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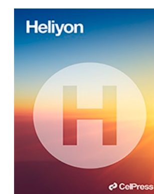

Research article

Study of gene polymorphisms in Toll-like receptor 2 in patients with acute lymphoblastic leukemia

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ABSTRACT

Objectives: Acute Lymphoblastic Leukemia (ALL) is a multifactorial disease that results from the interaction between multiple genetic factors. ALL is characterized by uncontrolled production of hematopoietic precursor cells of the lymphoid progenitors within the bone marrow. The development of hematological malignancies has been associated with malignant-like cells that express low levels of immunogenic surface molecules, thus, facilitating their escape from cellular antineoplastic immune responses. This risk may be partly influenced by variations in polymorphic genes that control immune function and regulation. Toll-like receptors (TLRs) are well known pattern recognition receptors playing key role in innate immune response. Abnormal expression and dysregulation of TLRs will provide an opportunity for cancer cells to escape from the immune system and enhance their proliferation and angiogenesis. Toll-like receptor 2 (TLR2) play an essential role in innate immunity. Single nucleotide polymorphisms (SNPs) are present in a number of TLR genes and have been associated with various disorders.

Methods: In this study, 265 subjects have been divided into two groups included 150 patients with ALL and115 healthy volunteers. All subjects were genotyped using TaqMan PCR techniques. In total, Five SNPs were statistically evaluated in the TLR2 (rs1898830 A/G, rs3804099 T/C, rs3804100 T/C, rs1339 T/C, and rs1337 C/G), which may influence the susceptibility of ALL. Minor allele frequency and genotype distribution were compared across the study groups, and the relative risk and differences between patients and controls were estimated. Moreover, the mRNA expression level was evaluated in patients with ALL and the matched healthy individuals by Real-Time Quantitative Reverse Transcription PCR (qRT-PCR).

Results: TLR2 rs1898830 A/G; rs3804099 T/C; rs3804100 T/C; rs1339 T/C, were significantly decrease the risk in our population, overall and for certain subtypes and ALL samples exhibited significant increase in the mRNA levels of TLR2.

Conclusions: This study shows that TLR2 could be an independent prognostic factor of ALL risks in the Saudi population. Suggesting that genetic variation in genes associated with an immune response may be important in the etiology of ALL. In addition, the results herein revealed that TLR2 overexpression is associated with ALL and has diverse biological significance in the context of the complex relationship between inflammation and cancer development. Therefore, these data could open further studies to explore the possible clinical relevance of TLRs as pathological markers for Leukemia and enhance the strategies regarding hematological malignancies prevention based on their gene expression.

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1. Introduction

The innate immune system utilizes different types of receptors that detect invasive pathogens in the host. These molecules are collectively designated as pattern recognition receptors (PRRs). Toll like receptor (TLR) are one of the major classes of receptors implicated in the pathogenesis of many diseases [1]. TLRs are widely expressed on immune and non-immune cells, including Hematopoietic stem cells (HSCs) [2]. A wide range of studies indicate that HSCs fate is strongly influenced by several classes of TLRs, since TLRs are expressed at early HSCs development and have significant functions in the long-term their repopulating, confirming the contribution of TLRs to HSCs hematopoiesis [3]. Generally, molecular studies revealed that enhanced or aberrant TLRs signaling is associated with defective hematopoiesis and hematopoietic malignancy [4]. Increasing evidence proved that Toll like receptor 2 (TLR2) is pathogenic factor that involved in tumorigenesis and associated with different hematological malignancies [5]. Moreover, genetic polymorphism of TLR2 found to be strongly linked with cancer progression [6].

In Saudi Arabia, the most common types of cancer are colorectal, Non- Hodgkin's Lymphoma, and leukemia [7]. In 2013, According to the Saudi Cancer Registry reports, childhood cancer accounted for 6 % of all cancer, and Acute Lymphoblastic Leukemia (ALL) accounts for the highest incidence of 31 % among all cancer types [8]. Over 15 years, the trend of Leukemia showed the likelihood of a rate increase, particularly in males with the highest incidence reported from the central region of Saudi Arabia. Furthermore, leukemia is one of the most common cancers among Saudi adults, in 2015, Saudi National Cancer Registry reported the incidence of Leukemia in KSA was 4.5 % of all female cancers and 7.5 % for men [9].

Leukemia is a type of blood cancer that emerges from a hematopoietic origin, defined as a complex multifactorial disease resulting from multiple genetic variants and hematologic disorders, and ALL is associated with clonal expansion of leukemic cells in the bone marrow, usually resulting in high numbers of cells of the affected lineage in circulating blood [10].

In Saudi Arabia, studies on single nucleotide polymorphisms in different proposed risk genes and increased risk of ALL are limited and restricted to the Glucocorticoids NR3C1 gene and isocitrate dehydrogenases 1 and 2 (IDH1/2) [11,12]. However, no previous studies examined SNPs in our studied gene regarding ALL.

Several SNPs in encoded genes for TLRs or in their signaling adapter proteins have been linked with the progression and the susceptibility of some human diseases. TLRs encoding genes are massively polymorphic and encoded to several variants of amino acid sites [13]. Hence, a growing body of literature has examined the genetic variations of TLRs since TLRs are essential targets for drug design and have been utilized as anti-viral and anti-cancer agents.

Some have coined that TLR2 genes were associated with the risk of different subtypes of Leukemia and Lymphoma [14], since genetic variations in TLR2 influenced susceptibility to marginal zone B-cell lymphoma [15].

However, our case-control study has identified promising candidate susceptibility genes supporting a polygenic model based on genetic variation and gene expression. In addition, it may have a clinical interest as an indicator of tumor aggressiveness and a prognostic indicator in Leukemia or other cancers.

2. Materials and methods

2.1. Criteria for sample selection

Human whole blood samples of total 265 Individuals were collected for the study. Gender and age-matched controls and cases were selected for the study. The study included 150 patients (60 females and 90 males) diagnosed with Acute Lymphoblastic leukemia (ALL) and having no other known pathologies or hematological disorders and previous cancer and 115 unrelated healthy individuals without any clinical signs of any type of cancer or other diseases of both genders (female and male) served as controls. The characteristics of ALL patients and control subjects are shown in Table 1.

Ethics approval

The study was approved by the medical ethics committee in King Khalid University Hospital and the ethics committee of King Saud University, Riyadh, Saudi Arabia (Ref. No. 20/0525/IRB).

2.2. Blood sample collection

Blood samples were collected by venipuncture in the presence of an anticoagulant, ethylenediaminetetraacetic acid (EDTA) from all subjects. Genomic DNA was isolated from whole blood using the commercial kit, DNeasy Blood & Tissue Kit (QIAGEN).

Table 1
The age characteristics of ALL patients and control subjects.

Patient				Control				P-value
Number of subjects	mean ± St.Dev	Min	Max	Number of subjects	Mean ± St.Dev	Min	Max	
150	22.45 ± 20.27	1	80	115	18.68 ± 15.53	2	85	$P = 0.10$

Table 2
Allelic and genotypic association of TLR2 polymorphism in controls and ALL patients, showing codominant, dominant, recessive, over dominant, and additive genetic models.

Locus	Model	Genotype	ALL % n = 150	Control% n = 115	OR (95 % CI)	*P*-value	AIC
rs1898830 A/G	Alleles	A	0.7	0.64	1	0.162	
		G	0.3	0.36	0.771 (0.535–1.111)		
	Codominant	AA	78 (52 %)	46 (40 %)	1		364.2
		AG	53 (35 %)	55 (48 %)	0.568 (0.336–0.960)	**0.035**	
		GG	19 (13 %)	14 (12 %)	0.800 (0.367–1.747)	0.576	
	Dominant	AA	78 (52 %)	46 (40 %)	1	**0.05**	363
		AG + GG	72 (48 %)	69 (60 %)	0.62 (0.38–1.01)		
	Recessive	AA + AG	131(87.3 %)	101 (87.8 %)	1.05 (0.50–2.19)	0.9	366.7
		GG	19 (12.7 %)	14 (12.2 %)	1		
	Over-dominant	AA + GG	97 (64.7 %)	60 (52.2 %)	1	**0.04**	362.5
		AG	53 (35.3 %)	55 (47.8 %)	0.60 (0.36–0.98)		
	Log-additive	—	—	—	0.78 (0.55–1.12)	0.18	364.9
rs3804099 T/C	Alleles	T	0.53	0.44	1	**0.042**	361.8
		C	0.46	0.55	0.700 (0.496–0.989)		
	Codominant	TT	50 (33.3 %)	22 (19 %)	1		361.8
		TC	61 (40.7 %)	59 (51.3 %)	0.455 (0.246–0.842)	**0.012**	
		CC	39 (26 %)	34 (29.6 %)	0.505 (0.256–0.997)	**0.049**	
	Dominant	CC	39 (26 %)	34 (29.6 %)	1	0.52	366.3
		TC + TT	111 (74 %)	81 (70.4 %)	1.19 (0.69–2.05)		
	Recessive	CC + TC	100 (66.7 %)	93 (80.9 %)	1	0.108	359.9
		TT	50 (33.3 %)	22 (19 %)	0.4731 (0.266–0.841)		
	Over-dominant	CC + TT	89 (59.3 %)	56 (48.7 %)	1	0.085	363.8
		TC	61 (40.7 %)	59 (51.3 %)	0.65 (0.40–1.06)		
	Log-additive	—	—	—	1.39 (0.99–1.94)	0.053	363
rs3804100 T/C	Alleles	T	0.63	0.36	1	**<0.001**	
		C	0.37	0.64	0.327 (0.229–0.467)		
	Codominant	TT	43 (28.7 %)	3 (2.6 %)	1		
		TC	104 (69.3 %)	77 (67 %)	0.094 (0.028–0.315)	**<0.001**	296
		CC	3 (2 %)	35 (30.4 %)	0.006 (0.001–0.031)	**<0.001**	
	Dominant	TT	43 (2.87 %)	3 (2.6 %)	1	**<0.001**	329.7
		TC + CC	107 (71.3 %)	112 (97.4 %)	0.07 (0.02–0.22)		
	Recessive	TT + TC	147 (98 %)	80 (69.6 %)	1	**<0.001**	319.6
		CC	3 (2 %)	35 (30.4 %)	0.05 (0.01–0.16)		
	Over-dominant	TT + CC	46 (30.7 %)	38 (33 %)	1	0.68	366.6
		TC	104 (69.3 %)	77 (67 %)	1.12 (0.66–1.88)		
	Log-additive	—	—	—	0.08 (0.03–0.18)	**<0.0001**	294.2
rs1339 T/C	Alleles	T	0.76	0.68	1		
		C	0.24	0.32	0.667 (0.454–0.980)	**0.038**	
	Codominant	TT	91 (60.7 %)	59 (51.3 %)	1		
		TC	47 (31.3 %)	39 (33.9 %)	0.781 (0.457–1.336)	0.367	364.9
		CC	12 (8 %)	17 (14.8 %)	0.458 (0.204–1.027)	**0.054**	
	Dominant	TT	91 (60.7 %)	59 (51.3 %)	1	0.13	364.4
		TC + CC	59 (39.3 %)	56 (48.7 %)	0.68 (0.42–1.12)		
	Recessive	TT + TC	138 (92 %)	98 (85.2 %)	1	0.081	363.7
		CC	12 (8 %)	17 (14.8)	0.50 (0.23–1.10)		
	Over-dominant	TT + CC	103 (68.7 %)	76 (66 %)	1	0.66	366.5
		TC	47 (31.3 %)	39 (33.9 %)	0.89 (0.53–1.49)		
	Log-additive	–	—	—	0.71 (0.50–1.01)	0.057	363.1
rs1337 C/G	Alleles	C	0.85	0.83	1		
		G	0.15	0.17	0.868 (0.541–1.393)	0.55	
	Codominant	CC	113	87 (75.7 %)	1		
		CG	30	18 (15.7 %)	1.283 (0.671–2.453)	0.451	366.4
		GG	7	10 (8.7 %)	0.539 (0.197–1.473)	0.228	
	Dominant	CC	113	87 (75.7 %)	1	0.95	366.7
		CG + GG	37	28 (24.4 %)	1.02 (0.58–1.79)		
	Recessive	CC + CG	143	105 (91.3 %)	1	0.19	365
		GG	7	10 (8.7 %)	0.51 (0.19–1.39)		
	Over-dominant	CC + GG	120	97 (84.3 %)	1	0.36	365.9
		CG	30	18 (15.7 %)	1.35 (0.71–2.56)		
	Log-additive	–	—	—	0.90 (0.59–1.36)	0.61	366.5

Abbreviations; OR, odds ratio; CI, confidence interval, n, number of individuals; **Boldfaced** values indicate a significant difference at the P ≤ 0.05 level.

2.3. Single nucleotide polymorphisms and genotyping

Polymorphisms of TLR2 gene (rs1898830 A/G, rs3804099 T/C, rs3804100 T/C, rs1339 T/C, and rs1337 C/G) were detected in all subjects by the TaqMan allelic discrimination.

In this research, we identified Five single nucleotide polymorphisms (SNPs) in TLR2 gene (rs1898830 A/G, rs3804099 T/C, rs3804100 T/C, rs1339 T/C, and rs1337 C/G) in all subjects by the TaqMan allelic discrimination through the dbSNP databases (https://www.ncbi.nlm. nih.gov/snp/). SNP were selected according to their minor allele frequency (MAF) ≥ 5 %.; Hardy-Weinberg equilibrium (HWE) *P* value cut-off >0.005. TLR2 genotyping of the Five SNPs was performed by VIC- and FAM-labelled allelic discrimination method, using assay-on demand TaqMan assays ordered from Applied Biosystems according to the manufacturer's instructions using ViiA™7, v.1.1. real-time PCR (Applied Biosystems, USA).

Real-time PCR was implemented in 10 **μl** a reaction system containing 0.26 **μl** 2x SNP Genotyping Assay, 5.5 **μl** 2xPower Taq Master Mix, 2.24 **μl** Nuclease-Free Water, and 2 **μl** DNA template (100 ng/**μl**). The PCR conditions for all SNPs were 1 cycle at 95 °C for 10 min followed by 40 cycles (95 °C for 15 s, 55 °C for 30 s, and 72 °C for 30 s) and a final extension at 72 °C for 5 min. For confirmation, about 5 % of the samples were randomly chosen for repeat genotyping.

2.4. mRNA relative quantification by quantitative PCR

A Reverse Transcription System Kit (Promega, Madison, USA) was used for cDNA synthesis and genomic DNA elimination. Quantitative PCR (qPCR) reactions were performed using TaqMan® gene expression assay (ThermoFisher) specific probes for TLR2 gene (Catalog # 4331182 and assay ID: Hs_00610101m1). For the reference gene, GAPDH was used as the normalizing endogenous control and its relative expression was performed using TaqMan® gene expression assay (Catalog #4331182 and Assay ID: Hs02758991_g1) (ThermoFisher). The reactions were performed in triplicate, using 25 ng of cDNA in Quantastudio flex ViiA™7 Real-Time PCR (Applied Biosystems, USA). The program of amplification was an initial denaturation at 95 °C for 5 min followed by 40 cycles of denaturation at 95 °C for 15 s and annealing at 60 °C for 60 s and 72 °C for 30 s, and a final extension for 10 min at 72 °C. The relative quantitation of the gene expression was calculated as the fold-change relative to the average expression of the controls using the comparative C_T method (the $2^{-\Delta\Delta Ct}$ method). Statistical analysis was performed using GraphPad Prism 9.

2.5. Statistical analysis

The control data was assessed for The Hardy-Weinberg equilibrium test to detect any deviation in the control samples. The chi-square analysis was used to compare Genotype distribution and allele frequency differences across groups. SNP genotypes were coded into three groups: homozygous (ancestral allele), heterozygous, and homozygous (minor allele) to assess the minor allele frequency (MAF), Odds ratios (OR) with 95 % confidence intervals (CI) were calculated to estimate the strength of the associations. The cutoff for significance was a two-tailed p-value ≤0.05. All statistical calculations were performed using SPSS version 22 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Association between TLR2 (rs1898830 A/G) gene variant in patients with ALL and control group

Frequencies of the TLR2 rs1898830 AA (wild type), AG (heterozygous), and GG (polymorphic homozygous) genotypes were 52 %, 35.3 %, and 12.7 % in ALL patients, and 40 %, 47.8 %, and 12.2 % in the healthy control group, respectively. Moreover, the frequency of the TLR2 rs1898830 A allele was 70 % and 64 % in the ALL patient and control groups, respectively.

The genotype and allele frequencies of the rs1898830 in TLR2 gene in ALL patients and the control group are shown in Table 2. A protective role of the genotypes AG vs. AA with (OR: 0.568; $\chi2 = 4.460$; CI: 0.336–0.960; $p = 0.035$), for the dominant model AG + GG vs. AA with (OR: 0.62; CI: 0.38–1.01; $p = 0.05$) and the Over-dominant models (OR: 0.60; CI: 0.36–0.98; $p = 0.04$) were observed against the disease. For the three significant models TLR2 rs1898830 A/G, the Over-dominant model was the best fit. However, the AIC and BIC for the dominant/co-dominant model was also low and very close to the Over-dominant models and, therefore, could also be a good fit model.

In addition, the genotype distributions did not significantly deviate from Hardy-Weinberg expectations for both groups (p > 0.05).

3.2. Association between TLR2 (rs3804099 T/C) gene variant in patients with ALL and control group

Frequencies of the TLR2 rs3804099 TT (wild type), TC (heterozygous), and CC (polymorphic homozygous) genotypes were 33.30 %, 40.7 %, and 26 % in ALL patients, and 19.1 %, 51.3 %, and 29.6 % in the healthy control group, respectively.

Moreover, frequency of the TLR2 rs3804099 T allele was 53 % and 46 % in the ALL patient and control groups, respectively. The genotype and allele frequencies of the rs3804099 in TLR2 gene in ALL patients and the control group are shown in Table 2.

Out of the five genetic models, one models showed significant association with decreased ALL risk. Under the codominant model, TC and CC genotypes were significantly associated with ALL in contrast to the TT genotype, conferred a 0.445-fold and 0.505-fold reduction in ALL progression. One copy of C allele is necessary to decrease the risk of developing ALL. As for the frequency of alleles, in comparison with the T allele, the C allele homozygote was positively associated with a decrease in ALL risk. In addition, the

genotype distributions did not deviate from Hardy-Weinberg expectations for both groups (p > 0.05).

3.3. Association between TLR2 (rs3804100 T/C) gene variant in patients with ALL and control group

Frequencies of the TLR2 rs3804100 T/T (wild type), T/C (heterozygous), and C/C (polymorphic homozygous) genotypes were 28.7 %, 69.3 %, and 2 % in ALL patients, and 2.6 %, 67 %, and 30.4 % in the healthy group, respectively. Moreover, the TLR2 rs3804100 T allele frequency was 63 % and 36 % in the ALL patient and control groups, respectively. The genotype and allele frequencies of the rs3804100 in TLR2 gene in ALL patients and the control group are shown in Table 2. The pooled ORs from the overall classification found a significant association between rs3804100 and decreased risk of ALL under the allelic model (C allele vs. T allele: (OR: 0.327; $\chi 2 = 38.69$; CI: 0.229–0.467; $p < 0.001$), under the recessive model TT + TC vs CC (OR: 0.05; CI: 0.01–0.16; $p < 0.001$), the dominant model TT vs TC + CC (OR: 0.07; CI: 0.02–0.22; $p = <0.001$), and the Log-additive model (OR: 0.08; CI: 0.03–0.18; $p < 0.001$).

There was a highly significant difference in the distribution of TLR2 rs3804100 genotypes (TT, TC and CC) for the Co-dominant model between the patient and control groups (OR: 0.094; $\chi 2 = 14.71$; CI: 0.024–0.315; $p = <0.001$) and (OR: 0.006; $\chi 2 = 36.48$; CI: 0.001–0.031; $p = <0.001$), respectively. For this SNP, the Log-the additive model was the best fit (AIC = 294.2). However, the AIC and BIC for the Co-dominant model were also low and very close to the additive model and, therefore, could also be a good fit model (AIC = 296). In addition, the genotype distributions deviate from Hardy-Weinberg expectations for control group (p > 0.01).

3.4. Association between TLR2 (rs1339 T/C) gene variant in patients with ALL and control group

Frequencies of the TLR2 rs1339 TT (wild type), TC (heterozygous), and CC (polymorphic homozygous) genotypes were 60.7 %, 31.3 %, and 8 % in ALL patients, and 51.3 %, 33.9 %, and 14.8 % in the healthy control group, respectively. Moreover, frequency of the TLR2 **rs1339** T allele was76 % and 68 % in the ALL patient and control groups, respectively. The genotype and allele frequencies of the rs1339 in TLR2 gene in ALL patients and the control group are shown in Table 2. There was a borderline of statistical significance in the distribution of TLR2 rs1339 in CC genotype between the patient and control groups (OR: 0.458; $\chi 2 = 3.70$; CI: 0.204–1.027; $p = 0.054$) was observed against the disease. Moreover, carriers of the minor allele C were at significantly reduced risk of ALL compared with carriers of the T allele (OR: 0.667; $\chi 2 = 4.29$; CI: 0.454–0.980; $p = 0.038$). In addition, the genotype distributions did not significantly deviate from Hardy-Weinberg expectations for both groups (p > 0.05).

3.5. Association between TLR2 (rs1337 C/G) gene variant in patients with ALL and control group

Frequencies of the TLR2 rs1337 CC (wild type), CG (heterozygous), and GG (polymorphic homozygous) genotypes were 75.3 %, 20 %, and 4.6 % in the ALL-patient group, and 75.7 %, 15.7 %, and 8.7 % in the control group, respectively. The frequency of the TLR3 rs1337 C allele was also observed to be 0.85 % in the ALL-patient group and 0.83 % in the control group (Table 2). However, a statistically significant difference in the distribution of TLR2 rs1337 genotypes, as well as in allele frequencies between the patient and control groups was not detected for all genetic models. In addition, the genotype distributions did not significantly deviate from Hardy-Weinberg expectations for both groups ($p > 0.05$).

3.6. Haplotype analyses of TLR2 gene polymorphisms and risk to ALL

Linkage disequilibrium block construction and haplotype analysis were performed to infer a better interpretation of haplotypes by converting the genotype data in a linkage format, pairwise D'values between SNPs were calculated at 95 % confidence interval using Haploview v4.2.

The ALL risk was reduced significantly among individuals who carried the haplotypes "ACCTC, GTCTC, GCCTC, GCCCG, ACCCG; rs1898830, rs3804099, rs3804100, and rs1339, and rs1337". The two common haplotypes in TLR2 ″ ATTTC and ACTTG; rs1898830, rs3804099, rs3804100, rs1339, and rs1337″ were found significantly associated with ALL (Table 3, Fig. 1).

Table 3
Association of significant haplotypes with acute lymphoblastic leukemia among Saudi Arabian population.

	Haplotype	Frequency	Case, Control Frequency	Chi Square	p Value
Chromosome 4					
TLR2	**ACCTC**[a]	**0.163**	**0.123, 0.215**	**8.211**	**0.0042**
	ATTTC[b]	**0.136**	**0.188, 0.068**	**16.153**	**5.84×10^{-5}**
	GTCTC[a]	**0.055**	**0.030, 0.088**	**8.451**	**0.0036**
	ACTTG[b]	**0.033**	**0.047, 0.015**	**4.157**	**0.0415**
	GCCTC[a]	**0.032**	**0.018, 0.049**	**3.889**	**0.0486**
	GCCCG[a]	**0.02**	**0.008, 0.035**	**4.866**	**0.0274**
	ACCCG[a]	**0.016**	**0.003, 0.033**	**7.576**	**0.0059**

[a] Protective haplotype.
[b] Risk haplotype. Sig. SNPs: Block of significant SNPs (Order of Significant SNPs Chromosome 4: rs3804099, rs3804100, and rs1339).

3.7. Association of TLR2 relative mRNA expression and ALL

The mRNA expression level of TLR2 was increased in ALL patients compared with the corresponding healthy donors. However, ALL patients detected a significant statistical difference between the **ΔCt** mean of TLR2 mRNA expression level (The mean values of the **ΔCt** in ALL vs. non-ALL; 2.002 ± 0.423 vs. 2.44 ± 0.63, *p*-value = 0.001). Remarkably, the $2^{-\Delta\Delta Ct}$ method revealed that TLR2 was significantly up-regulated in ALL patients (fold change: 143.73 ± 32.253, *p*-value: 0.0001) (Fig. 2).

4. Discussion

This research analyzes the relationship between genetic variations and allelic frequencies of the TLR2 gene in the development of ALL in the Saudi Arabian population. To our knowledge, no studies have explored the role of Toll gene variants in blood cancer risk in this population. Some SNPs in this study are newly evaluated in the context of leukemia. Studies suggest TLR gene variations can alter immune responses to pathogens and disease outcomes. Identifying novel susceptibility genes for hematological malignancies is crucial.

Human TLR2 is located in the long arm of chromosome 4 (4q31.3), including one coding exon and two non-coding exons. TLR2 has been recognized as a pathogenic factor implicated in tumorigenesis [5,16]. We observed a combined effect of a genetic variant of TLR2 since all four SNPs, rs1898830, rs3804100, rs3804099, and rs1339, showed a significant protective association against ALL, while Individuals carrying rs1337 C > G have a higher risk of ALL in the Saudi population.

TLR2 rs1898830 A/G has not been studied in detail in relation to ALL. In our study, statistical analyses of genotypic frequencies for the rs1898830 A/G revealed a significant difference between patients and controls in the examined population under the heterozygous genotype, dominant, and over-dominant model. Notably, the carrier status of one copy of the allele (G) is related to decreases in the risk of having ALL in the Saudi population.

The frequency of the wild allele (A) at rs1898830 in the TLR2 gene in healthy Saudis is 0.64. This is comparable to other populations, such as Europeans (0.67), Vietnamese (0.62), and British (0.63). However, it is higher than in populations like Bangladesh (0.41) and Sri Lanka (0.38), with Peru recording the lowest frequency (0.33). For the minor allele (G) at rs1898830 in the TLR2 gene, the frequency in healthy Saudis is 0.36. This is similar to the British (0.37) and Vietnamese (0.38) frequencies. However, many populations, such as Gambia (0.09), Nigeria (0.09), and Sierra Leone (0.10), have much lower frequencies (1000 Genomes Project Consortium, 2015).

The rs1898830, known as −15,607A/G, is in the first intron of the gene that plays a vital role in gene expression regulation. SNPs in introns can alter the susceptibility to disease and modulate the genotype-phenotype association [17]. Accordingly, rs1898830 might alter the expression and functionality of TLR2, which leads to an increase in innate immune cells number and enhance their function [18]. Thus, it may explain our cases' significant up-regulation of TLR2 expression.

Our results align with a study that reported genotype AG and allele G, as protective against the development of T2DM in the southern Chinese population [19]. Moreover, it was reported that rs1898830 was associated with neonatal severe hepatitis among the Chinese population, with findings indicating that subjects with the AG genotype had a lower risk of having the disease [20].

In contrast to our results, this SNP has been associated with the risk of different conditions in various populations. A study conducted in Lebanon revealed that G allele carriers were associated with decreased HDL-C levels and an increased risk of hypertension [16].

Additionally, African–American women with pelvic inflammatory disease carrying the AA genotype for TLR2 SNP rs1898830 had a three-fold increased rate of Bacterial vaginosis infection [21]. In addition, rs1898830, rs3804099, and rs3804100 of TLR2 may increase risk of Alzheimer's disease in a Northern Han Chinese population [22].

Contradictory to our results concerning other types of cancer or diseases, TLR2 (rs1898830) polymorphism is not associated with susceptibility to colon and rectal cancer in the USA [23], hepatocellular carcinoma [24], sepsis in Chinese Han population [25],

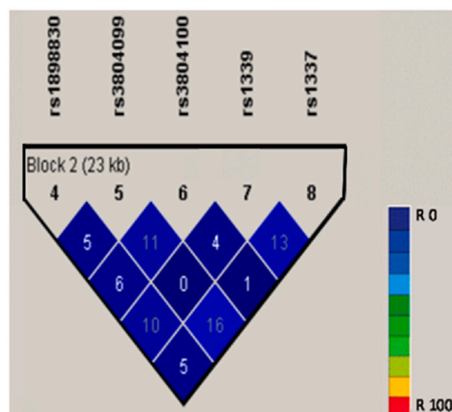

Fig. 1. Haploview Linkage disequilibrium (LD) plot of the 5 SNPs in 4q24, 4q31.3, and 4q35 locus. The pairwise correlation between the single nucleotide polymorphisms (SNPs) were measured as r2 and shown (x100) in each diamond.

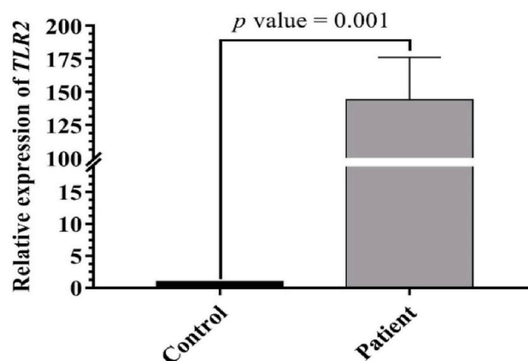

Fig. 2. TLR2 expression level in ALL and control.
TLR2 level varied significantly between patients and control ($p = 0.0001$).

Asthma [26] and Rheumatoid arthritis [27].

The TLR2 rs3804099 polymorphism is associated with a decreased risk of ALL. The heterozygous TC and CC genotypes, as well as the C allele, were found at significantly lower frequencies in ALL patients compared to controls. This association was particularly significant in males and patients aged over 18, where the C allele acted as a protective factor against ALL. The frequency of the T allele in the Saudi population (0.55) is comparable to that in Toscani in Italy (0.55), the European population (0.56), and the Iberian population in Spain (0.57). However, it is higher than in populations from Gambia (0.24), Luhya in Kenya (0.29), and African Americans (0.38). Conversely, the T allele frequency is lower in the Saudi population compared to East Asians (0.72) and Peruvians from Lima (0.71) (1000 Genomes Project Consortium, 2015).

Additionally, the association between ALL risk and individuals carrying rs3804100 SNP in all allelic and genetic models and based on the gender and age of the patient were found. Carriers of the minor allele "C" were at a significantly reduced risk of ALL compared with carriers of the T allele.

The TLR2 rs3804100 (Ser450Ser) allele distributions in our ethnic population differ from those in other HapMap populations. In healthy Saudis, the wild allele (T) frequency is 0.36, the lowest recorded in the 1000 Genomes Project, while the minor allele (C) frequency is 0.64, the highest recorded. Other populations, such as East Asians (0.76) and Europeans (0.94), have higher T allele frequencies (1000 Genomes Project Consortium, 2015). Our study found that TLR2 rs3804100 is closely associated with decreased susceptibility to ALL, similar to the association patterns of rs3804100 and rs3804099 SNPs in various diseases.

According to a meta-analysis study, the overall analysis of rs3804099 showed that this SNP could decrease gastric cancer risk significantly in Asians [5]. Similarly, rs3804099 CC homozygosity seemed to protect against colon cancer risk in the USA [23]. Moreover, our results contradict other data demonstrating that the TLR2 polymorphisms rs3804100 and rs3804099 failed to play a role in the progress of ALL in a Caucasian population [28]. In the Saudi Arabian population, for rs3804099 and rs3804100, it was found that the T allele frequency in rs3804100 increased the risk of colon cancer by more than threefold and was linked to early-onset colon cancer, while the TLR2 rs3804099 TT genotype increased the risk of colon cancer development by more than 3.8- and 5-fold in female patients and patients aged less than 57 years [29].

Along with that, TLR2 SNPs rs3804099 and rs3804100 showed no significant association between Helicobacter pylori infection also in the Saudi patients [30]. In the Egyptian population, individuals with the C allele of rs3804099 and A allele of rs1898830 had a 2.2 and 1.4-fold increased risk of developing Hepatocellular carcinoma [31]. It has been suggested that rs3804099 polymorphism culminates in synonymous mutation (Asn199Asn), and the genome-wide association studies have disclosed a substantial contribution of synonymous SNPs to human disease. The nucleotide variations influence TLR2 mRNA splicing phenotype and transcription factor binding, which changes the protein expression, conformation, and function [32].

The T allele and TT genotype frequencies of rs1339 at the TLR2 gene in healthy subjects were 0.68 and 0.513, respectively, like the data from multiple populations such as East Asians (0.69) and Vietnams (0.67). However, the frequency of the T allele in the healthy individuals was lower than in many populations, including the Gambians population (1.00), Esan in Nigeria (0.99), and Mende in Sierra Leone (0.99) (1000 Genomes Project Consortium, 2015).

The distribution of TLR2-rs1339 genotypes showed a significant association with ALL patients ($P < 0.5$). However, very few studies have reported the association between the T > C variant in rs1339 with different diseases. All these studies are in contrast with our results. rs1339 showed no association with childhood asthma in Caucasians [33], and Talaromycosis susceptibility among patients with AIDS in the Han Chinese population [34].

To date, no study has assessed the effect of TLR2 rs1337 on the risk of cancer among our population or any other population. In healthy subjects, the C allele frequencies of rs1337 at the TLR2 gene were 0.83. In this SNP, Peru, Mexican, and Pakistan populations were similar to our allele frequencies. In contrast, several populations have much higher T allele frequency than the Saudi population, such as; Nigerians, Gambians, and Japanese (1000 Genomes Project Consortium, 2015). The correlation between TLR2 rs1337 and ALL risk was affected by age. In patients aged >18, The heterozygous "CG" genotype showed more than a Five-fold higher risk for developing ALL compared to controls (OR: 5.33). The functional consequences of rs1337, located in the 3′-untranslated regions (3′-UTRs) of TLR2, may influence the expression of mRNAs. These changes in nucleotides owe the potential to influence diseases

susceptibility [35]. In our study, we found that TLR2 expression is much higher in patients with ALL, suggesting that TLR2 may play a significant role in disease etiology.

5. Conclusion

In this study, we investigated TLR2 expression in Saudi patients with acute lymphoblastic leukemia (ALL). Our findings revealed significantly higher TLR2 expression in these patients compared to normal individuals, likely due to the absence of drug treatment as the patients were newly diagnosed. This upregulation may influence the initiation and progression of ALL by increasing the production of inflammatory cytokines and chemokines. Blocking TLR2 activity has been shown to reduce cancer metastasis, and specific SNPs, like rs1337 in adult patients, may increase leukemia risk by affecting TLR2 expression. Therefore, TLR2 could serve as a biomarker for ALL due to its significant role in the disease's pathogenesis and development.

Data availability statement

The datasets generated and/or analyzed in the current study are not publicly available due to ethical concerns but can be obtained from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Fadwa M. Alkhulaifi: Software, Methodology, Conceptualization. **Rasha Alonaizan:** Writing – original draft, Data curation. **Suliman Alomar:** Writing – review & editing, Project administration.

Declaration of competing interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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