L-18 Polymorphisms Impose Considerable Impacts on Acute Myeloid Leukemia Occurrence

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Abstract

Background: Interleukin 18 (IL-18), a pro-inflammatory cytokine, play multiple roles in immune and inflammatory responses. It also closely associates with the development and treatment of cancers. Present study was aimed to explore the effects of rs1946518 and rs187238 polymorphisms in promoter region of *IL-18* gene for the acute myeloid leukemia (AML) susceptibility.

Methods: Present case-control study recruited 128 AML patients and 145 healthy individuals. TaqMan assay method was used to sequence the *IL-18* polymorphisms in all of the subjects. Serum concentration of IL-18 was detected by ELISA kit. Association between *IL-18* polymorphisms and AML susceptibility was assessed by χ^2 test. Odds ratios (ORs) and 95% confidence intervals (CIs) were utilized to present the association strength.

Results: G allele of rs1946518 distinctly associated with increased susceptibility of AML (*P*=0.045, OR=1.418, 95%CI=1.007-1.997). Higher frequency of rs187238 GG genotype in AML patients indicated that it significantly associated with enhanced AML susceptibility (*P*=0.017, OR=4.081, 95%CI=1.273-13.089). Meanwhile, positive association also has been discovered between rs187238 G allele and AML risk (*P*=0.001, OR=2.155, 95%CI=1.341-3.464). Besides, rs187238 GG genotype might obviously decrease the serum concentration of IL-18 in AML patients.

Conclusions: Minor allele of *IL-18* rs1946518 and rs187238 polymorphisms might positively associate with the AML susceptibility might via alter the IL-18 concentration.

Background

Acute myeloid leukemia (AML) is one of the malignant disease in myeloid hematopoietic stem cells (or progenitor cells) . This disease often present in adults. It is characterized by the hyperplasia of original and immature myeloid cells in bone marrow and peripheral blood. Clinical features of AML mainly include the reduction of erythrocyte, platelets (PLT), and normal white blood cells (WBC). Fatigue, shortness of breath, easy bruising and bleeding and increased of infection usually appear in the AML patients. Patients may die from the complications. AML incidence is increased with the ages, despite it is a relatively rare disease . AML had high mortality. It reduce the life quality and bring heavy economic burden for the family and society . Recent years, the treatment of AML had great progress . But, present therapy methods could not crude AML. Therefore, it is necessary to probe the AML pathogenesis, so as to find an effective therapy method for AML. Various factors have been identified correlated with the occurrence of AML . More and more evidence provided that immune system play a crucial role in the regulation of malignancy in hematopoietic system .

Interleukin (IL) 18 is a pro-inflammatory cytokine which belongs to IL-1 superfamily. *IL-18* gene is located at chromosome 11q23.1. IL-18 is produced by macrophages, other immune cells or circulating cancer cells. It had multiple functions, such as inducing the production of other cytokines, promoting the proliferation and differentiation of T-helper type I (Th1) cells, and enhancing the activity of natural killer

(NK) cells . Many studies indicated that IL-18 involved in the occurrence and development of autoimmune disease, chronic inflammatory disorders and even cancers . Besides, other studies found that IL-18 is up-regulated in the patients with non-Hodgkin's lymphadenoma, acute lymphoblastic leukemia (ALL) and/or chronic myelocytic leukemia (CML) . Polymorphisms in IL-18 gene might alter the expression level. However, the influence of *IL-18* polymorphisms for AML is not clear.

In this study, we selected two single nucleotide polymorphisms (SNPs), rs1946518 (-607T/G) and rs187238 (-137G/C), in the promoter region of *IL-18* gene to detect the association of it with the AML susceptibility. Meanwhile, the effects of *IL-18* SNPs for IL-18 serum level also measured in this study.

Methods

Study subjects

During January 2013 to June 2016, 128 AML patients were recruited from PanYu Central Hospital. AML patients were diagnosed by two pathologists according to WHO guidelines . Patients had detailed medical history, and without the histories of myelodysplastic syndrome (MDS), myeloproliferative diseases (MPS) and did not contact the medicines which might lead to the leukemia potentially. Healthy controls received regular examination, and matched with the cases both in age and gender. All subjects were Chinese Han population and older than 16 years.

This experimentation was approved by the ethic committee of PanYu Central Hospital. Written informed consents were signed by the patients and/or guardians.

Genotyping method

Blood samples were collected from the elbow vein and dealt with EDTA. Samples were divided into leukocytes and serum. Genomic DNA were extracted from leukocytes using the DNA extraction kit (TIANGEN biochemical technology (Beijing) co., LTD). *IL-18* rs1946518 and rs187238 polymorphisms were sequenced by TaqMan assay following previous study .

Serum concentration of IL-18

Serum IL-18 concentration in every subject was determined by ELISA kit (Abnova,Taibei) conforming to the manufacture's instruction.

Statistical analysis

Hardy-Weinberg equilibrium (HWE) test was used to assess the genotype and allele distribution. Genotype frequencies were calculated by direct counting. Continuous variables were assessed by t test or non-parametric test. Association between *IL-18* polymorphisms and AML susceptibility were evaluated by Chi-square test and presented by odds ratios (ORs) with corresponding 95% confidence intervals (CIs). All of the calculations were performed by PASW18.0. Statistically significant level set to 0.05 (two side).

Results

Characteristics of subjects

Similar distributions of age and gender exited between case and control groups (**Table 1**, *P*>0.05). But, erythrocyte, WBC, Haemoglobin B (Hb) and PLT were significantly increased in AML patients than that in healthy controls (*P*<0.001).

Association between *IL-18* SNPs and AML susceptibility

Genotype and allele distributions of rs1946518 and rs187238 SNPs were conforming to the HWE test in the control group (**Table 2**, *P*>0.05), suggesting that the study subjects could on behalf of the general population.

Higher frequencies of rs1946518 TG and GG genotypes were discovered in AML patients, but these difference had no statistical significance (**Table 2**, *P*>0.05). A slightly increased susceptibility has been brought by the rs1946518 GG genotype (*P*=0.055). G allele frequencies respectively were 45.70% in AML patients and 37.24% in controls. Significant difference existed in rs1946518 G allele between case and control groups, indicating a positive association between G allele and AML susceptibility (*P*=0.045, OR=1.418, 95%CI=1.007-1.997).

Rs187238 CC, CG and GG genotype frequencies were 67.19%, 23.44%, 9.38% in cases and 80.69%, 16.55%, 2.76% in controls, respectively. Significant association was discovered between GG genotype and enhanced AML susceptibility (*P*=0.017, OR=4.081, 95%CI=1.273-13.089). G allele was more frequently observed in AML patients than that in healthy controls, suggesting a distinct increased susceptibility for AML (*P*=0.001, OR=2.155, 95%CI=1.341-3.464). These results demonstrated that *IL-18* SNPs might act as a predictor for AML.

Effects of rs187238 genotypes for IL-18 serum concentration

Serum concentration of IL-18 was 112.19±92.31 pg/mL in AML patients and 139.47±94.51 pg/mL in healthy controls (*P*=0.067). Rs1946518 genotypes had no significant association with the susceptibility, then we did not explore the influence of rs1946518 genotypes for IL-18 concentration. In AML patients, IL-18 concentration was significantly higher in CC genotype carriers (132.78±59.02 pg/mL) than that in GG genotype carriers (97.41±34.53 pg/mL) (**Figure1**, *P*=0.026). There was no significant difference of IL-18 concentration between CG genotype carriers (107.64±43.59 pg/mL) and GG genotype carriers. But, IL-18 concentration had no significant difference between healthy individuals with CC, CG, GG genotype carriers (*P*>0.05).

Discussion

AML is a malignancy in blood system, that is the rapid growth of WBC which could interfere the production of normal blood cells in the bone marrow. Occurrence of AML result in the imbalance of

immune system, then promote the proliferation of tumor cells. Various studies considered that IL-18 closely associated with the immune situation, and the onset of malignancy. IL-18 is produced by hematopoietic and non-hematopoietic lineages . Pre-activated IL-18 will promote the proliferation and cytotoxicity of NK cells . Maha et al. indicated that IL-18 expression level correlated with the responses for the chemotherapy of AML . Other study also provided that the expression level of IL-18 in leukemic cells of ALL patients related to the diagnosis and prognosis of ALL . Polymorphisms in the gene promoter region might affect the expression level of protein which will lead to the abnormal physiological process. *IL-18* rs1946518 and rs187238 SNPs have been found correlated with various cancers, such as oral cancer, papillary thyroid cancer, breast cancer . These evidence suggested that IL-18 play an important role in the onset and development of AML. *IL-18* SNPs might contribute to AML onset. Due to very few studies focused on the association of *IL-18* SNPs and AML susceptibility, we performed this study.

In present study, we found that rs1946518 GG genotype slightly correlated with enhanced risk of AML. While, rs1946518 G allele significantly associated with 1.418 times increased susceptibility. Rocha and colleagues indicated that -607CC genotype significantly associated with human T-cell leukemia virus type 1 (HTLV-1) infection . But Wang et al. found that all of the genotypes and alleles of rs1946518 SNP had no significant association with the AML susceptibility . Rs187238 GG genotype distinctly correlated with 4.081 times enhanced risk of AML. Significantly higher frequency of rs187238 G allele existed in AML patients, demonstrating that rs187238 G allele obviously related to AML susceptibility approximately 2.155 times. A significant association has been discovered by Yalçın et al. between rs187238 SNP and the risk of chronic lymphocytic leukemias (CLL) and CML in Turkish population .

IL-18 concentration was down-regulated in AML patients when compared with the healthy individuals, despite the difference was not significant. AML patient with rs187238 GG genotype had significantly lower IL-18 serum level, in the comparison with CC genotype carriers. Serum concentration of IL-18 had similar distribution in the idiopathic recurrent miscarriage patients with different rs187238 genotypes . These results demonstrated that rs187238 SNP might contribute to the expression level of IL-18.

Inconsistent of our results with previous studies might caused by the discrepancies of region, ethnicity, sampling bias, sample size and/or environment factors. Several limitations existed in present study should be admitted. First of all, the test power was not high enough due to the small sample size. Second, only one ethnicity enrolled in this study might limit the application range of our results. Third, current results were not adjusted by confounding factors may affect the precision thereof. Finally, lack of the experiment on interactions between gene-gene or gene-environment factors will reduce the robustness of present results. Therefor, well designed studies with enlarged sample size and ethnicity numbers are needed in the future, so as to certify the mechanism of *IL-18* SNPs for AML susceptibility.

Conclusions

In summary, we suggested that minor alleles of *IL-18* rs1946518 and rs187238 SNPs might act as the risk factors for AML. Besides, rs187238 GG genotype correlated with elevated AML susceptibility might

via up-regulated IL-18 concentration. This retrospective study could not certify the pathogenesis of AML, thus, it should be further studied the influence of *IL-18* SNPs for the severity, process and recurrence of AML both in vivo and in vitro.

List Of Abbreviations

Interleukin 18 (IL-18)

acute myeloid leukemia (AML)

Odds ratios (ORs)

confidence intervals (CIs)

platelets (PLT)

white blood cells (WBC)

Interleukin (IL)

T-helper type I (Th1)

natural killer (NK)

acute lymphoblastic leukemia (ALL)

chronic myelocytic leukemia (CML)

single nucleotide polymorphisms (SNPs)

myelodysplastic syndrome (MDS)

myeloproliferative diseases (MPS)

Hardy-Weinberg equilibrium (HWE)

Haemoglobin B (Hb)

human T-cell leukemia virus type 1 (HTLV-1

chronic lymphocytic leukemias (CLL)

Declarations

Ethics approval and consent to participate

This study was supported by the Ethics Committee of PanYu Central Hospital and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

The subjects had been informed the objective. Certainly, written consents were signed by every subject in this study.

Consent for publication

We obtaining permission from participants to publish their data.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

H.Q., S.C. and W.X. conceived and designed the experiments; H.Q., S.C. and W.X. conceived and performed the experiments; H.Q., S.C. and W.X. prepared figures. H.Q., S.C. and W.X. wrote the main manuscript text. All authors reviewed the manuscript.

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Tables

Table 1. Characteristics of subjects

Characteristics	Case n=128(%)	Control n=145(%)	*P*
Age (years)	34.18±10.71	32.87±10.49	0.426
Gender (male)	68(53.13)	74(51.03)	0.730
Erythrocyte (g/L)	2.81±0.86	4.76±0.4	<0.001
WBC ($\times 10^9$/L)	24.91±19.72	6.11±1.19	<0.001
Hb ($\times 10^{12}$/L)	84.67±28.38	144.5±13.25	<0.001
PLT ($\times 10^9$/L)	32.82±22.71	243.35±30.89	<0.001

Notes: WBC, white blood cells; Hb, Haemoglobin B; PLT, platelets;

Table 2. Association between *IL-18* SNPs and AML susceptibility

Genotype/Allele	Case n=128(%)	Control n=145(%)	*P*	OR(95%CI)
rs1946518				
TT	40(31.25)	59(40.69)	-	-
TG	59(46.09)	64(44.14)	0.260	1.360(0.796-2.322)
GG	29(22.66)	22(15.17)	0.055	1.944(0.981-3.854)
T	139(54.30)	182(62.76)	-	-
G	117(45.70)	108(37.24)	0.045	1.418(1.007-1.997)
P_{HWE}	0.420	0.502		
rs187238				
CC	86(67.19)	117(80.69)	-	-
CG	30(23.44)	24(16.55)	0.089	1.686(0.921-3.087)
GG	12(9.38)	4(2.76)	0.017	4.081(1.273-13.089)
C	202(78.91)	258(88.97)	-	-
G	54(21.09)	32(11.03)	0.001	2.155(1.341-3.464)
P_{HWE}	0.001	0.059		

Figures

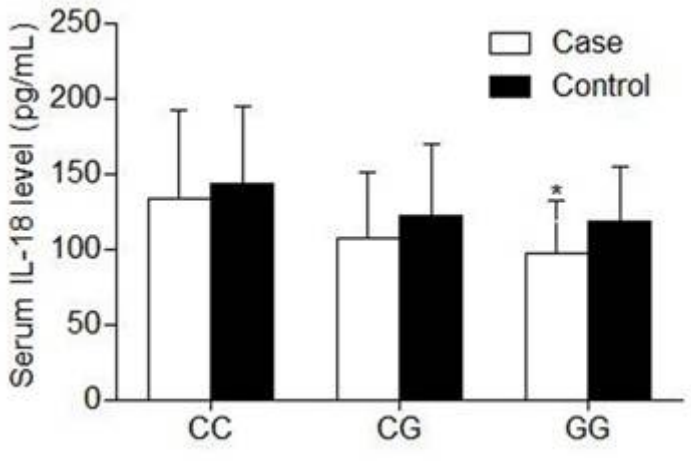

Figure 1

Effects of rs187238 genotypes for IL-18 serum concentration. In AML patients, IL-18 concentration was significantly higher in CC genotype carriers (132.78±59.02 pg/mL) than that in GG genotype carriers (97.41±34.53 pg/mL) (P=0.026). No significant difference of IL-18 concentration between healthy individuals with CC (142.85±51.77 pg/mL), CG (121.69±47.60 pg/mL), GG (118.31±36.54 pg/mL) genotype carriers.