitative and qualitative in folate metabolism are enhancing the risk factors for leukemia, (Hussain et al., 2012). The common functional polymorphisms in *MTHFR* gene; C677T and A1298C could affect the activity of the enzyme, (He et al., 2014). The rs1801133 (C677T) polymorphism appears at exon 4 and modifies amino acid substitution of alanine-valine at codon 222. The rs1801131 (A1298C) polymorphism present in exon 7 and varies the amino acid substitution of glutamine-alanine at codon 429. (Jiang et al., 2014) Limited studies have been contributed to perform the meta-analysis studies with C677T and A1298C polymorphisms in AML. (Qin et al., 2014) From Saudi Arabia, no genetic studies have been documented till now and based on prior studies; AML subjects were genotyped with C677T and A1298C polymorphisms in the *MTHFR* gene. Here, the present study aimed to investigate the relationship between C677T and A1298C polymorphisms in *MTHFR* gene and susceptibility to acute myeloid leukemia in the Saudi population.

MATERIAL AND METHODS

AML subjects: In this study, 100 AML cases and 100 healthy controls were recruited from the Department of Hematology and Oncology in Riyadh regional hospital. AML diagnosis was confirmed through (i) bone marrow examination, (ii) full blood count and with (iii) flow cytometry. Apart from this pathology tests, cytogenetic analysis, such as chromosomal report and fluorescent in situ hybridization was also performed to reconfirm the results. During January 2016-November 2017, the blood samples were collected for this study. The inclusion criteria for AML cases were based on the following norms such as (I) Saudi nationality, (II) adolescent male and female subjects, (III) disease diagnosed through histopathological/cytogenetic confirmation and (IV) written and signed consent inform. The exclusion criteria were (I) Non-Saudi, (II) Patient diagnosed with another type of cancers and (III) Unsigned consent form. Age matched controls (N=100) were selected in Saudi nationality without effecting any type of specific cancers. From Riyadh regional hospitals, ethical grant was received through the Ministry of Health Affairs along with the signed inform consent form from 200 participants elaborated in this study as per the Declaration of Helsinki. From all the participated subjects, 2 mL of the peripheral blood was collected in an EDTA vacutainer and stored in the freezer for the further molecular analysis.

Molecular analysis: Two hundred genomic DNA was extracted from blood samples using with the genomic DNA purification kit (Sigma-Aldrich) as per the companies' protocol. Isolated and purified genomic DNA were confirmed through 1% agarose gel electrophoresis and cleansed DNA samples was stored at -40C in the freezer. The rs1801133 (C677T) and rs1801131 (A1298C) polymorphisms were genotyped using polymerase chain reaction (PCR) and restriction fragment length polymorphisms (RFLP) analysis. Complete details of the primers, restriction enzymes and PCR sizes are shown in Table 1. The 50-microliter PCR master mix consisted of

Table 1. Involvement of primer sequences in this study

SNP	rs number	Primer sequences	Amino acid substitution	PCR product	Enzyme
C677T	rs1801133	F: TCACCGGATCATGGCCAGCA R: TTCCTTACTGGTCCTCACATCTC	Ala222Val	198bp	HinfI
A1298C	rs1801131	F: GAACTCCCTGAAAAGCTAAAGC R: GTTGGGCTCAAATATACGGTGG	Glu429Ala	145bp	MboII

4 µL of genomic DNA (40-60 ng/µL) and 30 µL of PCR mix containing 10X buffer, $MgCl_2$, dNTPs, and 10x Taq DNA polymerase. The 10 pmoles of 2 µL of forward and reverse primers were added to the master mix followed by the addition of 12 µL of distilled water. PCR reaction was standardized for the final volume of 50 µL. C677T and A1298C primers were adopted from earlier studies, (Khan et al., 2015, Tanyildiz et al., 2016). PCR conditions for C677T and A1298C polymorphisms were as follows. Initial denaturation and denaturation were carried out for 5 mins at 94°C and 94°C for 30 secs, followed by 35 cycles. The annealing temperatures for C677T were 56°C and 58°C secs for A1298C polymorphisms respectively. Extension and final extension were found to be 72°C for 45 sacks and 5 mins respectively.

The PCR products were digested for 16 hours at 37°C with both the restriction enzymes as *HinfI* and *MboII*. The digested products of *HinfI* enzyme when electrophoresed through 2% agarose gel indicated the normal homozygote (CC) as 198bp. Mutant homozygote (TT) will expose two bands of 175 bp and 23 bp, whereas the heterozygous (CT) genotype will inferred from three bands of 198 bp, 175 bp, and 23 bp. The A1298C polymorphism abolishes a *MboII* restriction site and digestion results in 100 and 45bp fragment in the presence of the 1298C allele; and 75,45 and 25bp fragments in the absence of the 1298A alleles. Both the fragments from the AC fragments as 100,75,45 and 25 bp respectively.

Statistical analysis: Clinical data were statistically analyzed using Openepi software.(Khan et al., 2015) To compare the observed and expected genotype frequencies, Hardy-Weinberg Equilibrium (HWE) was performed. Genotype differences between cases and controls were executed with the odds ratios, upper and lower limits of the 95% confidence intervals (95% CI) for C677T and A1298C polymorphisms. The overall values of $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Characteristics of the subjects: One hundred AML cases and 100 healthy controls were recruited in this study. Data on clinical traits between the cases and controls such as age and gender were recorded and documented in Table 2. The age range of AML cases were in between 19-82 years [mean 38.9 (15.1)] and in controls, it was 18-63 years' old [mean 39.91(2.06)]. There was a significant difference between the cases and controls in the age (p=.02). The AML patients comprised of 61 males and 39 females and in the control subjects there were 54 males and 46 females.

HWE tests and genetic analysis: Genotypic distribution between C677T and A1298C polymorphisms were in accordance with HWE (p<.05). Both the allelic and genotypic distributions between AML cases and controls in C677T and A1298C polymorphisms were shown in the Table 3. The results showed in Table 3 both the allelic and genotypic association in C677T (T vs C: OR-0.75 (95% CI:0.38-1.46); p=.39); CT vs CC: OR-0.72 (95% CI:0.35-1.46); p=.37) and A1298C polymorphisms (C vs A: OR-1.03 (95% CI:0.60-1.76); p=.89); AC vs AA: OR-1.04 (95% CI:0.57-1.89); p=.88) were not significant between AML cases and controls. The dominant, co-dominant and recessive models also failed to show the significant associations in both the polymorphisms (Table 3).

The current study has designed as case-control to explore the association between C677T and A1298C genetic polymorphisms in the *MTHFR* gene and its effect

Table 2. Anthropometric details of the patients involved in this study

	AML cases (n=100)	Controls (n=100)	p-Value
Age (Years)	38.9±15.1	39.9±12.06	0.02
Minimum and maximum ages	19-82	18-63	-
Males	61 (61%)	54 (54%)	-
Females	39 (39%)	46 (46%)	-
N/A= Not analyzed/ Not applicable			

Table 3. Genotype and allele frequency distribution between AML cases and controls with C677T and A1298C variants in the MTHFR gene

MTHFR (rs1801133)	AML Cases (n=100)	Controls (*n*=100)	Odds Ratio	(95% CI)	*p* value[a]
Genotype and allele	N (%)	N (%)			
CC	83 (83%)	78 (78%)			
CT	17 (17%)	22 (22%)	0.72	0.35-1.46	0.37
TT	00 (00%)*	00 (00%)*	1.00	0.02- 99.99	0.99a
CT+TT vs CC	17 (17%)*	22 (22%)*	0.73	0.36-1.47	0.37a
CT vs CC+TT	17 (17%)*	22 (22%)*	0.73	0.36-1.47	0.37a
TT vs CC+CT	00 (00%)*	00 (00%)*	1.00	0.02- 99.99	0.99a
C	183 (91.5%)	178 (0.89)			
T	17 (8.5%)	22 (0.11)	0.75	0.38-1.46	0.39
MTHFR (rs1801131)					
AA	67 (67%)	68 (68%)	Reference		
AC	33 (33%)	32 (32%)	1.04	0.57-1.89	0.88
CC	00 (00%)	00 (00%)	1.00	0.01-51.12	0.99a
AC+CC vs AA	33 (33%)*	32 (32%)*	1.04	0.58-1.88	0.88a
AC vs AA+CC	33 (33%)*	32 (32%)*	1.04	0.58-1.88	0.88a
CC vs AA+AC	00 (00%)*	00 (00%)*	1.00	0.01-50.88	0.99a
A	167 (83.5%)	168 (84%)	Reference		
C	33 (16.5%)	32 (16%)	1.03	0.60-1.76	0.89
* & a *p value* after Yates Continuity Correction.					

on AML disease in the Saudi population. The current findings showed non-significant association between the cases and controls. To the best of my knowledge, this is initial study implemented with the association of C677T (rs1801133) and A1298C (rs1801131) polymorphisms in the *MTHFR* gene in AML disease risk in the Saudi Arabia. C677T and A1298C polymorphisms in *MTHFR* gene is well-known genetic polymorphisms that have an effect on human diseases such as PIH, GDM and PCOS, (Wu et al., 2013, Khan et al., 2015, Carlus et al., 2016). AML is confirmed as biological and clinically heterogeneous cancer of bone marrow, characterized through the rapid growth of abnormal myeloid cells.(Passaro et al., 2017) AML is known to be genetic and molecular heterogeneous disorder characterized by uncontrolled proliferation and blocked maturation of abnormal myeloid precursors.(Zhang et al., 2018b).

The disease has been classified based on histologic, cytogenetic and molecular genetic characteristics. AML is known to have genetic and molecular changes that alter normal hematopoietic growth and differentiations results in the accumulation of large numbers of abnormal, immature myeloid cells in the bone marrow and cytogenetic- molecular morphologies becomes the cornerstones of the therapeutic plainings, (Bacher et al., 2010). Patients diagnosed with AML and CCAAT/ enhancer-binding protein alpha (*CEBPA*) mutations confirm the deficiency of leukopenia, which leads to infections. Fever and weight loss are common symptoms associated with *CEBPA* variants, (Ho et al., 2009, Mannelli et al., 2017).

Recent advanced molecular techniques such as second generation advanced technique, micro array and the detection of molecular markers and their characterization has been aided with AML, (Bacher et al., 2010).The relation with AML and C677T/A1298C polymorphisms has been documented with multiple molecular studies. The enzyme 5,10 methylenetetrahydrofolate reductase plays a crucial role by irreversibly reducing 5,10 methylenetetrahydrofolates to 5 methyltetrahydrofolate, the predominant circulatory form of folate, (Bănescu and Trifa, 2015). A couple of the genetic polymorphisms involved in this study plays a role in reducing the MTHFR enzymatic activity from 40-70% in homozygous or either heterozygous subjects, affecting the folate metabolism, (Yaliwal and Desai, 2012). Low levels of folic acid may

lead to elevated uracil incorporation into DNA and this reduced DNA repairs the capacity which then leads to altered DNA methylation, which may promote leukemogenesis, (Duthie et al., 2000). Through this mechanism, the connection was bonded between AML and *MTHFR* genetic polymorphisms, (Kaur and Kaur, 2016, Jin et al., 2018, Rai, 2016, Rai, 2018, Zhang et al., 2018a).

Till now only single meta-analysis study has been documented with negative association in AML.(He et al., 2014) Maximum studies carried out with AML and *MTHFR* gene showed the negative association with the combination of the C677T and A1298C variants, (Skibola et al., 1999, Deligezer et al., 2003, Chen et al., 2006, da Costa Ramos et al., 2006, Bolufer et al., 2007, Moon et al., 2007, Barbosa et al., 2008, Lightfoot et al., 2010, Vahid et al., 2010, Hussain et al., 2012, Huang et al., 2015, Liu et al., 2016., Lien et al., 2017). The individual combination of C677T, (Moon et al., 2007, Skibola et al., 1999) and A1298C, (Vahid et al., 2010, Zheng et al., 2013) variants showed the positive associations with AML. There are limited genetic studies on next-generation and exome sequencing studies have been documented with AML through the worldwide, (Ley et al., 2008, Koh et al., 2014, Ilyas et al., 2015, Heo et al., 2017, Zhang et al., 2018b).

Ley et al., (2008) had conducted an initial study to perform next-generation sequencing in AML patients whereas, Heo et al., (2017) performed whole exome sequencing analysis in 36 Korean patients and identified 11 novel mutations; among them five of them were previously documented. Zhang et al (Zhang et al., 2018b) performed the whole exome sequencing studies in pediatric AML children. The results concluded from this study confirm the novel insights into the genetic basis of treatment failure in AML children.

The strength of the current study was the incorporation of 100 AML cases and 100 healthy controls. This study has certain limitations such as skipping the body mass index, family history and other clinical details. I have skipped the cytogenetic analysis and FISH data of AML patients and healthy controls. I did not validate the genotyping results through Sanger sequencing. Although the purpose of recruiting the patients from the hospital is to ensure the complete geographical coverage of the Kingdom of Saudi Arabia, this study results may not reflect the trend of the entire Saudi population.

To the best of my knowledge, this is the first genetic study investigated the association of the C677T and A1298C genetic polymorphisms with AML risk in Saudi Arabia. These results confirm the negative association; therefore, the *MTHFR* gene polymorphisms may not be associated with susceptibility to AML in the Saudi population. However, earlier global results along with meta-analysis studies confirms the negative association. Further studies would be required in different ethnic populations, especially in Arabic countries to facilitate a meta-analysis-based investigation in the future. I strongly recommend employing next-generation sequencing, exome sequencing-based examination in a larger cohort of AML cases with elaborated clinical information of the patients.

Conflict of Interest

There is no conflict of Interest towards this manuscript

REFERENCES

Bacher, U., Schnittger, S. & Haferlach, T. 2010. Molecular Genetics In Acute Myeloid Leukemia. Current Opinion In Oncology, 22, 646-655.

Bănescu, C. & Trifa, A. P. 2015. Methylenetetrahydrofolate Reductase 677 C T Polymorphism Is Associated With Acute Myeloid Leukemia. Leukemia & Lymphoma, 56, 1172-1174.

Barbosa, C. G., Souza, C. L., Moura Neto, J. P. D., Arruda, M. D. G. B., Barreto, J. H., Reis, M. G. & Goncalves, M. S. 2008. Methylenetetrahydrofolate Reductase Polymorphisms In Myeloid Leukemia Patients From Northeastern Brazil. Genetics And Molecular Biology, 31, 29-32.

Bolufer, P., Collado, M., Barragán, E., Cervera, J., Calasanz, M.-J., Colomer, D., Roman-Gómez, J. & Sanz, M. A. 2007. The Potential Effect Of Gender In Combination With Common Genetic Polymorphisms Of Drug-Metabolizing Enzymes On The Risk Of Developing Acute Leukemia. Haematologica, 92, 308-314.

Bosshard, R., O'reilly, K., Ralston, S., Chadda, S. & Cork, D. 2018. Systematic Reviews Of Economic Burden And Health-Related Quality Of Life In Patients With Acute Myeloid Leukemia. Cancer Treatment Reviews.

Both, A., Krauter, J., Damm, F., Thol, F., Göhring, G., Heuser, M., Ottmann, O., Lübbert, M., Wattad, M. & Kanz, L. 2017. The Hypomorphic Tert A1062t Variant Is Associated With Increased Treatment-Related Toxicity In Acute Myeloid Leukemia. Annals Of Hematology, 96, 895-904.

Carlus, S. J., Sarkar, S., Bansal, S. K., Singh, V., Singh, K., Jha, R. K., Sadasivam, N., Sadasivam, S. R., Gireesha, P. & Thangaraj, K. 2016. Is MTHFR 677 C> T Polymorphism Clinically Important In Polycystic Ovarian Syndrome (PCOS)? A Case-Control Study, Meta-Analysis And Trial Sequential Analysis. Plos One, 11, E0151510.

Chen, B., Jiang, N., Ji, M., Hou, P., Lu, Z., Gao, C., Ding, J., Sun, Y., Wang, J. & Cheng, J. 2006. A New Method For 5, 10-Methylenetetrahydrofolate Reductase Single Nucleotide Polymorphisms Genotyping Used To Study Susceptibility Of Hematological Malignancy. Zhongguo Shi Yan Xue Ye Xue Za Zhi, 14, 1069-1073.

Da Costa Ramos, F. J., Cartaxo Muniz, M. T., Silva, V. C., Araújo, M., Leite, E. P., Freitas, E. M., Zanrosso, C. W., Hatagima, A., De Mello, M. P. & Yunes, J. A. 2006. Association Between The Mthfr A1298c Polymorphism And Increased Risk Of Acute

Myeloid Leukemia In Brazilian Children. Leukemia & Lymphoma, 47, 2070-2075.

Deligezer, U., Akisik, E. & Dalay, N. 2003. Genotyping Of The Mthfr Gene Polymorphism, C677t In Patients With Leukemia By Melting Curve Analysis. Molecular Diagnosis, 7, 181-185.

Duthie, S. J., Narayanan, S., Blum, S., Pirie, L. & Brand, G. M. 2000. Folate Deficiency In Vitro Induces Uracil Misincorporation And Dna Hypomethylation And Inhibits Dna Excision Repair In Immortalized Normal Human Colon Epithelial Cells. Nutrition And Cancer, 37, 245-251.

Fujita, S., Honma, D., Adachi, N., Araki, K., Takamatsu, E., Katsumoto, T., Yamagata, K., Akashi, K., Aoyama, K. & Iwama, A. 2018. Dual Inhibition Of EZH1/2 Breaks The Quiescence Of Leukemia Stem Cells In Acute Myeloid Leukemia. Leukemia, 32, 855.

He, H., He, G., Wang, T., Cai, J., Wang, Y., Zheng, X., Dong, Y. & Lu, J. 2014. Methylenetetrahydrofolate Reductase Gene Polymorphisms Contribute To Acute Myeloid Leukemia And Chronic Myeloid Leukemia Susceptibilities: Evidence From Meta-Analyses. Cancer Epidemiology, 38, 471-478.

Heo, S. G., Koh, Y., Kim, J. K., Jung, J., Kim, H.-L., Yoon, S.-S. & Park, J. W. 2017. Identification Of Somatic Mutations Using Whole-Exome Sequencing In Korean Patients With Acute Myeloid Leukemia. Bmc Medical Genetics, 18, 23.

Ho, P. A., Alonzo, T. A., Gerbing, R. B., Pollard, J., Stirewalt, D. L., Hurwitz, C., Heerema, N. A., Hirsch, B., Raimondi, S. C. & Lange, B. 2009. Prevalence And Prognostic Implications Of Cebpa Mutations In Pediatric Acute Myeloid Leukemia (Aml): A Report From The Children's Oncology Group. Blood, 113, 6558-6566.

Huang, L., Deng, D., Peng, Z., Ye, F., Xiao, Q., Zhang, B., Ye, B., Mo, Z., Yang, X. & Liu, Z. 2015. Polymorphisms In The Methylenetetrahydrofolate Reductase Gene (Mthfr) Are Associated With Susceptibility To Adult Acute Myeloid Leukemia In A Chinese Population. Cancer Epidemiology, 39, 328-333.

Hussain, S. R., Naqvi, H., Raza, S. T., Ahmed, F., Babu, S. G., Kumar, A., Zaidi, Z. H. & Mahdi, F. 2012. Methylenetetrahydrofolate Reductase C677T Genetic Polymorphisms And Risk Of Leukaemia Among The North Indian Population. Cancer Epidemiology, 36, E227-E231.

Ilyas, A. M., Ahmad, S., Faheem, M., Naseer, M. I., Kumosani, T. A., Al-Qahtani, M. H., Gari, M. & Ahmed, F. 2015. Next Generation Sequencing Of Acute Myeloid Leukemia: Influencing Prognosis. Bmc Genomics, 16, S5.

Jiang, N., Zhu, X., Zhang, H., Wang, X., Zhou, X., Gu, J., Chen, B. & Ren, J. 2014. The Relationship Between Methylenetetrahydrofolate Reductase Polymorphism And Hematological Malignancy. Clinical Laboratory, 60, 767-774.

Jin, H., Cheng, H., Chen, W., Sheng, X., Levy, M. A., Brown, M. J. & Tian, J. 2018. An Evidence-Based Approach To Globally Assess The Covariate-Dependent Effect Of The Mthfr Single Nucleotide Polymorphism Rs1801133 On Blood Homocysteine: A Systematic Review And Meta-Analysis. The American Journal Of Clinical Nutrition, 107, 817-825.

Kaur, A. & Kaur, A. 2016. Maternal Mthfr Polymorphism (677 C–T) And Risk Of Down's Syndrome Child: Meta-Analysis. Journal Of Genetics, 95, 505-513.

Khan, I. A., Shaik, N. A., Kamineni, V., Jahan, P., Hasan, Q. & Rao, P. 2015. Evaluation Of Gestational Diabetes Mellitus Risk In South Indian Women Based On Mthfr (C677t) And Fvl (G1691a) Mutations. Front Pediatr, 3, 34.

Koh, Y., Kim, D.-Y., Yook, J., Park, H., Lee, C.-S., Ahn, K.-S., Lee, H.-J., Kim, H. L., Jung, J. & Kim, H. J. 2014. Whole Exome Sequencing Of Acute Myeloid Leukemia Patients In Korea And Its Comparison With Tcga Results: Dramatic Difference Of Genomic Signatures According To Ethnicity. Am Soc Hematology.

Ley, T. J., Mardis, E. R., Ding, L., Fulton, B., Mclellan, M. D., Chen, K., Dooling, D., Dunford-Shore, B. H., Mcgrath, S. & Hickenbotham, M. 2008. Dna Sequencing Of A Cytogenetically Normal Acute Myeloid Leukaemia Genome. Nature, 456, 66.

Lien, S.-Y. A., Young, L., Gau, B.-S. & Shiao, S. P. K. 2017. Meta-Prediction Of Mthfr Gene Polymorphism-Mutations, Air Pollution, And Risks Of Leukemia Among World Populations. Oncotarget, 8, 4387.

Lightfoot, T. J., Roman, E., Smith, M. T. & Skibola, C. F. 2010. Acute Lymphoblastic Leukaemia In Children–Is There A Role For MTHFR? British Journal Of Haematology, 149, 797-798.

Liu, P., Zhang, M., Xie, X., Jin, J. & Holman, C. D. A. J. 2016. Polymorphisms Of 5, 10-Methylenetetrahydrofolate Reductase And Thymidylate Synthase, Dietary Folate Intake, And The Risk Of Leukemia In Adults. Tumor Biology, 37, 3265-3275.

Mannelli, F., Ponziani, V., Bencini, S., Bonetti, M. I., Benelli, M., Cutini, I., Gianfaldoni, G., Scappini, B., Pancani, F. & Piccini, M. 2017. Cebpa–Double-Mutated Acute Myeloid Leukemia Displays A Unique Phenotypic Profile: A Reliable Screening Method And Insight Into Biological Features. Haematologica, 102, 529-540.

Moon, H. W., Kim, T. Y., Oh, B. R., Min, H. C., Cho, H. I., Bang, S. M., Lee, J. H., Yoon, S. S. & Lee, D. S. 2007. Mthfr 677cc/1298cc Genotypes Are Highly Associated With Chronic Myelogenous Leukemia: A Case-Control Study In Korea. Leukemia Research, 31, 1213-1217.

Park, S., Choi, H., Kim, H. J., Ahn, J.-S., Kim, H.-J., Kim, S.-H., Mun, Y.-C. & Jung, C. W. 2018. Genome-Wide Genotype-Based Risk Model For Survival In Core Binding Factor Acute Myeloid Leukemia Patients. Annals Of Hematology, 97, 955-965.

Passaro, D., Di Tullio, A., Abarrategi, A., Rouault-Pierre, K., Foster, K., Ariza-Mcnaughton, L., Montaner, B., Chakravarty, P., Bhaw, L. & Diana, G. 2017. Increased Vascular Permeability In The Bone Marrow Microenvironment Contributes To Disease Progression And Drug Response In Acute Myeloid Leukemia. Cancer Cell, 32, 324-341. E6.

Qin, Y.-T., Zhang, Y., Wu, F., Su, Y., Lu, G.-N. & Wang, R.-S. 2014. Association Between Mthfr Polymorphisms And Acute Myeloid Leukemia Risk: A Meta-Analysis. Plos One, 9, E88823.

Rai, V. 2016. Methylenetetrahydrofolate Reductase C677t Polymorphism And Recurrent Pregnancy Loss Risk In Asian Population: A Meta-Analysis. Indian Journal Of Clinical Biochemistry, 31, 402-413.

Rai, V. 2018. Strong Association Of C677t Polymorphism Of Methylenetetrahydrofolate Reductase Gene With Nosyndromic Cleft Lip/Palate (Nscl/P). Indian Journal Of Clinical Biochemistry, 1-11.

Rashed, R., Shafik, R. E., Shafik, N. F. & Shafik, H. E. 2018. Associations Of Interleukin-10 Gene Polymorphisms With Acute Myeloid Leukemia In Human (Egypt). Journal Of Cancer Research And Therapeutics, 14, 1083.

Rose, D., Haferlach, T., Schnittger, S., Perglerova, K., Kern, W. & Haferlach, C. 2017. Subtype-Specific Patterns Of Molecular Mutations In Acute Myeloid Leukemia. Leukemia, 31, 11.

Seedhouse, C., Faulkner, R., Ashraf, N., Das-Gupta, E. & Russell, N. 2004. Polymorphisms In Genes Involved In Homologous Recombination Repair Interact To Increase The Risk Of Developing Acute Myeloid Leukemia. Clinical Cancer Research, 10, 2675-2680.

Skibola, C. F., Smith, M. T., Kane, E., Roman, E., Rollinson, S., Cartwright, R. A. & Morgan, G. 1999. Polymorphisms In The Methylenetetrahydrofolate Reductase Gene Are Associated With Susceptibility To Acute Leukemia In Adults. Proceedings Of The National Academy Of Sciences, 96, 12810-12815.

Smolkin, M. & Perrotta, P. 2016. Molecular Diagnostics For Coagulopathies. Diagnostic Molecular Pathology. Elsevier.

Tanyildiz, H. G., Yesil, S., Bozkurt, C., Çandir, M. O., Akpinar-Tekgündüz, S., Toprak, S., Yüksel, D. & Sahin, G. 2016. Are The Methylenetetrahydrofolate Reductase 1298 And 677 Gene Polymorphisms Related To Optic Glioma And Hamartoma Risk In Neurofibromatosis Type 1 Patients? The Turkish Journal Of Pediatrics, 58, 152.

Vahid, P., Farnaz, R., Zaker, F., Farzaneh, A. & Parisa, R. 2010. Methylenetetrahydrofolate Reductase Gene Polymorphisms And Risk Of Myeloid Leukemia. Laboratory Medicine, 41, 490-494.

Wingelhofer, B., Maurer, B., Heyes, E. C., Cumaraswamy, A. A., Berger-Becvar, A., De Araujo, E. D., Orlova, A., Freund, P., Ruge, F. & Park, J. 2018. Pharmacologic Inhibition Of Stat5 In Acute Myeloid Leukemia. Leukemia, 32, 1135.

Wu, X., Wang, X., Chan, Y., Jia, S., Luo, Y. & Tang, W. 2013. Folate Metabolism Gene Polymorphisms Mthfr C677t And A1298c And Risk For Down Syndrome Offspring: A Meta-Analysis. European Journal Of Obstetrics & Gynecology And Reproductive Biology, 167, 154-159.

Yaliwal, L. V. & Desai, R. M. 2012. Methylenetetrahydrofolate Reductase Mutations, A Genetic Cause For Familial Recurrent Neural Tube Defects. Indian Journal Of Human Genetics, 18, 122.

Zampini, M., Tregnago, C., Bisio, V., Simula, L., Borella, G., Manara, E., Zanon, C., Zonta, F., Serafin, V. & Accordi, B. 2018. Epigenetic Heterogeneity Affects The Risk Of Relapse In Children With T (8; 21) Runx1-Runx1t1-Rearranged Aml. Leukemia, 32, 1124-1134.

Zhang, R., Huo, C., Wang, X., Dang, B., Mu, Y. & Wang, Y. 2018a. Two Common Mthfr Gene Polymorphisms (C677t And A1298c) And Fetal Congenital Heart Disease Risk: An Updated Meta-Analysis With Trial Sequential Analysis. Cellular Physiology And Biochemistry, 45, 2483-2496.

Zhang, T.-J., Lin, J., Zhou, J.-D., Li, X.-X., Zhang, W., Guo, H., Xu, Z.-J., Yan, Y., Ma, J.-C. & Qian, J. 2018b. High Bone Marrow Mir-19b Level Predicts Poor Prognosis And Disease Recurrence In De Novo Acute Myeloid Leukemia. Gene, 640, 79-85.

Zheng, M., Yue, L., Zhang, H., Yang, C. & Xie, C. 2013. Association Of Single Nucleotide Polymorphism Of Methylenetetrahydrofolate Reductase Gene With Susceptibility To Acute Leukemia. Zhonghua Yi Xue Yi Chuan Xue Za Zhi, Zhonghua Yixue Yichuanxue Zazhi, Chinese Journal Of Medical Genetics, 30, 451-455.