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MDM2, P53 and P21 Gene Polymorphisms in Acute Myeloid Leukemia

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ABSTRACT

Background: P53 is a tumor suppressor gene that initiates apoptosis in response to severe DNA damage. MDM2 is a major negative regulator of P53. P21 is a cell cycle checkpoint gene functioning as a downstream effector of p53. A single nucleotide polymorphism (SNP) in the promoter of MDM2 gene, SNP309 can increase the expression level of MDM2, thereby causing an impairment of p53 tumor suppressor activity. A G-C exchange at p53 codon 72 polymorphism alters the primary structure of the p53 protein. Both polymorphisms have been implicated in cancer. As regards p21, a nonsynonymous polymorphism of Ser31Arg was shown to be associated with increased risk of cancer.

Aim of the Study: The aim of this work was to study the frequency of p53, p21, and MDM2 polymorphisms among AML patients and in apparently normal healthy controls to describe the prevalence of such mutations and to detect whether or not they have an implication on the development of AML.

Results: A significant association was found between p21 polymorphism and acute myeloid leukemia, while borderline significance was found with MDM2 and no association with p53 polymorphisms. When the MDM2, p53 and p21 polymorphisms were combined, no multiplicative joint effect concerning the risk of development of AML could be found, except for MDM2 and p21, where a borderline significance existed between the frequencies of the mutant versus the wild types, denoting a possible increased risk for the development of AML with the presence of those two mutations together.

Conclusion: In conclusion, it is suggested that the p21 Ser31Arg polymorphism may be a genetic susceptibility factor in the pathogenesis of AML. Future studies are recommended to investigate the biological role of P21 gene polymorphism in AML and to exploit its role in prognosis and therapy.

Key Words: *Acute myeloid leukemia – Gene polymorphism – MDM2 – P53 – P21.*

INTRODUCTION

Acute myeloid leukemia (AML) is a heterogeneous disease with numerous genetic abnormalities. In fact, genetic abnormalities are present in more than 90% of AML, and a majority of these genetic abnormalities are recurrent. Most acute myeloid leukemias appear to be the consequence of a combination between gene mutations and gene rearrangements that confer a proliferative and/or survival advantage to hematopoietic differentiation and subsequent apoptosis of cells, resulting in the accumulation of primitive cells and the development of leukemia **[1]**.

P53 is a tumor suppressor gene that initiates apoptosis in response to severe DNA damage. P21 (CDKN1A, Waf1) is a cell cycle checkpoint gene functioning as downstream effectors of p53 and acts as an inhibitor of cyclin-dependent kinase. In response to DNA damage, cell cycle arrest at the G1 to S phase is caused by p21 through p53 up-regulation **[2]**.

MDM2 is a crucial negative regulator of p53 through several mechanisms. MDM2 directly binds to p53, resulting in the inhibition of p53 transactivation activity **[3]**. MDM2 also acts as an ubiquitin protein ligase and controls p53 by targeting it for proteasomal degradation. Therefore, overexpression of MDM2 leads to the increased degradation of p53 and down-regulates its tumor suppressor function **[4]**.

The importance of MDM2 over expression, p53 and p21 inactivation in acute Myeloid Leukemia (AML) remains controversial, al-

though a few studies have suggested that p53 and MDM2 up-regulation affect the risk of AML. It is suggested that the MDM2 and TP53 variants interact to modulate responses to genotoxic therapy and are determinants of risk for t-AML. Recently, Xiong et al. **[5]** suggested that the MDM2 SNP309 homozygous GG genotype may be a genetic susceptibility factor in AML.

The p53 pathway is an important response to oncogenic stress, and p53 regulates its own intracellular levels through an autoregulatory feedback pathway with MDM2 **[6]**. MDM2 binds to p53 and inactivates it through ubiquitination. MDM2 is a proto-oncogene and loss of p53 function is caused by MDM2 overexpression, mutations, and other mechanisms, resulting in malignant transformation or carcinogenesis **[7]**. In the p53 pathway, p53, p21, and MDM2 play a crucial role together. Polymorphisms in p53-MDM2 **[8]** and p53-p21 **[9]** have been reported to be associated with other cancers, such as lung, esophageal, colorectal, breast, and gastric cancer. Based on this evidence, we investigated whether these gene polymorphisms and their gene-gene interaction may be important in AML. In this study for each polymorphism, a significant association with AML was observed only for the p21 polymorphism, although additional polymorphisms in the other genes have been linked to susceptibility for other cancers.

In this work we analyzed the frequency of p53, p21, and MDM2 polymorphisms among AML patients referring to the National Cancer Institute (NCI) and in apparently normal healthy controls aiming to describe the prevalence of such mutations.

MATERIAL AND METHODS

This study included 77 newly diagnosed AML patients who presented to the Medical and Pediatric Oncology Departments, NCI, Cairo University in the period between April 2010 and October 2011. Diagnosis was performed according to clinical, morphological, cytochemical and immunophentypic examination. The criteria for inclusion in this group were:

1- Egyptian origin residing in Egypt as judged by their names, language and places of birth.

2- Availability of biological material.

The recruited patients comprised 43 males and 34 females between the ages of 4 and 83 years with a median of 35 years.

A general population control group composed of 72 individuals comprising 53 males and 19 females was randomly selected from blood donors. The criteria for inclusion in the control group were:

1- Anonymous, healthy, and unrelated individuals.

2- Egyptian origin residing in Egypt as judged by their language and place of birth. Informed consent was obtained from all participants involved in the study or their parents.

The study was performed according to Helsinki declaration and theNCI IRB approved the study.

Cases were subjected to the following routine investigation:

1- Thorough history taking.

2- Full clinical examination, particularly for hepatomegaly, splenomegaly and lymphadenopathy.

3- Complete blood picture.

4- Bone marrow aspiration and morphological examination using Romanowsky stain, supplemented with cytochemical stains such as Myeloperoxidase (MPO), Sudan Black B Stain (SBB), Esterases and Acid Phosphatase when indicated.

5- Immunophenotyping by Flow cytometry: To confirm the diagnosis of AML with a wide panel of myeloid markers (MPO, CD13, CD33, CD117, CD14 and CD15), lymphoid markers (CD10, CD19, CD22, CD79a, CD20, Cyto μ, Kappa and Lambda for B lymphoid series, and CD3, CD2, CD4, CD8, CD7 and CD5 for T lymphoid series) and the stem cell marker CD34 as well as CD56 and HLADR on routine basis.

Genotyping:

DNA was isolated from peripheral blood and its concentration was measured as described by Gupta et al. **[9]**.

Genotyping for all studied loci was performed by PCR-RFLP method. Primers sequences, restriction enzymes and fragments obtained are presented in Table (1).

MDM2 T309G Genotyping:

The MDM2 T309G polymorphism was determined by using the method described by Hirata et al. **[10]**. Each PCR assay was performed using 100ng of genomic DNA, 0.2µM of each primer (Table 1), 1U of Hot Start Taq DNA polymerase (Quiagen), 200µM dNTP, 1.5mM MgC l2, 10mM Tris-HCl (pH 8.4), and 50mM KCl. After initial denaturation for 10 minutes at 95°C, the PCR was performed for 35 cycles of 45 seconds at 95°C, 45 seconds at 59°C, and 1 minute at 72°C. The last elongation step was extended to 7 minutes. The amplified fragments targeted the site of polymorphism: The 158-bp fragment for MDM2 T309G contained the T → G bp substitution at nucleotide 309 that creates a MspA1I restriction site. The digestion products were visualized with ethidium bromide after electrophoresis on 3.5% agarose gel at 100 volts for 30min. The MDM2 309TT wild type homozygous was identified by the presence of only a 158bp fragment. 309TG heterozygous was identified by 158, 112, and 46 bp fragments, and 309GG homozygous variant was identified by 112 and 46bp fragments (Fig. 1).

P53 Codon arg72pro Polymorphism:

PCR was performed in 25µL containing 100ng of genomic DNA, 0.5µmol/L of primers (Table 1), 200µmol/L dNTPs, 10mmol/L Tris-HCl (pH 8.3), 2.5mmol/L MgCl2, 50mmol/L KCl, and 1U of Hot Start Taq DNA polymerase (Quiagen). After initial denaturation for 10 minutes at 95°C, the PCR was performed for 35 cycles of 45 seconds at 95°C, 45 seconds at 58°C, and 1 minute at 72°C. The last elongation step was extended to 7 minutes. The Arg → Pro substitution abolishes a restriction site on digestion with BstUI restriction enzyme (10 U). The resulting restricted fragments were evaluated on a 3.5% agarose gel at 100 volt for 30 min **[7]**. showing 113bp and 86bp bands for the wild type and 199bp, 113bp and 86bp bands for heterozygous variant and the homozygous variant remains undigested showing 199bp (Fig. 2).

P21 codon 31 Ser/arg Polymorphism:

P21 codon 31 Ser/arg Polymorphism was characterized by the PCR-RFLP **[7]**. DNA fragment of 225bp was amplified in 25 µL containing 100ng of genomic DNA, 0.5µmol/L of primers (Table 1), 200µmol/L dNTPs, 10mmol/L Tris-HCl (pH 8.3), 2.5mmol/L MgCl2, 50mmol/L KCl, and 1 U of Hot Start Taq DNA polymerase (Quiagen). After denaturation for 10 minutes at 95°C, the PCR was performed for 35 cycles of 1 minute at 95°C, 1 minute at 58°C, and 2 minutes at 72°C. The last elongation step was extended to 7 minutes. The presence of polymorphic variant arg results in abolishing the restriction site of Blp I enzyme. The PCR product (5 to 10µL) was digested with Blp I (10U, 37°C), and subjected to electrophoresis on a 2.5% agarose gel at 100 volt for 30 min. The wild type (ser/ser) resulted in two smaller fragments (122 and 103bp) (Fig. 3) while the heterozygous variant resulted in 225,122,103bp and the homozygous variant prevents digestion resulting in only 225bp band.

Table (1): Primer sequence and PCR, PCR/RFLP fragment size.

Gene	Primer	Primer sequences	Enzyme	Fragment sizes
MDM2 SNP309	Forward	5'- CGCGGGAGTTCA GGG TAAG-3'	MspA1I (10 U)	158 bp (TT) wild 158,112,46 bp (TG) heterozygous
	Reverse	5-CTGAGTCAACCTG CC C A CTG-3'		112,46 bp (GG) homozygous
P53 codon 72	Forward	5'-TTGCCGTCCCAA GCAAT GGATGA-3'	BstUI (10U)	113,86 bp (GG) wild 199,113,86 bp (GC) heterozygous
	Reverse	5'-TCTGGGAAGGG ACAGA AGATGAC-3'		199 bp (CC) homozygous
P21 codon 31	Forward	5'ACCAGGGCCTTCC TTGT ATC-3'	BlpI (10U)	122,103 bp (ser/ser) wild 225,122,103 bp (ser/arg) heterozygous
	Reverse	5'-GTCACCCTCCAG TGGTG TCT-3'		225 bp (arg/arg) homozygous

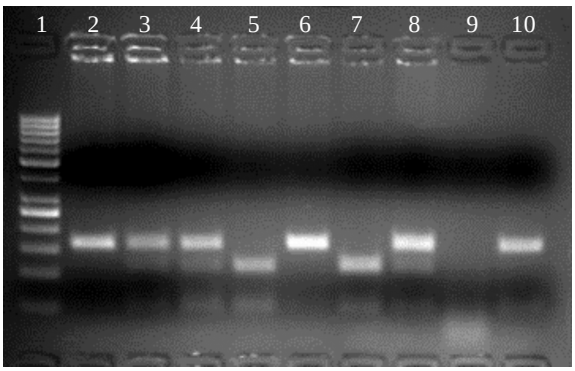

Fig. (1): MDM2 T309G polymorphism after MspA1I digestion.

Lane 1: 50 bp ladder

Lanes 2, 6, 10: Wild type (TT) showing one band at 158 bp

Lanes 3, 4, 8: Heterozygous variant (TG) showing bands at 158,112,46 bp

Lanes 5, 7: Homozygous variant (GG) showing bands at 112,46bp

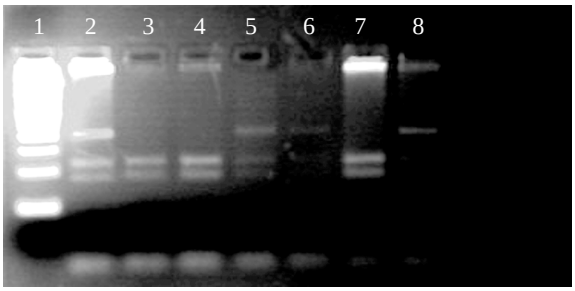

Fig. (2): P 53 arg72Pro polymorpsim after BstUI digestion.

Lane 1: 50 bp ladder

Lanes 2, 5, 8: Heterozygous (Arg/Pro) showing bands at 199,113,86 bp

Lanes 3, 4, 6, 7: Wild type (Arg/Arg) showing bands at 113, 86bp

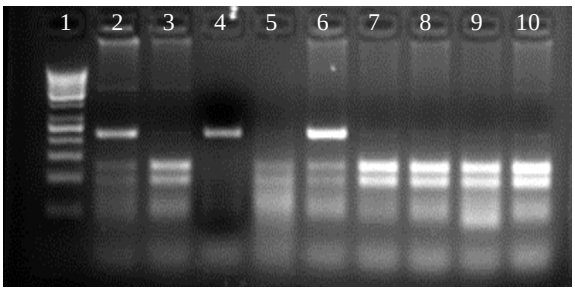

Fig. (3): P 21 Ser31arg gene polymorphism after digestion by BlpI.

Lane 1: 50 bp ladder

Lanes 2, 6: Heterozygous (Ser/Arg) showing bands at 225, 122,103bp

Lanes 3, 5, 7-10: Wild type (Ser/Ser) showing bands at 122,103bp

Lane 4: Homozygous variant (Arg/Arg) showing one band at 225.

Statistical methods:

Data was analyzed using SPSSwin statistical package version 17 (SPSS Inc., Chicago, IL). Numerical data were expressed as mean and standard deviation or median and range as appropriate. Qualitative data were expressed as frequency and percentage. Chi-square test (Fisher's exact test) was used to examine the relation between qualitative variables. For quantitative data, comparison between two groups was done using Mann-Whitney test (non-parametric t-test). Comparison between 3 groups was done using Kruskal-Wallis test (non-parametric ANOVA). Logistic regression was used for calculation of odds ratio (OR) with 95% confidence interval (CI) for risk estimation. A p-value <0.05 was considered significant.

RESULTS

This study was conducted on 77 cases of newly diagnosed AML who presented to the Adult and Pediatric oncology departments of the National Cancer Institute (NCI), Cairo University in the period from April 2010 to October 2011 as well as 72 age and sex comparable healthy individuals as a control group. The relative frequency of MDM2, P53 and P21 genes regarding the wild, heterozygous and homozygous types in the two studied groups was investigated.

The age of cases ranged from 4 to 83 years with a median of 34 years and a mean of 38±17.3 years. The patient group included 43 males (55.8%) and 34 females (44.2%).

The presenting total leucocytic count in the study cases ranged from 2 to 180 with a mean of 34.71±31.22 and a median of $29x10^9$/L. The platelet count ranged from 2 to 271 with a mean of 45±41 and a median of $33x10^{12}$/L. Hemoglobin ranged from 4 to 13, with a mean of 7.7±3.2, and a median of 7.3gm/dl. Blasts in peripheral blood ranged from 0 to 90% with a mean of 38.3%±28 and a median of 32%. The mean percentage of blasts in marrow was 69.2% ±18.5, the median was 72% and the range was 20-95%. Bone marrow megakaryocytes and platelets were reduced in 98.7% of AML cases.

Cytochemistry:

Myeloperoxidase was positive in 94.7% of cases while NASDA/NASDAF was positive/

partially inhibited in 18.2% of cases and positive /totally inhibited in 2.6% of cases.

Immunophenotyping:

The myeloid lineage markers including MPO were detected in 96.1% of cases, CD33 in 93.5% of cases and CD13 in 98.7% of cases. Also CD 117 was positive in 57% of cases and HLA-DR was positive in 71.4% of cases. Myeloid with monocytic markers including CD14 and CD64 were positive in 11.7% and 6.5% of cases respectively. Aberrant expression of lymphoid markers was detected in 19.5% of cases with CD7 showing the highest expression in (7.8%) of cases.

FAB classification:

The most commonly encountered FAB subtype was M2 (32.5%), followed by M1 (29.9%), M4 (18.2%), M3 (14.3%), M5 (2.6%) and finally both M0 and M7 (1.3%).

MDM2:

Table (2) demonstrates the frequency of MDM2 polymorphisms and compares mutant versus wild types in cases versus controls. Fig. (1) demonstrates the different PCR MDM2 fragments.

P53:

Table (3) demonstrates the frequency of p53 polymorphisms and compares mutant versus wild types in cases versus controls. (Fig. 2) demonstrates the different PCR p53 fragments.

P21:

Table (4) demonstrates the frequency of p53 polymorphisms and compares mutant versus wild types in cases versus controls. (Fig. 3) demonstrates the different PCR p53 fragments.

Combinations:

Combination between MDM2-P21 showed that the frequency of the mutant versus wild types was 78.1% versus 21.9% in AML group and 63.1% versus 36.9% in the control group with a *p*-value=0.061 which is a borderline significance (Table 5).

Also combination between P53-MDM2 showed that the frequency of the mutant versus wild types was 76.6% versus 23.4% in AML group and 72.3% versus 27.7% in the control group with a *p*-value=0.580 denoting no significant difference between the control group and the AML group.

Finally combination between P53-21 showed that mutant versus wild types frequencies were 67.8% versus 32.2% in AML group and 53.6% versus 46.4% in the control group with a *p*-value=0.103, also denoting no significant difference between the control group and the AML group.

From the previous results, when the MDM2, P53 and P21 polymorphisms were combined, no multiplicative joint effect existed concerning the risk of developing AML except for MDM2 and P21, where a borderline significance exists denoting a possible increased risk for AML.

Correlation of the clinical, hematological and immunophenotypic parameters with the three gene polymorphisms expression in AML cases showed that its expression was not related to any of these parameters.

Table (2): Comparison between the frequencies of MDM2 types in AML versus control groups.

	MDM2 (GG)	MDM2 (TG)	MDM2 (TT)	*p*-value	MDM2 (TG+GG)	*p*-value
AML	14 (20.6%)	33 (48.5%)	21 (30.9%)	0.083	47 (69.1%)	0.07
Control	6 (9.2%)	29 (44.6%)	30 (46.2%)		35 (53.8%)	
Total	20 (15%)	62 (46.6%)	51 (38.3%)		82 (61.7%)	

* TG+GG vs. TT

Table (3): Comparison between the frequencies of P53 types in AML versus control groups.

Group	p53 GG	p53 GC	p53 CC	*p*-value	p53 (GC+CC)	*p*-value
AML	36 (52.9%)	26 (38.2%)	6 (8.8%)	0.792	32 (47.1%)	0.820
Control	38 (55.1%)	27 (39.1%)	4 (5.8%)		31 (44.9%)	
Total	74 (54.0%)	53 (38.7%)	10 (7.3%)		63 (46.0%)	

* GC+CC vs. GG

Table (4): Comparison between the frequencies of P21 types in AML versus control groups.

Group	p21 arg/arg	p21 ser/arg	p21 ser/ser	*p*-value	P21 (ser/arg+arg/arg)	*p*-value
AML	3 (4.6%)	19 (29.2%)	43 (66.2%)	0.017	22 (38.8%)	0.008
Control	1 (1.4%)	9 (12.9%)	60 (85.7%)		10 (14.3%)	
Total	4 (3.0%)	28 (20.7%)	103 (76.3%)		32 (23.7%)	

* ser/arg+arg/arg vs. ser/ser

Table (5): Comparison between combined MDM2-P21 mutants versus wild types in AML compared to control.

Group	MDM2/P21 (mutant combination)	MDM2/P21 (both wild)	Total	*p*-value
AML	50 (78.1%)	14 (21.9%)	64 (100%)	0.061
Control	41 (63.1%)	24 (36.9%)	65 (100%)	
Total	91 (70.5%)	38 (29.5%)	129 (100%)	

DISCUSSION

In the present study, the presence of MDM2, p53 and p21 gene polymorphism in 77 newly diagnosed AML cases and 72 age and sex comparable healthy controls was analyzed.

To the best of the author's knowledge, no previous studies addressed the correlation of the 3 previous parameters in AML cases. Previous studies only investigated either one or two of them in relation with AML.

Numerous studies have investigated the association between the MDM2 SNP309 polymorphism and the risk of cancer but the results have been inconsistent. In the present study, common polymorphisms in MDM2 were examined to investigate whether or not they would affect the pathogenesis of AML in the Egyptian patients at NCI. A borderline significance was observed between AML cases and controls, with a *p*-value of 0.083. This result is inconsistent with a case-control study on the role of both polymorphisms p53 and MDM2 among 231 patients with AML and 128 normal controls from a northern Chinese population. They observed a nearly 3.52-fold increase in AML risk associated with the MDM2 GG genotype compared with the MDM2 TT genotype (p=0.001) **[5]**. This difference in results may be due to the larger number of patients in the Chinese study and/or to ethnic variation.

A meta-analysis of 21 case-control studies conducted on 14,770 cases with different tumor types and 14,524 controls from 25 published case-control studies to estimate the effect of SNP309 on tumor risk, as well as to quantify the potential between-study heterogeneity showed that ORs of a variety of cancers associated with the MDM2 GG and TG genotypes were 1.17 (95% CI=1.04-1.33) p=0.0002 and 1.15 (95% CI=1.03-1.28) p=0.0005. The analyses suggested that MDM2 SNP309 serves as a low-penetrance susceptibility tumor marker **[11]**. The present finding is partially consistent with the more recent report by Ellis et al. **[12]** who tested associations between patients with t-AML (n=171) and 2 common functional p53-pathway variants, the MDM2 SNP309 and the TP53 codon 72 polymorphism and showed that MDM2 SNP309G allele was associated with a modest increased risk in de novo AML but not in therapy-related AML. So far there exists one published leukemia study claiming that the MDM2 SNP309 G allele reduced the risk of the disease in a Singaporean Chinese population, which is in discordance with the present results **[13]**. The discrepancy between their studies and this one could perhaps be due to ethnic and/or geographic variations of the frequency of these alleles in different healthy populations **[14]**. It is noteworthy that the basal frequency of the G allele in Singaporean Chinese population is much higher than that in our studied healthy population. Hence, it appears that ethnicity and/or geography bias may influence the effect of the MDM2 G allele on AL risk, perhaps in combination with genetic background, carcinogen exposure in different populations, or just simply sample sizes.

Since the discovery by Bond et al., that SNP309 of the MDM2 gene can accelerate the onset of leukemia at a young age in the patients, there have been quite few studies assessing the impact of the G allele on timing of leukemia onset **[14]**. The evidence from studies of leukemia is controversial, showing an association with early onset in Caucasian and Black populations but not in Hispanic and Singporean populations. Hence, it appears that the SNP309 has common susceptibility across populations with different ethnicity-specific effects. Their work in northern Chinese population showed that the SNP309 had no effect on the timing of acute leukemia onset, which further consolidated the idea that the SNP309 has different ethnic effects. Other studies **[15,16]** investigating genetic polymorphisms have demonstrated similar ethnicity-specific effects, which might suggest interactions between gene polymorphisms and unidentified factors associated with ethnic status.

Another study who genotyped children (n=575) with de novo acute myeloid leukemia (AML) treated on three Children's Oncology Group protocols for the presence of SNP309 and healthy blood donors as control population, showed that MDM2 polymorphism increased the susceptibility to childhood AML where the variant G/G genotype was associated with an increased susceptibility to AML (OR 1.5; p=0.049) **[19]**. The difference in results may be due to different age groups as the patients in the present study have a mean of 38 years and a median of 34 years. This finding of increased risk of malignancy in association with the homozygous variant genotype is in agreement with a number of prior studies. Studies of solid tumors including renal cell carcinoma, colorectal cancer, pancreatic cancer, gastric carcinoma, lung cancer, soft tissue sarcoma and neuroblastoma have reported an increased risk of malignancy in persons with a homozygous variant genotype, with odds ratios typically between 1.5 and 2.0 **[14,17]**.

Numerous molecular epidemiological studies have attempted to demonstrate a correlation between inheritance of p53 codon 72 polymorphism and increased susceptibility to various cancers. The role of this polymorphism remains controversial, with some studies reporting increased risk associated with the Pro 72 allele; which results in the substitution of proline for arginine, for certain cancer types and others failing to reach such conclusions. Even meta-analyses of the various studies have not fully resolved this issue **[18,19]**. In the present study, it was found that there is no additional effect from the p53 Arg72Pro polymorphism in the pathogenesis of AML. This is consistent with a case-control study consisting of 231 AML patients and 128 normal individuals from a northern Chinese population (p=0.25) **[5]**. Also these results are lending weight to the conclusion of Nakano et al., that the p53 Arg72Pro was not associated with risk or clinical parameters of AML **[20]**. Similarly, there are also inconsistent associations between the codon 72 polymorphism with age of tumor onset **[21]**. Similarly another study of twenty-five patients with ALL and 65 patients with AML, both recently diagnosed mutation of the P53 gene was found in one patient of the 25 with ALL and in five patients of the 65 with AML **[22]**. Another study about therapy-related acute myeloid leukemia susceptibility, showed that the polymorphism alone cannot influence the risk of t-AML, an interactive effect was detected such that MDM2 TT TP53 Arg/Arg double homozygotes, and individuals carrying both a MDM2 G allele and a TP53 Pro allele, were at increased risk of t-AML (p-value for interaction is 0.009) **[12]**.

In the present study, the p21 codon 31 polymorphism was significantly associated with risk for AML. This result is consistent with the other studies that investigated this polymorphism in various types of cancers. A study on 104 cancer cervix patients and 160 control in Chinese southern women showed that the frequencies of p21 Arg/Arg, Ser/Arg, and Ser/Ser genotypes among patients were significantly different from those among controls (p<0.05) **[23]**. In another study on 53 bladder cancer patients, 119 healthy controls in Taiwanese patients, there was a significant difference in p21 codon 31 polymorphism between the control and the cancer patients (p<0.01). The arginine form was prominent in the cancer patients (per copy of the A allele, odds ratio=2.03, 95% confidence interval =1.23-3.37) **[24]**.

Also another study from northeastern Iran on 126 eosophageal squamous cell carcinoma cases and 100 controls showed that the frequencies of the wild type and variant genotypes for each of these SNPs were the same and equal to

78.57% for the wild type genotype and 21.43% for the variant genotype, respectively, among cases and 82% and 18%, respectively, among controls and this difference was not statistically significant (p=0.52) **[25]**.

When the MDM2, P53 and P21 polymorphisms were combined, the author did not find any multiplicative joint effect concerning the risk of developing AML except for MDM2 and p21 where a borderline significance existed between the frequency of the mutant versus the wild types and which was 78.1% versus 21.9% in the AML group and 63.1% versus 36.9% in the control group with a p-value=0.061, denoting a possible increased risk for AML. This is consistent with Xiong et al. **[5]**, where no multiplicative effect was found when they combine MDM2 GG and p53 Pro/Pro genotypes in the risk of AML in a case control study of 231 AML patients and 128 normal controls from a northern Chinese population.

The present results are inconsistent with another study about therapy-related acute myeloid leukemia susceptibility, which showed that the polymorphism alone cannot influence the risk of t-AML, an interactive effect was detected such that MDM2 TT TP53 Arg/Arg double homozygotes, and individuals carrying both a MDM2 G allele and a TP53 Pro allele, were at increased risk of t-AML (p-value for interaction is 0.009) **[11]**. The difference in results may be attributed to the fact that this study observed this interactive effect only in a certain type of patients e.g. In patients previously treated with chemotherapy but not in patients treated with radiotherapy, and in patients with loss of chromosomes 5 and/or 7, acquired abnormalities associated with prior exposure to alkylator chemotherapy.

In conclusion, this is the first report to the best of the author's knowledge to show the relation between AML and the 3 gene mutations in the P53 pathway where there is a borderline significant association between a functional polymorphism in MDM2 and AML, insignificant association between polymorphism in p53 and AML and a significant association between polymorphism in p21 and AML. It is thus suggested that the p21 codon 31 polymorphism might be a genetic susceptibility factor in the pathogenesis of AML; another study on higher numbers is needed to validate these findings.

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