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Dual biomarkers long non-coding RNA GAS5 and its target, NR3C1, contribute to acute myeloid leukemia

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ABSTRACT

Acute myeloid leukemia (AML) is a complex hematological neoplasm with poor prognosis. At present, overwhelming evidence indicates that different genetic abnormalities are relevant to the pathogenesis of AML. Nevertheless, its exact molecular mechanism is still unknown. Recently, it was reported that lncRNAs play crucial roles in tumorigenesis. But, their role in the molecular pathogenesis of AML has not been extensively explored. GAS5, one of the earliest known lncRNAs, has an essential role in the formation and progression of multiple human cancers. It was recently demonstrated that GAS5 acts as a riborepressor of the Glucocorticoid receptor) GR) and abnormal levels of GAS5 may alter response of hematopoietic cells to glucocorticoids. GAS5 can have interaction with the GR that encoded by *NR3C1* gene and inhibit its transcriptional activity.

To test whether the genetic variants can be associated with AML risk, we genotyped rs55829688 (T > C) polymorphism in *GAS5* and three *NR3C1* SNPs namely rs6195, rs41423247 and rs6189/rs6190 in a population of 100 Iranian AML patients and 100 healthy subjects.

The analysis of the data showed the frequency of alleles and genotypes of rs55829688 and rs6189/rs6190 polymorphisms did not differ between patients and healthy subjects. But, rs41423247 and rs6195 demonstrated a significant correlation with AML risk. The rs6195 was associated with higher AML susceptibility in the co-dominant (OR = 4.58, 95% CI = 2.11–9.981, $P < .0001$), dominant (OR = 4.55, 95% CI = 2.155–9.613, $P < .0001$), and over-dominant (OR = 4.43, 95% CI = 2.042–9.621, $P < .0001$) models. Also, the rs41423247 polymorphism was associated with higher risk of AML in co-dominant (OR = 2.07, 95% CI = 1.171–4.242, $P = .012$) and dominant (OR = 2.47, 95% CI = 1.192–5.142, $P = .010$) models. Furthermore, haplotype analysis (rs41423247, rs6189.rs6190, rs6195, and rs55829688 respectively) demonstrated that GGAT, CGGT, and GGGT haplotypes were associated with higher risk of AML in the studied population (*p*-values = .007, 0.042 and 0.044, respectively). The present study reveals a possible role for NR3C1 in the pathogenesis of AML.

1. Introduction

Acute myeloid leukemia (AML) refers to a complex hematological neoplastic disorder with poor prognosis, which is the results of clonal proliferation of immature myeloid progenitor cells in bone marrow and peripheral blood (Wu et al., 2015). This disease is the most frequent type of malignant myeloid disorder in adults with an incidence of approximately 2–4/100,000 per year (http://seer.cancer.gov/).

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Moreover, its incidence increases with population age (De and Abdul-Hay, 2016). Although the exact etiology of AML is unknown, it is accepted that interaction between different genetic factors and environmental factors such as chemical exposure, ionizing radiation and smoking leads to AML progression (Alazhary et al., 2015; Fei et al., 2015). Recently, extensive molecular studies have shown that various genetic abnormalities play important roles in the pathogenesis of AML (El et al., 2017). Currently, key genetic alterations including those in DNMT3A, WT1, FLT3-ITD, CEBPA and NPM1 have been discovered as prognostic factors to predict disease course and response of AML patients to therapeutic options. Nevertheless, these markers describe only a few prognostic factors for AML (Kapranov et al., 2007; Zhang et al., 2015; Lu et al., 2016).

More recently, genome and transcriptome studies have demonstrated a novel class of non-coding RNAs, termed long noncoding RNAs (lncRNAs), which are mRNA-like transcripts with a transcription length of greater than 200 base pairs and lack of protein-coding potential (Papaioannou et al., 2017). Accumulating evidences suggest that lncRNAs participate in a variety of biological processes such as cell cycle, chromatin remodeling, apoptosis, survival, regulation of transcription and post-transcriptional regulation(Mazraeh et al., 2020; Sayad et al., 2018b; Sayad et al., 2018a; Sayad et al., 2017a; Sayad et al., 2017b). Genetic alterations in the lncRNAs can change their structure, stability thus influencing related biological pathways (Xing et al., 2015). In the recent decades, increasing number of studies unveiled the key role of lncRNAs in a wide variety of cancers including hematologic malignancies. Therefore, understanding their biological process may increase the potential application of these molecules as biomarkers for diagnosis and therapy of these diseases (Gao and Wei, 2017; Li and Wang, 2016). Recently, it was acknowledged that lncRNAs have a critical role in the pathogenesis of AML. However, molecular mechanisms are still largely unknown (Zebisch et al., 2016).

The growth arrest-specific transcript 5 (*GAS5*) is one of the earliest known lncRNA that is located in the 1q25 region of the human genome and consists of 12 exons. It can exert an inhibitory effect on cell proliferation and stimulate apoptosis which thus acting as a tumor suppressor gene. Evidence indicates that GAS5 plays a potential role in the formation and progression of multiple human cancers, including breast cancer, prostate cancer, lung cancer, gastric cancer and cervical cancer which suggests GAS5 can be considered as a new diagnostic and prognostic biomarker and a therapeutic target (Gao et al., 2017; Pickard and Williams, 2015).

It is recently reported that GAS5 acts as a riborepressor of the Glucocorticoid receptor) GR(. It can interact with the GR and inhibit its transcriptional activity. GAS5 binds with DNA binding domain of the GR and competes with glucocorticoid response elements (GREs) for binding with GR, thus repressing the transcription activity induced by this receptor (Pickard and Williams, 2015; Lucafo et al., 2015; Kino et al., 2010). Glucocorticoids (GCs) are steroid ligands that exert their effects on target cells via GR (El-Fayoumi et al.). Glucocorticoids play a key role in stimulation of erythropoiesis and are also involved in the formation of platelets and white blood cells (WBCs) (Beato et al., 1995; Lucafò et al., 2018; Hattangadi et al., 2011; Lee et al., 2015; Schwingshackl et al., 2016). In many previous studies, it has been observed that a number of single nucleotide polymorphisms (SNPs) are presented in the GR gene (*NR3C1*) which mainly lead to alteration in its function in the responses to GCs and causes various diseases (El-Fayoumi et al., 2018; Gu et al., 2017).

Based on these evidences, we hypothesized that genetic variants in GAS5 and its target gene NR3C1 may play a key role in the susceptibility of AML, thus being important for prognosis and risk stratification of AML. Accordingly, to test this hypothesis, we conducted a case-control study to assess the possible effects of rs55829688 (T > C) polymorphism in *GAS5* and three *NR3C1* SNPs (rs6195, rs41423247 and rs6189/rs6190) in Iranian AML patients and healthy subjects.

Table 1
The clinical characteristics of AML patients.

	Cases(n = 100)	Healthy controls (n = 100)
Sex		
Female, n (%)	42	44
Male, n (%)	58	56
WBC count, $\times 10^9$/L (median, range)	9.2(0.11–665)	–
Platelet count, $\times 10^9$/L /L (median, range)	61 (5–1117)	–
FAB subtype		
AML M0	15	
AML M1	22	
AML M2	36	
AML M4	14	
AML M5	4	
AML M6	8	
AML M7	1	
Cytogenetics available		
Normal karyotype, n (%)	23	
Trisomy 8, n (%)	18	
Trisomy 11, n (%)	12	
Trisomy 13, n (%)	13	
Trisomy 21, n (%)	7	
Other trisomies, n (%)	3	
−7/del(7q), n (%)	8	
del(5q), n (%)	2	
del(9q), n (%)	3	
del(20q), n (%)	2	
Other deletions, n (%)	2	
Others or combinations of 2 of above aberrations, n (%)	7	

2. Materials and methods

2.1. The study population

A total of 100 AML patients with average age of 64.6 years (range: 4.3–80.1 years), and 100 healthy controls with average age of 58.3 years (range: 4.8–69.6 years) participated in this case-control study. All patients were referred to the Division of Medical Genetics, Tabriz Children's Hospital for diagnosis of AML between January 2016 and May 2018. AML was detected according to the World Health Organization (WHO) criteria and French-American-British (FAB) (Table 1). All patients had de novo AML without any myelodysplastic syndrome and their clinical specifications were available.

2.2. Genomic DNA extraction and genotyping

The rs55829688, rs6195, rs41423247 and rs6189/rs6190 SNPs were genotyped by restriction fragment length polymorphism (RFLP) method. For PCR, the genomic DNA of all study participants was extracted from peripheral blood samples using the DNA isolation kit (Gene All, Germany), according to the manufacturer's protocol. Primer sequences and specific annealing temperatures are listed in Table 2. PCR reactions were prepared in total volume of 25 μL containing 12.5 μL Ampliqon master mix, 1 μL of each primer (10 μM), 2 μL of template DNA (50 ng/μL) and 8.5 μL of sterile deionized water. Thermocycling conditions were as follows: initial denaturation at 95 °C for 5 min and 35 cycles of PCR consisting of denaturation at 95 °C for 30 s, specific annealing temperatures for 30 s and extension at 72 °C for 30 s, and final extension step of 72 °C for 5 min. Successful amplification was confirmed by detection of PCR product band on a 2% agarose gel using a 100 bp DNA ladder (Fermentase, Germany).

After PCR amplification, for subsequent digestion of PCR products each PCR reaction was incubated with an appropriate restriction enzyme. The BsrdI, TasI, *Bcl*I and *Mnl*I restriction enzymes (Fermentase, Germany) which were identified as appropriate enzymes for digestion

Table 2
Primer sequences and restriction enzymes used for genotyping of SNPs of the NR3C1 and GAS5 genes.

SNPs	Forward and reverse primers	PCR products	Annealing temperature	Restriction enzyme	Digest fragments (bp)
rS55829688	F: 5′-TGGCTTAGAAGTCCCAGTCA-3′ R: 5′- CGTCCCGGAAGTGAAATCC-3′	500 bp	59 °C	BsrdI	Allele C: 295,205
ER22/23EK (rs6189/rs6190)	F: 5′-TTGATTCGGAGTTAACTAAAAGG-3′ R: 5′-ATCCCAGGTCATTTCCCATC-3′	444 bp	60 °C	MnlI	Allele G: 163,143,50,50,35,3 Allele A: 178,163,50,50,3
BclI (rs41423247)	F: 5′-GAGAAATTCACCCCTACCAAC-3′ R: 5′- AGAGCCCTATTCTTCAAACTG-3′	418 bp	58 °C	BclI	Allele G: 263,155
N363S (rs6195)	F: 5′-CCAGTAATGTAACACTGCCCC-3′ R: 5′-TTCGACCAGGGAAGTTCAA-3′	357 bp	59 °C	TasI	Allele A:135, 73, 70, 60, 19 Allele G:135, 92,70,60

of PCR products by NEBCutter program (http://nc2.neb.com/NEBcutter2/) were used for genotyping of rs55829688, rs6195, rs41423247, and rs6189/rs6190, respectively. The detailed descriptive information of restriction enzymes is presented in Table 2. Briefly, 10 μL of PCR product was mixed with 1.5 U of restriction enzyme, 2 μL of 10× Buffer, 10 μL of deionized water, then preparations were incubated at the temperature required for each enzyme (as shown in Table 2). The digested DNA products and the DNA size marker were electrophoresed on a 3% agarose gel and stained with safe stain. To document the result, gel pictures were taken under ultraviolet light. The digested pattern of BsrdI endonuclease for rs55829688 amplification product was 500 bp for the TT; 295 bp and 205 bp for the CC and 500 bp, 295 bp, and 205 bp segments for the TC genotypes (Table 2). The digested pattern of TasI endonuclease for rs6195 amplification product was as follows: 135, 73, 70, 60 and 19 bp segments for the AA; 135, 92, 70 and 60 bp for the GG and 135, 92, 73, 70, 60 and 19 bp for the AG genotype (Table 2). *Bcl*I digested the rs41423247 amplification product in the following manner: 263 bp and 155 bp for the GG, 418 bp for the CC, 418 bp, 263 bp and 155 bp segments for the GC genotypes. *Mnl*I digested the rs6189/rs6190 amplification products had the following pattern: 163, 143, 50, 50, 35 and 3 bp and for AA; 178, 163, 50, 50 and 3 bp for GG and 178, 14 and 35 bp for the AG genotypes (Table 2).

2.3. Statistical analysis

Statistical analyses were carried out using the SPSS software (version16). Accordance with Hardy–Weinberg equilibrium (Lu et al.) was evaluated by Fisher's exact test to compare the observed genotype frequencies with the expected ones among the control subjects. Associations between each genotype and AML risk were assessed by measuring odds ratios (ORs) and 95% confidence intervals (CIs) in four different inheritance models including recessive, dominant, co-dominant, and over-dominant models. In addition, haplotype analysis was finally conducted using SNPStats online software (https://www.snpstats.net/snpstats/start.htm). The level of the statistical significance was set at P value < .05.

3. Results

In the present study, we genotyped lncRNA *GAS5* rs55829688 SNP and three *NR3C1* SNPs namely rs41423247, rs6189/rs6190a nd rs6195 in all study participants. Genotype distributions of four SNPs in AML patients and healthy subjects are given in Table 3. Statistical analysis showed that there were no significant deviations from Hardy–Weinberg equilibrium (Lu et al.) for rs6195, rs6189/rs6190 and rs55829688 polymorphisms either in cases or controls (P values > .05). However, distribution of rs41423247 genotype in patients (p = .00055) but not in controls (p = .16) showed a significant departure from Hardy–Weinberg equilibrium.

Subsequently, the associations of the genotypes with AML risk were calculated in four inheritance models (co-dominant, dominant, over-dominant and recessive models). There were no significant association between the rs6189/rs6190 and rs55829688 genotypes and AML in any of the inheritance models (Table 4). But, significant associations were observed between rs6195 and rs41423247 SNPs and AML risk. For the rs6195 A > G polymorphism, G allele carriers have a significantly increased AML risk (OR = 4.58, 95% CI = 2.11–9.981, P < .0001) in the co-dominant model. Similarly, significant associations were observed in the dominant (AG + GG vs. AA; OR = 4.55, 95% CI = 2.155–9.613, P < .0001) and over-dominant (AG vs. AA/GG; OR = 4.43, 95% CI = 2.042–9.621, P < .0001) models. For the rs41423247 C > G polymorphism, G allele carriers had susceptibility for AML (P = .012, OR = 2.078, 95% CI = 1.171–4.242) in co-dominant (CG vs. CC genotype) model. Similarly, significant association were observed in a dominant model (CG + GG vs. CC; P = .010, OR = 2.475, 95%CI = 1.192–5.142) (Table 4).

Table 3
Hardy-weinberg equilibrium statistics.

rs41423247		CC	CG	GG	P-value of HWE
	All subjects	40(20%)	125(62%)	35(18%)	0.0064
	Patients	13(13%)	68(68%)	19(19%)	0.00055
	Controls	27(27%)	57(57%)	16(16%)	0.16
rs6189.rs6190		GG	AG	AA	P-value of HWE
	All subjects	179(90%)	19(10%)	2(1%)	0.13
	Patients	88(88%)	11(11%)	1(1%)	0.34
	Controls	91(91%)	8(8%)	1(1%)	0.21
rs6195		AA	AG	GG	P-value of HWE
	All subjects	153(76%)	43(22%)	4(2%)	0.89
	Patients	64(64%)	33(33%)	3(3%)	0.76
	Controls	89(89%)	10(10%)	1(1%)	0.3
rs55829688		TT	TC	CC	P-value of HWE
	All subjects	126(63%)	68(34%)	6(3%)	0.51
	Patients	66(66%)	30(30%)	4(4%)	0.75
	Controls	60(60%)	38(38%)	2(2%)	0.23

3.1. Haplotype analysis and linkage disequilibrium

The results of the haplotype analysis demonstrated that theoretically there were 16 possible haplotypes, which were derived from four polymorphic sites; however, just the frequencies of nine haplotypes were > 1%. The results of haplotype analysis between AML patients and healthy controls are shown in Table 5. Haplotype analysis (rs41423247, rs6189/rs6190, rs6195 and rs55829688 respectively) indicated that GGAT, CGGT, and GGGT were significantly higher in AML patients and were associated with 1.6, 3.23 and 5.1 folds increase in the risk of AML, respectively(Table 5).

In addition, the linkage disequilibrium patterns of four SNPs

Table 4
Association between SNPs, rs41423247, rs6189.rs6190, rs6195 and rs55829688 and AML.

SNPs	Models	Alleles and genotypes	Frequency in AML group, number (%)	Frequency in control group, number (%)	Odds ratio (95% confidence intervals)	P value
rs41423247	Allele	G vs. C	106(53%)	89(44%)	1.406 (0.948–2.085)	0.109
	Co-dominant	GG vs. CC	19 (19%)	16 (16%)	2.466 (0.965–6.302)	0.057
		CG vs. CC	68 (68%)	57 (57%)	2.078 (1.171–4.242)	0.012
	Dominant	CC	13 (13%)	27 (27%)	2.475 (1.192–5.142)	0.010
		CG + GG	87 (87%)	73 (73%)		
	Recessive	CC + CG	81 (81%)	84 (84%)	1.231 (0.592–2.56)	0.710
		GG	19 (19%)	16 (16%)		
	Over dominant	CC + GG	32 (32%)	43 (43%)	1.603 (0.9–2.855)	0.143
		CG	68 (68%)	57 (57%)		
rs6189.rs6190	Allele	A vs. G	13(6%)	10(5%)	1.321 (0.5653–3.086)	0.668
	Co-dominant	AA vs. GG	1 (1%)	1 (1%)	1.034 (0.063–16.02)	0.743
		AG vs. GG	11 (11%)	8 (8%)	1.031 (0.74–25.433)	0.770
	Dominant	GG	88 (88%)	91 (91%)	1.333 (0.588–3.023)	0.489
		AG + AA	12 (12%)	9 (9%)		
	Recessive	GG + AG	99 (99%)	99 (99%)	1.000 (0.63–15.767)	0.751
		AA	1 (1%)	1 (1%)		
	Over dominant	GG + AA	89 (89%)	92 (92%)	1.421(0.546–3.698)	0.6306
		AG	11 (11%)	8 (8%)		
rs6195	Allele	G vs. A	39(20%)	12(6%)	3.795 (1.922–7.493)	0.00004
	Co-dominant	GG vs. AA	3 (3%)	1 (1%)	4.172 (0.424–41.02)	0.4169
		AG vs. AA	33 (33%)	10 (10%)	4.589 (2.11–9.981)	P < .0001
	Dominant	AA	64 (64%)	89 (89%)	4.551 (2.155–9.613)	*P* < .0001
		AG + GG	36 (36%)	11 (11%)		
	Recessive	AA + AG	97 (97%)	99(99%)	3.062(0.313–29.94)	0.3106
		GG	3 (3%)	1 (1%)		
	Over dominant	AA + GG	67 (67%)	90 (90%)	4.433 (2.042–9.621)	P < .0001
		AG	33 (33%)	10 (10%)		
rs55829688	Allele	C vs. T	38(19%)	42(21%)	0.8824 (0.540–1.441)	0.7078
	Co-dominant	CC vs. TT	4 (4%)	2 (2%)	1.818 (0.321–10.29)	0.798
		CT vs. TT	30 (30%)	38 (38%)	0.717 (0.396–1.298)	0.343
	Dominant	TT	66 (66%)	60 (60%)	0.772 (0.434–1.374)	0.464
		CT + CC	34 (34%)	40 (40%)		
	Recessive	TT + CT	96 (96%)	98 (98%)	2.042 (0.365–1141)	0.341
		CC	4 (4%)	2 (2%)		
	Over dominant	TT + CC	70 (70%)	62 (62%)	0.699 (0.388–1.259)	0.296
		CT	30 (30%)	38 (38%)		

(rs41423247, rs6189/rs6190, rs6195 and rs55829688 respectively) were analyzed by calculation of D' and r values (S1 and S2). Linkage disequilibrium analyses indicated no strong linkage between SNPs in the assessed population.

4. Discussion

Emerging evidence indicates that lncRNAs are critically involved in a variety of human tumor entities, including AML (Schmitt and Chang, 2016; Jariwala and Sarkar, 2016). Thus, the identification of cancer-associated lncRNAs might reveal novel prognostic biomarkers or new therapeutic strategies for the treatment of human cancer (Wei and Wang, 2015; Zebisch et al., 2016). GAS5 is one of the best-studied lncRNA that was discovered and structurally characterized in 1992 by Coccia et al. (Zheng et al., 2016).

As a tumor suppressor gene, *GAS5* plays a crucial role in apoptosis and its expression is significantly increased during growth arrest. Previous studies indicated that GAS5 expression is downregulated in many types of cancers, including colorectal cancer, gastric cancer, breast cancer, cervical cancer, glioblastoma, and non-small cell carcinoma (Yin et al., 2014; Sun et al., 2014; Mourtada-Maarabouni et al., 2009; Cao et al., 2014; Toraih et al., 2018; Qiao et al., 2013). Interestingly, in the study conducted by Tu et al., patients with higher GAS5 expression showed susceptibility to hepatocellular carcinoma, which indicates GAS5 may act as a proto-oncogene in this king of cancer (Tao et al., 2015). Nevertheless, the role of GAS5 in AML is unknown. Recently, Yan et al. have identified that the rs55829688 (T > C) in the promoter region of GAS5 is significantly associated with prognosis of AML. They reported that patients with rs55829688 CC genotype have higher GAS5 expression in peripheral blood mononuclear cells and harbored a longer platelets recovery than carriers of rs55829688T allele (Yan et al., 2017).

Table 5
Analysis for the Haplotype frequencies of GAS5 and NR3C1 between AML cases and controls.

rs41423247	rs6189.rs6190	rs6195	rs55829688	Total frequency	Frequency in AML group	Frequency in control group	OR (95% CI)	P value
C	G	A	T	0.4156	0.3373	0.492	1.00	
G	G	A	T	0.2898	0.3412	0.2424	1.6 (0.87–3.02)	0.007
G	G	A	C	0.1124	0.071	0.1573	0.42(0.16–1.09)	0.29
C	G	G	T	0.0332	0.0508	0.0163	3.23(0.5319.63)	0.042
G	G	G	T	0.0316	0.0492	0.0104	5.10 (0.58–44.6)	0.044
C	G	A	C	0.0298	0.0385	0.0174	2.28(0.36–14.2)	0.11
G	G	G	C	0.0281	0.0432	0.0141	3.16(0.46–21.7)	0.071
C	A	G	C	0.0143	0.0282	0.0139	2.18(0.2617.84)	0.19
G	A	A	T	0.0114	0.0117	0.0108	1.1(0.07216.75)	0.48

Furthermore, emerging evidence has suggested that GAS5 acts as a crucial mediator of GC in peripheral blood mononuclear cell. It is known that GCs exert their function by activating GRs. Thus, GAS5 may alter GCs effectiveness probably through interfering with the mechanism of GR autoregulation (Lucafo et al., 2015). Recently in some studies, GCs have been reported as a promoter for the formation of white blood and platelets (Schwingshackl et al., 2016). Generally, response to GCs is determined by the occurrence of genetic variants in the *NR3C1* gene. It was demonstrated that there are many polymorphisms in the GR gene (NR3C1) which leads to changes in GC expression in target tissues and subsequently cause various diseases. Furthermore, in gastric cancer, childhood acute lymphoblastic leukemia, and lung cancer, NR3C1 SNPs were significantly associated with the cancer susceptibility. The most commonly reported functional polymorphisms within this gene are rs41423247, rs6189/rs6190 and rs6195 (Kadmiel and Cidlowski, 2013; Gu et al., 2017; Cihan et al., 2017; Zhu et al., 2017).

The polymorphism rs41423247 C > G is located in the intron 2 of the *NR3C1* gene and leads to hypersensitivity to GCs (Kadmiel and Cidlowski, 2013). The rs6195 A > G and rs6189/rs6190 G > A are located in exon 2 of the *NR3C1* gene. The rs41423247 SNP is correlated with increased sensitivity of GR to GCs, thereby it increases the action of the hormone. The rs6195 SNP is associated with decrease GC sensitivity. The rs6189/rs6190 SNP affects the tertiary structure of the NR3C1 protein and causes decrease receptor capability of target gene transactivation (Panek et al., 2013). Extensive researches have linked these SNPs with different diseases. For example, Pietras et al. examined the effects of rs41423247 SNP on bronchial asthma risk in the Polish population and observed that carriers of allele G (GG + GC) have a higher risk for bronchial asthma (OR = 5.44, CI 95% = 2.05–14.41) (Pietras et al., 2011). Other studies indicated the association of rs41423247 polymorphism with abdominal obesity, memory, depression, and hypersensitivity to GC treatment (Hauer et al., 2011; Ackermann et al., 2013; Gałecka et al., 2013). In addition, in a previous study conducted by Lin et al., the rs6195 SNP was associated with the raised body mass index and coronary artery diseases (Lin et al., 2003). ElFayoumi et al. evaluated the effects of three *NR3C1* polymorphisms in development of ALL and the toxicity outcome, in terms of liver toxicity, glucose abnormality and infections, in Saudi Children and found that these SNPs were not associated with the development of ALL but they observed that the G allele of rs6195 was associated with the higher risk of toxicity of glucose abnormality. They also depicted that rs41423247 can be consider as a risk factor for liver toxicity and glucose abnormality (El-Fayoumi et al., 2018). Moreover, Akyildiz et al. reported that rs41423247 SNP is significantly related to the family history of breast cancer (Akyildiz et al., 2018). However, the role of *NR3C1* polymorphisms in AML has not been assessed previously.

Our study showed that *NR3C1* might be involved in the progression of AML and this finding further highlighted the importance of this gene in tumorigenesis process. For the first time, we found that rs6195 A > G and rs41423247 C > G in *NR3C1* gene were significantly associated with risk of AML. Moreover, in the rs6195 A > G polymorphism, polymorphic allele carriers had higher risk of AML in dominant, co-dominant and over-dominant models. Regarding the rs41423247 polymorphism, the frequency of the CG + GG was significantly higher in AML patients than in the healthy controls. We also investigated the frequencies of haplotypes. Being defined as a set of linked SNPs, haplotype analysis is a strong method for assessing the risk of a certain disorder (Crawford and Nickerson, 2005). Our results showed that GGAT, CGGT, and GGGT haplotypes exhibited statistically significant difference between patients and controls and were associated with higher risk of AML. The GGAT haplotype consists of the polymorphic allele of rs41423247 polymorphism (i.e. G) and the ancestral alleles of rs6189.rs6190, rs6195, and rs55829688 polymorphisms (i.e. G, A, and T). Similarly, the CGGT haplotype consists of the polymorphic allele of rs6195 polymorphism (i.e. G) and the ancestral alleles of rs41423247 rs6189.rs6190, and rs55829688 polymorphisms (i.e. C, G, and T) while GGGT haplotype has polymorphic alleles of rs41423247 and rs6195 (i.e. G and G) and the ancestral alleles of rs6189.rs6190 and rs55829688 polymorphisms (i.e. G and T). Therefore, these results indicate that rs41423247 and rs6195 polymorphisms have a strong link with AML. The G alleles of rs41423247 and rs6190 lead to increased sensitivity to the GCs. The rs41423247 polymorphism changes the processing of the primary GR transcripts through affecting the process of alternative *NR3C1* gene splicing and causes a genetic predilection to GCs sensitivity (Pietras et al., 2011). The rs6195 polymorphism causes alteration of Asparagine to Serine in codon 363 of GR protein. It leads to structural changes in the A/B region of the GR and functional changes within the functional domain AF1 which result in increased transactivation ability of GR. Here, we assume that increased sensitivity of GR to GCs due to GR variations (rs41423247 C > G, rs6195 A > G) may change the expression of GCs in the target tissue and affect their function in hematopoiesis (El-Fayoumi et al., 2018).

It should be noted that although the role of GAS5 and NR3C1 in multiple diseases has been reported, this is the first study investigating polymorphisms of GAS5 and its target gene in relation with AML risk. According to the study conducted by Yan et al., rs55829688 T > C SNP has a functional role in GAS5 expression (Yan et al., 2017). We also speculated that higher expression of GAS5 may inhibit GCs function in hematopoiesis and blood cell formation. However, our results showed no significant association between GAS5 rs55829688 and AML. But, a significant association was observed between the NR3C1 rs6190 and rs41423247 genotypes and risk of AML. Based on the present results, we speculate that the alteration of GAS5 may not affect the function of GCs in the hematopoiesis, while rs6190, rs41423247 SNPs might influence AML development through regulation of GCs expression.

In conclusion, the present population-based case–control study provided the first evidence that NR3C1 rs6190 and rs41423247 polymorphisms might affect the risk of AML in Iranian population. Our results may require confirmation in larger prospective studies in different ethnicities with gene expression and functional assays in future.

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Declration of Competing Interest

The authors declare that they have no competing interests. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed in the current study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

This study was approved by the ethics committee of Tabriz University of Medical Sciences, Tabriz, Iran.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yexmp.2020.104399.

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