RESEARCH ARTICLE

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DNA Repair Genes Polymorphisms: Impact on Acute Myeloid Leukemia Patients Outcome

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Abstract

Background: *ATM*; *XRCC6* and *LIG4* genes play an important role in repairing the double-strand DNA breaks and maintaining the genome stability. Single nucleotide polymorphisms (SNPs) in these genes could affect these genes expression and function. The aim of this study was to address the effect of SNP of the DNA repairing genes on corresponding gene expression as well as AML patient's outcome. **Subjects and Methods:** This is cross sectional study included 95 newly diagnosed AML patients. For all subjects included in our study SNPs and expression of *ATM* (rs189037G>A), *XRCC6* (rs2267437C>G) and *LIG4* (rs1805388C>T) genes were evaluated by RFLP and real time PCR. **Results:**The following SNPs in *ATM* (AA); *XRCC6* (GG); and *LIG4* (TT) are associated with down regulation of the corresponding genes (P<0.001). The lower expression of *ATM* and *LIG4* genes are associated with shorter OS and DFS. Cox regression multivariate analysis revealed that lower expression of *ATM* HR : 2.02 (CI: 1.12-3.64; p=0.020. **Conclusion:** The following SNPs of *ATM* (AA); *XRCC6* (GG); and *LIG4* (TT) are associated with down regulation of corresponding genes expression. *ATM* and *XRCC6* lower expression are predictors of OS while *ATM* is predictor of DFS and could be used for optimizing the AML therapy.

Keywords: AML- *ATM*- *XRCC6*- *LIG4*- Genes- Outcome

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Introduction

Acute myeloid leukemia (AML) is the most prevalent malignant myeloid neoplasm among adults; and results from clonal proliferation of immature myeloid cells and down regulation of myeloid differentiation. AML relapses are initiated by chemo-resistant residual leukemic cells. Based on cytogenetic findings AML was stratified into 3 categories: AML with recurrent cytogenetic abnormalities; AML with normal cytogenetic and AML with adverse cytogenetic abnormalities. Recurrently mutated genes in CN-AML were identified, such as NPM1, signal transduction genes (FLT3), or myeloid transcription factor genes (CEBPA, RUNX1) (Gabellier et al., 2020).

DNA damage and repair mechanisms influence not only the genetic predisposition to leukemia but are also very important for refractoriness to treatment. DNA repair capability plays a critical role in maintaining the stability of human genome and error-prone DNA repair might be a risk factor for several cancers (Renen et al.,2002). The most severe type of DNA damage in hemopoietic stem cells is double strand breaks lesions (Hopfner et al., 2009), which, if not repaired in a proper manner, may lead to neoplastic transformation due to chromosomal abnormalities (Belli et al.,2002). There are two mechanisms for double strand breaks lesion repair: homologous recombination (HR) and non-homologous end joining (Hopfner et al., 2009; House et al., 2014) and both are important for controlling the genomic instability of hemopoietic stem cells (Meh and Habe 2014). Common genetic polymorphisms in DNA repair genes might affect protein function and, thus, the capacity to repair damaged DNA, which in turn could lead to genetic instability and leukemogenesis (Kentsioti et al., 2019).

Single nucleotide Polymorphisms (SNPs) are normal common variation in the bases sequence in the DNA among normal populations which could result in changes in the genes expression and function. In this context SNPs might affect genes responsible for DNA repair namely Ataxia Telangiectasia Mutated (*ATM*); X-ray repair cross-complementing 6 (*XRCC6*); and *LIG4*. Several investigators define functional polymorphisms (FPs) as alterations in DNA that have an effect on the gene function (expression of mRNA or its protein); among these polymorphisms the most suggested ones included *ATM* (rs189037G>A), *XRCC6* (rs2267437C>G) and *LIG4* (rs1805388C>T). FPs can alter mRNA folding and consequently affect mRNA stability (Albert et al., 2011). Additionally, FPs may also alter protein conformations by modifying translocation elongation (Liao and Lee 2010).

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The *ATM* gene was mapped to chromosome 11q22–23 transcripts mRNA with a coding sequence of 9168 bp (Prokopcoved et al., 2007). The polymorphism rs189037 is located at the 5′UTR of the promoter region of *ATM* gene (Gu et al., 2016). *ATM* is a 350-kDa protein serine/threonine kinase, member of the phospho-inositide 3-kinase (PI3-kinase)-like family (PIKK) belongs to the core components of DNA-damage response machinery and acts as an intracellular sensor by recognizing double-strand breaks (DSBs).

XRCC group is a family of DNA repair genes with a main function in the repair of single-strand breaks and DNA-based damage. Multi genes in the XRCC family have been well studied in the process of carcinogenesis and have been deemed as promising genetic biomarkers (Gong et al., 2016). Many studies have reported the association between the rs2267437 *XRCC6* polymorphism and cancer susceptibility (Haitato et al .,2013). Promoter gene *XRCC6* −61 (rs2267437C>G) CG/GG genotype was a significantly associated with lower risk of AML (Wang et al 2010).

LIG4 gene, located on human chromosomes 13q33-34, encodes an ATP-dependent DNA ligase that joins single-strand breaks in a double-stranded polydeoxynucleotide in an ATP-dependent reaction. Polymorphisms of *LIG4* gene may influence DNA repair ability, thus altering the genetic stability and resulting in carcinogenesis. A growing number of studies have investigated the relevance of *LIG4* T9I (rs1805388) polymorphisms with cancer risk (Xie et al 2014).

The impact of dysregulation of DNA damage response genes on evolution of various subtypes of acute myeloid leukemia (AML) patients as well as patients outcome were previously reported (Esposito et al 2014, Nieborowska-Skorska et al 2017, Elizabeth et al 2018). However, its frequency and clinical impact on Egyptian cohort of AML patients was not previously addressed.

The aim of this study is to evaluate the functional DNA repair genes SNPs and its expression on AML patients' outcome.

Materials and Methods

Patients

This is cross sectional study conducted on 95 AML patients (50 male; 45 female) with mean age 42.0 years (age range 21-58 years) at diagnosis before start of therapy. The diagnosis was based on morphological examination of blood and bone marrow smear (blast cells ≥20%) which confirmed by immunophenotyping. Cytogenetic t(8;21); t(15;17); inv16; t(16;16); t(6;9); and genetic assessments (FLT3; NPM) was done for the aim of risk stratification.

The clinical data of the investigated patients are presented in Table 1. All non-AML-M3 patients received cytosine arabinoside plus anthracyclines-based regimens as described previously (Jiao et al., 2009). The response for treatment was evaluated in accordance of the revised recommendations that was postulated by International Working Group for therapeutic trials in AML (Cheson et al 2003).

The follow-up time for the AML patients included in the current study was 24 months.

All included patients were given informed-consent according to declaration of Helsinki before inclusion in the study. The study was approved Mansoura faculty of medicine local ethical committee.

Endpoints definitions: Overall survival is defined as the time from diagnosis until death or end of the study. DFS is defined as the time from remission until disease relapse, death or end of the study (Gourgou-Bourgade et al.,2015).

Methods

Genotype analysis

The method used to determine *ATM* (rs189037G>A), *XRCC6* (rs2267437C>G) and *LIG4* (rs1805388C>T) polymorphisms detection includes DNA extraction/amplification by polymerase chain reaction (PCR)/restriction fragment length polymorphism (RFLP).

Genomic DNA extraction

Total genomic DNA was extracted from the bone marrow cells of AML patients using the Mini QIA amp DNA isolation kit (QIAGEN) following the standard procedures according to the manufacturer's instructions.

Genotyping

All patients were Genotyped for *ATM* rs189037 G>A (5-UTR), *XRCC6* rs2267437C>G (-61), *LIG4* rs1805388 C>T (T9I) by PCR- restriction fragment length polymorphism(RFLP) method. The amplified DNA were digested with restriction enzyme endonuclease enzymes The primers and restriction enzymes were as follow: for *ATM* rs189037 (G > A) F: 5'-GCTGCTTGGCGTTGCTTC-3'- R: 5'-CATGAGATTGGCGGTCTGG-3'- MscI; for *XRCC6* rs2267437(C>G) 3′TCTCCACTCGGCTTTTCTTCCA5′ 5′TCTCCCTCCGCTTCGCACTC 3′ - Ban I; and for *LIG4* rs1805388 (C>T) 3′TCTGTATTCGTTCTAAAGTTGAACA 5′ - 5′TGCTTTACTAGTTA AACGAGAAGAT 3′- HpyCH4 III.

Each 25-µl PCR reaction contained 25 ng of genomic DNA, 12.5 µl of 2X PCR Master Mix (50 units/ml of Taq DNA polymerase supplied in a proprietary reaction buffer (pH 8.5), 400-µM dATP, 400-µM dGTP, 400-µM dCTP, 400-µM dTTP and 3mM MgCl) (Promega corporation, Fitchburg, WI, USA) and 10nM of each primer. The following thermal cycling conditions were used: an initial denaturation at 95°C for 10 minutes, followed by 35- cycles of 95°C for 45 seconds, a specific annealing temperature for 30 seconds and enzyme extension at 72°C for 45 seconds. The final extension was done at 72°C for 10 minutes. PCR was performed using Master cycler Pro Vapo Protect Technology (Eppendorf, Hamburg, Germany).

The PCR products were digested with restriction endonuclease at 37°C for 90 minutes and then visualized by electrophoresis in a 3% on agarose gel after staining with ethidium bromide.

Gene expression Quantitation

Total RNA extraction

Total RNA extractions isolated from obtained mononuclear cells (bone marrow), obtained from AML patients. This was performed with TRizol Reagent™ (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's protocol. Then the total RNA was purified by using RNA purification kit (Invitrogen, USA). RNA was treated with amplification grade DNase I to eliminate any residual genomic DNA from the sample and further purified with the RNeasy® Micro Kit (Qiagen Inc., Germany). RNA quality and quantity were analyzed using a spectrophotometer, NanoDrop ND-1000 (Nanodrop Technologies, Delaware, USA).

cDNA synthesis

The complementary DNA (cDNA) was synthesized from total RNA using QuantiTech Reverse Transcriptase Kit (Qiagen, USA) according to the manufacturer's protocol. cDNA samples were stored at −20 °C until further use.

Quantitative real-Time PCR

The steady state mRNA of the indicated genes (*ATM*, *XRCC6* and *LIG4*) was quantified using the QuantiTect SYBR Green PCR Kit (Qiagen, USA). Expression level of the housekeeping gene, GAPDH, was used for normalization. The following PCR parameters were used to detect fold changes in genes expression, 94°C for 5 min, 40 cycles (94°C for 30 sec, 60°C for 30 sec, 72°C for 45 sec). For gene expression quantification, the comparative Ct method was used. Gene expression levels for each sample were normalized to the expression level of the housekeeping gene encoding the housekeeping enzyme within a given sample (ΔCt). Each sample was performed in duplicate and results were evaluated by using $2^{-\Delta\Delta CT}$ method as relative gene expression values. The primers for Real Time PCR were as follow: for *ATM* rs189037 (G>A) Forward: 5′-TGTGACTTTT CAGGGGATTTG-3′ (sense) and Reverse: 5′- ATAGGAATCAGGGCTTTTGGA-3′; For *XRCC6* rs2267437(C>G) Forward: 5′-TCATGGCAACTCCAGAGCAG, -3′ Reverse: 5′-AACCTTGGGCAATGT CAGGT-3′ and for *LIG4* rs1805388 (C>T) forward: 5′- CCGCAGGAAACC ATCAAGATC-3′; reverse: 5′- TTCTCG TTTAACTG GCCTCGG-3′.

Statistical Methods

The sample size was calculated statistically. The collected data was analyzed using Statistical package for Social Science (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp). Kruskal Wallis test was used to assess the statistical significance of the difference in gene expressions between genotypes followed by pairwise comparisons. The ROC Curve (receiver operating characteristic) was used to evaluate the sensitivity and specificity for quantitative diagnostic measures. The optimum cut off point was defined as that which maximized the AUC value. AUC greater than 0.9 has high accuracy, while 0.7–0.9 indicates moderate accuracy, 0.5–0.7, low accuracy and 0.5 a chance result (Fischer et al., 2003).

Kaplan–Meier curve was used to identify the impact of DNA repair genes polymorphisms on AML patients overall survival (OS) analysis and the statistical significance of differences among curves was determined by Log-Rank test. Cox regression analysis of factors potentially related to survival was performed to identify which one is independent factor might jointly have a significant influence on survival. A p value is considered significant if <0.05.

Results

The AML patients Characteristics

The clinical, demographic as well as laboratory data of studied cohort of AML patients are shown in Table 1.

Effect of DNA repair genes (ATM; XRCC6; LIG4) polymorphisms on corresponding genes expression

ATM gene expression showed significant difference between studied *ATM* SNPs (p<0.001), this difference was significantly lower gene expression in GA and AA genotypes when compared to GG one (p=0.002, <0.001

Table 1. Studied AML Patient's Characteristics

Patient (n=95)	Characteristics
Female/Male (%)	50/45 (52.6 /47.8)
Mean (range) age at diagnosis (years)	42 (21- 58)
Blood blast cells % median (range)	32 (5–92)
Peripheral WBC at diagnosis (109 /L)	28.0(5.0- 120)
Bone marrow blasts% median (range)	55 (24–92)
Cytogenetic risk groups	
Favorable	35 (36.8)
Intermediate	45 (47.4)
Unfavorable	15 (15.8)
FAB subtypes	
MO	3 (3.2)
M1	15 (15.8)
M2	16 (16.8)
M3	5 (5.3)
M4	32 (33.7)
M5	13 (13.7)
M6	8 (8.4)
M7	3 (3.2)
Molecular genetic findings	
FLT3+	20 (21.1%)
NPM+	50 (55.6)
FLT3+/NPM+	12 (12.6)
FLT3- /NPM-	8 (8.4)
Induction of remission	
Responder	55 (57.9)
Non-responder	40 (42.1)
Outcome	
Survived	56 (58.9)
Died	39 (41.1)

respectively). Also, *XRCC6* gene expression showed significant difference between studied *XRCC6* SNPs (p=0.015), this difference was significantly lower in GG genotype when compared to CG and CC ones (p=0.011, 0.042 respectively). Likewise, *LIG4* gene expression showed significant difference between studied *LIG4* genotypes (p<0.001), this difference was significantly lower in TT genotypes when compared to CT and CC ones (p=0.002, 0.004 respectively) (Table 2).

Impact of Studied DNA repair genes expression on induction remission response of studied AML patients

ROC curve was conducted to determine the optimal *ATM*, *XRCC6*, *LIG4* expression levels that can predict non remission. *ATM*, *XRCC6*, LIGS expression levels showed low accuracy AUCs (=0.591, 0.520 and 0.525 respectively) (data not shown).

Impact of studied DNA repair genes polymorphism on AML OS and DFS

The impact of DNA repair genes polymorphism on the OS and DSF of the AML patients after exclusion of the effect of FLT3. The statistical analysis revealed that the *ATM* AA genotype is associated with shortest OS and DFS as compared to other genotypes, GA or GG (p<0.001, =0.043 respectively). Likewise; GG genotype of *XRCC6* had the shortest OS and DFS as compared to CC and CG ones (p=0.003, 0.042 respectively). On the other hand, *LIG4* gene polymorphism did not affect significantly OS or DFS in AML cases (Figure 1A,B, C; Figure 2A,B,C).

Cox proportional analysis to address the factors predicting studied AML patients OS

COX regression analysis was carried out for prediction of factors affecting OS, using age, gender, TLC, bone marrow blasts percentage, NPM, FLT3, cytogenetic,

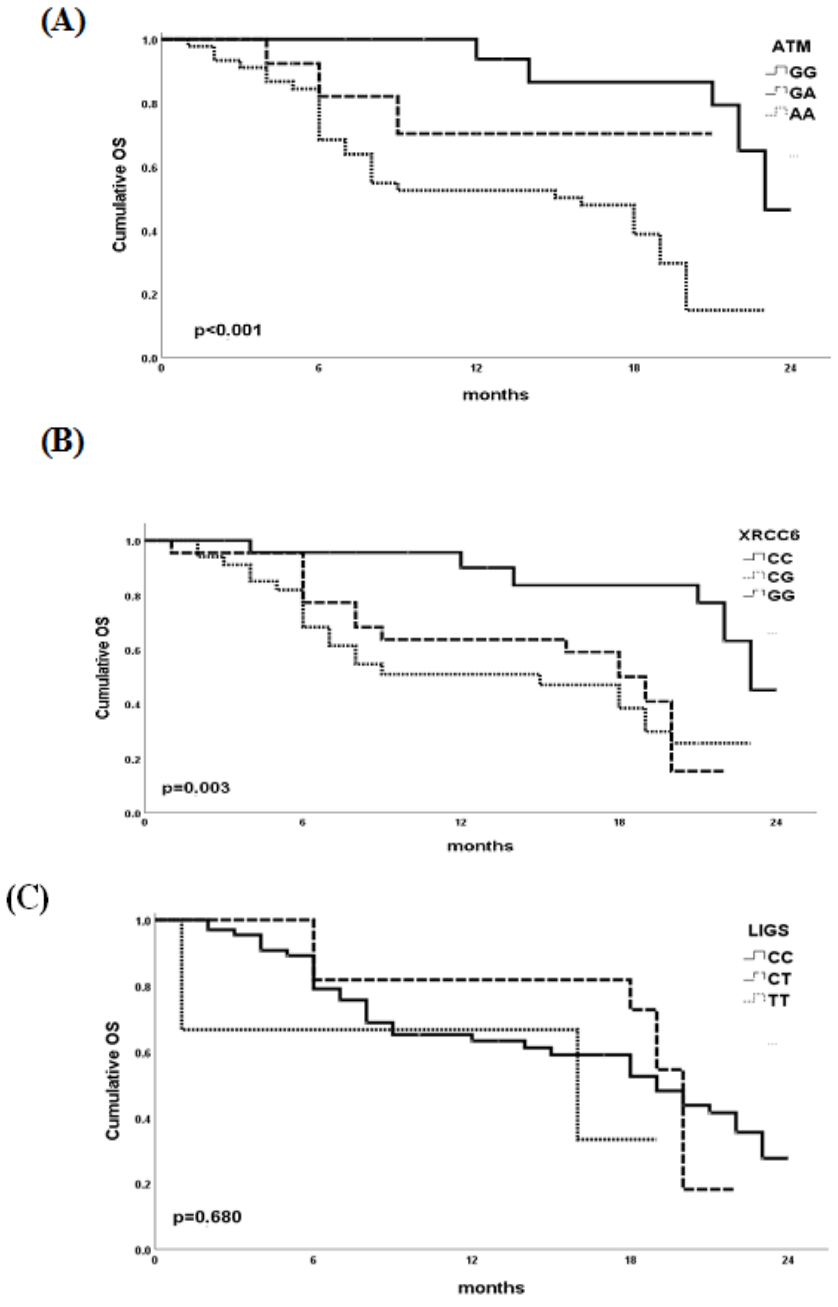

Figure 1. Kaplan Meier Curves of AML OS according to (A) *ATM,* (B) *XRCC6* and (C) *LIG4* Genotypes.

Table 2. Expression of the Studied DNA Repair Genes (*ATM, XRCC6, LIG4*) in Relation to Genotypes in Studied AML Patients.

Genotypes of studied genes	Gene expression	P value	Pairwise comparisons	
	Median (Range)		Comparison between	P value
ATM gene expression				
AA	0.0012 (0.0001- 0.0015)	<0.001	GG vs GA	0.002
GA	0.0140 (0.0014- 0.0160)		GG vs AA	<0.001
GG	0.0190 (0.001- 0.033)		GA vs AA	0.133
XRCC6 gene expression				
GG	0.0200 (0.001- 0.188)	0.015	CC vs CG	0.011
CG	0.0245 (0.011- 0.260)		CC vs GG	0.129
CC	0.0980 (0.011- 0.240)		CG vs GG	0.042
LIG4 gene expression				
CC	0.072 (0.001- 0.240)	<0.001	CC vs CT	0.002
CT	0.020 (0.025- 0.026)		CC vs TT	0.004
TT	0.023 (0.016- 0.019)		CT vs TT	0.742

SNP, single nucleotide polymorphism

ATM, *XRCC6* and *LIG4* genes expression as covariates. Higher BM blasts, mutated FLT3, lower *ATM* and lower *XRCC6* genes expressions were associated with shorter OS, while mutated NPM was associated with longer OS in univariable analysis. However, in multivariable analysis, only mutated FLT3, lower *ATM* and *XRCC6* genes expressions were considered poor prognostic factors for shorter OS in studied AML cases (Table 3).

Cox proportional analysis to address the factors predicting studied AML patients DFS

COX regression analysis was conducted for prediction of factors affecting DFS, using age, gender, TLC, bone marrow blasts percentage, NPM, FLT3, cytogenetic findings, *ATM*, *XRCC6* and *LIG4* genes expressions as covariates. Higher BM blasts, mutated FLT3, lower *ATM* expression were associated with shorter DFS, in univariable analysis. However, in multivariable analysis, only lower *ATM* gene expression was considered poor prognostic factor for shorter DFS in studied AML cases (Table 4).

Discussion

The DNA repair system is necessary for maintenance of genomic integrity during DNA replication and essential for reversal of DNA damage. Polymorphisms in DNA repair genes can alter the repair capacity resulting in unrepaired damage, which may lead to unregulated cell growth and development of cancer (Choi et al., 2016). Despite an improvement in prognosis classification, mostly based on the identification of gene mutations such as NPM1, FLT3, or CEBPA, outcomes in AML remain heterogeneous, underlying the wide diversity of this AML subset. In this study, we assessed the impact of DNA repair genes polymorphisms on corresponding genes expression as well as the impact of these genes expression on overall survival.

The *ATM* AA SNPs; *XRCC6* GG SNPs; and *LIG4* TT SNPs are associated with down regulation of corresponding genes. These findings are in agreement with the results in previous reports (Gabellier et al., 2020). Previous study on MDS patients found that the rs228593, rs2267437 and rs1805388 functional polymorphisms

Table 3. COX Regression Analysis to Address Factor that could Predict AML Patients OS.

Parameters	Univariable		Multivariable	
	P value	HR (95% CI)	P value	HR (95% CI)
Age (years)	0.778	1.01 (0.97-1.04)		
Sex (male vs Female)	0.233	0.70 (0.39-1.26)		
WBCs X10^9/L	0.183	1.01 (0.99-1.02)		
BM blast cells%	0.029	1.02 (1.00-1.03)	0.568	1.01 (0.99-1.02)
NPM	<0.001	0.24 (0.12-0.52)	0.955	0.98 (0.41-2.30)
FLT3	<0.001	2.81 (1.62-8.60)	<0.001	3.85 (1.01-6.72)
Cytogenetic risk group	0.571	1.18 (0.66-2.11)		
ATM gene expression	0.006	2.15 (1.25-3.69)	0.02	2.02 (1.12-3.64)
XRCC6 gene expression	<0.001	2.38 (1.67- 5.30)	<0.001	2.55 (1.33-5.0)
LIG4 gene expression	0.23	1.58 (0.75-3.31)		

Adjustment factors included, BM blast cells%; NPM; FLT3; *ATM* gene expression ; and *XRCC6* gene expression. HR, hazard ratio; CI, confidence interval.

Table 4. COX Regression Analysis for Prediction of Factors Affecting DFS

	Univariable		Multivariable	
	P value	HR (95% CI)	P value	HR(95% CI)
Age (years)	0.26	1.03 (0.98-1.09)		
Sex (male vs Female)	0.422	0.71(0.31- 1.64)		
WBCs X10^9/L	0.123	1.01(0.99- 1.03)		
BM Blast cells%	0.007	1.04(1.01- 1.06)	0.09	1.02 (0.997-1.05)
NPM	0.071	0.41 (0.16- 1.08)		
FLT3	0.014	2.68(1.37- 6.01)	0.088	3.14 (0.842-11.70)
		1.29 (0.56- 2.94)		
ATM gene expression	<0.001	2.34(1.92- 4.70)	<0.001	2.21 (1.346- 6.22)
XRCC6 gene expression	0.157	1.75(0.81- 3.81)		
LIG4 gene expression	0.615	1.38(0.40-4.75)		

Adjustment factors included, BM blast cells%; NPM; FLT3; ATM expression ; XRCC6 expression; and LIG4 expression; HR, hazard ratio; CI, confidence interval.

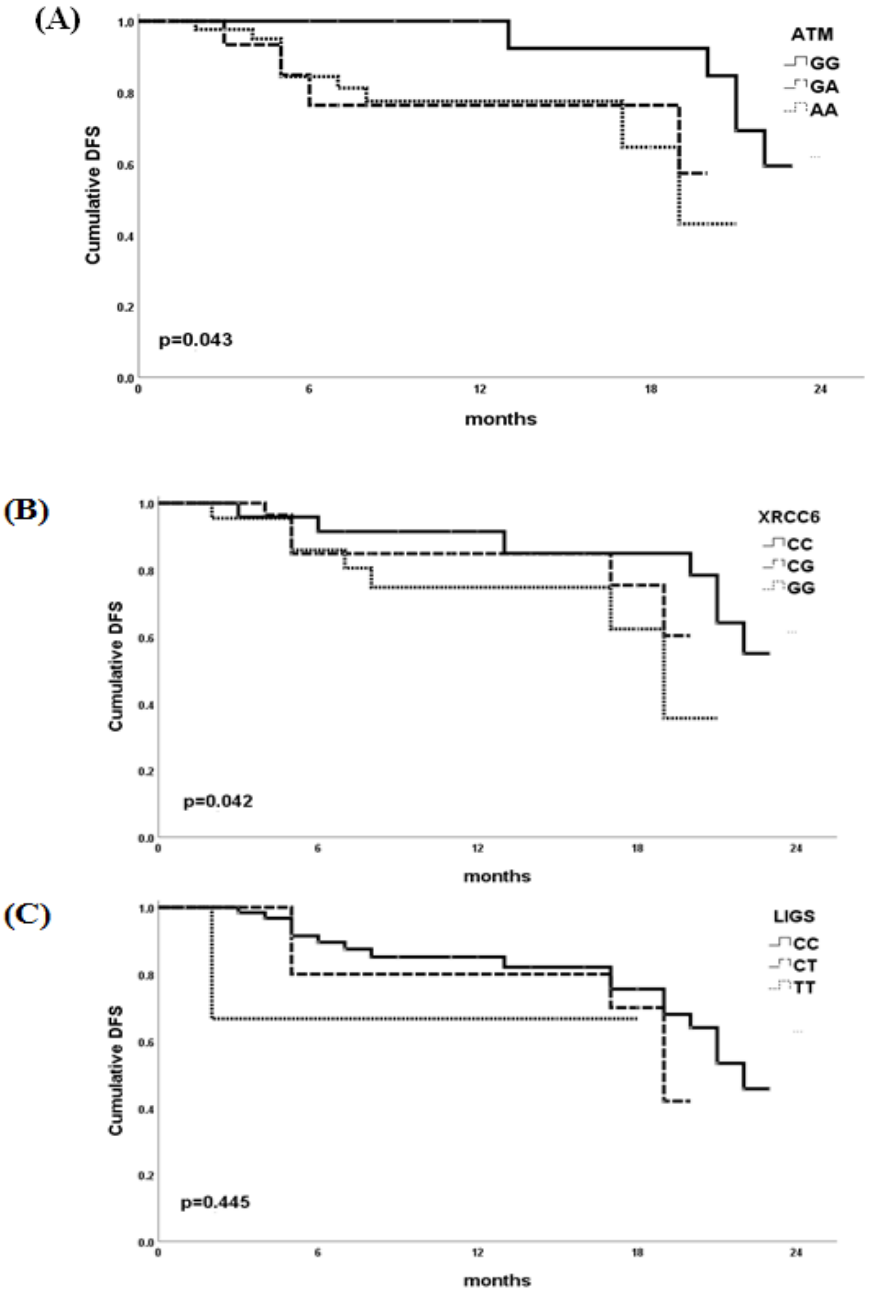

Figure 2. Kaplan Meier Curves of AML DFS According to (A) ATM, (B) XRCC6 and (C) LIG4 Genotypes.

probably alter the level of expression of the *ATM*, *XRCC6* and *LIG4* genes, respectively, being important in the maintenance of genomic stability (Junior et al., 2016).

Previous report stated that the *LIG4* rs1805388 polymorphism results in a non-synonymous amino acid change from threonine to isoleucine at the N-terminal of the *LIG4* protein that is essential for its activity (Grawunder et al., 1998). The rs1805388 (A3V) polymorphism in the N-terminal of *LIG4* mildly but reproducibly reduce adenylation and ligation activities (2-3 fold) (Girard et al., 2004) and increase the hydrophobic nature of this region of the protein (O`Driscoll et al., 2002).

The optimal *ATM*, *XRCC6*, *LIG4* expression levels that can predict non remission showed low accuracy AUCs (0.591, 0.520 and 0.525 respectively). Similar published results regarding post chemotherapy response analysis in AML patients heterozygous for *ATM* 4138 C>T (rs3092856) (Shi et al., 2011).

The impact of SNPs of studied genes on the overall survival revealed that *ATM* AA genotypes is associated with shorter OS followed by GA and the GG and the differences were statistically significant. Similar findings were reported in lymphoid malignancies, as well as a variety of solid tumors like colorectal cancer (Choi et al.,2016; Dimberg and Skarstedt 2016). Likewise, *XRCC6* GG genotypes is associated with the shortest OS and DFS as compared to other SNPs. In the other hand; *LIG4* SNPs had no significant impact on OS or DFS. Similar findings were reported by Park et al (2021). Important finding was previously reported which stated that mice deficient for these genes are viable, but their cells have defects in DNA end joining, These defects occur because these genes are normally related to the maintenance of genomic stability via a mechanism that involves the reduction of chromosomal rearrangements (Zompi et al 2021).

Rs189037 is a single nucleotide polymorphism (SNP) located in the promoter region (non-coding region) of *ATM* gene. Rs189037 polymorphism can't directly influence amino acid coding of *ATM* protein. So, it is possible to participate in splicing, intervention, modification, determination process changing RNA stability and then influences the expression level of *ATM* protein (Wang et al., 2010).

To figure out how this polymorphism regulates the *ATM* mRNA expression, Chen et al. (2010) analyzed the sequences around the SNP rs189037 and demonstrated that AP-2a binds to the SNP site to repress *ATM* transcription (Leask et al., 1991), according to the bioinformatics analysis by Leask et al. (1991).

To date, the *ATM* protein kinase has been straightly considered to be a DNA damage sensor and one of therapeutic targets for cancer (Kalil et al., 2012). Accumulated evidence has demonstrated that *ATM* protein is activated immediately in response to DNA double strand breaks (DSB) caused by either genetically programmed or the appearance of selected exogenous factors (Van Dyck et al., 1999; Williams et al., 2007). With the purpose of rapid and accurate sense and repairing damage in cellular lesion, the signal is generated to recruit *ATM* kinase to DSB sites and cause the phosphorylation of multiple *ATM* substrates (Williams et al., 2007). Cells have formed complex molecular networks to sense DSBs and coordinate their repair. Individuals who have deficiencies in the abilities above might lead to the cell death, somatic mutations, and carcinogens (Van Dyck et al., 1999).

ATM promotes the recombination of DSB intermediates and prevents broken DNA ends from chromosome mutation (Lim et al., 2000). *ATM* mutation leads to the cancer-predisposing genetic disorder ataxia-telangiectasia (A-T) which belongs to genomic instability syndromes (Shiloh et al., 2003).

Univariate analysis using age; sex, NPM; FLT3; WBCs, blast cells; *ATM*; *XRCC6*; *LIG4* genes expression were examined and that significant parameter in univariate analysis were introduced into multivariate analysis which revealed that genes expression of *ATM* and *XRCC6* are independent predictor of OS. These findings are in agreement with that reported by Junior et al (2016) who assessed the impact of panel of DNA repair genes in MDS patients. Also, previous report stated that *ATM* alterations are present at diagnosis in about 25% of individuals with chronic lymphocytic leukemia; these alterations are associated with a peculiar gene expression pattern and a shorter treatment-free interval (Guarini et al., 2012).

Multivariate Cox proportional hazards to predict DFS reveal that *ATM* expression was significantly independent predictor of AML DFS. Similar results in MDS patients were reported (Junior et al., 2016). The limitation of this study is the small sample of patients included.

In conclusion the following genotypes of *ATM* (AA), *XRCC6* (GG), and *LIG4* (TT) are associated with down regulation of corresponding genes expression. The lower *ATM* and *XRCC6* genes expression is predictor of OS while *ATM* gene expression is predictor of DFS. *ATM* gene expression could be used for optimizing the AML therapy at diagnosis and could be a target for AML therapy.

Author Contribution Statement

Salah Aref Conception and supervision; Nadia El Menshawy Interpretation and analysis of data; Tarek Abou Zeid Interpretation and analysis of data; Enas Gouda Preparation of the manuscript; Revision for important intellectual; Nora Abdel Aziz Laboratory work.

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Availability of data

The data of the present study is available upon request to the corresponding author

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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