Safwat *et al.*
Egyptian Journal of Medical Human Genetics (2022) 23:43
https://doi.org/10.1186/s43042-022-00253-5

Egyptian Journal of Medical Human Genetics

RESEARCH Open Access

Interleukin-4 gene intron 3 VNTR polymorphism in adult acute myeloid leukemia

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Abstract

Background: The proliferation of acute myeloid leukemia (AML) blast into the bone marrow microenvironment is controlled by cytokines. Interleukin-4 (IL-4) has recently been discovered to suppress the development and persistence of AML cells selectively. Intron three of the Interleukin-4 (IL-4) gene contains a 70-bp minisatellite region polymorphism that may influence gene transcriptional activity and subsequently affect the production level of IL4. We investigated the IL-4 gene intron three variable number tandem repeat (VNTR) polymorphism as a molecular marker in AML associated with clinical and laboratory variables and a prognostic factor for therapeutic response and disease outcome.

Results: IL-4 gene intron three minisatellite regions polymorphism was assessed in 60 adult AML patients and 60 healthy controls, comparable concerning age and gender, using polymerase chain reaction. Three study marker genotypes were detected in AML patients; P1/P1 (3%), P1/P2 (40%), and P2/P2 (56.7%). The frequency of P2 alleles was significantly more in AML patients than in healthy controls (76.7% versus 25%; $P < 0.001$). Compared to the heterozygous group and P1/P1 carriers, AML patients with the homozygous P2/P2 genotype had a higher total leucocytic count and increased blast percentages in bone marrow or peripheral blood, besides a lower platelet count. P2P2 genotype was also significantly associated with poor therapeutic response, higher susceptibility to disease recurrence and shorter overall survival and disease-free survival.

Conclusion: The IL-4 intron 3 VNTR polymorphism could be included in the molecular risk stratification of AML to predict poor disease. This information can be utilized in incorporating biological therapy into the present therapeutic protocols to enhance chemotherapy regimens' current low response rates.

Keywords: AML, IL4, VNTR

Background

Several molecular and cytogenic abnormalities characterize acute myeloid leukemia (AML). Since cytokines and growth factors are generated in the medullary microenvironment, and they regulate cell survival, proliferation, and differentiation, polymorphisms in their respective genes may influence cancer susceptibility [1].

Interleukin 4 (IL4) is a well-known T-helper 2 (Th2) cytokine. It is a pleiotropic cytokine that has a dichotomous role in inducing cancer. According to some studies, IL-4 prevents carcinogenesis by promoting apoptosis. In contrast, other researchers disagree with this theory, claiming that IL-4 enhances tumor growth, spread, and metastasis [2].

The IL4 gene is located in the cytokine cluster region on the long arm of chromosome 5 (5q31-33), with four exons and three introns. Polymorphisms affecting the intron-3 minisatellite 70 bp region may influence its transcriptional activity, resulting in three-repeat allele, two-repeat allele, or the rare four-repeat allele [3].

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Even though an increasing number of studies have been designed to investigate how IL4-intron 3 VNTR polymorphism can influence human cancer risk, only one study has examined the association between the IL-4 intron-3 VNTR polymorphism and different types of leukemias [4].

In the present study, we evaluated the different genotypes of IL4- intron 3 VNTR polymorphism in Egyptian adult AML patients and investigated its association with clinical and laboratory data, chemotherapy response, and disease outlook.

Methods

Patients selection

This prospective study was conducted on 60 newly diagnosed adults with AML recruited from the Hematology Unit of Ain Shams University hospital during the years 2018–2019 and 60 healthy controls of age and sex-matched.

- Inclusion criteria

  Newly diagnosed AML patients aged 16–60 years who are candidates for induction chemotherapy
- Exclusion criteria
  Relapsed and resistant AML cases were excluded
  Patients not fit for chemotherapy or on palliative chemotherapy
  Pregnancy and patients had concomitant severe liver, heart, and kidney comorbidities.

The control group is hospital-based. The control group consists of healthy adults who attend our hospital outpatient clinic for routine medical check-ups and submit blood samples for routine laboratory workups. Subjects with no markers in their check-ups were enrolled in the study as controls with similar proportion to the age and sex of cases. Their ages ranged from 38 to 45 years (Mean 41.08 ± 2.18 years). Forty subjects were male and 20 were female in a 2:1 ratio.

All patients and controls were asked to give informed consent before participation in the study. This study followed the guidelines set by our university's local ethical and scientific committees.The procedures followed the ethical principles outlined in the 1964 Helsinki Declaration.

Patients were diagnosed based on complete history, clinical examination, and laboratory investigations, including a complete blood count, an LH 750 (Beckman Coulter), Leishman-stained PB films examination, and bone marrow (BM) aspiration and examination. In addition, flow-cytometric immuno-phenotyping was performed to segregate patients into different FAB subtypes using an EPICS XL Coulter flow cytometer. Also, karyotyping and fluorescence in situ hybridization (FISH) were performed to demonstrate patients' cytogenetic risk categories.Cytogenetic risk groups were determined regarding the 2016 NCCN guidelines [5].

Sample collection

Peripheral blood and bone marrow samples were collected on ethylene diamine tetra-acetic acid (EDTA) (1.2 mg/ml) for morphological and immune-phenotypic evaluation and study of the IL4 gene polymorphism. BM aspirates were collected in sterile, preservativefree, lithium heparin-coated vacutainer tubes for cytogenetic analysis.

Polymerase chain reaction (PCR) technique

DNA was extracted using a whole blood genomic DNA extraction kit (QIAamp DNA blood mini kit supplied by Qiagen, Hilden, Germany); according to the manufacturer's protocol, all extracts were stored at − 20 C. A spectrophotometer was used to measure the absorbance of extracts at 260 nm and 280 nm to determine their concentration and purity. PCR was carried out in a volume of 25 μL reaction mixture containing genomic DNA, primers, Taq polymerase, MgCl2, 10 × reaction buffer, and deoxy-ribonucleoside triphosphate mix using QIAGEN Taq ready to use PCR master mix kit (cat. nos. 201443).

Amplification was performed with primers; the forward primer sequence was 5′ TAG GCT GAA AGG GGG AAA GC 3′ and for the reverse primer was 5′ CTG TTC ACC TCA ACT GCT CC 3′. Amplification was performed in a Biometra T-professional PCR System thermocycler (Analytik Jena AG) using an initial denaturation at 95 °C for 5 min, 30 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 45 s, extension at 72 °C for 1 min and final extension at 72 °C for 10 min.

All PCR amplification products were electrophoresed on a 2% agarose gel stained with ethidium bromide and visualized by UV BioDoc Analyze Darkhood transilluminatior (Biometra, Analytik Jena AG), the PCR product was 183 bp for the P1 allele and 253 bp for the P2 allele.

Treatment regimen

All patients were induced with standard chemotherapy protocols according to the 2016 NCCN guidelines to receive the standard 3 + 7 protocol [6].

Upon achieving complete remission (CR), consolidation to prevent subsequent relapse was given. The type of consolidation conferred depends on the risk of relapse, which is largely dictated by cytogenetics, and the availability of a matching sibling donor (MSD) to undergo allogeneic hematopoietic stem cell transplantation

(allo-SCT). Patients with a low risk of relapse received high-dose cytarabine for 4 cycles and those lacking a donor received the same consolidation protocol, while patients with MSD and demonstrating intermediate or poor risk cytogenetics were allo-transplanted and received high-dose Cytarabine until they have access to transplantation facility with a maximum of 4 doses [7].

Assessment of response

On day 28 of the induction cycle, all patients underwent bone marrow examination to determine the status of remission. The follow-up period lasted for up to 24 months with a median of 9 months. Patients were divided into responders attaining CR and resistant cases according to the European Leukemia Net (ELN 2017) [8]. CR was achieved when the BM examination was normal with less than 5% blast cells and disappearance of Aeur rods, recovery of absolute neutrophil count (ANC) > 1000/μL, and platelets > 100,000/μL, the extramedullary disease had resolved and cytogenetic aberrations disappeared. Relapse was defined as the re-emergence of leukemic cells in the bone marrow (≥ 5%) or the peripheral blood or as the appearance of a new extramedullary site of disease in patients with previously reported CR [8].

The European Leukemia Net (ELN) defined primary refractory AML as failure to achieve CR after two courses of intensive induction chemotherapy, excluding patients with aplastic aplasia or death due to an indeterminate cause [9].

Minimal residual disease (MRD) is thus defined as the persistence of leukemic cells after chemotherapy and is responsible for relapse onset. Quantitative MRD frequency assessment can provide important prognostic information after chemotherapy [9].

Overall survival (OS) was determined from the date of diagnosis to the date of death or the last known date to be alive. In contrast, disease-free survival (DFS) was calculated from CR to the date of relapse or the last follow-up [10].

Statistical analysis

The data were collected, updated, coded, and fed into the Statistical Package for Social Science (IBM SPSS™) version 20. The normality of data was assessed using Kolmogrov–Smirnov test. Qualitative data are presented as numbers and percentages. Normally distributed data were expressed as mean, standard deviations, and ranges, while skewed distribution data were presented as median with interquartile range (IQR). To compare quantitative variables between two groups, we used Student t test when data were parametric and Mann–Whitney test when skewed. Qualitative data were compared using Chi-square and Fisher exact when the predicted number was found in any cell less than 5. Kaplan–Meier Analysis using Log Rank test was used to compare median OS and DFS between different groups. Results that reached a level of $P < 0.05$ were considered statistically significant.

Results

The main demographic and clinicopathological characteristics of the studied cohort are presented in Table 1. The polymorphism of the study gene in AML cases and controls is depicted in Table 2. The genotype distribution of the intron 3 VNTR in IL4 gene was consistent with the Hardy–Weinberg equilibrium both in the control group and in patients ($P > 0.05$).

Table 1 Demographic and clinicopathological characteristics of AML patients

Variables	AML patient ($n = 60$)
Age (years), Mean ± SD	43.1 ± 14.3
Sex, *n* (%)	
Male	42 (70)
Female	18 (30)
Extramedullary involvement, *n* (%)	
Positive	34 (56.7)
Negative	26 (43.3)
TLC ($\times 10^3$/μL), Median (IQR)	19.5 (5.8–58)
Hemoglobin (g/dL), mean ± SD	7.5 ± 2.2
Platelets ($\times 10^3$/μL), Median (IQR)	40.5 (15–79)
PB blasts (%), Median (IQR)	32 (5–63)
Initial BM blasts (%), Median (IQR)	69 (50–85)
FAB subtype, *n* (%)	
M0	3 (5.0)
M1	12 (20)
M2	32 (53.3)
M4	11 (18.3)
M5	0 (0.0)
M6	0 (0.0)
M7	2 (3.3)
Immunophenotyping, *n* (%)	
HLA-DR	53 (88.3)
CD34	49 (81.7)
MPOX	50 (83.3)
Cytogenetic risk groups, *n* (%)	
Favorable	7 (11.66)
Intermediate	38 (63.33)
Poor	5 (8.33)
NA*	10 (16.66)

MPOX myeloperoxidase, *NA* not applicable, *ten patients (16.66%) non-provided data Or failed cytogenetic analysis, Favorable cytogenetic markers: t(8;21), inv(16); Intermediate cytogenetic markers: normal karyotype, trisomy 8; Unfavorable cytogenetic markers t(11q23), t(9;11), monosomy7

Table 2 Genotype and allele frequencies of IL4 in patients with AML and controls

Variables	Patients ($n=60$)	Control ($n=60$)	*P* value
Genotype, *n* (%)			< 0.001
P1P1	2 (3.3)	42 (70.0)	
P1P2	24 (40)	6 (10)	
P2P2	34 (56.7)	12 (20)	
Alleles, *n* (%)			< 0.001
P1	28 (23.3)	90 (75.0)	
P2	92 (76.7)	30 (25.0)	

Statistically significant discrepancies were observed between cases and controls in IL-4 intron 3 minisatellite region polymorphism, where the frequencies of P1P2 and P2P2 genotypes were significantly higher in the AML patients than controls (40%, 56.7% vs 10%, 20%, $P<0.001$, respectively). On the contrary, the homozygous P1P1 genotype was significantly prevalent among the control group, with a frequency of 70% compared to 3.3% for the study group ($P<0.001$) (Table 2).

Moreover, P2 allele demonstrated a significantly higher frequency in the AML cohort versus the controls (76.7% vs. 25%, P value <0.001). Conversely, P1 allele showed a higher frequency among controls with a statistically significant difference (75% vs. 23. 3%, $P<0.001$) (Table 2).

Correlation between intron3 VNTR -IL4 genotypes and clinical and laboratory parameters of AML patients

To investigate the impact of intron 3 VNTR-IL4 on the clinical and laboratory data of the studied patients, they were stratified based on their genotypes. Clinical and laboratory characteristics were compared between the P2P2 genotype. The combined P1P2 and P1P1 genotypes are presented in Table 3.

This comparison demonstrated that the P2P2 genotype was significantly associated with older age ($P=0.004$), higher total leucocytic count ($P=0.025$),

Table 3 Clinical and laboratory data among homozygous carriers of P2 allele versus heterozygous group and non-carriers

Variables	P1P1 and P1P2 ($n=26$)	P2P2 ($n=34$)	*P* value
Age (years), Mean ± SD	37.1 ± 11.50	47.6 ± 14.70	**0.004**
Sex, *n* (%)			0.909
Male	18 (69.2)	24 (70.6)	
Female	8 (30.8)	10 (29.4)	
Extramedullary involvement, *n* (%)			0.700
Positive	12 (46.2)	14 (41.2)	
Negative	14 (53.8)	20 (58.8)	
TLC ($\times 10^3$/μL), Median (IQR)	12.6 (4.5–30)	28 (15–130)	**0.025**
Hemoglobin (g/dL),			0.120
Mean ± SD	7.82 ± 2.21	7.2 ± 2.1	
Platelets ($\times 10^3$/μL), $\times 10^9$/L), Median (IQR)	66 (18 -141)	19 (13–62)	**0.010**
PB blasts (%)			**0.005**
Median (IQR)	20 (2–49)	58 (33–69)	
BM blasts (%),Median (IQR)	60 (38–80)	80 (67–85)	**0.023**
FAB Subtype, *n* (%)			0.973
M0, M1	6 (23.1%)	8 (23.5%)	
M2	14 (53.8%)	19 (55.9%)	
M4/5, M6, M7	6 (23.1%)	7 (20.6%)	
Immunophenotyping, *n* (%)			
HLA-DR	22 (84.6)	31 (91.2)	0.432
CD34	21 (80.8)	28 (82.4)	0.874
MPOX	20 (76.9)	30 (88.2)	0.244
Cytogenetic risk groups, *n* (%)*			0.32
Favorable	4 (19)	3 (10)	
Intermediate and poor	16 (61.53)	27 (90)	
Complete remission	23 (88.55)	22 (64.7%)	**0.035**

Bold values indicate significant *p* value

*Six patients (23%) from (P1P2 + P1P1) group and four patients (11.76%) from P2P2 group were excluded due to non -provided data or failed cytogenetic analysis

MPOX myeloperoxidase; Favorable cytogenetic markers: t(8;21), inv(16); Intermediate cytogenetic markers: normal karyotype, trisomy 8; Unfavorable cytogenetic markers t(11q23), t(9;11), monosomy7

lower platelet count ($P=0.010$), and a higher count of PB and BM blasts than those with P1P2 and P1P1 genotypes ($P=0.005$ and 0.023, respectively). However, all the other studied parameters were similar to the studied subgroups (Table 3).

Prognostic impact of intron 3 VNTR-IL4 genotypes in AML patients

When evaluating disease outcomes in our leukemic patients according to their genotypes (P2P2 vs. P1P2 + P1P1 genotypes), CR was found to be a common fate in patients who harbored P1P2 and P1P1 genotypes. Our analysis showed that 23 patients (88.5%) of this subgroup had achieved CR. In contrast to this observation, among the 34 patients included in the P2P2 subgroup, 22 patients (64.7%) showed CR, a *P* value = 0.035 (Table3).

Therefore, it is evident that patients with the P2P2 genotype had a poor disease outcome manifested in their high rate of treatment resistance. However, the combined (P1P2 + P1P1) genotypes showed a favorable impact on disease outcome as most of the patients in this group showed an excellent therapeutic response and attained CR.

Impact of IL4 SNP on the outcome

After a mean follow-up interval of 12 months, patients with P2P2 had lower OS in comparison with patients with P1P1, P1P2 (OS = 38.1% vs. 44.4%), 95% CI = 3.987–20.013 vs. 1.876–4.124, $P=0.527$) (Table 4, Fig. 1).

Regarding DFS, patients with P2P2 had lower DFS in comparison with patients with P1P1, P1P2 (DFS = 59.10% vs. 75%), 95% CI = 13.351–21.674 vs. 14.099–24.901, $P=0.557$) (Table 5, Fig. 2).

Correlation between OS, DFS with other variables

No statistical significance was detected between OS, DFS with age, TLC, HBG, PLT, peripheral blasts, initial aspirate, extramedullary involvement, diagnosis, HLA-DR, CD34, MPO, and PCR products (Tables 6, 7).

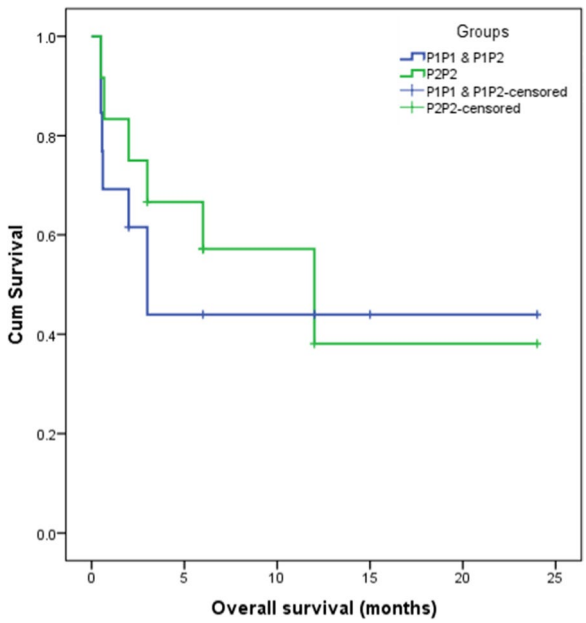

Fig. 1 Kaplan–Meier curves of OS of patients with P2P2 (38.1%) (green line) and patients with P1P1 and P1P2 (44.40%) (blue line) after 12 months of follow-up

Discussion

Blood cell and hematopoietic stem cell progenitors found in the medullary cavities are susceptible to their environment. Cytokines play a fundamental role in transducing extracellular signals and impulses by binding to their respective receptors on the cell surface [11].

In the context of leukemia, abnormal cytokine levels and aberrant response to them result in perturbed bone marrow niche architecture, which enhances leukemogenesis and disease progression [12]. In AML, these events are well documented [11].

Deregulated cytokine production is attributed to polymorphisms and VNTRs in cytokine genes. These polymorphisms critically influence genetic susceptibility to cancers in several ways, such as affecting

Table 4 Overall survival of the studied AML patients

Groups	Total N	N of events	OS (months)		95% CI		Survival at (%) months			Log Rank Test	
			Median	SE	Lower	Upper	3 months (%)	6 months (%)	12 months (%)	X^2	*P* value
P1P1 and P1P2	26	14	3	0.573	1.876	4.124	61.50	44.40	44.40	0.399	0.527
P2P2	24	12	12	4.088	3.987	20.013	66.70	57.10	38.10		

OS overall survival, *SE* standard error, *N* number

Table 5 Disease-free survival of the studied AML patients

Groups	Total N	N of events	PFS (months)		95% CI		PFS at (%) months			Log Rank Test	
			Median	SE	Lower	Upper	3 months (%)	6 months (%)	12 months (%)	X^2	*P* value
P1P1 and P1P2	18	2	19.500	2.756	14.099	24.901	75.00	75.00	75.00	0.345	0.557
P2P2	22	6	17.513	2.123	13.351	21.674	90.00	78.80	59.10		

DFS disease-free survival, *SE* standard error, *N* number

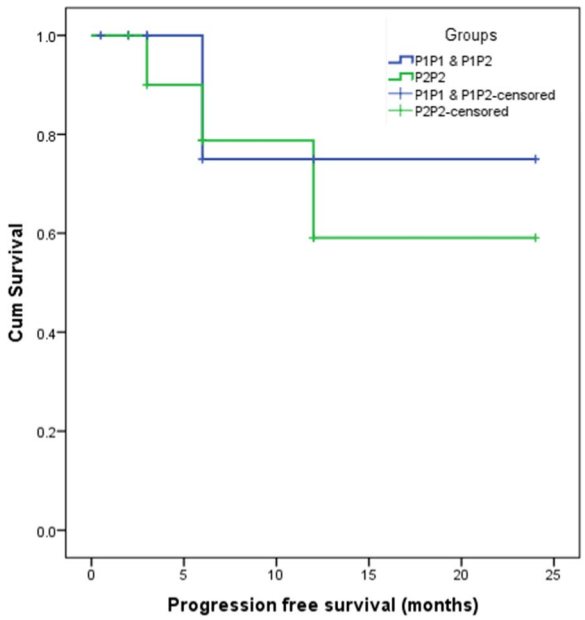

Fig. 2 Kaplan–Meier curves of DFS of patients with P2P2 (59.10%) (green line) and patients with P1P1 and P1P2 (75%) (blue line) after 12 months of follow-up

the level and the function of cytokines engaged in immune responses, disrupting nuclear factor binding to targeted genes, and altering apoptosis [13–15].

IL-4 is a crucial cytokine that controls proliferation, differentiation, and apoptosis in hematopoietic and non-hematopoietic cell lineages. Recent investigations have confirmed the essential role of IL4 in the survival of AML cells [16].

The polymorphism of the minisatellite region in the third intron of the IL-4 gene has attracted increased attention due to its role in changing gene expression. Thus, the amount of IL4 produced [16]. Currently, this IL4 gene polymorphism has been identified as a risk factor affecting susceptibility to carcinogenesis [15]. However, its role as a genetic marker in AML is still undetermined. To date, it is still unknown whether this polymorphism has prognostic value in the stratification of AML risk, as it highlights the factors contributing to the provocation and impairment of blast cells, which are the fundamental basics in AML cell biology with potential treatment outcomes [11].

Therefore, we performed this work to reveal its role and provide a deeper evaluation of its association with clinical and laboratory data and its potential prognostic impact on AML patients.

There are three alleles for IL4 gene VNTR polymorphism, namely, P1 allele, three repeats; P2 allele, two repeats; and P3 allele, four repeats. The P1 allele is the most standard allele, and the P3 allele is the rarest one [17].

In the current study, P1/P2 and P2/P2 genotypes were frequently detected in leukemic patients, and the P2 allele was significantly associated with the disease. These findings are inconsistent with a study by Ahmed et al. [4], in which patients of different kinds of leukemias have been recruited. They observed a higher incidence of allele loss in leukemic patients, and they concluded that P1/P1 and P1/P2 genotypes are collectively associated with leukemogenesis. Moreover, their study confirmed a higher frequency of P1 allele in leukemic patients. This difference stems from the observed heterogeneity of various types of leukemia in the patients enrolled in their study.

Duan et al. [15] performed a meta-analysis to investigate IL-4 intron 3 VNTR polymorphism and its relationship to cancer risk. They concluded that the P2 allele might be linked to a lower risk of cancer compared to the P1 allele. However, some reservations were raised regarding that meta-analysis. The pooled data were obtained from studies on different cancer types, accounting for heterogeneity. Various types of malignancies might trigger different host responses, and the interplay between environmental factors and the host might also affect susceptibility to different types of cancer [18].

In the present work, patients with the P2P2 genotype had higher leukocytosis, moderate anemia, marked thrombocytopenia, and higher blast percentages compared to heterozygous and non-carriers of the P2 allele. They also had inferior disease outcomes than the other group since they were less responsive to therapy and had a higher incidence of relapse.

It has been evidenced that the IL-4 intron 3 VNTR variant can alter messenger ribonucleic acid splicing

Table 6 Correlation of overall survival with other variables

		Total N	N of events	OS (months)		95% CI		Survival at				Log Rank test		
				Mean	SE	Lower	Upper	1 m. (%)	3 m. (%)	6 m. (%)	12 m. (%)	X^2	*P* value	Sig
Overall		50	26	12.346	1.565	9.279	15.412	76.0	55.3	50.6	43.4	–	–-	–
Age	≤ 45 yrs	24	10	14.719	2.226	10.355	19.082	87.5	56.6	56.3	56.3	1.817	0.178	NS
	> 45 yrs	26	16	10.379	2.082	6.298	14.46	65.4	46.2	46.2	33.0			
TLC	TLC < 20	27	13	13.31	2.100	9.195	17.425	77.8	62.6	58.4	45.4	0.481	0.488	NS
	TLC ≥ 20	23	13	11.242	2.308	6.718	15.766	73.9	46.6	41.4	41.4			
HGB	< 7.5	26	11	14.399	2.169	10.148	18.649	76.9	68.8	59.6	52.2	1.587	0.208	NS
	> 7.5	24	15	10.292	2.163	6.053	14.531	75.0	41.7	41.7	34.7			
PLT	< 40	27	15	10.972	2.168	6.724	15.221	74.1	54.6	44.6	35.7	0.722	0.395	NS
	≥ 40	23	11	13.638	2.265	9.199	18.077	78.3	56.5	56.5	50.2			
PB blasts	< 32	26	14	12.11	2.112	7.971	16.249	73.1	61.1	52.4	39.3	0.013	0.910	NS
	> 32	24	12	12.725	2.29	8.237	17.213	79.2	48.9	48.9	48.9			
Initial aspirate	< 69	25	10	14.989	2.191	10.694	19.283	80.0	67.8	63.2	55.3	2.517	0.113	NS
	> 69	25	16	9.708	2.101	5.591	13.826	72.0	42.7	37.9	31.6			
Extramedullary involvement	Negative	31	17	11.876	1.942	8.07	15.681	77.4	57.0	49.4	39.6	0.124	0.725	NS
	Positive	19	9	13.474	2.55	8.476	18.471	73.7	52.6	52.6	52.6			
Diagnosis	M0	2	2	1.5	0.5	0.52	2.48	50.0	0.0	0.0	0.0	5.682	0.338	NS
	M1	8	3	9.7	2.397	5.002	14.398	75.0	60.0	60.0	60.0			
	M2	27	15	11.601	2.112	7.461	15.74	74.1	51.0	47.1	40.3			
	M3	3	1	20	3.266	13.599	26.401	66.7	66.7	66.7	66.7			
	M4	8	4	13.5	3.742	6.166	20.834	87.5	62.5	50.0	50.0			
	M7	2	1	3.5	1.768	0.035	6.965	50.0	50.0	50.0	50.0			
HLA-DR	Negative	8	2	19.025	3.054	13.038	25.012	87.5	87.5	87.5	70.0	2.942	0.086	NS
	Positive	42	24	11.085	1.702	7.75	14.42	73.8	48.9	43.4	38.6			
CD34	Negative	11	4	16.073	3.138	9.922	22.224	81.8	72.7	72.7	58.2	1.414	0.234	NS
	Positive	39	22	11.263	1.764	7.806	14.721	74.4	50.0	44.1	39.2			
MPOX	Negative	8	4	12.875	3.939	5.155	20.595	75.0	50.0	50.0	50.0	0.000	0.988	NS
	Positive	42	22	12.285	1.698	8.956	15.613	76.2	56.3	50.9	42.4			
PCR product	P1P1	1	0	–	–	–	–	100.0	100.0	100.0	100.0	1.561	0.458	NS
	P2P2	29	14	13.285	2.039	9.289	17.282	72.4	61.9	58.0	46.4			
	P1P2	20	12	10.438	2.421	5.693	15.184	80.0	43.1	36.9	36.9			

resulting in different splice variants. There is strong evidence that IL4 VNTR polymorphism may alter IL-4 synthesis, with the P1 allele boosting IL-4 expression compared to the P2 allele [19].

Together with ours, this finding raises questions regarding the mechanism by which IL4 affects leukemic cells growth and survival and whether a P2P2 genotype with lower IL4 expression causes more malignant clone and resistant disease.

According to a recent study, IL4 is an inhibitor of AML cells that appears as the first hit to leukemic cells because it showed the most selective suppression of their growth while retaining normal bone marrow cells. The authors of this study referred to its antileukemic effect to STAT6 being a downstream mediator of IL4 signaling and a crucial signaling pathway in macrophage function and activation. In a STAT 6-dependent manner, IL4 selectively triggers programmed cell death of AML cells [20].

Indeed, our patients with the P2P2 genotype and lower IL4 expression had higher blast percentages, increased therapy resistance, a higher incidence of relapse, shorter OS, and a worse disease outcome when compared to the heterozygous and non-carrier of the P2 allele.

Several reports indicate that IL-4 is the central cytokine of T-helper 2 cells that enhances the differentiation and function of CD4 and CD8-T cells [21]. Moreover, some tumors contained IL4 in their microenvironment, primarily expressed by tumor-infiltrating leucocytes [22, 23].

Table 7 Correlation of progression free survival with other variables

		Total N	N of events	PFS (months)		95% CI		Survival at				Log Rank test		
				Mean	SE	Lower	Upper	1 m. (%)	3 m. (%)	6 m. (%)	12 m. (%)	X^2	*P* value	Sig
Overall		40	8	18.31	1.68	15.03	21.60	100.0	93.8	78.1	65.1	–	–	–
Age	≤ 45 yrs	22	3	20.14	1.97	16.27	24.01	100.0	100.0	78.6	78.6	0.997	0.318	NS
	> 45 yrs	18	5	16.65	2.51	11.72	21.58	100.0	87.5	78.8	52.5			
Sex	Females	24	3	10.41	0.83	8.79	12.03	100.0	88.2	79.4	79.4	0.090	0.764	NS
	Males	16	5	17.79	2.19	13.50	22.08	100.0	100.0	78.6	58.9			
TLC	TLC < 20	21	4	18.49	2.22	14.13	22.85	100.0	100.0	85.7	61.2	0.179	0.672	NS
	TLC ≥ 20	19	4	18.08	2.50	13.19	22.97	100.0	86.7	69.3	69.3			
HGB	< 7.5	21	4	18.57	2.27	14.13	23.01	100.0	100.0	78.6	65.5	0.062	0.803	NS
	> 7.5	19	4	18.23	2.43	13.46	22.99	100.0	87.5	78.8	65.6			
PLT	< 40	21	4	17.71	2.57	12.68	22.74	100.0	93.3	76.4	61.1	0.088	0.767	NS
	≥ 40	19	4	18.79	2.21	14.47	23.12	100.0	94.1	79.6	68.3			
PB blasts	< 32	19	5	16.69	2.47	11.85	21.53	100.0	93.3	77.8	51.9	0.625	0.429	NS
	> 32	21	3	19.94	2.09	15.84	24.04	100.0	94.1	78.4	78.4			
Initial aspirate	< 69	20	3	19.20	2.40	14.51	23.89	100.0	100.0	84.6	67.7	0.492	0.483	NS
	> 69	20	5	17.40	2.39	12.71	22.10	100.0	88.2	72.2	61.9			
Extramedullary involvement	Negative	25	7	16.78	2.10	12.67	20.88	100.0	95.0	71.2	55.4	1.599	0.206	NS
	Positive	15	1	22.25	1.68	18.97	25.53	100.0	91.7	91.7	91.7			
Diagnosis	M0	1	0	–	–	–	–	100.0	100.0	100.0	100.0	0.139	0.933	NS
	M1	7	0	–	–	–	–	100.0	100.0	100.0	100.0			
	M2	20	5	17.59	2.35	12.99	22.20	100.0	88.2	74.7	62.2			
	M3	3	1	20.00	3.27	13.60	26.40	100.0	100.0	100.0	66.7			
	M4	8	2	16.80	3.94	9.07	24.53	100.0	100.0	60.0	60.0			
	M7	1	0	–	–	–	–	100.0	100.0	100.0	100.0			
HLA-DR	Negative	7	1	21.00	2.60	15.91	26.09	100.0	100.0	100.0	75.0	0.602	0.438	NS
	Positive	33	7	17.79	1.94	13.99	21.60	100.0	92.3	72.9	63.8			
CD34	Negative	10	1	21.60	2.15	17.39	25.81	100.0	100.0	100.0	80.0	1.477	0.224	NS
	Positive	30	7	17.17	2.09	13.08	21.26	100.0	91.7	70.1	60.1			
PCR product	P1P1	1	0	–	–	–	–	100.0	100.0	100.0	100.0	0.482	0.786	NS
	P2P2	22	5	18.06	2.15	13.85	22.27	100.0	94.1	81.6	61.2			
	P1P2	17	3	18.32	2.80	12.83	23.81	100.0	92.9	69.6	69.6			

In support of these reports, IL-4 expressed by tumors or T cells had been observed to improve tumor elimination with innate immunity acting in synergy with CD8 + T cells to decrease tumor load [24].

Similarly, Gitlitz et al. [25] concluded that IL-4 with granulocyte macrophage-colony stimulating factor (GM-CSF) boosts the quantity and performance of antigen-presenting cells in cancer patients. Moreover, the anti-tumor activity of IL4 has been shown in experiments on various cancers such as colon, breast, and renal carcinoma [26, 27].

In contrast to the findings above, other studies indicated that IL-4 produced by the tumor hinders apoptosis, causing the immortalization of malignant cells [16]. It has been reported that IL-4 induces the development and metastasis of head and neck squamous carcinoma [28–30].

Based on previous findings, it can be confirmed that IL-4 is a potent tumor immune modulator with both tumor-promoting and tumor-suppressing features. How IL-4 will interact either way is primarily determined by the type of tumor-clearing effector cell (adaptive/innate) and the kind of tumor cell studied. That is why a single molecule can adversely influence different tumor models and how innate and adaptive immunity will respond [24].

Since IL4 has contradictory effects on tumor immunity, gene sequence variations can still affect gene expression and function. It will significantly benefit investigating the association between IL-4 polymorphism variants and the risk of several human cancers. In addition to determining

the molecular mechanisms underlying cell responses to IL-4 in various cancer types, it may provide valuable information for designing new risk stratification models and novel treatments for cancer patients.

Conclusions

The IL4 intron 3 VNTR polymorphism could be considered a novel genetic marker in AML predicting poor disease outcomes. Incorporating this molecular predictor into future AML therapeutic protocols improves patients' counseling and risk stratification. Moreover, it could pave the way for advancing biological therapies that use the IL-4 effect on AML cells to improve overall disease outcomes.

Further studies on a larger scale of patients with more extended follow-up periods are needed to validate our observations and confirm the effect of this polymorphism on the clinical outcome.

Abbreviations

AML: Acute myeloid leukemia; IL-4: Interleukin-4; VNTR: Variable number of tandem repeat; PCR: Polymerase chain reaction; Th2: T-helper 2; BM: Bone marrow; FISH: Fluorescence in situ hybridization; EDTA: Ethylene diamine tetra-acetic acid; CR: Complete remission; MSD: Matched sibling donor; OS: Overall survival; DFS: Disease-free survival; allo-SCT: Allogeneic hematopoietic stem cell transplantation; GM-CSF: Granulocyte macrophage-colony stimulating factor.

Acknowledgements

Not applicable.

Authors' contributions

NA contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript. MR worked out almost all of the technical details, supervised the work and revised the final manuscript. AM collected the patients' data, contributed to the interpretation of the results regarding clinical outcome and therapeutic response, shared in writing and revising the manuscript. HS collected the patients' data, contributed to the interpretation of the results regarding clinical outcome and therapeutic response, shared in writing and revising the manuscript. All authors read and approved the final manuscript.

Funding

None.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate

The procedures applied in this study were approved by the Ethical Committee of Human Experimentation of Ain Shams University and are in accordance with the Helsinki Declaration of 1975. FWA 000017585. All patients and controls were asked to give an informed written consent before participation in the study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interests.

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Received: 21 May 2021 Accepted: 29 January 2022
Published online: 04 March 2022

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