ORIGINAL ARTICLE

Association study of rs2243188 (interleukin-19) and rs933717 (MAP1LC3B) polymorphisms with Juvenile systemic lupus erythematosus and Juvenile lupus nephritis in Egyptian population

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ABSTRACT

Background: *An autoimmune connective tissue illness that affects several organs and lowers a patient's quality of life is the juvenile systemic lupus erythematosus (JSLE) and associated juvenile lupus nephritis (JLN). Interleukin-19 (IL-19) has both anti- and pro-inflammatory actions. LC3-associated phagocytosis (LAP) regulates immune responses to dying cells. Polymorphisms disrupting both mechanisms have been correlated to autoimmune diseases.* ***Objective****: to determine whether single-nucleotide polymorphisms (SNPs) of rs2243188 (IL-19) and/or rs933717 (MAP1LC3B) exhibit meaningful relationship with JSLE and /or JLN in the Egyptian population.* ***Methodology:*** *forty-seven JSLE patients and fifty matched healthy controls (HC) were included. SNPs identification was done using allelic discrimination real-time polymerase chain reaction (PCR) technology.* ***Results:*** *Genotyping of rs933717 and rs2243188 showed no statistical difference between the two studied groups with (P=0.206 and 0.468 respectively). Similarly, there was no significant difference in allele frequencies in neither rs933717 nor rs2243188 (P= 0.362 and 0.552 respectively). Twenty-nine out of the 47 JSLE patients (61.7%) displayed JLN symptoms. Similarly, JLN revealed no significant difference in either the genotype distribution or the allele frequency in the dominant/negative model for both genes. Likewise, there was no significant difference in the frequency of any allele concerning rs933717 or rs2243188, (P= 0.309 and 0.196 respectively).* ***Conclusion:*** *Interleukin-19 rs2243188 and MAP1LC3B rs933717 polymorphisms do not seem to play any role in the pathogenesis of JSLE or JLN in Egyptian population.*

INTRODUCTION

Juvenile-onset systemic lupus erythematosus (JSLE), sometimes referred to as pediatric lupus (pSLE) or childhood SLE (cSLE) is a less common form of systemic lupus erythematosus (SLE) that can exhibit severe morbidity and possible mortality [1]. The disease mainly affects females reaching nine times the incidence in males [2]. It is an autoimmune illness that affects several organs and tissues disrupting their normal functions. About half of SLE patients experience a variety of symptoms, such as serositis, neurologic problems and importantly lupus nephritis (LN)[3]. The condition is marked by the generation of numerous autoantibodies production, complement activation and immune complex deposition [4]. The etiology and course of SLE are complex and involve both hereditary and environmental variables, yet the precise mechanism behind SLE remains unclear.

Cytokines are tiny water-soluble intercellular signaling proteins, peptides or glycoproteins that are mainly produced by immune cells. The degree of cytokine production can be influenced by polymorphisms in the regulatory areas of cytokine itself and cytokine receptor genes, which may play significant roles in the pathophysiology of SLE [5]. Interleukin-19 (IL-19) is a member of the IL-10 family of cytokines secreted mainly by monocytes and plays a

major role as a proinflammatory cytokine[6]. As a result, IL-19 functioning as a signal transduction factor, is involved in tissue damage and local inflammatory response[5]. Moreover, according to previous research, rs2243188 SNP locus in the IL19 gene is highly associated with SLE [7].

Autophagy is a homeostatic mechanism that has been preserved throughout evolution, it is responsible for lysosomal destruction of un-needed transported cytoplasmic components. According to some research, peripheral T and B lymphocytes in SLE patients may have dysregulated autophagy[8–11]. LC3-associated phagocytosis (LAP) regulates immune responses to dying cells, studies observed that inhibition of LAP develops SLE like illness. Furthermore, Martinez et al. [12] supported the theory that SLE could have a hereditary component involving LC3. MAP1LC3B gene encodes microtubule-associated protein LC3B which is a pivotal protein and the main marker of autophagy, it is involved in synthesis and maturation of autophagosomes and controls immune response to dying cells [13]. LC3B-II is higher in lupus-prone mice and SLE patients [10]. Rs933717, proved as an associated rSNP-related gene polymorphism that includes MAP1LC3B, which encodes LC3B (proximal transcriptional regulation) and FBXO31 (distal transcriptional regulation and RNA binding protein-mediated regulation) [14]. The aim of this case-control study is to identify the possible association between rs2243188 (IL-19) and rs933717 (FBXO31-MAP1LC3B) polymorphisms and the risk of JSLE & JLN in the Egyptian population.

METHODOLOGY

Patients and control subjects

This case control study was conducted in the National Research Centre, Centre of Excellency and Outpatient Clinic of Rheumatology Department, Kasr Alainy Faculty of Medicine. In this research, a total of 47 patients with JSLE (7 males and 40 females, age range 6-16 years) were included as cases, while 50 age and sex matched as healthy controls (HC). The diagnosis for SLE fulfilled European League Against Rheumatism/American College of Rheumatology (EULAR/ACR) Classification Criteria for SLE [15]. JLN patients (29 patients) were included if they met any one of the subsequent requirements: (a) 0.5 g/day of persistent proteinuria, (b) active cellular casts, and (c) LN biopsy evidence [16]. Patients' history was taken in addition to full clinical examination, recent routine laboratory data was obtained from patients' records. JSLE activity was assessed using Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2k) [17]. If a patient had any of the following conditions, they were not allowed to participate: 1) other autoimmune disorders; or 2) any significant systemic disorders. Inclusion criteria for HC included: 1) not meeting any of the SLE criteria; 2) no history of immune disease or direct family history; 3) no significant sickness or history of illness in the family.

Ethical consideration

The study followed the World Medical Association code of ethics approved by Declaration of Helsinki in 2015. The study was approved by Ethics Committee of National Research Centre Cairo, Egypt with approval number 16288 and an informed written consent was obtained from parents prior to data collection.

DNA extraction and genotyping

DNA genomic materials were obtained from Ethylenediaminetetraacetic acid EDTA blood samples using and following manufacturer's protocol of QIAamp DNA Blood Kit (Qiagen, Hilden, Germany). The extracted genomic materials were stored at −20°C until analysed. Applied Biosystems TaqMan™ SNP Genotyping Assay (Applied Biosystems, Cat No: 4351379) and TaqMan™ Genotyping Master Mix (Cat No.43-713-53) were used for allelic discrimination of both rs2243188 and rs933717 polymorphisms. PCR conditions were initial activation at 95ºC for 10 minutes, followed by 40 cycles of denaturation for 15s at 95ºC and annealing/extension for 1min at 60ºC using Rotor-Gene Q real time PCR system (Qiagen, Hilden, Germany). Florescence data gathering and final analysis was performed using Rotor-Gene Q programmed software at the extension step. Negative and positive controls were genotyped in each assay and 10% randomly selected samples were duplicated and showed 100% concordance rate to ensure quality control[18,19].

Statistical Analysis

Data were coded and entered using the statistical package for the Social Sciences (SPSS) version 28 (IBM Corp., Armonk, NY, USA). Data was summarized using mean, standard deviation, median, minimum and maximum in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. Comparisons between quantitative variables were done using the non-parametric Mann-Whitney test[20]. For comparing categorical data, Chi square ($\chi 2$) test was performed. Exact test was used instead when the expected frequency is less than 5 [21]. P-values less than 0.05 were considered as statistically significant.

RESULTS

All subjects were from the Egyptian population. Forty-seven JSLE patients, 40 females and 7 males with age range 6 to 16 years. Fifty healthy control (HC) age and gender matched were included.

Genotype distribution and allele frequency were investigated for both loci to detect any susceptibility to JSLE, as presented in table 1. Genotype distribution for rs933717 and rs2243188 showed no significant

difference between patient group and controls (p value is 0.206 and 0.468 respectively).

Of note, there was not enough search for JSLE dominant and recessive models for both gene polymorphisms for the Egyptian population, hence the two dominant inheritance models were analyzed. Studying rs933717 locus with respective to model 1 (CC the dominant model and TT+CT the recessive one) and model 2 (TT the dominant and CC+CT the recessive model) on one hand, and analyzing rs2243188 with respective to both models with CC the dominant for model 1 and AA the dominant for model 2, results showed no statistical difference between the two models as per Table 1. However, model 2 was further studied in this work as it revealed less P-value than model 1, as well as most previous search considered model 2 with TT the dominant genotype for rs933717 and AA the dominant one for rs2243188 for SLE in other geographic districts. As mentioned earlier, there was not enough research for the dominant inheritance model for both loci in the Egyptian population. Therefore, both inheritance models were studied. Although both models revealed no significant difference, model 2 for rs933717 and rs2243188 showed relatively more of significance than model 1. In other words, the former revealed a P-value of model 2 (0.087) less than that of model 1 (1) likewise, the latter has a P-value of model 2 (0.259) less than that of model 1 (0.907). Therefore, model 2 was used for further correlations in the current search for having the lower P-value.

Table 1: Genotype frequencies and the dominant inheritance models for both rs933717 and rs 2243188 in JSLE patients and HC

		HC		JSLE		P value
		Count	%	Count	%	
rs 933717	TT	6	12.0%	12	25.5%	0.206
	CT	40	80.0%	31	66.0%	
	CC	4	8.0%	4	8.5%	
rs 933717 model 1	CC	4	8.0%	4	8.5%	1
	TT+CT	46	92.0%	43	91.5%	
rs 933717 model 2	TT	6	12.0%	12	25.5%	0.087
	CC+CT	44	88.0%	35	74.5%	
rs 2243188	AA	2	4.0%	5	10.6%	0.468
	CA	24	48.0%	20	42.6%	
	CC	24	48.0%	22	46.8%	
rs 2243188 model 1	CC	24	48.0%	22	46.8%	0.907
	AA+CA	26	52.0%	25	53.2%	
rs 2243188 model 2	AA	2	4.0%	5	10.6%	0.259
	CC+CA	48	96.0%	42	89.4%	

JSLE: juvenile systemic lupus erythematosus HC: Healthy controls

In Table 2, there was no significant difference in the frequency of neither the C allele for both loci nor for the T or A allele for rs933717 and rs 2243188 respectively with P-values equal to 0.362 for the former and 0.552 for the latter (P > 0.05).

Table 2: Allele frequencies of rs933717 and rs2243188 in JSLE patients and HC

		HC		JSLE		P value
		Count	%	Count	%	
rs933717 alleles	allele T	52	52.0%	55	58.5%	0.362
	allele C	48	48.0%	39	41.5%	
rs2243188 alleles	allele A	28	28.0%	30	31.9%	0.552
	allele C	72	72.0%	64	68.1%	

JSLE: juvenile systemic lupus erythematosus HC: Healthy controls

As shown in table 3, there was no significant difference between rs933717 model 2 genotypes within JSLE group with respective to age, JSLE age of onset, disease duration and SLEDAI-2K (P>0.05).

Table 3: Comparison of current age, JSLE age of onset, duration, and SLEDAI-2K in the JSLE group for rs933717 with respective to model 2

JSLE	rs933717 model 2										P value
	TT					CC+CT					
	Mean	SD	Median	Min.	Max.	Mean	SD	Median	Min.	Max.	
Current Age(years)	11.92	3.20	12.75	6.00	16.00	11.40	2.94	11.00	4.50	16.00	0.524
JSLE age of onset (years)	8.29	2.45	8.75	4.50	12.00	8.01	2.89	8.00	2.00	14.00	0.816
JSLE duration(years)	2.65	1.39	3.25	0.50	4.20	2.77	1.54	2.00	0.50	6.30	0.853
SLEDAI-2K	5.92	6.53	2.00	0.00	22.00	4.51	5.20	2.00	0.00	26.00	0.667

JSLE: juvenile systemic lupus erythematosus SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity Index 2000

Similarly, rs2243188 model 2 genotypes showed no significant differences in age, JSLE age of onset, disease duration and SLEDAI-2K (P>0.05) as demonstrated in table 4.

Table 4: Comparison of current age, JSLE age of onset, duration, and SLEDAI-2K in the JSLE l group for rs2243188 with respective to model 2

JSLE	rs2243188 model 2										P value
	AA					CC+CA					
	Mean	SD	Median	Min.	Max.	Mean	SD	Median	Min.	Max.	
Current Age(years)	13.50	1.87	14.00	11.00	16.00	11.30	3.02	11.50	4.50	16.00	0.119
JSLE age of onset(years)	9.90	2.36	11.00	6.00	12.00	7.86	2.75	8.00	2.00	14.00	0.088
JSLE duration(years)	2.86	2.02	2.00	1.50	6.30	2.72	1.44	2.35	0.50	6.00	0.854
SLEDAI-2K	3.60	3.58	2.00	2.00	10.00	5.02	5.73	2.00	0.00	26.00	0.675

JSLE: juvenile systemic lupus erythematosus SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity Index 2000

Out of the 47 JSLE patients in the experimental group, 29 patients (or 61.7% of the total) displayed JLN symptoms. Association between JLN with rs933717 and rs2243188 loci was studied and results are demonstrated as per table 5.

Table 5 shows that there was no significant difference for distribution of genotype frequency for the JLN patients and HC. Genotype distribution in the dominant/ recessive models for both loci was calculated and no significant difference was detected.

Table 5: Genotype frequencies and the dominant inheritance models for both rs933717 and rs2243188 in JLN patients and HC

		HC		JLN		P value
		Count	%	Count	%	
rs933717	TT	6	12.0%	8	27.6%	0.237
	CT	40	80.0%	19	65.5%	
	CC	4	8.0%	2	6.9%	
rs933717 model 1	CC	4	8.0%	2	6.9%	1
	TT+CT	46	92.0%	27	93.1%	
rs933717 model 2	TT	6	12.0%	8	27.6%	0.080
	CC+CT	44	88.0%	21	72.4%	
rs2243188	AA	2	4.0%	4	13.8%	0.264
	CA	24	48.0%	14	48.3%	
	CC	24	48.0%	11	37.9%	
rs2243188 model 1	CC	24	48.0%	11	37.9%	0.385
	AA+CA	26	52.0%	18	62.1%	
rs2243188 model 2	AA	2	4.0%	4	13.8%	0.185
	CC+CA	48	96.0%	25	86.2%	

JLN: juvenile lupus nephritis HC: Healthy controls

Table 6 shows that there was no significant difference in the frequency of neither the C or T allele of rs933717 with P value of 0.309 nor the C or A allele for rs2243188 with P value of 0.196.

Table 6: Allele frequencies of rs933717 and rs2243188 in JLN patients and controls

		HC		JLN		P value
		Count	%	Count	%	
rs933717 alleles	allele T	52	52.0%	35	60.3%	0.309
	allele C	48	48.0%	23	39.7%	
rs2243188 alleles	allele A	28	28.0%	22	37.9%	0.196
	allele C	72	72.0%	36	62.1%	

JLN: juvenile lupus nephritis HC: Healthy controls

DISCUSSION

JSLE is a complicated condition that can exhibit an extensive range of signs and symptoms, often resembling other prevalent pediatric disorders. Diagnosing the patient early and receiving the specialized care needed on time is typically challenging[22]. The underlying cause of immune system abnormalities in SLE is complicated with number of variables playing roles in its development including genetics, environment, infections, and medications[23]. The involved pathophysiology includes improper immune system activation with aberrant expression of cytokines and autoantibodies[24].

Our case control study was performed to identify the association between two under-investigated polymorphisms; rs2243188 (IL-19) and rs933717 (FBXO31-MAP1LC3B), that might play roles in important immunological pathways of JSLE or JLN pathogenesis.

IL-19 has a complicated role in SLE through both its anti- and pro- inflammatory actions as a signal transducer for the STAT3 and STAT1 pathways as well as an inducer for other inflammatory cytokines synthesis and production[6].

Our results concerning rs2243188 (IL-19) showed no significant difference between JSLE patients and HC neither in genotypes nor in allele frequency distributions. Likewise, JLN showed no significant difference with the HC. These results are contradicting Ni et al[5] who declared that the IL19 gene rs2243188 SNP locus C allele was highly associated with SLE in Chinese patients in addition to increase of LN susceptibility risk by 2.201-fold higher with CC genotype compared to other genotypes. Similarly, Wang et al.[19] noticed that the Dominant pattern (CC+CA) was significantly lower in the HC. On the other hand, Lin et al.[6] discovered in his studies on Chinese groups that the C allele was significantly lower in SLE and LN groups, besides this polymorphism did not correlate with IL-19 serum levels despite the significant difference between SLE various subclasses. As a consequence of the dual pro- and anti- inflammatory roles of IL-19 and the controversial results of the mentioned studies, we cannot predict the exact effect of IL-19 rs2243188 polymorphism on pathogenesis of JSLE.

Autophagy is a highly suggested candidate mechanism in SLE pathogenesis[25]. Dysregulated autophagy whether deficiency or overactivity both lead to disturbance of immune system with possible autoimmunity and inflammation[11]. MAP1LC3B (rs933717) polymorphism is suggested as a causative agent in increased LC3B-II autophagy leading to SLE pathogenesis[14].

For MAP1LC3B (rs933717), our results did not show any significant statistical difference in all models between T and C alleles neither for JSLE nor for JLN and this was contradictory to Qi et al.[14] research group. Their study demonstrated an increased suitability to SLE with a Chinese rs933717C variant. Moreover, they stated that the same rs933717 C allele was considered a risk allele and was associated with higher expression of MAP1LC3B in addition to increasing luciferase (reporter gene) activity on cell lines up to 3.8 folds. Their search also revealed that Lupus-prone mice and SLE patients tend to show overexpression of MAP1LC3B mRNA. However, they are doubtful that rs933717 is a solo player in these changes.

According to Gheita et al[26], the Egyptian juvenile onset is rather around 8.6% of SLE cases, this low prevalence of JSLE explains the small sample size gathered in the present study. This was one of the limitations to our study that might affect statistical significance and hence generalization of the results.

Other limitations include the limited data According to current work, these two polymorphisms and their association with SLE were previously studied only in adult Chinese population[5,6,14,19]. Therefore, it is not necessarily to have the same correlation with SLE patients from other populations [7]. Moreover, our study was the first to be carried on Egyptian JSLE patients. Diverse inheritance patterns in different races and cultures in addition to nature selection may contribute to the disparity between the results[18,19,27]. Along with the fact that SLE pathogenesis is multifactorial and

polygenic, individual genetic variations are common and not yet fully discovered [31].

We recommend further comprehensive investigations addressing concerned polymorphisms in variable races and JSLE with larger sample size. Additional pathway testing may provide not only a better understanding of the pathogenic inflammatory and autophagy related mechanisms associated with JSLE but also identification of novel targets for treatment.

CONCLUSION

Finally, our findings revealed that in the Egyptian community, the genotype and allele frequencies for both loci (rs2243188 & rs933717) do not show a statistical significance as a protective or risk factor against JSLE or JLN in our sample of Egyptian pediatric population.

Declarations:
Consent for publication: Not applicable
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Competing interests: The author(s) declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.
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