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Single Nucleotide Polymorphism rs6859219 (C>A) in the *ANKRD55* Gene is Associated with Rheumatoid Arthritis in the Iranian Population.

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Introduction:

Rheumatoid arthritis (RA) has multifactorial etiology and numerous genetic and environmental factors have been related to an increased risk of RA. Recently, genome-wide association studies (GWAS) suggested a large number of single nucleotide polymorphisms (SNPs) loci affecting susceptibility to RA. One of these loci is rs6859219 (C>A), a functional polymorphism in the *ANKRD55* gene which was associated with the expression of ANKRD55 and IL6ST. A functional study demonstrated that rs6859219 polymorphism is associated with DNA methylation of 5 proximal CpG sites which this methylation was correlated with the expression of IL6ST and ANKDR55 in CD^{4+} T cells. They noted that this intronic locus, known as an enhancer, physically interacts with the IL6ST promoter. IL6ST gene encodes the common cytokine receptor gp130. The receptor systems for IL6, LIF, OSM, CNTF, IL11, CTF1, and BSF3 can utilize IL6ST for initiating signal transmission. By way of example, binding of IL6 to IL6R induces IL6ST homodimerization and formation of a high-affinity receptor complex which activates Janus kinases, leading to the phosphorylation of IL6ST tyrosine residues and finally activation of STAT3. Therefore, dysregulation in IL6, IL6ST, and STAT3 pathways could result in an autoimmune condition. In the current study, we evaluated the possible association between rs6859219 (intronic variant) in the ANKRD55 gene with RA risk in the Iranian population.

Method:

A total of 118 unrelated subjects with RA as a case group and 115 unrelated healthy subjects as a control group were included in this case-control study. Subjects in the case group were recruited from the Alzahra hospitals, Isfahan, Iran. All the RA patients met the diagnostic criteria created by the American College of Rheumatology (ACR). Controls were also selected from the same population with no signs and personal and family history of RA or other immunological and autoimmune conditions. The study was approved by the university ethics board and all participants gave written informed consent.

Approximately, 3 ml of the blood sample was collected into EDTA anticoagulant tubes from each contributor and stored at − 20°C for DNA isolation. DNA was extracted using a DNA isolation kit (GeNet Bio; Korea) consistent with the instruction manual. The real-time polymerase chain reaction high-resolution melting (HRM) method was used to determine rs6859219 polymorphism genotypes. HRM was performed using HOT FIREPol EvaGreen HRM Mix (no ROX) HRM PCR kit which contains HOT FIREPol® DNA Polymerase, 5x EvaGreen® HRM buffer, 12.5 mM MgCl2, dNTPs, Bovine serum albumin (BSA), and EvaGreen dye (Solis BioDyne Estonia).

Results:

Table 1: Baseline characteristics of RA patients and control subjects participated in the study

Characteristics	Patients	Controls	*P*
Total number	118	115	
Age at now	47.3983 ± 9.801	46.0304±12.430	0.238
Gender n (%)			
Male	35(29.7%)	37(32.2%)	0.678
Female	83(70.3%)	78(67.8%)	
Age of onset	42.9492±9.012	--	--
BMI	25.9568±2.353	23.6487±3.353	<0.001*
SBP	121.3559±12.258	120.3478±9.839	0.490
DBP	79.0678±8.058	79.0000±8.589	0.950
Positive family history n (%)	19 (16%)	0	--

Data are mean±SD, or n (%). *P value < 0.05. RA: Rheumatoid arthritis; BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure. SD: Standard deviation

Table 2: Laboratory characteristics of patients with RA and controls group

	Patients (118)	Controls (115)	*P*
ESR (mm/h)	38.1780±25.894	15.9739±6.813	<0.001*
CRP (mg/l)	17.4331±18.862	4.4452±2.586	<0.001*
White blood cell (10^9/1)	7194.067±2211.48101	6607.217±1467.486	0.018*
Hemoglobin (HB)	12.510±1.141	14.156±1.447	<0.001*
PLT(10^9/1)	260.449±61.044	249.2000±66.258	0.179
Creatinine (mg/dL)	1.022±0.183	0.8690±0.191	<0.001*
BUN	17.131±4.747	16.008±4.477	0.065
FBS	95.966±15.310	91.982±22.012	0.109
HDL	49.449±7.572	49.652±11.414	0.873
LDL	109.694±29.332	108.443±36.647	0.773
TG	165.050±47.023	157.191±68.789	0.309

Table 3: Association between genotypes and allele frequency with RA risk

Genotype group	Patients (n = 118) n (%)	Controls (n = 115) n (%)	OR (95%CI)	*P* value
AA	35(30%)	66(57%)	Reference	---
AC	18(15%)	32(28%)	1.06 (0.48, 2.27)	0.99
CC	65(55%)	17(15%)	7.12 (3.51,15.05)	<0.001*
Allele				
A	88(37%)	164(71%)	Reference	---
C	148(63%)	66(29%)	4.16 (2.78, 6.28)	<0.001*
Dominant inheritance				
AA	35(30%)	66(57%)	Reference	---
CC+AC	83(70%)	49(43%)	3.17 (1.79, 5.69)	<0.001*
Recessive inheritance				
AA+AC	53(45%)	98(85%)	Reference	---
CC	65(55%)	17(15%)	7.00 (3.62, 14.11)	<0.001*

Conclusion:

There was a significant difference in the genotype and allele frequencies of rs6859219 between patients and controls (P<0.001). Logistic regression analysis demonstrates that CC genotype and C allele increased the risk of RA (OR for genotype= 6.72; 95%CI [1.75-25.64]/ OR for allele=4.31; 95%CI [2.19-8.47]). Moreover, in the patient group, there was a significant correlation between mean erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) concentration with rs6859219 polymorphism (P< 0.05). Our findings propose a substantial correlation between rs6859219 polymorphism and RA risk and clinicopathological characteristics of this disease in the Iranian population.