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FOXP3 Polymorphism and Susceptibility to Pediatric Acute Lymphocytic Leukemia (ALL): A Preliminary Data

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Abstract

FOXP3 (forkhead box P3) polymorphism is associated with many inflammatory diseases and cancers. Acute lymphoblastic leukemia (ALL) is the most common type of pediatric malignancies.This study was designed to investigate the impact of FOXP3 (-3279C/A and -2383C/T) gene polymorphism on the susceptibility of Egyptian children to ALL. A total of 128 subjects with ALL and 124 healthy controls were enrolled in this study. Genotyping of FOXP3 (-3279C/A and -2383C/T) were performed by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP). There was a significant increase ($P<0.01$) in FOXP3 (-3279CC) genotype, while FOXP3 -3279CA genotype was significantly decreased in ALL patients compared to controls. Insignificant change in FOXP3 (-2383C/T) genotypes was demonstrated between both groups. FOXP3 (-2383CC) genotype was significantly decreased ($p<0.05$) in treatment responders compared to non-responders and a significant increased ($p<0.05$) in relapsed patients comparing to the non-relapsed group. Taken together, our pilot study pointed to the potential role of FOXP3 (-3279C/A) gene polymorphisms in Egyptian children ALL susceptibility. An additional prospective large scale study should be carried out to support our findings.

Keywords: FOXP3; SNP; pediatric ALL; RFLP.

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1. Introduction

Pediatric hematopoietic leukemia is one of the highest mortality rates throughout the globe [1]. Acute lymphoblastic leukemia (ALL) is the most common type of pediatric hematopoietic leukemia in children, accounting for 30-35% of all pediatric cancers [2,3]. It represents the 11^{th} and 10^{th} most frequent cause of cancer occurrence and death worldwide; respectively [4]. Regulatory T cells (Tregs) were defined as immunosuppressive $CD4^+$ T cells expressing $CD25^+$ on their surface [5]. They are characterized by the expression of the transcription factor forkhead box P3 (FOXP3) [6]. They are reported to be associated with tumor progression and reduced survival in cancer patients [7,8]. In leukemia, Tregs are reported to have a dual role either by suppressing anti-tumor immune responses mediated by T-cells or regulating the malignant immune cell, directly or by interfering with T-cell help [9]. FOXP3, the master transcriptional activator for Tregs, is important for its development and maintenance of function [10,11]. FOXP3 gene; 1296 bp in size, is located at the short arm of the X-chromosome (Xp11.23) and consisted of 11 exons encoding a protein of 431 amino acids and 3 non-coding exons [12]. Single nucleotide polymorphisms (SNPs) in the FOXP3 gene might change its expression level and impair the suppressive function of Tregs [13]. In the promoter region of FOXP3, there are five SNPs: -924A/G (rs2232365), -1383C/T (rs2232364), -2383C/T (rs3761549), -3279C/A (rs3761548) and -3499A/G (rs3761547) [14]. Two of them (-3279C/A and -2383C/T) have been reported to be associated with cancer risk [13, 15]. In the light of the vital role of Treg cells in cancer, the current study is performed to investigate the genetic association between FOXP3 (-3279C/A) and (-2383C/T) gene polymorphism and susceptibility to ALL in the Egyptian children.

2. Subjects and Methods

2.1 Patients and controls

This study consisted of 128 Pediatric ALL patients with a mean age of 7.18 ± 0.38 collected from the Department of pediatric oncology at South Egypt Cancer Institute and Hospital, Assiut University, Egypt. They were categorized by risk depending on the assignment protocol [16]. The patient's hematological, biochemical data and immunophenotyping were recorded from pediatric ALL files with the help of the medical oncologist during follow up. Treatment based on Total XIIIB regimen [17]. One hundred and twenty-four age- and sex-matched donors' healthy individuals free of any chronic diseases and without a family history of leukemia were included as control individuals. The blood samples of healthy individuals used in the present study were part of the samples taken for clinical diagnostic tests in the hospital. All protocol investigations were done in accordance with the Human Ethical Clearance Committee guidelines for Clinical Researches according to Helsinki Declaration (1964) and performed with the understanding of the human subject. Research approved by the Ethical Clearance Committee (http://www.aun.edu.eg/faculty-medicine/Ethical Committee.php) at South Egypt Cancer Institute and Hospital, Assiut University (IORG: IORG0006563 and IRB: IRB00007877). Informed consent was obtained from a parent and/or legal guardian for every patient before blood collection and the ethics committee/institutional review board has approved the consent procedure.

2.2 DNA Isolation

From each individual, 3 ml venous blood was collected on ethylene-diamine-tetra-acetic acid (EDTA) sterile vacutainer. Genomic DNA was extracted from whole blood samples by the GentraPuregene Blood Kit (Qiagen Company, Hilden, Germany) according to the manufacturer's instructions. Extracted DNA was applied to 1% agarose gel electrophoresis to confirm its integrity. The concentration of DNA in all samples was measured by using a nanodrop 2000c (Thermo Fisher Scientific, Waltham, MA, USA).

2.3 Genotyping of FOXP3

FOXP3 (-3279C/Aand -2383C/T) primer sequences and DNA fragments characterizing each genotype/allele were summarized in Table (1). Genotyping of FOXP3 (-3279C/A and -2383C/T) were performed by PCR-based restriction fragment length polymorphism (PCR-RFLP) method as previously described [15, 18]. The reaction was performed in one tube with a final reaction volume of 25 µl. PCR mixtures consisted of 2x DreamTaq Green Master Mix (Fermentas, Thermo Fisher Scientific Inc.), 10pmoles of each primer, and 200 ng of DNA. All polymerase chain reactions (PCR) were performed in 2720 thermal cycler (Applied Biosystems). The amplification conditions for -3279C/A were: 5 minutes at 94°C and 30 cycles of denaturing for 30s at 94°C, annealing for 30s at 68°C and extension for 30s at 72°C then a single final extension for 10 minutes at 72°C. For -2383C/T, initial denature at 95°C for 3 minutes followed by 30 cycles (denaturing at 94°C for 30s, annealing at 60°C for 30s, and extension at 72°C for 1 minutes) then single final extension at 72°C for 10 minutes was performed. Using proper restriction enzyme, PCR products were digested and visualized by 4% agarose gel electrophoresis in 0.5X Tris-acetate-EDTA (TAE) buffer with ethidium bromide staining (10 mg/ml). The PCR products/fragments were estimated by comparing them with 100bp/25bp DNA ladder (Fermentas).

2.4 Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Science (SPSS) version 21 (IBM Corporation, USA). Comparisons between both groups were performed by independent T-test, and the results were presented as the mean ± standard error. Categorical variables were presented as frequencies (%). Chi-squared (X^2) tests were performed to compare allele, genotype and haplotype distribution. The odds ratio (OR) and 95% confidence intervals (CI) were calculated to measure the relative risks in both control and ALL patients.

Table 1: Primers used in the genotyping of FOXP3 SNPs

Position	Primer sequence	PCR Product	Enzyme	Restriction product
FOXP3				
-3279C/A (rs3761548)	Forward: 5′-CCTCTCCGTGCTCAGTGTAG-3′. Reverse: 5′-CTCACCTAGCCCAGCTCTTG-3′.	300 bp	*PstI*	CC: 300bp CA:300/159/ 141 bp AA:159/141 bp
-2383C/T (rs3761549)	Forward: 5′-GCCTGGCACTCTCAGAGCTT-3′. Reverse: 5′-GTCTGTGGAGGCTCCGAACA-3′.	942 bp	*BsrI*	CC:528/213/164 bp CT:565/377/213/164 bp TT:565/377

The correlation between variables was determined using Spearman's correlation test. The online tool SNPstats (https://www.snpstats.net/start.htm) was used to perform haplotype reconstruction from population genotype

data and Linkage Disequilibrium (LD) parameters (D′ andr^2). All values were two-tailed, and *P* values <0.05 were considered to be statistically significant.

3. Results

3.1 Patients' characteristics

The demographic, hematological and biochemical characteristics of pediatric ALL patients and healthy controls enrolled in this study are summarized in Supplementary Table (1) while clinical characteristics of ALL patients are presented in Supplementary Table (2).

3.2 Association between FOXP3polymorphism and ALL

Genotype distribution and allelic frequency of FOXP3 SNPs (-3279C/A and-2383C/T) in ALL patients and healthy subjects were presented in Table (2). Analysis of FOXP3 -3279C/A polymorphism revealed that -3279CC genotype was the most frequent genotype in female patients while -3279CA genotype was the most frequent one at female controls. Comparing with female controls showed a significant increase ($p<0.01$) in -3279CC genotype and a significant decreased ($p<0.001$) in -3279CA genotype in female patients. In both genders, the -3279C allele was more frequent in pediatric ALL children compared to A allele. No statistically significant difference in allele frequency between patients and controls. FOXP3 -3279CC and AA genotype could be considered as risk factors for ALL (OR= 3.07; OR=1.66; respectively) and -3279CA genotype might be a protective genotype (OR=0.27). After the segregation of patients and controls, the -3279C allele might be considered as a risk factor for the disease in female (OR= 1.26) and male (OR= 1.13) patients. On the other side, the -3279A allele might be considered as a protective factor from pediatric ALL in females (OR=0.79) and (OR=0.87) in males. Genotyping of FOXP3 -2383C/T showed that the CT genotype and C allele were the most frequent while the TT genotype and T allele were the least in both groups. Insignificant change in the distribution of all genotypes/alleles between patients and control groups was demonstrated. -2383CT genotype (OR= 1.72) in females and C allele (OR= 1.26) in males could be considered as risk factors for ALL**.** Haplotype frequencies of the FOXP3 (-2383C/T) and (-3279C/A) SNPs in ALL patients and controls were summarized in Table (3). Four haplotypes were emerged (CC, CT, AC and AT) from the estimates of haplotype frequencies. The highest frequency of haplotypes in ALL patients group was CC. Insignificant change in haplotype distribution was demonstrated between both groups/genders. The haplotype estimates (D'=0.053, r^2=0.001) for males and (D'=0.085, r^2=0.006) for females indicate the absence of LD between FOXP3 (-2383C/T and -3279C/A) in the Egyptian population.

Table 2: Demographic, hematological and biochemical characteristics of ALL patients and healthy controls.

Laboratory Investigations	Control group (N=124) (Mean± SE)	ALL group (N=128) (Mean± SE)	P-value
Demographic Data			
Age (year)	6.67 ± 0.30	7.18 ±0.38	NS
Gender (M/ F)	58/66	44/84	NS
Hematological Data			
WBCs (10^3/µL)	8.04 ± 0.18	46.53 ± 6.60	**P<0.001**
Hemoglobin (g/dl)	11.49 ± 0.11	8.15 ± 0.21	**P<0.001**
PLT (10^3/µL)	316.76 ± 5.94	81.60 ± 7.40	**P<0.001**
Biochemical Data			
AST (IU/L)	25.16 ± 0.60	43.65 ± 5.71	**P<0.01**
ALT (IU/L)	22.56 ± 0.45	32.24 ± 3.72	**P<0.01**
ALB (g/L)	43.72 ± 0.41	36.00 ± 1.17	**P<0.001**
Bilirubien (mg/dl)	0.66 ± 0.01	0.86 ± 0.25	NS
Urea (mg/dl)	24.33 ± 0.75	38.98 ± 15.56	NS
Creatinine (mg/dl)	0.35 ± 0.01	0.61 ± 0.16	NS
LDH (IU/L)	305.52 ± 4.44	1803.71 ± 217.98	**P<0.001**
Sodium (mEq/L)	137.26 ± 0.17	130.82 ± 2.70	NS
Potasium (mEq/L)	4.25 ± 0.03	4.61 ± 0.41	NS
Calcium (mg/dl)	9.37 ± 0.04	9.21 ± 0.13	NS

All data are presented as mean ± SE. WBCs: White blood cells, PLT: Platelet, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALB: Albumin, LDH: Lactate dehydrogenase, IU/L: International Units per liter, g/L: Gram per liter, mg/dL: Milligrams per Deciliter, mEq/L: MilliequivalentsPerLitre, NS: not significant.

Table 3: Clinical characteristics of ALL patients

Data	Total patients (N=128) N (%)
WBC	
Low risk <50,000/Ml	89 (69.5%)
High risk >50,000/Ml	39 (30.5%)
Age at diagnosis	
Low risk 1-10 years	75 (58.6%)
high risk >10 years	53 (41.4%)
Response for treatment	
Responders	98 (76.6%)
Non responders	30 (23.4%)
Immunophenotype	
B-ALL	107 (83.6%)
T-ALL	21 (16.4%)
Relapse	
No relapse	102 (79.6%)
Relapsed	26 (20.4%)
Outcome	
Alive	118 (92.2%)
Dead	10 (7.8%)
CNS II	
Yes	83 (72.8%)
No	31 (27.2%)
CNS III	
Yes	42 (40.8%)
No	61 (59.2%)

Table 4: Genotype distribution and allelic frequency of the FOXP3 SNPs (-2383C/T,and -3279C/A) in controls and ALL patients

Position	Control group (N=124)	ALL group (N=128)	OR (95% CI)	P-value
-3279C/A (rs3761548) (N,%)				
	Female (66)	**Female (84)**		
C/C	11 (17%)	32 (38%)	**3.07** (1.40 - 6.73)	**P<0.01**
C/A	41 (62%)	26 (31%)	0.27 (0.13 - 0.53)	**P<0.001**
A/A	14 (21%)	26 (31%)	**1.66** (0.78 - 3.52)	NS
CA/AA	55 (83%)	52 (74%)	0.32 (0.14 - 0.71)	**P<0.01**
C	63 (48%)	90 (54%)	**1.26** (0.80 - 1.99)	NS
A	69 (52%)	78 (46%)	0.791 (0.50 - 1.24)	NS
	Male (58)	**Male (44)**		
C	73 (63%)	58 (66%)	**1.13** (0.63 – 2.03)	NS
A	43 (37%)	30 (34%)	0.87 (0.49 - 1.56)	NS
-2383C/T (rs3761549) (N,%)				
	Female (66)	**Female (84)**		
C/C	20 (30%)	21 (25%)	0.76 (0.37 - 1.57)	NS
C/T	34 (52%)	51 (61%)	**1.45** (0.75 - 2.79)	NS
T/T	12 (18%)	12 (14%)	0.75 (0.31 - 1.79)	NS
CT/TT	46 (70%)	63 (67%)	**1.30** (0.63 - 2.68)	NS
C	74 (56%)	93 (55%)	0.97 (0.61 - 1.53)	NS
T	58 (44%)	75 (45%)	**1.02** (0.65 - 1.62)	NS
	Male (58)	**Male (44)**		
C	61 (53%)	51 (58%)	**1.26** (0.72 - 2.20)	NS
T	55 (47%)	37 (42%)	0.79 (0.45 - 1.37)	NS

P: p value (significant), NS: Not significant

Table 5: Haplotype frequencies of the FOXP3 SNPs (-2383C/T and -3279C/A)in controls and ALL patients

Haplotype	Gender	Control group	Patients group	OR (95% CI)	P-value
CC	Female	25.2%	33.6%	1.00	----
	male	32.2%	41.5%	1.00	----
CT	female	22.4%	21.7%	0.52 (0.24 – 1.14)	NS
	male	30.7%	24.3%	0.58 (0.28 – 1.20)	NS
AT	female	21.4%	24.6%	0.91 (0.47 – 1.76)	NS
	Male	16.7%	16.3%	0.70 (0.35 – 1.39)	NS
AC	Female	30.7%	19.9%	0.66 (0.28 – 1.55)	NS
	Male	20.3%	17.7%	0.79 (0.40 – 1.56)	NS

P: p value (significant), NS: Not significant

3.3 Association between FOXP3 SNPs and ALL clinical manifestation

Associations of the most common clinical findings of the disease and FOXP3 gene polymorphisms in ALL patients were presented in Table (4). Analysis of FOXP3 (-3279C/A) revealed that -3279CC genotype was significantly increased ($p<0.05$) and CA was significantly decreased ($p<0.001$) in patients' high age risk compared those with low risk. On the other side, the -3279CA genotype showed a significant reduction ($p<0.05$) in relapsed patients compared to non-relapsed ones. Moreover, results of FOXP3 (-2383C/T) SNP pointed that CC genotype was significantly decreased ($p<0.05$) in treatment responders compared to non-responders and a significant increased ($p<0.05$) in relapsed patients comparing to the non-relapsed group.

4. Discussion

FOXP3 is an immunological regulator and is involved in the regulation, activation and differentiation of regulatory T cells [19]. The absence of a functional FOXP3 gene product might result in an abnormal production of Treg cells [20]. FOXP3 polymorphisms have been investigated as candidate risk factors in autoimmune diseases and various tumors [21]. Several studies investigated the association between the FOXP3 promoter polymorphisms (-3279C/A and-2383C/T) and the risk of cancer [15, 19, 21]. In the current work, analysis of FOXP3 -3279 C/A polymorphism revealed that -3279CC genotype is the most frequent in ALL female patients with a significant decrease in CA genotype compared to female controls. The C allele is more frequent over A allele in both genders in the patient group compared to the control group. Thus, FOXP3 -3279CC and AA genotypes might be considered as risk factors for ALL in females. In accordance with these results, Piao and his colleagues [21] reported -3279CC genotype as a risk factor in the development of hepatic veno-occlusive disease after allogeneic hematopoietic stem cell transplantation suggesting this SNP to be considered as a candidate marker for predicting the severe complications after allogeneic hematopoietic stem cell transplantation for pediatric ALL patients. In other types of cancer, conflicting results about the role of FOXP3 gene polymorphism in neoplastic cells were demonstrated. The AA genotype and A allele were associated with a higher risk of prostate cancer incidence [22]. The meta-analysis results of Cheng and his colleagues [13] and Chen and his colleagues [23] stressed on the importance of -3279C/A SNP and its

association with increased cancer risk in various types of malignancies. In disagreement with these results, Cezar- dos- Santos and his colleagues [24] in the Brazilian population, reported that-3279AA genotype may exert a protective role against human papilloma virus infection in females. Concerning other diseases, the C allele is over A allele in patients with multiple sclerosis, although AA genotype and A alleles might be considered as risk factors for the disease in the Southern Brazilian population [25]. In agreement to this results, -3279A allele was found to be predisposed to the development of late acute cellular rejection and ulcerative colitis [26-27]. Controversy, no allelic association of-3279C/A SNP was observed with autoimmune thyroid disease and Crohn's disease susceptibility in South-Indian and Chinese population; respectively [28-29]. In the Egyptian population, a statistically significant increase in -3279CC genotype and C allele was previously recorded in patients compared to controls, suggesting that this SNP may increase the susceptibility of Psoriasis Vulgaris [30]. On the other hand, the current study showed that FOXP3 -2383CT genotype and C allele were the most frequent in the patient group compared to other genotypes (CC, TT) and T allele with a significant increase in FOXP3 -2383CT genotype in ALL patients compared to controls. This study suggested that -2383CT genotype in females and C allele in males to be considered as risk factors for ALL in children. This SNP was investigated in leukemia patients after allogeneic hematopoietic stem cell transplantation [31]. -2383TT genotype was found to be in higher frequencies comparing to other genotypes suggesting this genotype as an independent risk factor for the occurrence of acute Graft-versus-host disease as a complication after transplantation. -2383 CT and TT genotypes were reported to be associated with a higher risk of developing brain tumors in Iranian patients [32]. Insignificant change in the distribution of all genotypes of FOXP3 -2383C/T between Hepatocellular Carcinoma patients and control was previously demonstrated in the Egyptian population [15]. Supporting to the previous data, the meta-analysis of Chen and his colleagues [23] pointed out that FOXP3 -2383C/T polymorphism was not associated with increased cancer risk in the Chinese population. In autoimmune thyroid disease and Crohn's disease, no allelic association of -2383C/T SNP with susceptibility to diseases was reported in the South-Indian population [28]. Polymorphisms in FOXP3 could affect binding capability for transcription factors and, hence, FOXP3 expression and Treg function [33]. Interestingly, Nam and his colleagues [31] pointed that -2383C/T SNP is situated in a DNA binding site for activating enhancer-binding protein 4 (AP4), and it is predicted that SNP in FOXP3 -2383C/T will lead that AP4 cannot bind to DNA with the T allele of -2383C/T SNP. Therefore, although the understanding of the molecular mechanism of AP4 and FOXP3 in Tregs is limited, the modification of AP4 binding affinity in individuals homozygous for -2383T allele may induce less clonal expansion of Tregs and relative proliferation of effector lymphocytes.The findings of this study have to be seen in light of some limitations. The primary limitation to the generalization of these results is the sample size. Further study including larger sample size is important to improve our data. Although limited studies on pediatric ALL helped us to identify new gaps in the prior literature, it emphasized the need for further development in the area of this disease.

Table 6: Association of the most common clinical findings of the disease and FOXP3&ROR-γ gene polymorphisms in ALL patients

	WBCs –risk		Age risk		Response		ALL-Type		Relapse		CNSII		CNSIII	
	Low	**High**	**Low**	**High**	**Yes**	**No**	**B-cell**	**T-cell**	**Yes**	**No**	**Yes**	**No**	**Yes**	**No**
	83	35	75	53	98	30	107	21	26	102	83	31	42	61
	FOXP3 (-3279C/A) (rs3761548)													
CC	38	16	28	**30***	44	13	46	12	12	46	36	16	19	30
	45.8%	45.7%	37.3%	**56.6%**	44.9%	43.3%	43.0%	57.1%	46.2%	45.1	43.4%	51.6%	45.2%	49.2%
CA	20	8	**28*****	4	21	12	29	3	**12***	20	21	8	11	14
	24.1%	22.9%	**37.3%**	7.5%	21.4%	40.0%	27.1%	14.3%	**46.2%**	19.6%	25.3%	25.8%	26.2%	23.0%
AA	25	11	19	19	33	5	32	6	2	36**	26	7	12	17
	30.1%	31.4%	25.3%	35.8%	33.7%	16.7%	29.9%	28.6%	7.7%	35.3%	31.3%	22.6%	28.6%	27.9%
	FOXP3 (-2383C/T) (rs3761549)													
CC	18	11	24	10	**21***	13	26	8	**12***	22	23	6	13	10
	21.7%	31.4%	32%	18.9%	**21.4%**	43.3%	24.3%	38.1%	**46.2%**	21.6%	27.7%	19.4%	31.0%	16.4%
CT	53	18	40	36	62	14	66	10	12	64	48	20	22	42
	63.9%	51.4%	53.3%	67.9%	63.3%	46.7%	61.7%	47.6%	46.2%	62.7%	57.8%	64.5%	52.4%	68.9%
TT	12	6	11	7	15	3	15	3	2	16	12	5	7	9
	14.5%	17.1%	14.7%	13.2%	15.3%	10.0%	14.0%	14.3%	7.7%	15.7%	14.5%	16.1%	16.7%	14.8%

5. Conclusion

In conclusion, our preliminary data throws light on the potential role of FOXP3 (-3279C/A) gene polymorphisms in ALL susceptibility in Egyptian children. Analysis of selected SNPs denotes that FOXP3 -3279CC, AA genotype, -3279C allele, and -2383CT genotype in female patients in addition to -2383C allele in males are considered as a risk factor for ALL in Egyptian population. Taking a deep look into clinical manifestations of the diseases, FOXP3 -2383CC genotype showed a remarkable decrease in treatment responder patients and an increase in relapsed ones. Collectively, the study results might help in understanding the FOXP3 genetic predisposition in pediatric ALL subjects which could affect their immunological status and the implication of these detected mutations in ALL risk.

6. Recommendations

We highly recommended to conduct further study on larger sample size and to consider measurement of FOXP3 protein expression. More clarification in the role of FOXP3 polymorphisms in controlling the expression of FOXP3 in ALL patients and its correlation with the detected mutation is quite important.

7. Conflict of interest

The authors declare that they have no conflict of interest.

8. Role of the funding source

No funding source

References

[1]. S.C.Bernard, E.H. Abdelsamad, P.A. Johnson, D.L. Chapman, andM.Parvathanen, “Pediatric Leukemia: Diagnosis to Treatment–A Review,”J Cancer Clin Trials, vol. 2, pp.30, 2017.

[2]. M.A. Smith, S.F.Altekruse, P.C. Adamson, G.H.Reaman, andN.L. Seibel, “Declining childhood and adolescent cancer mortality,”Cancer, vol. 120, pp.2497-506, 2014.

[3]. P.Bhatia, S.Masih, N.Varma, D.Bansal, andA.Trehan, “High Expression of Lung Resistance Protein mRNA at Diagnosis Predicts Poor Early Response to Induction Chemotherapy in Childhood Acute Lymphoblastic Leukemia,”Asian Pac J Cancer Prev, vol. 16, pp.6663-8, 2015.

[4]. A.Miranda-Filho, M.Piñeros, J.Ferlay, I.Soerjomataram, A.Monnereau, andF.Bray, “Epidemiological patterns of leukaemia in 184 countries: a population-based study,”LancetHaematol, vol. 5, pp.e14-e24,2018.

[5]. S.Sakaguchi, “Regulatory T cells: key controllers of immunologic self-tolerance,”Cell, vol. 101, pp.455-8, 2000.

[6]. A.Huynh, M.DuPage, B.Priyadharshini, P.T.Sage, J.Quiros, C.M.Borges, N.et al., “Control of PI(3) kinase in Treg cells maintains homeostasis and lineage stability,”NatImmunol, vol. 16, pp.188-96,2015.

[7]. Y. Tang, X.Xu, S.Guo, C. Zhang , Y. Tang , Y.Tian , et al., “An increased abundance of tumor-

infiltrating regulatory T cells is correlated with the progression and prognosis of pancreatic ductal adenocarcinoma,".PLoS ONE, vol. 9, pp. e91551, 2014.

[8]. J. Shou,Z.Zhang,Y. Lai, Z. Chen,andJ.Huang, "Worse outcome in breast cancer with higher tumor-infiltrating FOXP3+Tregs: a systematic review and metaanalysis,"BMC Cancer, vol. 16, pp. 687, 2016.

[9]. C.A.LindqvistandA.S.Loskog, "T regulatory cells in B-cell malignancy – tumour support or kiss of death?,"Immunology, vol.135, pp.255-60, 2012.

[10]. J.D.Fontenot, M.A.Gavin,andA.Y.Rudensky, "FOXP3 programs the development and function of CD4+CD25+regulatory T cells,"NatImmunol, vol. 4, pp.330-6, 2003.

[11]. Y.Feng, A.Arvey, T.Chinen, J.van der Veeken, G.Gasteiger, andA.Y.Rudensky."Control of the inheritance of regulatory T cell identity by a cis element in the FOXP3 locus,"Cell, vol. 158, pp.749-63, 2014.

[12]. M.E.Brunkow, E.W.Jeffery, K.A.Hjerrild, B.Paeper, L.B.Clark, S.A.Yasayko, et al., "Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse,"Nat. Nat Genet, vol. 27, pp.68-73, 2001.

[13]. Z.Cheng, Y.Guo,andL.Ming, "Functional FOXP3 polymorphisms and the susceptibility to cancer: An update meta-analysis,"Medicine (Baltimore), vol. 97, pp.e11927, 2018.

[14]. W. M.Bassuny, K.Ihara, Y.Sasaki, R.Kuromaru, H.Kohno, N.Matsuura ,et al., "A functional polymorphism in the promoter/enhancer region of the FOXP3/Scurfin gene associated with type 1 diabetes,"Immunogenetics, vol. 55, pp.149- 56, 2003.

[15]. R.M.Talaat, S.M.Seada, G.I.Mohamed, A.T.Abdel-Moez,andM.Mokhles, "Polymorphism of FOXP3 in Egyptian Patients with Hepatocellular Carcinoma (HCC),"EC Gastroenterology and Digestive System, vol. 5.2, pp.97-106, 2018.

[16]. M.Smith, D.Arthur, B.Camitta, A.J.Carroll, W.Crist, P.Gaynon,et al.,"Uniformapproach to risk classification and treatment assignment forchildren with acutelymphoblasticleukemia,"JClinOncol, vol. 14, pp.18-24, 1996.

[17]. S.Kishi, W.Yang, B.Boureau, S.Morand, S.Das, P.Chen, E.H.et al., "Effects of prednisone and genetic polymorphisms on etoposide disposition in children with acutelymphoblasticleukemia,"Blood, vol. 103, pp.67-72, 2004.

[18]. N.Inoue, M.Watanabe, M.Morita, R.Tomizawa, T.Akamizu, K.Tatsumi, et al., "Association of functional polymorphisms related to the transcriptional level of FOXP3 with prognosis of autoimmune thyroid disease,"ClinExpImmunol, vol.162, pp.402-6, 2010.

[19]. L.L.JiangandL.W.Ruan, "Association between FOXP3 promoter polymorphisms and cancer risk: A meta-analysis. OncolLett, vol. 8, pp.2795-99,2014.

[20]. G.Roncador, J.F.Garcia, J.F Garcia, L.Maestre, E.Lucas, J.Menarguez, et al., "FOXP3, a selective marker for a subset of adult T-cell leukemia/lymphoma,"Leukemia, vol. 19,pp.2247-53.2005.

[21]. Z.Piao, H.J.Kim, J.Y.Choi, C.R.Hong, J.W.Lee, H.J.Kang, et al., "Effect of FOXP3 polymorphism on the clinical outcomes after allogeneic hematopoietic stem cell transplantation in pediatric acute leukemia patients,"IntImmunopharmacol, vol. 31, pp.132-9, 2016.

[22]. N. Chatrabnous, A. Ghaderi, A. Ariafar, M.S. Razeghinia, M. Nematiand A. Jafarzadeh, " Serum concentration of interleukin-35 and its association with tumor stages and FOXP3 gene polymorphism

in patients with prostate cancer,"Cytokine, vol. 113, pp. 221-7 , 2019.

[23]. Y. Chen, X. Qi, C. Bian, C. Ling, T. Yi, X. Mu, et al., " The association of FOXP3 gene polymorphisms with cancer susceptibility: a comprehensive systemic review and meta-analysis,"Biosci Rep, vol. 39, pp.BSR20181809, 2019.

[24]. F. Cezar-Dos-Santos, R.S. Ferreira, N.C.M.Okuyama, K.P. Trugilo, M.M. Sena, É.R. Pereira, et al.," FOXP3immunoregulatory gene variants are independent predictors of human papillomavirus infection and cervical cancer precursor lesions,"J Cancer Res ClinOncol, vol. 145, pp. 2013-25,2019

[25]. T. Flauzino, D.F. Alfieri, W.L. de Carvalho Jennings Pereira, S.R. Oliveira, A.P. Kallaur, M.A.B. Lozovoy, et al., " The rs3761548 FOXP3 variant is associated with multiple sclerosis and transforming growth factor β1 levels in female patients,"Inflamm Res, vol. 68, pp.933-43, 2019.

[26]. H. Thude, P. Tiede, M. Sterneck, B. Nashan, M. Koc, "Impact of TBX21, GATA3, and FOXP3 gene polymorphisms on acute cellular rejection after liver transplantation,"HLA, vol. 93, pp. 97-101, 2019.

[27]. S.L. Xia, S.J. Ying, Q.R. Lin, X.Q. Wang, W.J. Hong, Z.J. Lin, et al.,"Association of Ulcerative Colitis with FOXP3 Gene Polymorphisms and Its Colonic Expression in Chinese Patients," Gastroenterol Res Pract, vol. 2019, pp. 4052168, 2019.

[28]. N. Fathima, P. Narne and M. Ishaq, "Association and gene-gene interaction analyses for polymorphic variants in CTLA-4 and FOXP3 genes: role in susceptibility to autoimmune thyroid disease,"Endocrine, vol. 64, pp.591-604, 2019.

[29]. S. Xia, D. Zhang, S. Zheng, C. Wu, Q. Lin, S. Ying, et al.,"Association of Crohn's disease with Foxp3 gene polymorphisms and its colonic expression in Chinese patients,"JClin Lab Anal, vol. 33, pp. e22835, 2019.

[30]. M.A. Elsohafy, A.A. Elghzaly, H.M. Abdelsalam and M.A. Gaballah,"Assessment of the Possible Role of FOXP3 Gene (rs3761548) Polymorphism in Psoriasis Vulgaris Susceptibility and Pathogenesis: Egyptian Study,"IndianDermatol Online J, vol. 10, pp. 401-5, 2019.

[31]. M.Nam, S.Shin, K.U.Park, I.Kim, S.S.Yoon, T.K.Kwon,et al., "Association of FOXP3 Single Nucleotide Polymorphisms With Clinical Outcomes After Allogenic Hematopoietic Stem Cell Transplantation,"Ann Lab Med,vol. 38,no.6 pp.591– 8, 2018.

[32]. M. Moradi, S. Naeimi, S. Asadzade and A. Rahi," Genetic association study of promoter variation rs3761549 in the FOXP3 gene of Iranian patients diagnosed with brain tumour,"J Cell Biochem, vol. 10.1002/jcb.28473, 2019.

[33]. J.M. Oda, B.K. Hirata, R.L. Guembarovski,M.A. Watanabe, "Genetic polymorphism in FOXP3 gene: imbalance in regulatory T-cell role and development of human diseases,"J Genet, vol. 92, pp.163-71, 2013.