Cancer Genetics xxx (xxxx) 1–4

Contents lists available at ScienceDirect

Cancer Genetics

journal homepage: www.elsevier.com

Journal Cover Image

Genetic polymorphisms of tumor necrosis factor receptor-associated factor 3 and their association with acute lymphoblastic leukemia

Fadwa M Alkhulaifi [a], Hana Hakami [a, *], Jamilah Alshammari [a], Safa A Alqarzae [a], Aeshah Almuhaini [b], Sheka Y Aloyouni [c], Suliman Alomar [a, d, *]

[a] Zoology Department, College of Sciences, King Saud University, Riyadh, Saudi Arabia
[b] Biology Department, College of Sciences, King Abdulaziz University, Jeddah, Saudi Arabia
[c] Genetics section, Research Department, Natural and Health Sciences Research Center, Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia
[d] Doping Research Chair, Zoology Department, College of Sciences, King Saud University, Riyadh, Saudi Arabia

ARTICLE INFO

Keywords:
Acute lymphoblastic leukemia
Single nucleotide polymorphisms
TRAF3
Gene expression

ABSTRACT

Background: Acute lymphoblastic leukemia (ALL) is an aggressive hematological malignancy driven by genetic and immunological dysregulation. Tumor necrosis factor receptor-associated factor 3 (TRAF3) plays an essential role in regulating immune signaling, lymphocyte homeostasis, and NF-κB pathways. Although TRAF3 alterations have been implicated in various immune disorders and hematologic cancers, the contribution of TRAF3 genetic polymorphisms to ALL susceptibility remains insufficiently understood.

Purpose: To investigate the association between three TRAF3 single nucleotide polymorphisms (SNPs); rs33980500, rs13210247, and rs1131877, and ALL susceptibility in Saudi patients, and to evaluate TRAF3 mRNA expression levels in ALL compared to healthy individuals.

Methods: A case-control study was conducted involving 150 newly diagnosed ALL patients and 115 age- and sex-matched healthy controls. Genotyping of the selected TRAF3 SNPs was performed using TaqMan allelic discrimination assays. TRAF3 mRNA expression in peripheral blood white cells was quantified through RT-qPCR. Statistical analyses included genotype/allele frequency comparisons, odds ratios, Hardy-Weinberg equilibrium testing, linkage disequilibrium assessment, and haplotype construction.

Results: None of the analyzed SNPs showed a statistically significant association with ALL across genotype, allele, or haplotype models. Linkage disequilibrium between the SNPs was absent. However, TRAF3 mRNA expression was significantly upregulated in ALL patients, exhibiting a 3.88-fold increase compared with controls ($p < 0.05$).

Conclusion: Although the examined TRAF3 SNPs were not associated with ALL susceptibility in this cohort, the marked elevation of TRAF3 mRNA expression suggests a potential role for TRAF3-related signaling in ALL pathogenesis. TRAF3 expression may represent a promising biomarker warranting further investigation.

Background

Tumor necrosis factor receptors (TNFRs) are central to immune signaling, regulating innate and adaptive immunity, inflammation, apoptosis, and tissue homeostasis via TNF-α interactions [1,2]. TNFR-associated factors (TRAFs), particularly TRAF3, act as intracellular adaptors controlling immune responses, cell survival, and NF-κB signaling through both canonical and noncanonical pathways [3,4].

TRAF3 inhibits canonical NF-κB by limiting TRAF2 recruitment and regulates noncanonical NF-κB via inducing kinase (NIK) degradation [5–8]. Furthermore, TRAF3 RING finger domain provides E3 ubiquitin ligase activity, enabling modulation of protein interactions and type I interferon production [9,10]. TRAF3 also regulates the production of type I interferon (IFN-I) in a complex double role, influencing both innate and adaptive immune responses [9]. TRAF3 is critical for T- and B-cell, and myeloid cell homeostasis; disruptions compromise immunity, cell cycle, and metabolism, increasing susceptibility to infections, inflammatory diseases, and hematological malignancies [11–14].

Interestingly, TRAF3 knockout mouse model displayed a declined survival rate with lethality by postnatal day 10 due to a general failure in body organs and a dropped white blood cell count, highlighting TRAF3 role in blood immune cells and survival [15,16]. Human studies

* Corresponding authors.
E-mail addresses: hhakami1@ksu.edu.sa (H. Hakami), syalomar@ksu.edu.sa (S. Alomar).

https://doi.org/10.1016/j.cancergen.2026.01.012
Received 2 January 2026; Received in revised form 24 January 2026; Accepted 26 January 2026
2210-7762/

show that TRAF3 loss-of-function or mutations can lead to immunodeficiency, autoimmunity, and increased vulnerability to infections and to inflammatory conditions [17,18]. Additionally, loss-of function mutations in human TRAF3 are associated with hematopoietic malignancies particularly lymphomas and myelomas [11,19]. Nevertheless, a limited number of studies have investigated the link of TRAF3 SNPs to myeloma, diabetes cardiovascular complications, and human papillomavirus (HPV)-related lesions [19–21].

Objectives

Although TRAF3 SNPs have been implicated in immunological disorders and cancers [22,23], their roles in lymphoblastic leukemias remains unclear. This study investigated the association of three TRAF3 SNPs (rs33980500, rs13210247, and rs1131877) with ALL in Saudi Arabian individuals and evaluated TRAF3 mRNA expression levels in this disease context.

Study design

A case–control study enrolled 150 ALL patients and 115 age- and sex-matched healthy controls. Blood-derived DNA and RNA were analyzed for TRAF3 SNP genotyping and expression profiling.

Results

The genotype and allele frequencies of rs33980500 SNP on TRAF3 in ALL and control groups

TRAF3 rs33980500 genotypes (CC, CT, and TT) were similarly distributed in ALL patients (84 %, 7.3 %, 8.6 %) and controls (79 %, 12 %, 8 %), with no significant differences (Table 1). The distribution of the CT/TT genotypes showed no significant association with ALL susceptibility. Furthermore, the allele frequencies (C: 88 % vs. 85 %; T: 12 % vs. 15 %) were also not significantly different.

The genotype and allele frequencies of rs13210247 SNP on TRAF3 in ALL and control groups

TRAF3 rs13210247 genotypes (AA, AG, and GG) showed similar distributions in ALL and control individuals, with AA at 87.7 % vs. 82.6 % AA, and AG at 21.3 % vs. 17.39 %, and no GG genotype detected in either group (Table 1). Allele A frequencies were 89.3 % in ALL and 91.3 % in the controls. Differences in genotype and allele frequencies were statistically insignificant ($p = 0.42$ and 0.25, respectively).

The genotype and allele frequencies of rs1131877 SNP on TRAF3 in ALL and control groups

TRAF3 rs1131877 genotypes (TT, TC, and CC) showed similar distributions in ALL patients (59.3 %, 31.3 %, 9.3 %) and controls (55.6 %, 33.9 %, 10.4 %) with T allele frequencies of 75 % and 73 %, respectively (Table 1). No genotype or allele was significantly associated with ALL risk ($p = 0.53$).

Table 1
Association analysis between the genotypes of TRAF3 rs33980500, rs13210247 and rs1131877 loci in ALL. The association analysis for TRAF3 rs33980500 (C/T) genotype and allele frequencies is shown in the upper section of the table, while the association analysis for the genotypes and allele frequencies of TRAF3 rs13210247 (A/G) and rs1131877 (T/C) are presented in the second and third sections of the table, respectively. Both association analyses are for genetic models with the AIC and BIC scores in patients with ALL and control group. ALL; acute lymphoblastic leukemia, OR; odds ratio, CI; confidence interval, * means significant for $p \leq 0.05$.

SNP	Genetic model type	Genotype/variant	ALL $n = 150$		Control $n = 115$		ALL vs. Control			
			Count	%	Count	%	OR (95 % CI)	*p*-value	AIC	BIC
rs33980500	**Codominant**	CC	126	84	91	79	**Ref**			
		CT	11	7.3	14	12	0.57 (0.25 –1.3)	0.2	536.5	1435
	Dominant	TT	13	8.6	10	8	1 (0.42- 2.36)	1		
		C/C	126	84	91	79.1	1	0.3	535.8	1430.7
	Recessive	C/T-T/T	24	16	24	20.9	0.72 (0.39- 1.35)			
		C/C—C/T	137	91.3	105	91.3	1	1	536.2	1431.2
	Over-dominant	T/T	13	8.7	10	8.7	1 (0.42- 2.36)			
		C/C-T/T	139	92.7	101	87.8	1	0.2	534.5	1429.5
	Log-additive	C/T	11	7.3	14	12.2	0.57 (0.25- 1.3)			
		-	-	-	-	-	0.82 (0.13–5.22)	0.8	536.5	1431.4
	Allele frequency	C	263	88	196	85	**Ref**			
		T	37	12	34	15	0.81 (0.49- 1.34)	0.4	-	-
rs13210247	**Codominant**	AA	118	78.6	95	82.6	**Ref**			
		AG	32	21.3	20	17.39	1.28(0.69- 2.39)	0.42	524.3-	1419.2-
		GG	0	0	0	0	-	-		
	Allele frequency	A	268	89.3	210	91.3	**Ref**			
		G	32	10.3	20	8.7	0.77 (0.5- 1.20)	0.25	-	-
rs1131877	**Codominant**	TT	89	59.3	64	55.6	**Ref**			
		TC	47	31.3	39	33.9	0.87 (0.51–1.47)	0.6	536.6	1435.1
		CC	14	9.3	12	10.4	0.84 (0.36–1.93)	0.68		
	Dominant	T/T	89	59.3	64	55.6	1	0.56	536.4	1431.3
		T/C-C/C	61	40.7	51	44.4	0.86 (0.51–1.44)			
	Recessive	T/T-T/C	136	90.7	103	89.6	1	0.80	534.7	1429.6
		C/C	14	9.3	12	10.4	0.89 (0.39–2.05)			
	Over-dominant	T/T-C/C	103	68.7	76	66.1	1	0.77	536.5	1431.4
		T/C	47	31.3	39	33.9	0.88 (0.51–1.51)			
	Log-additive	-	-	-	-	-	0.39 (0.05–3.36)	0.4	535.8	1430.8
	Allele frequency	T	225	75	167	73	**Ref**			
		C	75	25	63	27	1.13 (0.75–1.69)	0.53	-	-

The linkage disequilibrium of the analyzed SNPs on TRAF3 gene and their haplotype frequencies

LD analysis showed no disequilibrium between rs13210247 and rs33980500 (correlation coefficient $r^2 = 0$) (Suppl. Table 1, and Fig. 1A). Haplotype analysis of rs1131877, rs13210247, and rs33980500 revealed no significant differences between ALL patients and controls, as the data shown in Suppl. Table 1, noting that the cumulative frequency of the five major haplotypes exceeded 90 %. The haplotype distribution revealed that; the wildtype T-A-C haplotype was the most frequent in both study groups, with (~53 %). In addition, the two haplotypes, C-A-C and T-G-C showed no significant association with ALL risk, as well as any other haplotypic combination across the three analyzed SNPs (Suppl. Table 1).

The expression levels of TRAF3 mRNA in ALL

TRAF3 mRNA expression was significantly higher in ALL patients than controls, with lower ΔCt values, exhibiting 4.78 ± 0.49 in ALL compared to 6.5 ± 0.5 among the controls ($p = 0.005$, Fig. 1B-C). Consistent with this, the 2^-ΔΔCt analysis revealed a 3.88-fold upregulation in the expression of TRAF3 mRNA in ALL group, relative to control group ($p = 0.019$, Fig. 1C).

Conclusions

ALL is a genetically complex malignancy in which immune-regulatory genes such as TRAF3 may play a role [24,25]. Although TRAF3 polymorphisms have been linked to immune disorders and cancers [19,22,26–28], their association with ALL remains unclear. We evaluated three TRAF3 SNPs (rs33980500, rs13210247, and rs1131877) and TRAF3 mRNA expression in Saudi ALL patients. None of the SNPs or their haplotypes were associated with ALL, and no LD was observed. While rs33980500 showed a slightly lower mutant allele frequency in ALL, this requires validation in larger cohorts. In contrast, TRAF3 mRNA was significantly upregulated in ALL, indicating dysregulated TRAF3 signaling and supporting its potential utility as a prognostic biomarker. The marked upregulation of TRAF3 expression in the absence of determined associated genetic variants suggests that TRAF3 dysregulation is predominantly driven by alternative non-genetic mechanisms. Aberrant activation of immune and inflammatory signaling pathways characteristic of ALL, including NF-κB, interferon, and cytokine-mediated transcriptional programs, may enhance TRAF3 transcription as part of a compensatory or feedback regulatory response independent of germline variation. Additionally, epigenetic remodeling, as well as post-transcriptional or post-translational regulatory processes, may contribute to increased TRAF3 expression in the absence of detectable sequence alterations.

In summary, this study suggests a role for TRAF3 in ALL among Saudi patients, as evidenced by elevated TRAF3 mRNA expression despite no significant SNP associations. These findings support the involvement of TRAF3 in immune regulation and leukemogenesis.

Declarations

Ethical approval

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

Funding

This study was supported by the Ongoing Research Funding Program- Research Chairs (ORF-RC-2025-4216) at King Saud University, Riyadh, Saudi Arabia. Also, the study was funded by Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2026R713), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

Availability of data and materials

The raw data used in this study are available upon reasonable request by contacting the corresponding author (syalomar@ksu.edu.sa).

CRediT authorship contribution statement

Fadwa M Alkhulaifi: Writing – original draft, Validation, Methodology, Investigation. **Hana Hakami:** Writing – review & editing, Writing – original draft, Visualization, Formal analysis. **Jamilah Alshammari:** Writing – original draft. **Safa A Alqarzae:** Methodology. **Aeshah Almuhaini:** Writing – original draft. **Sheka Y Aloyouni:** Funding acquisition. **Suliman Alomar:** Supervision, Resources, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

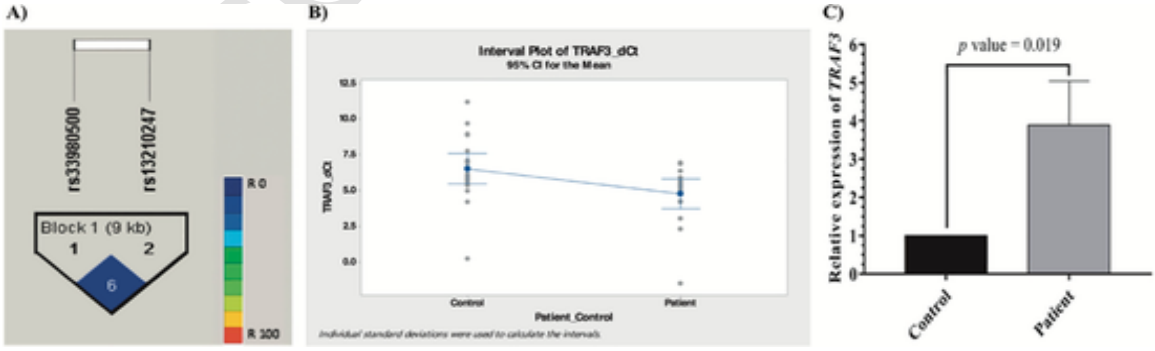

Fig. 1. Linkage disequilibrium between the analyzed SNPs on TRAF3 gene and the mRNA gene expression in relation to ALL. A. LD plot of the two SNPs in TRAF3 gene. The pairwise correlation between the SNPs were measured as r^2 and shown (x100) in each diamond. B. Expression of TRAF3 mRNA in peripheral blood in ALL and healthy individuals. A significant higher expression of TRAF3 in whole blood samples of ALL patients compared to healthy volunteers as indicated in dCT ($p < 0.005$). C. The relative expression of TRAF3 mRNA levels in ALL to control showed a signicant 4-fold differences, as calculated through 2^(-ΔΔCt) ($p = 0.019$).

Acknowledgement

We thank all participants and the medical staff at KKUH for their assistance with consent and blood sample collection.

Data availability

Data will be made available on request.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cancergen.2026.01.012.

References

[1] Kucka K, Wajant H. Receptor oligomerization and its relevance for signaling by receptors of the tumor necrosis factor receptor superfamily. Front Cell Dev Biol 2020;8:615141.
[2] Gough P, Myles IA. Tumor necrosis factor receptors: pleiotropic signaling complexes and their differential effects. Front Immunol 2020;11:585880.
[3] Ha H, Han D, Choi Y. TRAF-mediated TNFR-family signaling. Curr Protoc Immunol 2009 Nov. Chapter 11:11 9D 1- 9D 9.
[4] Shi JH, Sun SC. Tumor necrosis factor receptor-associated factor regulation of nuclear factor kappaB and mitogen-activated protein kinase pathways. Front Immunol 2018;9:1849.
[5] Bista P, Zeng W, Ryan S, Bailly V, Browning JL, Lukashev ME. TRAF3 controls activation of the canonical and alternative NFkappaB by the lymphotoxin beta receptor. J Biol Chem 2010 Apr 23;285(17):12971–8.
[6] Sun SC. The noncanonical NF-kappaB pathway. Immunol Rev 2012 Mar;246(1):125–40.
[7] Sanjo H, Zajonc DM, Braden R, Norris PS, Ware CF. Allosteric regulation of the ubiquitin:NIK and ubiquitin:TRAF3 E3 ligases by the lymphotoxin-beta receptor. J Biol Chem 2010 May 28;285(22):17148–55.
[8] Liao G, Zhang M, Harhaj EW, Sun SC. Regulation of the NF-kappaB-inducing kinase by tumor necrosis factor receptor-associated factor 3-induced degradation. J Biol Chem 2004 Jun 18;279(25):26243–50.
[9] Tseng PH, Matsuzawa A, Zhang W, Mino T, Vignali DA, Karin M. Different modes of ubiquitination of the adaptor TRAF3 selectively activate the expression of type I interferons and proinflammatory cytokines. Nat Immunol 2010 Jan;11(1):70–5.
[10] Ely KR, Kodandapani R, Wu S. Protein-protein interactions in TRAF3. Adv Exp Med Biol 2007;597:114–21.
[11] Bishop GA, Stunz LL, Hostager BS. TRAF3 as a multifaceted regulator of B lymphocyte survival and activation. Front Immunol 2018;9:2161.
[12] Arkee T, Hostager BS, Houtman JCD, Bishop GA. TRAF3 in T cells restrains negative regulators of LAT to promote TCR/CD28 signaling. J Immunol 2021 Jul 1;207(1):322–32.
[13] Lalani AI, Moore CR, Luo C, Kreider BZ, Liu Y, Morse 3rd HC, et al. Myeloid cell TRAF3 regulates immune responses and inhibits inflammation and tumor development in mice. J Immunol 2015 Jan 1;194(1):334–48.
[14] Hornick EL, Bishop GA. TRAF3: guardian of T lymphocyte functions. Front Immunol 2023;14:1129251.
[15] Xu Y, Cheng G, Baltimore D. Targeted disruption of TRAF3 leads to postnatal lethality and defective T-dependent immune responses. Immunity 1996 Nov;5(5):407–15.
[16] He JQ, Zarnegar B, Oganesyan G, Saha SK, Yamazaki S, Doyle SE, et al. Rescue of TRAF3-null mice by p100 NF-kappa B deficiency. J Exp Med 2006 Oct 30;203(11):2413–8.
[17] Zhang C, Han X, Sun L, Yang S, Peng J, Chen Y, et al. Novel loss-of-function mutations in TNFAIP3 gene in patients with lupus nephritis. Clin Kidney J 2022 Nov;15(11):2027–38.
[18] Rae W, Sowerby JM, Verhoeven D, Youssef M, Kotagiri P, Savinykh N, et al. Immunodeficiency, autoimmunity, and increased risk of B cell malignancy in humans with TRAF3 mutations. Sci Immunol 2022 Aug 12;7(74):eabn3800.
[19] Sood N, Hamide A, Dubashi B, Nisha Y, Mathaiyan J, Munirajan AK, et al. Impact of genetic polymorphisms in NF-kB2 and TRAF3 genes on response to bortezomib-based therapy in multiple myeloma patients. Asian Pac J Cancer Prev 2024 Mar 1;25(3):829–37.
[20] Zhou Z, Zeng C, Nie L, Huang S, Guo C, Xiao D, et al. The effects of TLR3, TRIF and TRAF3 SNPs and interactions with environmental factors on type 2 diabetes mellitus and vascular complications in a Han Chinese population. Gene 2017 Aug 30;626:41–7.
[21] Xiao D, Liu D, Wen Z, Huang X, Zeng C, Zhou Z, et al. Interaction between susceptibility Loci in MAVS and TRAF3 genes, and high-risk HPV infection on the risk of cervical precancerous lesions in chinese population. Cancer Prev Res (Phila) 2019 Jan;12(1):57–66.
[22] Huffmeier U, Uebe S, Ekici AB, Bowes J, Giardina E, Korendowych E, et al. Common variants at TRAF3IP2 are associated with susceptibility to psoriatic arthritis and psoriasis. Nat Genet 2010 Nov;42(11):996–9.
[23] Yu X, Chen Y, Chen J, Fan Y, Lu H, Wu D, et al. Shared genetic architecture between autoimmune disorders and B-cell acute lymphoblastic leukemia: insights from large-scale genome-wide cross-trait analysis. BMC Med 2024 Apr 15;22(1):161.
[24] Tebbi CK. Etiology of Acute Leukemia: a Review. Cancers (Basel) 2021 May 8;13(9).
[25] Iacobucci I, Mullighan CG. Genetic Basis of Acute Lymphoblastic Leukemia. J Clin Oncol 2017 Mar 20;35(9):975–83.
[26] Lin M, Ji X, Lv Y, Cui D, Xie J. The Roles of TRAF3 in Immune Responses. Dis Markers 2023;2023:7787803.
[27] Li MY, Chong LC, Duns G, Lytle A, Woolcock B, Jiang A, et al. TRAF3 loss-of-function reveals the noncanonical NF-kappaB pathway as a therapeutic target in diffuse large B cell lymphoma. Proc Natl Acad Sci U S A 2024 Apr 30;121(18):e2320421121.
[28] Yariswamy M, Yoshida T, Valente AJ, Kandikattu HK, Sakamuri SS, Siddesha JM, et al. Cardiac-restricted Overexpression of TRAF3 Interacting Protein 2 (TRAF3IP2) Results in Spontaneous Development of Myocardial Hypertrophy, Fibrosis, and Dysfunction. J Biol Chem 2016 Sep 9;291(37):19425–36.