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Association of *FOXO3* (rs17069665) gene polymorphism and childhood acute lymphoblastic leukemia in Egypt

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ABSTRACT

Background: Acute lymphoblastic leukemia (ALL) is the most common malignancy in children. Genetic variations, particularly gene polymorphisms, have been closely linked to increased susceptibility to ALL. One of those genes is Forkhead box O3 (*FOXO3*), which is considered a potential tumor suppressor gene.
Aim: This study intended to examine the potential significance of the *FOXO3* (rs17069665) single nucleotide polymorphism (SNP) as a risk factor for childhood ALL, in addition to its effect on the laboratory results, clinical manifestations and the clinical outcome after induction of chemotherapy.
Subjects and methods: Sixty-six newly diagnosed ALL children and 70 healthy children of matched age and sex as controls were recruited. *FOXO3* (rs17069665) polymorphism was detected using TaqMan real time PCR.
Results: Higher frequencies of the (AG) genotype and G-allele of *FOXO3* (rs17069665) variant were present in ALL patients in comparing with the controls (16.7 % vs. 4.3 %, $p = 0.017$ and 11.4 % vs. 2.1 %, $p = 0.003$, respectively). The frequencies of the *FOXO3* (rs17069665) SNP reflected a noticeably higher risk of ALL under diverse genetic models, including the co-dominant model (AG vs. AA, OR = 2.55), dominant (AG + GG vs. AA, OR = 2.81), and allelic (G-allele vs. A-allele, OR = 2.9) models. The single case of c-MYC mutation was observed with the (GG) genotype. No significant association between *FOXO3* (rs17069665) SNP polymorphism and response to chemotherapy was found.
Conclusion: Our findings showed that the *FOXO3* (rs17069665) polymorphism was associated with a greater incidence of ALL in Egyptian children, which might be a potential biomarker for ALL susceptibility.

1. Introduction

Acute lymphoblastic leukemia (ALL), the most prevalent cancer in children, is caused by the uncontrolled expansion of clonal lymphoid cells, most primarily pre-B cells (80 %–85 %), mature B cells (<5 %), and T cells (10 %–15 %). Childhood ALL has a peak incidence between 2 and 5 years (Neaga et al., 2021; Schwab and Harrison, 2018).

In Egypt, ALL accounts for 20 % of childhood malignancies, with an incidence of four per 100,000 cases every year (Abobakr et al., 2023). The etiology of ALL is not yet known; however, pediatric ALL is related to environmental factors, ionizing radiation, genetic syndromes, and genetic susceptibility (Abd El Hassib et al., 2021).

Numerous genetic subtypes of ALL are distinguished by significant chromosomal abnormalities, which are the hallmark of ALL, such as t (9,22) [BCR-ABL1], rearrangement of mixed lineage leukemia, and Philadelphia-like ALL (Terwilliger and Abdul-Hay, 2017). However, chromosomal aberrations by themselves frequently do not cause leukemia; additional genetic mutations, such as TP53 mutations, IKZF1 alterations, DUX4, MEF2D9, ZNF384-rearranged ALL, and PAX5, must contribute to carcinogenesis and have an impact on risk assessment and prognosis (Zhang et al., 2020; Brady et al., 2022).

Detecting genetic mutations helps stratify patients and tailor targeted therapies to specific molecular changes (Lejman et al., 2021). Target identification and creation of more potent drugs became possible by new technology. The ultimate objective is to substitute more targeted and efficient treatment for traditional chemotherapy (Ivanov et al., 2023).

Recent Genome-Wide Association Studies have discovered many genetic variants related to an elevated ALL risk, including single nucleotide polymorphisms (SNPs) in key genes (Fernandes et al., 2022).

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The Forkhead box O (FOXO) subfamily comprises the Forkhead transcription factors FOXO1, FOXO3, FOXO4, and FOXO6. *FOXO3* mediates many biological processes, such as proliferation, cell cycle progression, apoptosis, DNA damage, and carcinogenesis (Ozel Turkcu et al., 2014).

The *FOXO3* gene can be regulated by the Phosphatidyl inositol-4,5-bisphosphate 3-kinase-protein kinase B (PI3K-AKT) pathway (Temerik et al., 2023). Increased PI3K-AKT activity is associated with *FOXO3* gene down-regulation, which has been observed in various tumor types, including gastric, ovarian, breast, and prostate cancers (Temerik et al., 2023; Mirzaie et al., 2019). As a tumor suppressor gene, *FOXO3* inactivation will encourage cancer cells to evade apoptosis and become resistant to treatment (Yang et al., 2020). Furthermore, it has been demonstrated that in these kinds of malignancies, overexpression of *FOXO3* suppresses cell proliferation and stops tumor growth (Temerik et al., 2023; Mirzaie et al., 2019). Therefore, FOX proteins constitute potential targets for the diagnosis and treatment of various cancers (Odemis et al., 2021).

The relationship between the *FOXO3* gene and the emergence of malignancies has been investigated in previous research, it was found that reduced *FOXO3* gene expression was more prevalent in patients with acute myeloid leukemia (AML) and ALL than healthy controls (Temerik et al., 2023; Mirzaie et al., 2019; Zhou et al., 2019). However, *FOXO3* SNP (rs17069665) was not investigated before except for two previous studies done on Chinese children; the first found a link between this polymorphism and ALL development (Yang et al., 2020), while the second study found a link between it and the risk of rhabdomyosarcoma (Zhang et al., 2024).

While *FOXO3* gene expression was found to be significantly lower in ALL cases compared to controls in a previous study investigating the gene's expression in Egyptian children with newly diagnosed ALL (Temerik et al., 2023), no prior research on the *FOXO3* SNP (rs17069665) on Egyptian children with ALL has been done so far to our knowledge.

There is a need for more studies in this field to examine the possibility that the *FOXO3* SNP (rs17069665) polymorphism could be a biomarker for ALL susceptibility and play a role in the pathophysiology of ALL, potentially leading to the development of targeted treatments or preventive strategies. Thus, the current study aimed to assess the relationship between the *FOXO3* (rs17069665) variant and ALL susceptibility in Egyptian children, as well as the effect of this connection on the clinical outcome of leukemia patients.

2. Subjects and methods

2.1. Study subjects

This comparative cross-sectional study included 66 newly diagnosed ALL Egyptian children and 70 healthy age- and sex-matched children as a control group. The study was conducted over two years, 2022 and 2023 at Benha University and Benha Children Specialist Hospitals, Egypt. Based on the diagnostic criteria of National Cancer Comprehensive Network guidelines for ALL and the World Health Organization classification, the detection of >20 % lymphoblasts in the bone marrow confirms the diagnosis of ALL (Arber et al., 2016; Brown et al., 2021). Confirmation of diagnosis was done by immunophenotyping (IPT), which classified ALL into B-ALL or T-ALL subtypes. The inclusion criteria involved children ≤16 years newly diagnosed with ALL before starting any medication. Exclusion criteria comprised patients aged >16 years, patients who began treatment, and patients with any other malignancies at the time of this study.

A full history of all patients was recorded, and clinical signs relevant to ALL were assessed, such as fever, bone ache, bleeding tendency, lymphadenopathy, hepatomegaly, and splenomegaly. All patients were treated according to the ST Jude Children's Research Hospital total XV protocol, as shown in Fig. 1 (Yilmaz et al., 2023). Follow-up was performed using bone marrow aspirate to evaluate patients' responses to chemotherapy on day 14 and day 28 of induction. Complete remission (CR) after the induction phase was determined by the evidence of the absence of lymphoblasts in peripheral blood and cerebrospinal fluid (CSF) and <5 % blasts in the marrow smears on day 28, along with hematologic recovery (absolute neutrophil count ≥1000/μl and platelet count ≥100,000/μl in the peripheral blood) and no sign of extramedullary disease (Ali et al., 2022).

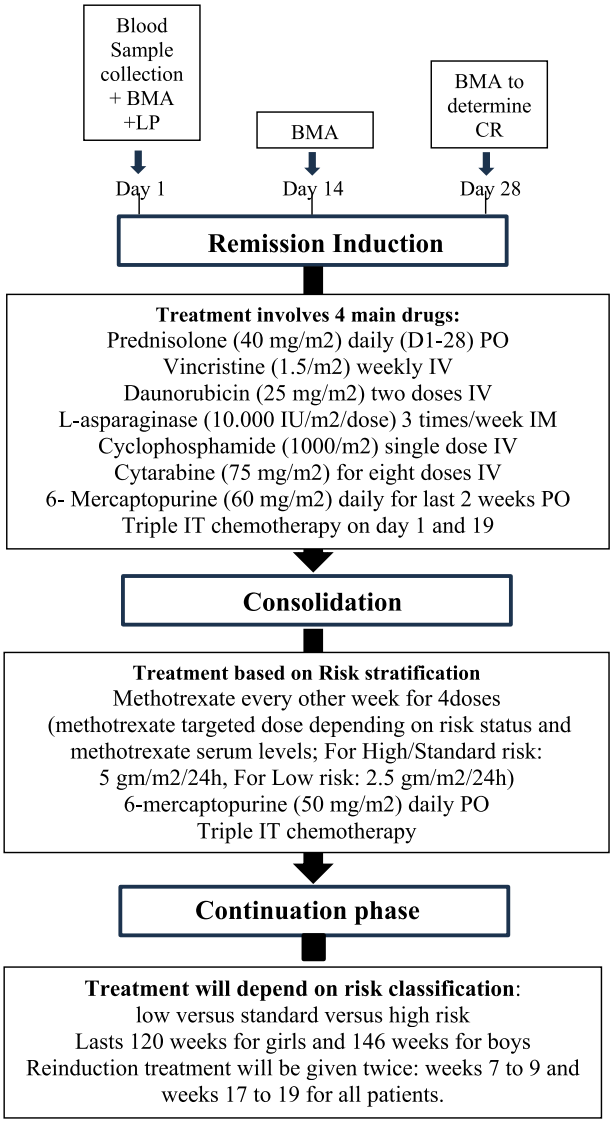

Fig. 1. Flow chart on the standard care and follow-up according to the ST Jude Children's Research Hospital total XV protocol.

2.2. Samples and methods

2.2.1. Samples

Six milliliters of blood were withdrawn from each participant. Each blood sample was separated into 3 sections: the first one was evacuated in tubes containing K3-EDTA, mixed well, and then divided into 2 aliquots, one of which was utilized for the IPT and complete blood count and the other of which was kept at −20 °C for later *FOXO3* (rs17069665 A > G) SNP detection. The second was evacuated in a sodium-heparin-containing tube for conventional karyotyping and gene mutation analysis. The third was evacuated in a plain tube, allowed to clot, then centrifuged, and the sera were collected for further clinical chemistry tests, including serum alanine aminotransaminase (ALT), aspartate

aminotransaminase (AST), total and direct bilirubin, lactate dehydrogenase (LDH), creatinine and urea. Bone marrow aspiration was performed for the preparation of smear slides for morphology. Lymphoblasts were counted in bone marrow and peripheral blood samples. A lumbar puncture was also performed to collect a sample of CSF from the spinal column and checked under a microscope for lymphoblasts that may spread to the brain and spinal cord.

2.2.2. Genomic DNA extraction and genotyping

Genomic DNA was extracted using the Gene JET whole-blood purification kit (Catalog # K0781; Thermo Scientific, EU) according to manufacturer instructions. The *FOXO3* (rs17069665 A > G) genotyping was carried out using TaqMan PCR SNP genotyping kits (Applied Biosystems, Singapore). PCR amplification was conducted using Step One Real-Time PCR [Thermal Cycling Block S/N (271003648); Applied Biosystem]. The experiment used a premade genotyping mix, a genotyping assay, and DNase/RNase-free water. The reaction also included 2.0 μl of DNA that had been adjusted to a concentration of 10 ng/ml. The reaction mixture had a total volume of 10.0 μl. The isolated DNA was amplified using sets of primers designed to detect the target polymorphism; their sequences were presented in Table S1. The sample was subjected to the following protocol: Heating at 95 °C for 10 min to denature the DNA. Then, it was subjected to 40 cycles of heating at 92 °C for 15 s, followed by cooling to 60 °C for 1 min to allow the primers to bind to the target DNA sequence and for the polymerase to extend the primers (annealing and extension), in which fluorescence was acquired and detected by using a Stepone Real-Time PCR instrument. To confirm genotyping results, a comprehensive search in published studies performed on the same SNP was done before carrying out the experiment (Yang et al., 2020; Zhang et al., 2024).

2.2.3. Statistical analysis

Quanto calculator software program (version 1.2.4) was used for sample size calculation (Gauderman et al., 2006), with respect to the previous studies by Yang et al. and Puckett and Chan (Yang et al., 2020; Puckett and Chan, 2023).

Using the goodness of fit Chi-square test, the chosen SNP in the controls was examined for Hardy-Weinberg equilibrium (HWE). Quantitative data were summarized as medians and ranges. Numbers and percentages were used to summarize the categorical data. The independent Mann-Whitney *U* test was used to compare quantitative data between the groups under study. Fisher's exact and chi-square tests were used to compare categorical data. When comparing more than two groups, the Kruskal-Wallis test was employed. The odds ratios were computed along with their 95 % confidence interval. Significant *p* values were those with a value of <0.05.

2.3. Ethical consideration

The present study was conducted with the Code of Ethics of the World Medical Association, in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments. This study has been approved by the local Ethics Committee of Benha University, Egypt (MoHP No. 0018122017/Certificate No: 1017), Study No.: MS. 20-7-2022. Written informed consent was provided by all children's legal guardians prior to their inclusion in the study, and assent from all children >12 years old was also taken.

3. Results

This study included 66 children newly diagnosed with ALL: 46 males (69.7 %) and 20 females (30.3 %) with median age 6 years (range 1–16), and 70 healthy children: 47 males (67.1 %) and 23 females (32.9 %), with median age 6 years (range 1–16 years). No significant difference between the two groups regarding age and sex was detected ($p = 0.94$ and $p = 0.74$, respectively).

At the time of diagnosis, as shown in Table 1, fever, bleeding, and bone ache were the most common reported symptoms. On the assessment of bone marrow, more than half of ALL patients had hypercellular bone marrow packed with blast cells. While on evaluation of CSF, all patients were blast-free. The IPT results showed B-ALL predominance in the whole cohort, while T-ALL constituted only one-fourth of ALL patients. Karyotyping analysis revealed karyotyping abnormality in only one-third of patients, including numerical and structural abnormalities. The results of some lab measurements of the ALL patients at diagnosis were compared to the controls and presented in Table 2.

Considering the clinical outcome following chemotherapy induction, out of the 66 assessed ALL patients, 62 (93.9 %) patients had CR, and only four (6.1 %) patients had no remission on day 28. The patients were further followed up over one year, and we recorded that 61 (92.4 %) patients had a morphological leukemia-free state, four (6.1 %) patients died, and one (1.5 %) patient was lost from the follow-up.

Table 3 showed that the frequency of variant (G allele) was (11.4 % vs. 2.1 %) among the patients and controls, respectively ($p = 0.003$). Using various genetic models, ALL patients were strongly associated with the *FOXO3* (rs17069665 A > G) SNP compared to healthy controls. Table 3 displayed three models: the allelic model (G allele vs. A allele,

Table 1
Clinical and laboratory characteristics of ALL patients ($N = 66$).

Parameter	N (%)
Symptoms	
Fever	32 (48.5 %)
Bone ache	30 (45.5 %)
Bleeding	32 (48.5 %)
Splenomegaly	19 (28.8 %)
Hepatomegaly	6 (9.1 %)
Lymphadenopathy	16 (24.2 %)
Blast%, median (range)	
Bone marrow blast	92 (30–99)
Peripheral blood blast	71 (22–95)
Cellularity	
Normocellular	14 (21.2 %)
Hypocellular	10 (15.2 %)
Hypercellular	42 (63.6 %)
CSF, N (%)	
Free of Lymphoblasts	66 (100 %)
Immunophenotyping ALL type	
B – ALL	49 (74.2 %)
Pro B	5 (7.7 %)
Common B	41 (62.1 %)
Pre B	3 (4.5 %)
T – ALL	17 (25.8 %)
Pre T	12 (18.2 %)
Cortical T	2 (3 %)
Medullary T	3 (4.5 %)
karyotyping	
Normal	46 (69.7 %)
Abnormal	20 (30.3 %)
Numerical abnormality	
Hypo-diploidy	6 (30 %)
Hyper-diploidy	5 (25 %)
Structural abnormality	9 (45 %)
Gene mutation	
c-MYC	1 (5 %)

Parameters were expressed by frequency (percentage), or median (range); CSF: Cerebrospinal fluid.

Table 2
Laboratory results in the studied groups.

Parameter	ALL (N = 66)	Control (N = 70)	p-value
TLC ($\times 10^9$/L)	22.15 (0.5–686.5)	8.95 (5.2–14.9)	0.001*
Hemoglobin (g/dl)	9.0 (6.10–14.20)	12.60 (11.50–15.70)	<0.001*
Platelets ($\times 10^9$/L)	36 (8.0–299.0)	269.5 (179.0–400.0)	<0.001*
ALT (U/L)	23 (6.0–302.0)	29 (15.0–38.0)	0.001*
AST (U/L)	29 (11.0–223.0)	32 (15.0–39.0)	0.029*
Total bilirubin (mg/dl)	0.6 (0.20–3.10)	0.7 (0.30–0.90)	0.13
Direct bilirubin (mg/dl)	0.2 (0.10–2.20)	0.2 (0.10–0.30)	0.64
LDH (U/L)	715 (300.0–5600.0)	199.5 (143.0–330.0)	<0.001*
Creatinine (mg/dl)	0.40 (0.30–0.80)	0.40 (0.30–0.70)	0.185
Urea (mg/dl)	20.0 (14.0–30.0)	19.0 (15.0–29.0)	0.002*

Parameters were expressed by median (range); Mann-Whitney *U* test was used, *Significant *p*-value; TLC: Total leukocytic count, ALT: Alanine aminotransaminase, AST: Aspartate aminotransaminase, LDH: Lactate dehydrogenase.

OR = 2.9, $p = 0.003$), the dominant (AG + GG vs. AA, OR = 2.81, $p = 0.007$), and the co-dominant (heterozygous comparison AG vs. AA, OR = 2.55, $p = 0.017$*).

The *FOXO3* (rs17069665 A > G) SNP was classified into wild-type, heterozygous and homozygous variant genotypes (AA, AG + GG, respectively). The two groups were compared to determine if they were different concerning lab measurements, demographic and clinical characteristics, and the patients' response following the induction phase of chemotherapy, as presented in Table 4. The (AG + GG) group had a considerably higher c-MYC mutation than the (AA) group ($p = 0.029$). Otherwise, no significant variation was detected considering the other parameters among the different genotype groups ($p > 0.05$).

A logistic regression analysis was conducted to predict the ALL diagnosis. In the univariate analysis, several variables were examined individually for their association with ALL diagnosis. Age, sex, TLC, LDH, and *FOXO3* (rs17069665) AG + GG genotype were included in the analysis as shown in Table 5.

Among these variables, TLC, LDH, and *FOXO3* (rs17069665) AG + GG genotypes showed significant associations with ALL diagnosis, as indicated by their *p*-values. In the multivariate analysis, these variables were included to adjust for potential confounding factors. After adjusting for these variables, the associations between TLC, LDH, and *FOXO3* (rs17069665) AG + GG genotype with ALL diagnosis remained significant.

4. Discussion

FOXO3 is a tumor suppressor gene that is expressed in a variety of organs, including B- and T-lineage cells. Numerous target genes, including those involved in apoptosis, DNA repair, and cell cycle regulation, are transcriptionally activated by this gene. According to recent reports, *FOXO3* also plays a significant role in modulating the response to physiologic oxidative stress, which helps to maintain the hematopoietic stem cell pool (Mirzaie et al., 2019).

The *FOXO3* (rs17069665 A > G) variant allele was specifically chosen for our investigation due to its high frequency and known impact on reducing *FOXO3* expression and function. Rs17069665 is an A to G transversion substitution in intron 1 of *the FOXO3* gene. The rs17069665 site overlaps a transcription factor binding site in the *FOXO3* promoter, and the G allele was assumed to abolish a transcription factor E site (Donlon et al., 2017). This eventually causes the cell cycle to become uncontrolled, which results in lymphoproliferation and the development of tumors and malignancies, including ALL (Kikuno et al., 2022; Wang et al., 2014).

During the current work, analysis of the *FOXO3* (rs17069665 A > G) polymorphism showed that patients displayed greater percentages of (AG) and (AG + GG) genotypes than controls (p 0.017, and 0.007, respectively), carrying a 2.5- and 3-fold risk of developing ALL (OR = 2.55, $p = 0.017$ and OR = 2.81, $p = 0.007$, respectively). Additionally, patients had a variant G-allele frequency substantially greater than controls, with an estimated 3-fold increased risk of ALL (OR = 2.9, $p = 0.003$).

Similarly, in the Yang et al. case-control study, which involved a total of 425 ALL cases and 1339 healthy non-ALL controls, the *FOXO3* gene rs17069665 A > G influenced the risk of ALL in Chinese children (GG vs. AA+AG, OR = 1.76, $p = 0.043$). They proposed that the polymorphism of *FOXO3* could serve as a possible biomarker for susceptibility to ALL

Table 4
The association between *FOXO3* (rs17069665) SNP and the patients' characteristics and clinical outcome.

Parameter	AA (N = 53)	AG + GG (N = 13)	p-value
Sex			
Male	36 (67.9 %)	10 (76.9 %)	1.00
Female	17 (32.1 %)	3 (23.1 %)	
Peripheral blood blast (%)	70 (22–95)	75 (22–95)	0.42
BM blast (%)			
On admission	92.0 (30–99)	90 (52–98)	0.38
Day 14	2.0(0.0–93)	2.0 (0.0–80.0)	0.27
Day 28	1.0(0.0–91)	1.0 (0.0–45.0)	0.12
BM cellularity			
Normocellular	13 (24.5 %)	1 (7.7 %)	0.17
Hypocellular	7 (13.2 %)	3 (23.1 %)	
Hypercellular	33 (62.3 %)	9 (69.2 %)	
IPT			
B-ALL	39 (73.6 %)	10 (76.9 %)	1.00
T-ALL	14 (26.4 %)	3 (23.1 %)	
Karyotyping			
Normal	37 (69.8 %)	9 (69.2 %)	0.87
Abnormal	16 (30.2 %)	4 (30.8 %)	
Gene mutation			
c-MYC	0 (0.0 %)	1 (7.7 %)	0.029*
Response to ttt			
CR	50 (94.3 %)	12 (92.3 %)	0.13
No CR	3 (5.7 %)	1 (7.7 %)	
Follow Up			
MLFS	49 (92.5 %)	12 (92.3 %)	0.14
Lost follow up	1 (1.9 %)	0 (0.0 %)	
Died	3 (5.7 %)	1 (7.7 %)	

Parameters were expressed as frequency (percentage), or median (range). *Significant p-value; BM: Bone marrow, IPT: Immunophenotyping, CR: Complete remission, MLFS: Morphological leukemia free state.

Table 3
Genetic models of *FOXO3* (rs17069665) SNP and the risk of ALL.

		ALL (N = 66)†		Control (N = 70)†			
Model		N	%	N	%	p-value	OR (95 % CI)
	AA	53.0	80.3	67.0	95.7	–	Reference
Co-dominant	AG	11.0	16.7	3.0	4.3	0.017*	2.55 (1.18–5.52)
	GG	2.0	3	0.0	0	0.14	–
Dominant	AG + GG	13.0	19.7	3.0	4.3	0.007*	2.81 (1.33–5.92)
Allelic	A	117.0	88.6	137.0	97.9	–	Reference
	G	15.0	11.4	3.0	2.1	0.003*	2.90 (1.43–5.88)

* Significant *P*-value.
† Patients and controls following HWE with *p*-values of 0.161 and 0.855, respectively.

Table 5
Univariate and multivariate analysis to predict ALL diagnosis.

	Univariate analysis			Multivariate analysis		
	P	OR	95 % CI	P	OR	95 % CI
Age	0.995	1	0.955–1.048	–	–	–
Sex	0.749	0.929	0.590–1.461	–	–	–
TLC	<0.001*	1.058	1.025–1.091	0.008*	1.157	1.039-1.288
LDH	<0.001*	1.014	1.010-1.019	<0.001*	1.02	1.011-1.029
FOXO3 (rs17069665) AG + GG genotype	0.007*	2.812	1.334-5.925	0.039*	2.806	1.715-11.006

* Significant P-value; OR: Odds ratio; CI: Confidence interval; TLC: Total leucocyte count; LDH: Lactate dehydrogenase.

(Yang et al., 2020). However, apart from this study, there hasn't been any reporting of the correlation between the *FOXO3* (rs17069665 A > G) polymorphism and ALL.

The *FOXO3* gene rs17069665 was also investigated in Zhang et al. study on rhabdomyosarcoma in Chinese patients; it was found that rs17069665 (GG vs. AA+AG, adjusted OR = 2.96; 95%CI [1.10–3.32]; $p = 0.010$) was related to the increased rhabdomyosarcoma risk. Furthermore, rs17069665 ($p < 0.001$) was linked to a lower overall survival rate in these patients. Functional research revealed that rs17069665 may modify the bindings to MYC, CTCF, and/or RELA, which may impact the transcription and expression of *FOXO3* (Zhang et al., 2024).

In a case-control study by Temerik et al., the expression of the *FOXO3* gene was evaluated in bone marrow samples from Egyptian children ($N = 53$) who were recently diagnosed with ALL in comparison to 30 healthy controls. The *FOXO3* gene was substantially less expressed in ALL cases than in controls, with a median of 0.33 versus 1 in the investigated groups, respectively (Temerik et al., 2023).

When Zhou et al. examined *FOXO3* expression in de novo AML patients, they discovered that the gene's expression in de novo patients was substantially lower than in control samples ($p = 0.009$). The survival time of *FOXO3* high patients was longer than that of *FOXO3* low patients in non-M3 AML ($p = 0.002$) (Zhou et al., 2019). Previous research in AML patients revealed that a higher rate of spontaneous cell proliferation was linked to enhanced PI3K activity and inactivated *FOXO3* in leukemic cells (Kubota et al., 2004; Chapuis et al., 2010).

After stratifying by gender, blast counts, marrow cellularity, gene mutation, IPT, karyotype, and other clinical outcome, additional analyses were carried out to investigate the relationship between the *FOXO3* (rs17069665 A > G) polymorphism and ALL susceptibility. Regarding karyotyping analysis, our study showed no significant difference between the different genotypes of *FOXO3* (rs17069665), suggesting that this particular genetic variation does not directly influence the chromosomal alterations commonly observed in ALL. While the c-MYC mutation was significantly associated with a GG genotype ($p = 0.029$), this could not be relied upon because it was only one case.

Similar to our findings, Yang et al. reported no association between *FOXO3* (rs17069665 A > G) genotypes and IPT. Moreover, they also noted that there were no associations between *FOXO3* (rs17069665 A > G) genotypes and karyotyping abnormalities, either hypodiploid, hyperdiploid, or normal karyotyping (Yang et al., 2020).

Also, Bandari et al. found no association between *FOXO3* expression and IPT ($p = 0.09$) (Bandari et al., 2021). The investigation conducted by Temerik et al. revealed a noteworthy inverse relationship between the patient's bone marrow and peripheral blast percentage and peripheral *FOXO3* measurement. The results of this study indicated that the *FOXO3* gene is a tumor suppressor, which suggests that treating ALL may involve targeting this gene (Temerik et al., 2023).

Regarding response to treatment, our results revealed no significant association between *FOXO3* (rs17069665 A > G) gene polymorphism and response to treatment ($p = 0.13$). This suggests that the polymorphism does not appear to impact the response to treatment, indicating that it may not be a critical factor in determining how patients respond to standard ALL therapies. Furthermore, the *FOXO3* (rs17069665) polymorphism over a one-year follow-up period showed no significant association with patient outcomes, such as disease progression or remission ($p = 0.14$).

Mirzaie et al. examined the *FOXO3* gene's mRNA expression level at three distinct stages in 70 patients with B-ALL and T-ALL and compared them with 70 healthy controls. Using real-time PCR, they discovered that the newly diagnosed ALL patients had lower *FOXO3* expression than the control, maintenance, and relapse groups. No statistically significant differences were seen across the groups, indicating that *FOXO3* expression is impacted by chemotherapy (Mirzaie et al., 2019). It has been previously discovered that *FOXO3* inactivation results in B-cell resistance to apoptosis, suggesting that it could be a therapeutic target for this illness (Ticchioni et al., 2007). Moreover, therapy-resistant T-ALL patients exhibited *FOXO3* cytoplasmic localization; these patients' T-ALL cells apparently inactivate *FOXO3* to evade apoptosis (Ausserlechner et al., 2013). According to these results, maintaining appropriate levels of *FOXO3* expression is essential for preventing leukemogenesis (Mirzaie et al., 2019).

FOXO3 has been demonstrated by Dewar et al. to be a reliable biomarker for leukemogenesis mediated by BCR-ABL. Additionally, they discovered that in cases of Philadelphia-positive ALL, where *FOXO3* is down-regulated, proteasomal inhibition of *FOXO3* by bortezomib may be a viable therapeutic strategy (Dewar et al., 2011).

The predictive power of various parameters for ALL diagnosis was examined using logistic regression analysis. The univariate study examined a wide range of variables. The results indicated that there were significant associations between ALL diagnosis and high TLC, high LDH level, and *FOXO3* (rs17069665) AG + GG genotype (odds ratio 1.058, 1.014, and 2.812, respectively), with the *FOXO3* (rs17069665) AG + GG genotype having the highest odds ratio.

These characteristics remained significant as independent predictors of ALL throughout the multivariate analysis; however, the most important risk factor, *FOXO3* (rs17069665) AG + GG genotype, had 2.8-fold higher odds of developing ALL in comparison to the *FOXO3* (rs17069665) AA genotype.

According to our knowledge, this is the first work in Egypt to examine the relationship between the *FOXO3* (rs17069665) gene polymorphism and the risk of pediatric ALL. From a research standpoint, our results pave the way for further studies to explore the mechanisms by which these factors contribute to ALL pathogenesis, potentially leading to the development of targeted therapies or preventive strategies.

The study's primary constraint is the comparatively small sample sizes of the ALL and control groups. To confirm our results and determine the frequency of this SNP in different populations while considering ethnic variation, more research is advised, also we recommend performing gene sequencing in a few samples for quality control. Moreover, further research is needed to fully understand the potential of the *FOXO3* candidate gene for cancer susceptibility and response to treatment. Gene-gene and gene-environment interactions should be the main areas of attention for this research.

5. Conclusion

The *FOXO3* (rs17069665 A > G) polymorphism contributes

pathologically to the ALL predisposition. This implies that a significant genetic factor impacting the risk of getting ALL may be inherited *FOXO3* dysfunction. Additionally, the results suggest that *FOXO3* genotyping is a promising potential marker for routine clinical application, especially for individuals at risk of developing ALL.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.genrep.2024.102015.

Abbreviations

ALL	Acute Lymphoblastic Leukemia
FOXO3	Forkhead Box O3
PCR	Polymerase Chain Reaction
AST	Aspartate transaminase
ALT	Alanine transaminase
LDH	Lactate dehydrogenase
IPT	Immunophenotyping
EDTA	Ethylene diamine tetra acetic acid
CSF	Cerebrospinal fluid
SNP	Single nucleotide polymorphism
TLC	Total leukocyte count
HWE	Hardy-Weinberg equilibrium
PI3K-AKT	Phosphatidyl inositol-4,5-bisphosphate 3-kinase-protein kinase B pathway
AML	acute myloid leukemia
CR	complete remission
BMA	bone marrow aspirate
LP	lumbar puncture
PO	per-oral
IV	intravenous
IT	intrathecal

CRediT authorship contribution statement

Dalia Mohamed Abd El Hassib: Data curation, Conceptualization. **Magda Abd el-Aziz Zidan:** Supervision, Project administration. **Samar Mahmoud Elbahy:** Writing – original draft. **Nahla Saieed Aboesha:** Methodology, Formal analysis. **Amira M.N. Abdelrahman:** Writing – review & editing, Validation.

Declaration of competing interest

The authors wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Data availability

Data will be made available on request.

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