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Association between TP53 polymorphisms and chronic lymphocytic leukemia

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Abstract. – OBJECTIVE: The aim of this study was to explore the association between TP53 gene polymorphisms (rs8068934 A>G and rs218698 C>T) and chronic lymphocytic leukemia (CLL).

PATIENTS AND METHODS: CLL patients who received treatment in our hospital were enrolled in this study as the disease group. Meanwhile, healthy subjects were taken as the control group. Peripheral blood samples were collected to detect TP53 gene polymorphisms at rs8068934 and rs218698, and the haplotype analysis was performed. The expression of TP53 was detected via reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Furthermore, the survival conditions were analyzed.

RESULTS: The allele distribution at rs8068934 (p=0.046) and rs218698 (p=0.028) of TP53 gene was different between control group and disease group. A allele frequency at rs8068934 and T allele frequency at rs218698 were significantly higher in disease group (p<0.05). The genotype distribution at rs218698 of TP53 gene in disease group was also different from that in control group (p=0.038). The results demonstrated that CC genotype frequency in disease group was significantly lower than that in control group (p<0.05). Besides, the distribution of dominant model at rs8068934 (p=0.042) and recessive model at rs218698 (p=0.033) in disease group exhibited remarkable differences from control group, in which AA+AG frequency (dominant model) at rs8068934 and CC+CT frequency (recessive model) at rs218698 in disease group were significantly higher. Meanwhile, the distribution of AT (p=0.029) and GC (p=0.007) haplotypes at rs8068934 and rs218698 in disease group was evidently different from that in control group. The results indicated that disease group showed significantly higher frequency of AT haplotype and lower frequency of GC haplotype (p<0.05). Moreover, TP53 gene polymorphisms at rs8068934 were significantly associated with the levels of white blood cells (WBC) (p=0.000) and platelets (PLT) (p=0.035). Patients with GG genotype had significantly higher level of WBC, while those with AG genotype showed significantly lower level of PLT (p<0.05). TP53 gene polymorphisms at rs218698 were associated with the level of red blood cells (RBC) (p=0.000). Patients with CT genotype had a remarkably lower level of RBC (p<0.05). There were significant correlations of TP53 gene polymorphisms at rs8068934 (p=0.000) and rs218698 (p=0.000) with the expression of TP53. The expression of TP53 was lower in people with AA genotype at rs8068934 but higher in people with TT genotype at rs218698 (p<0.05). Furthermore, TP53 gene polymorphisms at rs8068934 (p=0.000) and rs218698 (p=0.000) were markedly associated with patients' survival.

CONCLUSIONS: TP53 polymorphisms are significantly correlated with the occurrence and progression of CLL.

Key Words:
Chronic lymphocytic leukemia (CLL), Gene polymorphisms, TP53.

Introduction

Leukemia is a common malignant tumor of the hematopoietic system, which is manifested as ineffective hematopoiesis of hematopoietic stem cells. Therefore, there are very few functional blood cells in the body. Meanwhile, it is often accompanied by such severe symptoms as anemia, infection and bleeding occur[1,2]. Chronic lymphocytic leukemia (CLL) is a category of

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chronic leukemia, with increasing monoclonal B lymphocytes[3]. These lymphocytes are tumor cells with hypo-function and inability to resist stimuli such as external infection. As a result, patients' immunity is relatively low, and lymphocyte infiltration occurs in various organs. This may eventually lead to severe corresponding symptoms[4,5]. Therefore, it is of great significance to elucidate the pathogenesis of CLL for the prevention of the disease.

Currently, the balance between the expressions of oncogenes and tumor suppressor genes is one of the key internal factors controlling cell canceration in the body[6,7]. As the most important tumor suppressor gene studied so far, TP53 gene mutates in over half of tumor patients[8]. The mutation, single nucleotide polymorphism, copy number variation and epigenetic alteration of the TP53 gene are closely related to the occurrence of malignancies, such as leukemia[9,10]. A previous report sequenced TP53 hot-spot exons 5-8 (DNA-binding domain) and deletion status in patients with lymphoblastic leukemia and showed the frequencies of TP53 alterations for those relapsed cases[11]. To date, few studies have investigated the changes in TP53 gene polymorphisms at rs8068934 and rs218698 in CLL. Based on data from previous research and our preliminary data, we chose the positions of rs8068934 and rs218698 for further exploration.

In this study, the differences in TP53 gene polymorphisms at rs8068934 and rs218698 were detected in normal subjects and CLL patients. Haplotype analysis was performed, and the expression of TP53 gene and its influence on prognosis were further analyzed. Our study aimed to explore the association between TP53 gene polymorphisms (rs8068934 A>G and rs218698 C>T) and CLL.

Patients and Methods

General Data

A total of 200 healthy subjects and 200 CLL patients in the last 5 years were collected as the control group and disease group, respectively. The selection of patients was based on the guideline proposed by the Union for International Cancer Control (UICC). General data (name, age, gender, etc.), disease history, family history and drug allergy history were collected in both groups. The mean age in control group and disease group was (35.23±4.21) and (32.45±5.84) years old, respectively. There were no statistically significant differences in such general data as age and gender between the two groups (p>0.05). CLL was diagnosed and confirmed by two hematologists with senior titles *via* microscopic observation of bone marrow and peripheral blood smears. This investigation was approved by the Ethics Committee of Yantaishan Hospital.

Sample Collection and Preprocessing

A total of 5-6 mL of peripheral blood was first drawn by the on-duty nurse from the elbow vein in both groups. Next, collected blood samples were centrifuged at 3000 rpm for 8 min within 1.5 h. Mid-layer nucleated cells were then isolated into new 1.5 mL centrifuge tubes for extraction of genomic deoxyribonucleic acid (DNA) and detection of TP53 expression.

DNA Extraction

Genomic DNA was extracted from peripheral blood samples in both groups using the genomic DNA extraction kit (Qiagen, Hilden, Germany). An appropriate amount of mid-layer nucleated cell samples was taken, added with 20-30 μL of proteinase K and mixed evenly. Subsequently, 200 μL of buffer was added and mixed upside down, followed by incubation at 65°C for 15 min. Then, the mixture was added with absolute ethanol and shaken evenly for 30 s. Next, the flocculent precipitate was transferred into an absorption column and centrifuged for 35 s. After discarding the waste liquor, the absorption column was placed back into the collecting tube. 500 μL of deproteinized solution was added, followed by centrifugation. Then, the mixture was washed twice with 700 μL of washing buffer, and centrifuged. Finally, preheated elution buffer was added into the absorption column, placed for 2 min and centrifuged, and the resulting solution was the genomic DNA.

Polymerase Chain Reaction (PCR) Amplification and Analysis of TP53 Gene Polymorphisms

Polymorphic regions at TP53 gene loci rs8068934 and rs218698 were amplified *via* PCR. Primers were designed using Prime Premier 5 by the company, and the validity was verified, as follows: rs8068934 forward (5'→3'): CCCTTTCTTGCGGAGATTCTCT, reverse (5'→3'): ACAGCTTTGAGGTGCGTGTTT. rs218698 forward (5'→3'): GAGGTTGGCTCTGACTGTACC, reverse (5'→3'): TCCGTC-

Table I. Allele distribution at rs8068934 and rs218698 of TP53 gene in both groups.

Locus	Allele	Control group	Disease group	OR	95% CI	χ^2	*p*
rs8068934	A	207 (0.517)	235 (0.588)	1.32	1.01-1.75	3.96	0.046
	G	193 (0.482)	165 (0.412)				
rs218698	C	214 (0.535)	183 (0.458)	0.73	0.550.96	4.84	0.028
	T	186 (0.465)	217 (0.542)				

CCAGTAGATTACCAC. Reaction products were obtained *via* denaturation, annealing and extension. PCR products were sent to Zhejiang Biotechnology Co., Ltd. (Hangzhou, China) for sequencing, and TP53 gene polymorphisms at rs8068934 and rs218698 were finally analyzed.

Detection of TP53 Gene Expression via RT-qPCR

The expression of TP53 gene was detected *via* RT-qPCR. Total RNA was extracted from peripheral blood nucleated cells using TRIzol (Invitrogen, Carlsbad, CA, USA). Subsequently, extracted RNA was reversely transcribed into complementary deoxyribose nucleic acid (cDNA). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as the internal reference in the quantitative analysis of the TP53 gene expression. Specific PCR conditions were as follows: 95°C for 5 min, (95°C for 30 s, 57°C for 40 s, and 72°C for 30 s) ×45 cycles, and 72°C for 5 min. TP53 gene amplification primers: forward (5'→3'): CAGCACATGACGGAGGTTGT, reverse (5'→3'): TCATCCAAATACTCCACACGC; GAPDH: forward (5'→3'): CGCTCTCTGCTCCTCCTGTTC, reverse (5'→3'): ATCCGTTGACTCCGACCTTCAC.

Analysis of Related Clinical Indexes

Clinical indexes, including white blood cells (WBC), red blood cells (RBC), hemoglobin (Hb) and platelet (PLT) in disease group were analyzed in the Clinical Examination Room of Laboratory Department using a full-automatic blood routine analyzer (Mindray BC-2900, Shenzhen, China).

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 22.0 software (IBM Corp., Armonk, NY, USA) was used for all statistical analysis. Measurement data were compared using *t*-test. Analysis of variance was performed for comparison among groups, followed by Post-Hoc Test (Least Significant Difference). SHEsis website was conducted for polymorphism analysis, and log-rank test for survival analysis. To test the population homogeneity of the study subjects, the allele frequencies were tested against Hardy-Weinberg equilibrium by the χ^2-test. $p<0.05$ was considered statistically significant.

Results

Allele Distribution at rs8068934 and rs218698 of TP53 Gene in Both Groups

All allele frequencies did not deviate from Hardy-Weinberg equilibrium. As shown in Table I, the allele distribution at rs8068934 ($p=0.046$) and rs218698 ($p=0.028$) of TP53 gene was significantly different between control group and disease group. A allele frequency at rs8068934 and T allele frequency at rs218698 were significantly higher in disease group ($p<0.05$).

Genotype Distribution at rs8068934 and rs218698 of TP53 Gene in Both Groups

As shown in Table II, the genotype distribution at rs218698 of TP53 gene in disease group was different from that in control group ($p=0.038$). CC

Table II. Genotype distribution at rs8068934 and rs218698 of TP53 gene in both groups.

Locus	Genotype	Control group	Disease group	χ^2	*p*
rs8068934	AA	55 (0.275)	68 (0.340)	4.17	0.124
	AG	97 (0.485)	99 (0.495)		
	GG	48 (0.240)	33 (0.165)		
rs218698	CC	52 (0.260)	32 (0.160)	6.51	0.038
	CT	110 (0.550)	119 (0.595)		
	TT	38 (0.190)	49 (0.245)		

Table III. Analysis of TP53 gene polymorphisms at rs8068934 and rs218698 in both groups

	Locus	Genotype	Control group	Disease group	χ^2	p
Dominant model	rs8068934	AA+AG	152 (0.760)	167 (0.835)	6.32	0.042
		GG	48 (0.240)	33 (0.165)		
	rs218698	CC+CT	162 (0.810)	151 (0.755)	3.85	0.146
		TT	38 (0.190)	49 (0.245)		
Recessive model	rs8068934	AA	55 (0.275)	68 (0.340)	3.79	0.150
		AG+GG	145 (0.725)	132 (0.660)		
	rs218698	CC	52 (0.260)	32 (0.160)	6.84	0.033
		CT+TT	148 (0.740)	168 (0.840)		
Heterozygous model	rs8068934	AA	55 (0.275)	68 (0.340)	2.44	0.295
		AG	97 (0.485)	99 (0.495)		
	rs218698	CC	52 (0.260)	32 (0.160)	2.95	0.229
		CT	110 (0.550)	119 (0.595)		
Homozygous model	rs8068934	AA	55 (0.275)	68 (0.340)	2.1	0.350
		GG	48 (0.240)	33 (0.165)		
	rs218698	CC	52 (0.260)	32 (0.160)	2.23	0.328
		TT	38 (0.190)	49 (0.245)		

Table IV. Haplotype analysis of TP53 gene loci rs8068934 and rs218698 in both groups.

Haplotype	Control group	Disease group	OR	95% CI	χ^2	p
AC	111.31 (0.278)	111.72 (0.279)	1.005	0.738-1.369	0.001	0.974
AT	95.69 (0.239)	123.28 (0.308)	1.417	1.036-1.937	4.785	0.029
GC	102.69 (0.257)	71.28 (0.178)	0.628	0.447-0.882	7.249	0.007
GT	90.31 (0.226)	93.72 (0.234)	1.049	0.755-1.459	0.082	0.774

genotype frequency in disease group was remarkably lower than that in control group (p<0.05).

Analysis of TP53 Gene Polymorphisms at rs8068934 and rs218698 in Both Groups

The distribution of dominant model at rs8068934 (p=0.042) and recessive model at rs218698 (p=0.033) in disease group showed statistically significant differences from that in control group, in which AA+AG frequency (dominant model) at rs8068934 and CC+CT frequency (recessive model) at rs218698 in disease group were higher than control group (p<0.05, Table III).

Haplotype Analysis of TP53 Gene Loci rs8068934 and rs218698 in Both Groups

The distribution of AT (p=0.029) and GC (p=0.007) haplotypes at rs8068934 and rs218698 in disease group was evidently different from that in control group. The results indicated that disease group exhibited significantly higher frequency of AT haplotype and lower frequency of GC haplotype (p<0.05, Table IV).

Table V. Associations of TP53 gene loci rs8068934 and rs218698 with clinical indexes in disease group.

	Genotype	WBC (×109/L)		Hb (g/L)		RBC (×1012/L)		PLT (×109/L)	
		Disease group	p	Disease group	p	Disease group	p	Disease group	p
rs8068934	AA	18.42±2.14	0.000	97.23±7.42	0.274	3.24±0.84	0.342	167±21	0.035
	AG	17.45±2.93		87.41±4.21		3.26±0.93		145±24	
	GG	27.41±3.21		92.15±5.35		3.21±0.76		178±19	
rs218698	CC	19.23±1.62	0.164	88.89±6.14	0.264	3.20±0.43	0.000	162±12	0.374
	CT	17.94±2.51		91.09±4.05		2.34±0.23		161±18	
	TT	18.46±2.74		89.23±4.85		3.76±0.55		159±25	

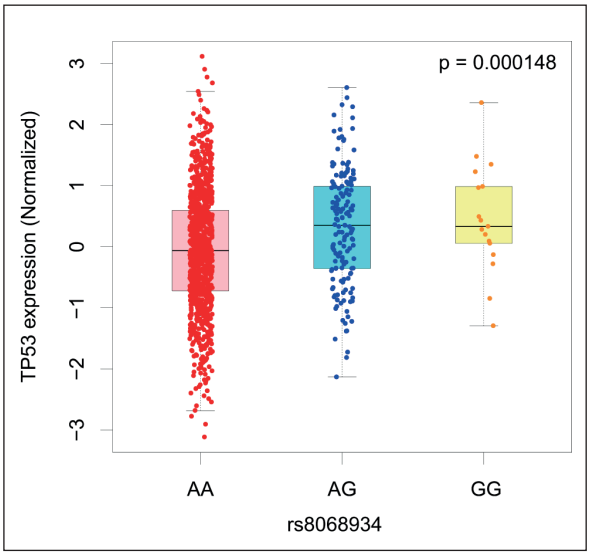

Figure 1. Association between TP53 gene polymorphisms at rs8068934 and gene expression.

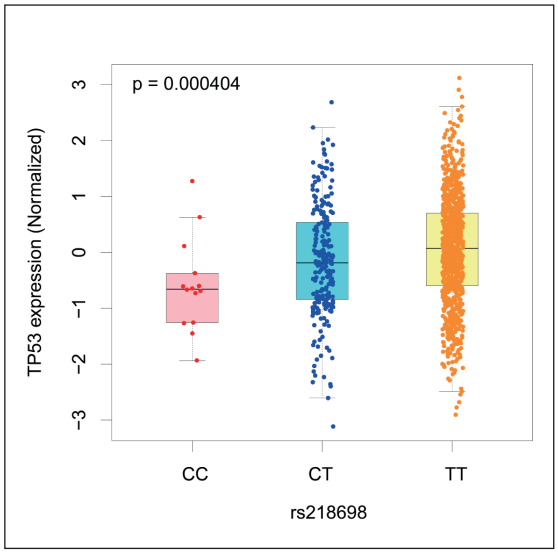

Figure 2. Association between TP53 gene polymorphisms at rs218698 and gene expression.

Associations of TP53 Gene Loci rs8068934 and rs218698 with Clinical Indexes in Disease Group

TP53 gene polymorphisms at rs8068934 were significantly associated with the levels of WBC (p<0.001) and PLT (p=0.035) in patients. Patients with GG genotype exerted significantly higher level of WBC, while those with AG genotype had lower level of PLT (p<0.05). TP53 gene polymorphisms at rs218698 were associated with the level of RBC (p<0.001), and patients with CT genotype had remarkably lower level of RBC (Table V).

Associations of TP53 Gene Polymorphisms at rs8068934 and rs218698 with Gene Expression

There were significant correlations of TP53 gene polymorphisms at rs8068934 (p<0.001) and rs218698 (p<0.001) with the expression of TP53. The expression of TP53 was lower in people with AA genotype at rs8068934 but higher in people with TT genotype at rs218698 (p<0.05, Figures 1 and 2).

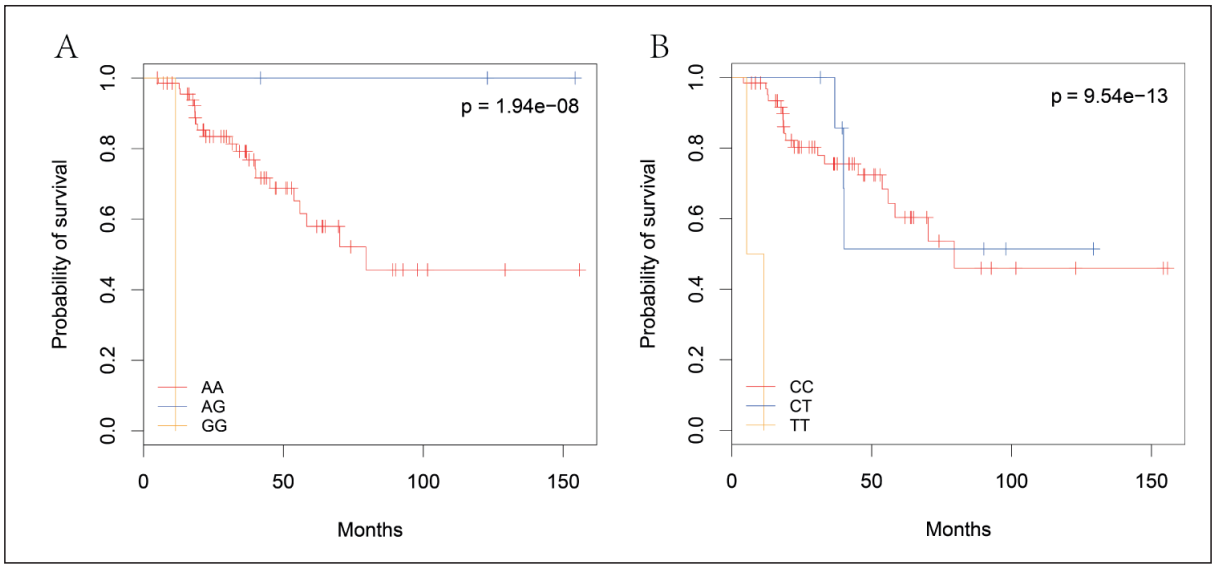

Figure 3. Associations of TP53 gene polymorphisms at rs8068934 (**A**) and rs218698 (**B**) with patients' survival.

Associations of TP53 Gene Polymorphisms at rs8068934 and rs218698 with Patients' Survival

TP53 gene polymorphisms at rs8068934 (p<0.001) and rs218698 (p<0.001) were markedly associated with patients' survival (Figure 3).

Discussion

CLL is a common malignant tumor of the hematopoietic system, seriously threatening human health. It is mainly characterized by chronic ineffective hematopoiesis in the bone marrow[12-14]. A variety of factors have been detected involved in the occurrence of CLL, including race factors (the risk of CLL in Europeans is generally higher than Asians), environmental factors (chronic stimulation of low-frequency magnetic field may be one of the factors), and family factors (people with a family history of leukemia or CLL have a higher risk of CLL)[15,16]. However, many causes of CLL have not been studied thoroughly yet. Based on gene polymorphisms, in this paper, the causes of CLL were explored from the genetic perspective. Our findings might provide a solid theoretical basis for clarifying the specific pathogenesis of CLL. Previous studies have demonstrated that people with specific genotypes are more susceptible to CLL. All these findings are of great significance for the prevention or early intervention of CLL, as well as the decrease of its morbidity and mortality rates.

TP53 is the most important initiating factor or influencing factor discovered for the occurrence of malignant tumors[17]. 53 kDa protein encoded by TP53 plays an important regulatory role in tumor cell proliferation, apoptosis, division, differentiation, migration and metastasis, maintenance of stem cell characteristics and initiation of cell cycle. TP53 can affect multiple functions of various cancer cells[18,19]. Meanwhile, its gene polymorphisms are associated with the occurrence of many types of leukemia[20,21]. In this study, the distribution of TP53 gene polymorphisms was compared between CLL patients and healthy people. The results indicated that the allele distribution at rs8068934 (p=0.046) and rs218698 (p=0.028) of TP53 gene was statistically different between control group and disease group. A allele frequency at rs8068934 and T allele frequency at rs218698 were significantly higher in disease group. The genotype distribution at rs218698 of TP53 gene in disease group was also different from that in control group (p=0.038). CC genotype frequency in disease group was remarkably lower than control group. The above results demonstrated that TP53 gene polymorphisms indeed had an influence on the occurrence of CLL, which might be one of the susceptible factors.

According to further analysis, the distribution of dominant model at rs8068934 (p=0.042) and recessive model at rs218698 (p=0.033) in disease group exhibited significant differences from that in control group. AA+AG frequency (dominant model) at rs8068934 and CC+CT frequency (recessive model) at rs218698 in disease group were higher than control group. The distribution of AT (p=0.029) and GC (p=0.007) haplotypes at rs8068934 and rs218698 in disease group was evidently different from that in control group, in which disease group had higher frequency of AT haplotype and lower frequency of GC haplotype. Summarily, the effect of TP53 gene polymorphisms on the occurrence of CLL might result from multiple loci and genotypes, rather than a single factor.

Furthermore, the associations between TP53 gene polymorphisms and the levels of clinical indexes were analyzed. It was indicated that TP53 gene polymorphisms at rs8068934 were significantly associated with the levels of WBC (p<0.001) and PLT (p=0.035). Patients with GG genotype had significantly higher level of WBC, while those with AG genotype showed lower level of PLT. TP53 gene polymorphisms at rs218698 were associated with the level of RBC (p<0.001), and patients with CT genotype had remarkably lower level of RBC. The above findings suggested that TP53 gene polymorphisms might affect the development of CLL *via* affecting the levels of WBC, PLT and RBC.

Finally, our results showed that there were significant correlations of TP53 gene polymorphisms at rs8068934 (p<0.001) and rs218698 (p<0.001) with the expression of TP53. TP53 expression was lower in people with AA genotype at rs8068934 but higher in people with TT genotype at rs218698. In addition, TP53 gene polymorphisms at rs8068934 (p<0.001) and rs218698 (p<0.001) were markedly associated with patients' survival. The novelty of this study was that TP53 gene polymorphisms could affect the prognosis of CLL, which might be realized by regulating gene expression.

Conclusions

Altogether, these results showed that, TP53 polymorphisms are significantly correlated with the occurrence and progression of CLL.

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Conflict of Interests

The authors declare that they have no conflict of interest.

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