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Original article

Experimental study of A66G-single nucleotide polymorphism in the *MTRR* gene and acute myeloid leukemia

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ABSTRACT

Acute Myeloid leukemia (AML) is the most common leukemia among adult population which begins with the bone marrow, but it frequently spreads to the blood stream as well. One of the common polymorphisms in AML is A66G in Methionine Synthase Reductase (MTRR), which converts isoleucine into methionine residue in the protein chain, the methionine/homocysteine cycle is disrupted. Limited studies were documented between A66G polymorphism in *MTRR* gene with AML. This study aimed to investigate the A66G polymorphism in *MTRR* gene with AML patients in the Saudi population. Peripheral blood was collected from 100 AML patients and 100 controls and DNA was isolated. Using A66G primers, polymerase chain reaction was performed followed by restriction fragment length polymorphism analysis. The mean age of both cases and controls was found to be an average of 39 years. Allele (G vs A: OR-3.41 [95 %CI: 1.87–11.24; p = 0.0001) and genotype analysis (GG vs AA: OR-3.43 [95 %CI: 1.45–8.11]; p = 0.0004) has shown the association. In conclusion, A66G polymorphism has a strong genetic role in the AML patients in Saudi population.

1. Introduction

Acute myeloid leukemia (AML) is a clonal hematopoietic stem cell malignancy characterized by an influx of immature progenitor cells that are halted in their developmental state and cause the reduced proliferation of red blood cells and the production of immature forms of white blood cells. AML morphology, immunophenotype, cytogenetic and epigenetic markers, and treatment response (such as patient outcomes) are all very varied (Döhner et al., 2021). AML is the most common a kind adult leukemia, with a high relapse and mortality rate (nearly 50–65 %). There are reports that there are leukemia initiating cells present, which are able to self-renew and create all of the bulk leukemia cell progenies that are employed for complications in AML treatment, resulting in a recurrence (He et al., 2021). Cancer is a very well-known disease in the field of cancer research, and AML has been a prime study for how cancer develops and progresses. The translocation (8;21) (q22;q22) AML disease entity is prominent in several genetic and biochemical subtypes of the disease. Functional studies have revealed that the oncofusion protein AML1/ETO (also known as RUNX1/ RUNX1T1) has a specific function, but they have also revealed other aspects of the protein's functions (Rejeski et al., 2021). AML accounts for 10–20 % of infant leukemia, and the 5-year survival rate for AML is less than 30 % in adults. Furthermore, elderly AML patients are poorly tolerated and contagious (Yuan et al., 2021). According to the French and American classifications, AML is divided into eight subtypes, M0-M7, based on the type of leukemia that develops and the degree of maturity of the leukemia (Lafuente et al., 1993). Although many AML experts prefer the 3 + 7 regimen, some do not. A bleak outlook until 2015 has given way to a very optimistic outlook, thanks to many new medications approved by the US Food and Drug Administration that have significantly advanced AML treatment options (Kantarjian et al., 2021).

It has been demonstrated that AML and/or *t*-AML is related to polymorphism genes such as base excision repair, detoxifying genes. NAD(P) glutathione reductase and quinine oxidoreductase S-transferases (Seedhouse et al., 2004). Methionine Synthase Reductase (MTRR) ensures that sufficient amounts of activated cobalamin are present in the remethylation of homocysteine (Hcy) to methionine. As the cobalt(I)alamin and methyl cobalt (III)alamin cofactors cycle. This enzyme's inactive form cob(II)ala-

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min results from cob(I)alamin's strong reductant properties. Cob (II)alamin is reductively methylated by MTRR using S-adenosylmethionine as the methyl donor to form methyl cob(III) alamin In order to retain cobalamin in an active state, MTRR performs a key role, and may be a significant predictor of tHcy levels (Gaughan et al., 2001). A housekeeping gene on chromosome 5p15.2-p15.3, the human MTRR is situated on chromosome 5. The A66G (rs1801394) SNP is the most frequent change in the MTRR gene, and it causes a change from isoleucine to methionine to be inserted at position 22. (I22M). Possible effects of MTRR polymorphisms on vitamin B12 cellular metabolism and blood transport (Basir, 2019; Guéant-Rodriguez et al., 2005). Previous studies have established a genetic link between the A66G polymorphism in the MTRR gene and AML (Gemmati et al., 2004). Furthermore, A66G polymorphisms have been examined in a diverse range of diseases, including lymphoid malignancies and tumors (Matsuo et al., 2001; Ma et al., 1997). Saudi Arabia has documented limited studies with leukemia (Jastaniah et al., 2016; Roberts et al., 1992; Bawazir et al., 2019), and no studies with A66G polymorphism have been conducted. The current study aimed to investigate genetic association studies with A66G polymorphism studies in AML in Saudi subjects.

2. Materials and methods

2.1. AML patients selection

This study protocols and consent forms was sanctioned from Jazan University. All patients have signed the informed consent form. This study was conducted as per the guidelines of the Declaration of Helsinki. In this case-control study, a total of 200 subjects were recruited from Riyadh Regional Laboratory, Riyadh and Saudi Arabia. The 200 subjects were divided as 100 AML cases and 100 controls. The inclusion and exclusion criteria of the study subjects were defined in the previous study (Farasani, 2019). The laboratory confirmed the diagnosis of AML patients using cytogenetics and histopathology. AML was indicated through bone marrow testing, a complete blood count, and flow cytometry. Furthermore, cytogenetics and FISH testing were used to confirm the findings. Patients with other cancers and non-Saudi participants with unsigned consent forms were exclusion criteria for AML cases. The healthy controls' inclusion criteria were that they had not been diagnosed with any sort of cancer or other ailment. Non-Saudi subjects were excluded as a criterion.

Table 1
Anthropometric information on the patients in this study.

Anthropometric measurements	AML cases (n = 100)	Controls (n = 100)	p-Value
Age (Years)	38.9 ± 15.1	39.9 ± 12.06	0.60
Minimum and maximum ages	19–82	18–63	–
Males (Gender)	61 (61 %)	54 (54 %)	–
Females (Gender)	39 (39 %)	46 (46 %)	–

2.2. Analysis of genomic DNA and PCR

In this study, 2 ml of blood was collected in an EDTA vacutainer as described (Khan et al., 2015). Genomic DNA was extracted from peripheral whole blood using genomic DNA purification kit (Bogari et al., 2021) for 200 subjects. The procedure was followed as per the norms of kit protocol. Later on, NanoDrop was used to measure the DNA quality (Al-Otaiby et al., 2021). A66G polymorphism in the MTRR gene was genotyped using the specific primers. The amplification was carried out in 50 µl of reaction volume using polymerase chain reaction (PCR) with PCR master mix, primer set, purified water, and DNA (Alshammary and Khan, 2021). The PCR protocol consists of 35 cycles, beginning with denaturation (95^0C), then annealing (58^0C), and finally extension (72^0C). Initial denaturation and final extensions were also involved (Saif and Khan, 2022). At 37 °C for 18 h, *NSPI* enzymes digested the 135 bp of PCR products. The samples were run on an ethidium bromide stained using a 3 % agarose gel. According to the 135 bp, it validates the AA-genotype, while GG-genotype confirms 90 and 45 bp.

2.3. Statistical analysis

In this study, *t*-test was performed between AML cases and control (Table 1). Hardy Weinberg Equilibrium analyses (HWE) was studied in A66G Polymorphism. Genotype and allele frequencies were carried out between AML cases and control. A $p \leq 0.05$ were confirmed as statistically significant (Khan et al., 2019).

3. Results

This study includes 100 AML cases and 100 controls whose ranges between 19 and 82 in cases and 18–63 in controls. It is shown in Table-1 that AML patients and non-AML patients (controls) were compared for their clinical features. The mean age for AML (38.91 + 15.1) vs controls (39.9 + 12.06) couldn't show the association (p = 0.60). AML cases range in age from 19 to 82, while control subjects in this study ranged in age from 18 to 63. AML cases included sixty-one percent male and thirty-nine percent female patients, whereas controls had fifty-four percent male and fourty-six percent female patients, according to a new study.

HWE analysis was performed in A66G polymorphism in the MTRR gene and analysis confirms the A66G polymorphism was found to be in accordance (χ^2 = 18.26 and p = 0.000019) after analyzing with one degree of freedom. In this study, table-2 defines the allele and genotype frequencies including with the different genetic mode of inheritances of the rs1801394 (A66G) polymorphism in two studied groups as AML cases and controls. The AML cases consists of 44 % of AA, 35 % of AG and 21 % of GG of genotype frequencies whereas in the controls, AA genotypes were found to be 77 % and AG and GG genotypes was 15 % and 8 % respectively. The allelic frequencies between A and G alleles were found to be

Table 2
Genotype distribution frequencies between A66G polymorphism and AML cases.

A66G	AML cases (n = 100)	Controls (n = 100)	ORs	95 % CIs	Pvalue
AA	44 (44 %)	77 (77 %)	Position	Position	Position
AG	35 (35 %)	15 (15 %)	4.08	2.01–8.29	0.0001
GG	21 (21 %)	08 (08 %)	4.59	1.87–11.24	0.0004
AA vs AG + GG	44 (44 %)	77 (77 %)	3.43	1.45–8.11	0.003
AA + AG vs GG	79 (79 %)	92 (92 %)	0.32	0.13–0.77	0.009
A allele	123 (61.5 %)	169 (84.5 %)	Position	Position	Position
G allele	77 (38.5 %)	31 (15.5 %)	3.41	2.11–5.5	0.0001

61.5 % and 38.5 % in the AML cases and 84.5 % and 15.5 % was found in the controls for A and G alleles respectively. The genotype frequencies for AG vs AA (OR-4.08 [95CI:2.08–8.29]; p = 0.0001) and GG vs AA (OR-4.59 [95CI: 1.87–11.24] p = 0.0004) was associated between cases and controls. Dominant model showed the statistical association (OR-3.43 [95CI: 1.45–8.11] p = 0.003) and recessive model showed the negative association (OR-0.32 [95CI: 0.13–0.77] p = 0.009). Allelic association was also documented (G vs A: OR-3.41 [95CI:2.11–5.5]; p = 0.0001). In this study, the power and sample size calculation were found to be 78 % (Table 2).

4. Discussion

This study was evaluated the affect of A66G polymorphism in the *MTRR* gene in Saudi patients confirmed with AML. Till now, there was no genetic studies were documented and current study confirms the initial study in the Saudi population with strong association in genotypes and allele frequencies (p = 0.0001; p = 0.0004 and p = 0.003). The mean age for AML and control subjects was found to be on an average of 39 years.

Leukemia is frequently affected by DNA abnormalities, including as translocations, inversions, and deletions. It's widely believed to have a pivotal part in leukemia's evolution. It's generally agreed that DNA methylation has a major effect on how genes are expressed. Involvement of the folic acid metabolic route in DNA methylation, DNA repair, and DNA synthesis is crucial. The enzyme methionine synthase (MS) is involved in the remethylation of homocysteine to methionine, which is part of the folate metabolic pathway. The functional cofactor form of MS in the remethylation of homocysteine to methionine is cobalamin, and MTRR is a part of this route that is necessary for its reductive methylation. Lower affinity for the MS-causing *MTRR* gene has been linked to a genetic variant called *MTRR* A66G (rs1801394). Human disorders, like male infertility and cancer, have been related to abnormal folate metabolism (Gaughan et al., 2001; Brosselin et al., 2009; Fang et al., 2014; Ren et al., 2019). The story of folate metabolism begins with the intake of folic acid in the form of diet (Kalpana, 2021). An enzyme designated methionine synthase reductase (MTR) is encoded by the *MTRR* gene. By using the aid of this enzyme, MTR is able to combine the amino acid homocysteine with methionine, making use of the B vitamins B12 and B2. As high homocysteine levels severely affect heart health, metabolism, mental health, prenatal development, and more, effective homocysteine clearance is a vital part of the methylation cycles. The relation between homocysteine and A66G polymorphism has been documented in multiple studies (Gaughan et al., 2001; Botto et al., 2003; Laraqui et al., 2006).

The *MTRR* gene is important in maintaining the MTR gene's current state, and a study discovered a link between cancer risk and genetic diversity within the *MTRR* gene. This most common polymorphism involves the substitution of isoleucine for methionine at chromosome 5 position 22 (5p15.2-p15.3) (A66G; rs1801394). The hypothesis is that homocysteine levels are lower in those who have the 66GG genotype. A previous *meta*-analysis studies conducted in several types of cancers with A66G polymorphism in the *MTRR* gene and a positive association was confirmed with an increased risk (Wang et al., 2017). A *meta*-analysis study in leukemia was discovered that the GG genotype in the *MTRR* A66G polymorphism reduced the incidence of leukemia in the Caucasian population (Fang et al., 2014). Previous studies with A66G polymorphism in AML confirmed both positive and negative associations (Kim et al., 2009; Gra et al., 2008) A66G polymorphism was found to be associated with Asian populations in AML, while Caucasian populations confirmed the negative association (Kim et al., 2009; Gra et al., 2008). This study was conducted solely on Saudi adults and confirmed the positive correlation.

In our study, RFLP analysis was implemented as this is one of the accurate techniques to identify the restriction bands after digesting at room temperature as per the advised temperature. Restriction endonucleases act according to certain nucleotide sequences in the DNA molecule being cleaved, depending on the enzyme utilized. Most enzyme recognition sites are between 4 and 6 nucleobases long. As a general rule, shorter recognition sequences yield more pieces. Fragments of varied sizes may be created if molecules vary in nucleotide sequence. It is possible to separate the pieces using gel electrophoresis. Enzymes that are derived from bacteria that vary greatly in their genera are known to be part of the cell's defense against invading viruses. The enzymes are known by the genus, species, and order of discovery. One of the limitations of this study is lower sample size and performed this study with A66G polymorphism.

5. Conclusion

The present study concludes as A66G polymorphism in the *MTRR* gene was found to be significantly associated in the adult Saudi population. A66G polymorphism was found to be an important role in cancers specially leukemia and there are only limited studies were restricted. It will be important for researchers to examine AML in a global population in the future.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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