International Journal of Immunogenetics

WILEY

INTERNATIONAL JOURNAL OF IMMUNOGENETICS

ORIGINAL ARTICLE

Risk Genes and Anti-C1q Autoantibodies in Upper Egyptian Patients With Systemic Lupus Erythromatosis—High Frequency of HLA-DRB1*04:05–DQA1*03–DQB1*02 Risk Haplotype in Lupus Nephritis Patients

Mostafa I. EL-Amir[1] | Mohamed Ali El-Feky[1,2] | Abdelkader Ahmed Hashim[3] | Mohammed H. Hassan[4] | Marwa Abdelhady[5] | Wael Abd El Mohsen Abady[6] | Amr Mohamed ElKaber[6] | Jorma Ilonen[7]

[1]Department of Medical Microbiology and Immunology, Faculty of Medicine, South Valley University, Qena, Egypt | [2]Department of Medical Microbiology and Immunology, Faculty of Medicine, Assiut University, Assiut, Egypt | [3]Department of Internal Medicine, Faculty of Medicine, South Valley University, Qena, Egypt | [4]Department of Biochemistry, Faculty of Medicine, South Valley University, Qena, Egypt | [5]Department of Internal Medicine, Faculty of Medicine, Luxor University, Luxor, Egypt | [6]Department of Physical Medicine, Rheumatology, and Rehabilitation, Faculty of Medicine, South Valley University, Qena, Egypt | [7]Immunogenetics Laboratory, Institute of Biomedicine, University of Turku, Turku, Finland

Correspondence: Mostafa I. EL-Amir (mostafaismail81@yahoo.com)

Keywords: anti-C1q | CTLA-4 gene (rs 231775) | HLA | SLE

ABSTRACT

This study was performed to determine anti-C1q serum level, genetic polymorphism in cytotoxic T lymphocyte–associated antigen 4 gene (CTLA-4 gene) (rs 231775), and HLA class II genes in susceptibility and early prediction of systemic lupus erythematosus (SLE) and lupus nephritis (LN) in Upper Egyptian patients. A total of 60 unrelated cases of SLE (30 cases with LN) and 60 healthy controls were studied for HLA-DQB1, HLA-DQA1, and HLA-DRB1 (DR4 subtypes) alleles. Anti-C1q level was estimated by ELISA. CTLA-4 gene genotypes were detected by PCR-RFLP. The means of age of SLE patients without nephritis and LN patients were 24 ± 5.09 and 32 ± 7.26, respectively. Most of the patients were females (93.3%). Anti-C1q serum level was significantly higher in LN patients (24.11 ± 4.26) versus SLE patients without nephritis (18.17 ± 1.35) (p value < 0.001). The AA genotype of the CTLA-4 gene was significantly higher in patients with LN versus SLE patients without nephritis (53.5% vs. 26.5%; p value = 0.035). (DR7)—DQA1*02-DQB1*0303 haplotype was higher in SLE patients versus the control group and showed the highest odds ratio (7.37) with a significant p value (0.031). Odds ratios of DRB1*0405–DQA1*03–DQB1*0302 and DRB1*0405–DQA1*03–DQB1*02 were 6.263 and 4.214, respectively. DRB1*0405–DQA1*03–DQB1*02 haplotype was detected in 11.7% of LN patients versus 1.7% of SLE patients without nephritis (OR = 8.82, p value = 0.02). DRB1*0405–DQA1*03–DQB1*02 haplotype, in addition to CTLA-4 gene (AA genotype), and high anti-C1q serum level can predict the progression of SLE Upper Egyptian patients to LN.

1 | Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease affecting multiple organs such as joints, skin, lungs, brain, and kidneys (Di Battista et al. 2018). Kidney affection mainly 3–5 years after the onset of SLE causes lupus nephritis (LN) (Di Battista et al. 2018).

Although multiple genetic and environmental factors are implicated in disease pathogenesis, the role of genetic factors in disease pathogenesis is supported by a high rate of SLE concordance in monozygotic twins (24%–35%) and positive family history (AlSaleh et al. 2008). Hyperactivity in T cell, B cell, and defects in regulatory T cell were demonstrated in SLE patients due to genetic predisposition. HLA Class II alleles show strong

association with SLE autoantibodies as HLA genes are involved in antigen presentation. Other non-HLA genes are implicated in disease pathogenesis as cytotoxic T lymphocyte–associated antigen 4 gene (CTLA4) and interferon regulatory factor 8 (IRF8) (Lessard et al. 2012). Several autoantibodies are directed against the nucleus, cytoplasm, and cell surface self-antigen. Due to loss of tolerance to self-antigen, autoreactive T cells infiltrate the tissue causing tissue damage (Moulton and Tsokos 2011). In addition to genetic factors, multiple environmental factors and toxic substances such as alcohol, cigarette smoking, occupational hazards, ultraviolet rays, certain medications, and infections are implicated in inducing SLE onset and enhancing complications (Mak and Tay 2014).

During the active stage of the disease, different complement proteins are activated due to immune complex deposition in the renal glomeruli associated with a decrease in the level of complement in the plasma. Kidney involvement leads to LN which is one of the most severe manifestations of SLE. C1q antibodies are considered as a biomarker of renal involvement. The strong correlation between the presence of anti-C1q and LN has been reported (Moroni et al. 2001).

CTLA-4 gene is located in chromosome 2q33. CTLA-4 gene has a crucial role in peripheral T cell tolerance. CTLA-4 downregulates T cell function through binding with B7 presented on APCs. Polymorphism in the CTLA-4 gene (+49 A/G, rs 231775) was implicated in several autoimmune diseases such as Graves' disease (Kouki et al. 2000), Rheumatoid arthritis (Osorio et al. 2004), and Type I diabetes (Mosaad et al. 2012). Early development of SLE in Japanese associated with (GG) genotype (Sugimoto et al. 2008).

The HLA system consists of protein antigens expressed on human cells and plays an important role in the immune response. HLA-DRB1 and HLA-DQB1 genes are considered the most important genes implicated in SLE. Due to extensive polymorphisms associated with HLA genes, susceptibility alleles vary across different ethnic groups and geographic regions (Morris et al. 2012). HLA-DRB1*15 and HLA-DRB1*03 are considered the most common genetic risk factors associated with SLE in Caucasians. The presence of the DRB1*15–DQB1*0602 haplotype increases the risk of LN among northern Italians (Marchini et al. 2003).

This study aimed to determine the level of serum anti-C1q, genetic polymorphism in CTLA-4 (+49 A/G, rs 231775), and HLA class II risk genes among Egyptian patients with SLE without nephritis and patients with LN.

2 | Material and Methods

A total of 60 unrelated cases of SLE (30 cases with LN) and 60 healthy controls were enrolled in this study after written consent. Four patients were males (three males were diagnosed as LN while only one diagnosed as SLE without nephritis). All patients were subjected to full history and clinical examination. All of the patients were diagnosed by the revised 1982 American Rheumatism Association criteria for the classification of SLE. The presence of four criteria or more ensures the diagnosis (Tan et al. 1982). The study was approved by the Ethics Committee, Qena Faculty of Medicine, South Valley University, and conducted in agreement with the Declaration of Helsinki. Ethical approval code: SVU-MED-MIC007-4-24-11-1014.

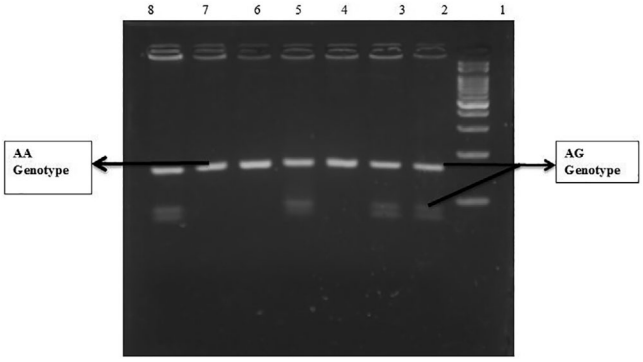

FIGURE 1 | Restriction fragment length polymorphism (RFLP) of CTLA-4 gene (+49 A/G, rs 231775). Lane 1 showing 100 bp DNA ladder from 100 to 1000 bp. Lane 2 showing AG genotype while Lane 7 showing AA genotype.

A total of 4 mm of EDTA blood were collected from every participant for HLA class II typing and allele polymorphism in the CTLA-4 gene (+49 A/G, rs 231775). DNA extraction was done by the salting out method as described previously by Miller et al. (1988). DNA concentration was adjusted to 20 ng/μL. DNA absorbance at 260 and 280 nm (A260/A280) was measured by UV spectrophotometer to determine sample purity. A good quality DNA sample should have an A260/A280 ratio of 1.7–2.0. Also, 2 mm of peripheral venous blood were collected into sterile plain tubes to obtain serum for analysis of anti-C1q antibodies. All samples were preserved at −20°C until molecular and ELISA analysis.

For detection of allele polymorphism in the CTLA-4 gene (rs 231775), the sequence of forward primer was 5′ GCT CTA CTTCCT GAA GAC CT 3′ and the sequence of reverse primer was 5′AGT CTC ACT CAC CTT TGC AG 3′. PCR volume in each reaction was 25 μL (12.5 μL master mix [Bioline, Catalogue no. BIO-25043, UK], 1 μL forward primer, 1 μL reverse primer, 2 μL extracted DNA, and 8.5 μL H_2O). The PCR was performed using a thermal cycler (Peqstar, VWR International, UK). The reaction conditions were initiated by denaturation at 94°C for 4 min followed by 35 cycles of 94°C for 30 s, 56°C for 30 s, 72°C for 30 s, and finally extension step at 72°C for 5 min.

After amplification of the CTLA-4 gene, the PCR product was separated by Gel electrophoresis (Multisub Horizontal Gel System, Cleaver Scientific, UK), and a band of 162 bp was detected by UV. BbvI restriction enzyme (New England BioLabs, USA) was used. The total reaction volume for restriction enzyme was 25 μL (8 μL of the PCR product, 2.5 μL NE Buffer, 2 μL restriction enzyme, and 12.5 μL H2O), and incubated at 37°C for 60 min. As shown in Figure 1, the genotypes were classified as follows: AA Genotype not affected by restriction enzyme (162) base pairs (bp), AG genotype (162 bp, 88 bp, and 74 bp), and GG genotype (88 bp, and 74) (Figure 1).

Assessment of anti-C1q level in the serum was performed using a human anti-C1q kit (Biospes, China), according to the manufacturer's instructions. Results were obtained by using a microtiter

plate reader (Infinite F50, Tecan, Austria) at an optical density of 450 nm. The results were expressed in U/L.

Genotyping of HLA genes was based on sequence-specific hybridization using lanthanide labelled oligonucleotide probes as described earlier (Kiviniemi et al. 2007). HLA-DQB1 alleles were identified using a homogeneous assay. By using DELFIA method (PerkinElmer Life and Analytic Sciences Wallac, Turku, Finland), HLA-DQA1 alleles and DRB1*04 subtypes were identified using hybridization on streptavidin-coated microtiter plates where labelled probes were detected by time-resolved fluorometry. These methods enabled us to identify the following alleles: DQB1* 03:01, 03:02, 03:03, 02, 04, 06:02, 06:03, 06:04, and 05:01; DQA1*02:01, 03, and 05; and DRB*04:01, *04:02, *04:03/06, *04:04, 04:05, 04:07, and 04:08. Alleles which determined by known linkage disequilibrium (LD) were written within parentheses

Significance was evaluated with Fisher's exact test (two-sided) or the standard χ^2 test when appropriate. Odds ratios (ORs) were calculated with Woolf's formula using Haldane's modification when some of the analysed frequencies were zero. The results were considered statistically significant when the p values were smaller than 0.05. A t-test was used to determine significant difference between the means of anti-C1q serum levels among different groups.

3 | Results

Female patients were significantly higher than the control group (93.3% in patients vs. 58.3% in control; p value less than **0.001**). In SLE patients without nephritis, one case was male (3.5%), while in LN, three patients were males (10%). The means of the age of SLE patients without nephritis and LN patients were 24 ± 5.09 and 32 ± 7.26, respectively. The albumin creatinine ratio was less than 30 mg/L in all patients with SLE without nephritis, while it was higher than 300 mg/L in all patients with LN.

Anti-C1q in patients (21.14 ± SD 4.33) was significantly higher than in the control group (3.04 ± SD 0.72) with a significant p value ($p <$ **0.001**). Also, a significant difference was observed between anti-C1q in LN patients (24.11 ± SD 4.26) versus anti-C1q in SLE patients without nephritis (18.17 ± SD 1.35) (p value **< 0.001**).

According to CTLA-4 (rs 231775) genotyping, 40% of the patients were (AA) genotype versus 36.6% in the control group (p value = 0.851); 56.6% of the patients were (AG) genotype versus 63.4% in the control group (p value = 0.576). The percentages of the A allele and G allele were similar between patients and controls (68.4% and 31.6%, respectively) with insignificant p values, as shown in Table 1.

As shown in Table 2, the AA genotype was found in 53.5% of patients with LN versus 26.5% in SLE patients without nephritis, with a significant p value ($p =$ **0.035**). The GG genotype was not detected in LN cases (0%), while 6.5% of SLE cases were positive for the GG genotype. A total of 76.6% of LN cases were positive for the A allele versus 60% of SLE cases, with a p value = **0.05**.

As shown in Table 3, **HLA-(DR3)—DQA1*05–DQB1*02** haplotype was found in 24.1% of the patients. This haplotype was also one of the most common haplotypes in the background population (17.5%) (OR = 1.5, and p = 0.204). **HLA-(DR7)—DQA1*02–DQB1*0303** showed the highest odds ratio (OR = 7.37) with a significant difference between patients and controls (p = **0.031**).

DRB1-0405 allele showed a significant difference between SLE patients and controls with a high odds ratio (OR = 5.15, p = **0.01**). **DRB1*0405–DQA1*03–DQB1*0302** haplotype and **DRB1*0405–DQA1*03–DQB1*02** haplotype were associated with high odds ratios (OR = 6.263 and 4.214, respectively) with insignificant p values (p = 0.055 and **0.053**, respectively).

DR (15)—DQB1*0602 haplotype was detected in 10% of the patients, while only 4.1% of the controls were positive for this haplotype with an insignificant p value (p = 0.078).

DR (15)—DQB1*0601 and (DR14)—DQB1*0503 haplotypes were not detected in patients but were found in 5% of the controls with a significant p value = 0.013 and 3.3% of the controls with a significant p value = **0.044**, respectively. DR (1/10)—DQB1*0501 haplotype was found in 12.5% of the controls versus 5% of the patients with a significant p value (p = **0.04**). Although DR (13)—DQB1*0604 showed a low odds ratio (OR = 0.237), an insignificant p value was detected with this haplotype (p = 0.053).

As shown in Table 4, DRB1*0405–DQA1*03–DQB1*02 showed a significant difference between LN patients and SLE patients without nephritis. A total of 11.7% of LN patients were positive for this haplotype versus 1.7% with a significant p value and high odds ratio (p = **0.02**; OR = 8.82). Although the DRB1*0405–DQA1*03–DQB1*0302 haplotype was higher in SLE patients without nephritis (8.3%) versus LN patients (1.7%), an insignificant p value (0.09) was detected.

4 | Discussion

SLE is an autoimmune disease characterized by the affection of multiple organs due to the production of autoantibodies. HLA molecules present antigen to the immune system, which potentially triggers an immune response in genetically susceptible individuals. Also, other non-HLA genes, such as the CTLA-4 gene that regulates immune tolerance, are implicated in disease pathogenesis. In our study, 93.3% of the cases were females with a significant p value. A similar study conducted on northeastern Italian SLE patients over the period 2012–2020 found that 85% of the patients were female (Zen et al. 2023). Some hormones as oestrogen and prolactin were implicated as activators of the immune system. Oestrogen modulates lymphocytes and dendritic cell activation while prolactin level is high in the serum of patients with SLE (Webb et al. 2018; Legorreta-Haquet et al. 2022).

In our study, the mean of age of SLE patients without nephritis was (24 ± SD 5.09), while that of LN patients was (32 ± SD 7.26). This result was similar to Chung (Chung et al. 2021), who found that the peak age for SLE patients was between 25 and 39 years. Due to the effect of sex hormones, different studies from different countries all over the world concluded that women of

TABLE 1 | Genotype and allele frequencies of CTLA-4 (rs 231775) in Egyptian patients with SLE and controls.

	SLE = 60 (%)	Control 60 (%)	OR (95%Cl)	*p* value
Genotype				
AA	24 (40)	22 (36.6)	1.15 (0.551–2.405)	0.851
AG	34 (56.6)	38 (63.4)	0.757 (0.364–1.575)	0.576
GG	2 (3.4)	0 (0)	6.86 (0.15–Inf)	0.154
Allele				
A	82 (68.4)	82(68.4)	1 (0.580–1.723)	1
G	38 (31.6)	38 (31.6)	—	—

TABLE 2 | Genotype and allele frequencies of CTLA-4 (rs 231775) in Egyptian patients with LN and SLE without nephritis.

	LN = 30(%)	SLE without nephritis = 30(%)	OR (95%Cl)	*p* value
Genotype				
AA	16 (53.5)	8 (26.5)	3.143 (1.066–9.267)	**0.035***
AG	14 (46.5)	20 (67)	0.437 (0.154–1.243)	0.118
GG	0 (0)	2 (6.5)	0.05 (0–27.31)	0.15
Allele				
A	46 (76.6)	36 (60)	2.190 (0.994–4.828)	**0.05***
G	14 (23.4)	24 (40)	—	—

Abbreviations: 95% CI, confidence intervals; OR, odds ratio.
*Significant p value ≤ 0.05.

TABLE 3 | Frequency of various HLA-DR/DQ genotypes among Egyptian patients with SLE and controls.

Haplotypes	SLE patients = 60(%)	Controls = 60(%)	OR (95%Cl)	*p* value
(DR3)—DQA1*05–DQB1*02	29 (24.1)	21 (17.5)	1.5 (0.8–2.8)	0.204
(DR7)—DQA1*0201–DQB1*02	17 (14.1)	12 (10)	1.4 (0.67–3.26)	0.322
(DR7)—DQA1*02–DQB1*0303	**7 (5.8)**	**1 (0.8)**	**7.37 (0.893–60.86)**	**0.031***
DR(15)—DQB1*0602	12 (10)	5 (4.1)	2.55 (0.87–7.49)	0.078
(DR11/12/13)—DQA1*05–DQB1*0301	14 (11.6)	18 (15)	0.74 (0.35–1.58)	0.44
(DR8)—DQB1*04	7 (5.8)	5 (4.1)	1.42 (0.43–4.62)	0.55
DR(1/10)—DQB1*0501	**6 (5)**	**15 (12.5)**	**0.36 (0.138–0.985)**	**0.04***
DR(13)—DQB1*0603	3 (2.5)	7 (5.8)	0.414 (0.104–1.64)	0.196
DR(13)—DQB1*0604	**2 (1.7)**	**8 (6.7)**	**0.237 (0.049–1.142)**	**0.053**
DR(13)—DQB1*0609	**1 (0.8)**	**1 (0.8)**	1 (0.062–16.174)	1
DR(16)—DQB1*0502	**1 (0.8)**	3 (2.5)	0.328 (0.034–3.196)	0.313
DRB1*0402–DQA1*03–DQB1*0302	**4 (3.3)**	**4 (3.3)**	1 (0.244–4.094)	1
DRB1*0405–DQA1*03–DQB1*02	**8 (6.7)**	**2 (1.7)**	**4.214 (0.876–20.27)**	**0.053**
DRB1*0405–DQA1*03–DQB1*0302	**6 (5)**	**1 (0.8)**	**6.263 (0.742–52.83)**	**0.055**
DRB1-0405	**14 (11.7)**	**3(2.5)**	**5.151 (1.44–18.42)**	**0.01***
DRB1*0403–DQA1*03–DQB1*0302	3 (2.5)	7 (5.8)	0.414 (0.104–1.640)	0.196
DR(15)—DQB1*0601	**0 (0)**	**6 (5)**	**0.07 (0–1.30)**	**0.013***
(DR14)—DQB1*0503	**0 (0)**	**4 (3.3)**	**0.11 (0–2.02)**	**0.044***

Abrreviations: 95% CI, confidence intervals; OR odds ratio.
*Significant p value ≤ 0.05.

TABLE 4 | Frequency of various HLA-DR/DQ genotypes among Egyptian patients with LN and SLE without nephritis.

	LN = 30(%)	SLE without nephritis = 30(%)	OR (95% CI)	*p* value
(DR3)—DQA1*05–DQB1*02	15 (25)	14 (23.3)	1.143 (0.41–3.14)	0.79
(DR7)—DQA1*0201–DQB1*02	9 (15)	8 (13.3)	1.179 (0.38–3.62)	0.77
(DR7)—DQA1*02–DQB1*0303	2 (3.3)	**5 (8.3)**	0.357 (0.06–2)	0.22
DR(15)—DQB1*0602	7 (11.7)	**5 (8.3)**	1.522 (0.42–5.47)	0.5
(DR8)—DQB1*04	2 (3.3)	**5 (8.3)**	0.357 (0.06–2)	0.22
DRB1*0402–DQA1*03–DQB1*0302	3 (5)	1 (1.7)	3.23 (0.31–32.88)	0.3
DRB1*0405–DQA1*03–DQB1*02	7 (11.7)	1 (1.7)	8.82 (1.01–76.96)	**0.02***
DRB1*0405–DQA1*03–DQB1*0302	1 (1.7)	**5 (8.3)**	0.172 (0.01–1.57)	0.09

Abbreviations: 95% CI, confidence intervals; OR odds ratio.
*Significant p value ≤ 0.05.

childbearing age were at higher risk for developing SLE compared with women in other age groups (Chiu and Lai 2010; Feldman et al. 2013).

According to anti-C1q, a significant difference was observed between anti-C1q levels in patients with LN and non-Lupus patients. Several studies concluded that monitoring anti-C1q antibodies can predict the renal flares in LN patients, which might be valuable in clinical management (Hegazy et al. 2012).

Genetic factors are important in the pathogenesis of SLE. Although CTLA-4 gene +49 A/G polymorphism has a crucial role in different autoimmune diseases, in our results, 40% of the patients were AA genotype versus 36.6% of the controls with an insignificant p value. Kishk (Kishk et al. 2021) concluded no difference in genotypes and allele distribution between SLE patients and controls in the Egyptian population.

The (GG) genotype was not detected in our control (0%) and only 3.4% of the patients were positive for this genotype with an insignificant p value. The (GG) genotype was defined as a risk factor for the development of SLE in Asians but not in Europeans (Lee et al. 2005). The European population showed a low frequency of GG genotypes in SLE patients and controls. The percentage of GG genotypes in the Portuguese population was low in SLE patients and controls (9.3% and 8.6%, respectively) with an insignificant p value (Barreto et al. 2004). Similar results were obtained from the Spanish population (Aguilar et al. 2003).

In our study, the AA genotype was higher in patients with LN (53.5%) versus SLE patients without nephritis (26.5%) with a significant p value (p = **0.035**). Ulker et al. (2009) found a relationship between the development of SLE and the AA genotype in the Turkish population. Although, in our study, the AA genotype is not associated with significant differences between patients and controls, it seems to be associated with the appearance of kidney complications in SLE patients. The main limitation of this study is the low number of patients, so this result needs to be confirmed with a large number of patients with LN.

In our study, **(DR7)—DQA1*02-DQB1*0303** haplotype confers a risk for SLE with the highest odds ratio (7.37) among different analysed haplotypes. Hrycek et al. (2005) found that the HLA-DRB1*07 allele was the only allele that was higher in SLE patients than in Controls with a significant p value among the Poland population of Caucasoid origin. Unlike our study, in the previous study, the author didn't differentiate between (DR7)—DQA1*02-DQB1*0303 and (DR7)—DQA1*0201–DQB1*02 haplotypes.

As found in our study, DRB1*0405–DQA1*03–DQB1*0302 and DRB1*0405–DQA1*03–DQB1*02 haplotypes were implicated as risk factors for SLE. Similar to our result, Wadi et al. (2014) found that DR4 was significantly higher among SLE patients than in controls in the Al-Qassim region, Saudi Arabia. In Mexicans, DRB1*0405 allele was ten times more frequent in SLE patients than in controls (Salgado-Galicia et al. 2020).

In our study, DRB1*0405–DQA1*03–DQB1*02 haplotype was significantly associated with patient progression to LN. Several studies found an association between the presence of the DR4 allele and high levels of auto-antibodies in SLE patients (Yao et al. 1994; El Sherbini et al. 2009).

Although different studies considered DR (15) and DR (3) as risk alleles for the pathogenesis of SLE (Niu et al. 2015), in our study, DR(15)—DQB1*0602 was marginally associated with a high odds ratio and an insignificant p value according to a study published by Marchini et al. (2003). Although 29% of SLE patients were positive for (DR3)—DQA1*05–DQB1*02 haplotype, 17.5% of the background population were positive for this haplotype, causing an insignificant p value.

In our study, several haplotypes, such as DR (1/10)—DQB1*0501, (DR14)—DQB1*0503, and DR (13), were associated with low frequency among SLE patients. Several studies among different populations found the protective role of these haplotypes (Niu et al. 2015; Wang et al. 2022; Vasconcelos et al. 2009).

5 | Conclusion

Egyptians are known to be of mixed ethnic origin. We found that in Egyptian patients with SLE, the HLA-DR/DQ haplotypes associated with SLE susceptibility and protection were

similar to some Saudi Arabian and European populations. (DR7)–DQA1*02-DQB1*0303, DRB1*0405–DQA1*03–DQB1*0302, and DRB1*0405–DQA1*03–DQB1*02 haplotypes were associated with a higher risk in the occurrence of SLE in Egyptian patients. Also, DRB1*0405–DQA1*03–DQB1*02 haplotype, which is implicated in different autoimmune diseases in Egyptians, was associated with the progression of SLE patients to LN.

Acknowledgements

The authors have nothing to report.

Data Availability Statement

The authors have nothing to report.

References

Aguilar, F., B. Torres, J. Sanchez-Roman, A. Nunez-Roldan, and M. F. Gonzalez-Escribano. 2003. "CTLA-4 Polymorphism in Spanish Patients With Systemic Lupus Erythematosus." *Human Immunology* 64: 936–940. https://doi.org/10.1016/s0198-8859(03)00171-x.

AlSaleh, J., V. Jassim, M. ElSayed, N. Saleh, and D. Harb. 2008. "Clinical and Immunological Manifestations in 151 SLE Patients Living in Dubai." *Lupus* 17, no. 1: 62–66. https://doi.org/10.1177/0961203307084297.

Barreto, M., E. Santos, R. Ferreira, et al. 2004. "Evidence for CTLA-4 as a Susceptibility Gene for Systemic Lupus Erythematosus." *European Journal of Human Genetics* 12: 620.

Chiu, Y. M., and C. H. Lai. 2010. "Nationwide Population-Based Epidemiologic Study of Systemic Lupus Erythematosus in Taiwan." *Lupus* 19: 1250–1255.

Chung, M. K., J. S. Park, H. Lim, C. H. Lee, and J. Lee. 2021. "Incidence and Prevalence of Systemic Lupus Erythematosus Among Korean Women in Childbearing Years: A Nationwide Population-Based Study." *Lupus* 30, no. 4: 674–679. https://doi.org/10.1177/0961203320984845.

Di Battista, M., E. Marcucci, E. Elefante, et al. 2018. "One Year in Review 2018: Systemic Lupus Erythematosus." *Clinical and Experimental Rheumatology* 36, no. 5: 763–777.

El Sherbini, H. M., A. K. El Garf, and S. S. El Din Mahmoud. 2009. "Human Leukocyte Antigen and Autoantibodies Association With Juvenile Systemic Lupus Erythematosus." *The Egyptian Journal of Immunology* 16: 107–114.

Feldman, C. H., L. T. Hiraki, J. Liu, et al. 2013. "Epidemiology and Sociodemographics of Systemic Lupus Erythematosus and Lupus Nephritis Among US Adults With Medicaid Coverage, 2000–2004." *Arthritis and Rheumatism* 65: 753–763. https://doi.org/10.1002/art.37795.

Hegazy, A., A. F. Barakat, M. A. El Gayyar, and L. F. Arafa. 2012. "Prevalence and Clinical Significance of Anti-C1q Antibodies in Cutaneous and Systemic Lupus Erythematosus." *Egyptian Journal of Medical Human Genetics* 13, no. 2: 167–171.

Hrycek, A., U. Siekiera, P. Cieslik, and W. Szkrobka. 2005. "HLA-DRB1 and -DQB1 Alleles and Gene Polymorphisms of Selected Cytokines in Systemic Lupus Erythematosus." *Rheumatology International* 26, no. 1: 1–6. https://doi.org/10.1007/s00296-004-0503-8.

Kishk, R. M., M. A. Abdellatif, R. E. Eldesouki, M. Fawzy, S. A. Abdelhady, and M. M. Fouad. 2021. "Cytotoxic T Lymphocyte Antigen 4 Gene +49 A/G (rs231775) Polymorphism and Susceptibility to Systemic Lupus Erythematosus." *Current Rheumatology Reviews* 17, no. 2: 247–251. https://doi.org/10.2174/1573397116666201119145153.

Kiviniemi, M., R. Hermann, J. Nurmi, et al. 2007. "A High-Throughput Population Screening System for the Estimation of Genetic Risk for Type 1 Diabetes: An Application for the TEDDY (The Environmental Determinants of Diabetes in the Young) Study." *Diabetes Technology & Therapeutics* 9, no. 5: 460–472.

Kouki, T., Y. Sawai, C. A. Gadrine, M. E. Fisfalen, M. L. Alegre, and L. J. DeGroot. 2000. "CTLA-4 Gene Polymorphism at Position 49 in Exon 1 Reduces the Inhibitory Function of CTLA-4 and Contributes to the Pathogenesis of Graves' Disease." *Journal of Immunology* 165: 6606. https://doi.org/10.4049/jimmunol.165.11.6606.

Lee, Y. H., J. B. Harley, and S. K. Nath. 2005. "CTLA-4 Polymorphisms and Systemic Lupus Erythematosus (SLE): A Meta-Analysis." *Human Genetics* 116: 361. https://doi.org/10.1007/s00439-004-1244-1.

Legorreta-Haquet, M. V., P. Santana-Sanchez, L. Chavez-Sanchez, and A. K. Chavez-Rueda. 2022. "The Effect of Prolactin on Immune Cell Subsets Involved in SLE Pathogenesis." *Frontiers in immunology* 13: 1016427. https://doi.org/10.3389/fimmu.2022.1016427.

Lessard, C. J., I. Adrianto, J. A. Ice, et al. 2012. "Identification of IRF8, TMEM39A, and IKZF3-ZPBP2 as Susceptibility Loci for Systemic Lupus Erythematosus in a Large-Scale Multiracial Replication Study." *American Journal of Human Genetics* 90: 648–660.

Mak, A., and S. H. Tay. 2014. "Environmental Factors, Toxicants and Systemic Lupus Erythematosus." *International Journal of Molecular Sciences* 15: 16043–16056. https://doi.org/10.3390/ijms150916043.

Marchini, M., R. Antonioli, A. Lleo, et al. 2003. "HLA Class II Antigens Associated With Lupus Nephritis in Italian SLE Patients." *Human Immunology* 64: 462–468. https://doi.org/10.1016/s0198-8859(03)00017-x.

Miller, S. A., D. D. Dykes, and H. F. Polesky. 1988. "A Simple Salting Out Procedure for Extracting DNA From Human Nucleated Cells." *Nucleic Acids Research* 16, no. 3: 1215. https://doi.org/10.1093/nar/16.3.1215.

Moroni, G., M. Trendelenburg, and N. D. Papa, et al. 2001. "Anti-C1q Antibodies May Help in Diagnosing a Renal Flare in Lupus Nephritis." *American Journal of Kidney Diseases* 37: 490–498. https://doi.org/10.1053/ajkd.2001.22071.

Morris, D. L., K. E. Taylor, M. M. A. Fernando, et al. 2012. "International MHC and Autoimmunity Genetics Network. Unraveling Multiple MHC Gene Associations With Systemic Lupus Erythematosus: Model Choice Indicates a Role for HLA Alleles and Non-HLA Genes in Europeans." *American Journal of Human Genetics* 91: 778–793.

Mosaad, Y. M., A. A. Elsharkawy, and B. S. El-Deek. 2012. "Association of CTLA-4 (+49A/G) Gene Polymorphism With Type 1 Diabetes Mellitus in Egyptian Children." *Immunological Investigations* 41, no. 1: 28–37. https://doi.org/10.3109/08820139.2011.579215.

Moulton, V. R., and G. C. Tsokos. 2011. "Abnormalities of T Cell Signaling in Systemic Lupus erythematosus." *Arthritis Research & Therapy* 13: 207. https://doi.org/10.1186/ar3251.

Niu, Z., P. Zhang, and Y. Tong. 2015. "Value of HLA-DR Genotype in Systemic Lupus Erythematosus and Lupus Nephritis: A Meta-Analysis." *International Journal of Rheumatic Diseases* 18, no. 1: 17–28. https://doi.org/10.1111/1756-185X.12528.

Osorio, Y., J. Fortea, H. Bukulmez, et al. 2004. "Dense Genome-Wide Linkage Analysis of Rheumatoid Arthritis, Including Covariates." *Arthritis and Rheumatism* 50: 2757. https://doi.org/10.1002/art.20458.

Salgado-Galicia, N. A., S. Hernández-Doño, D. Ruiz-Gómez, et al. 2020. "The Role of Socioeconomic Status in the Susceptibility to Develop Systemic Lupus Erythematosus in Mexican Patients." *Clinical Rheumatology* 39, no. 7: 2151–2161. https://doi.org/10.1007/s10067-020-04928-5.

Sugimoto, K., S. Fujita, H. Yanagida, et al. 2008. "Clinical Manifestations and Analyses of the Cytotoxic T-Lymphocyte Associated-4 Gene in Two Japanese Families With Systemic Lupus Erythematosus." *Clinical and Experimental Nephrology* 12: 149. https://doi.org/10.1007/s10157-007-0019-0.

Tan, E. M., A. S. Cohen, J. F. Fries, et al. 1982. "The 1982 Revised Criteria for the Classification of Systemic Lupus Erythematosus." *Arthritis and Rheumatism* 25: 1271–1277. https://doi.org/10.1002/art.1780251101.

Ulker, M., V. Yazisiz, N. Sallakci, et al. 2009. "CTLA-4 Gene Polymorphism of Exon 1(+49 A/G) in Turkish Systemic Lupus Erythematosus Patients." *International Journal of Immunogenetics* 36, no. 4: 245–250. https://doi.org/10.1111/j.1744-313X.2009.00856.x.

Vasconcelos, C., C. Carvalho, C. Pereira, et al. 2009. "HLA in Portuguese Systemic Lupus Erythematosus Patients and Their Relation to Clinical Features." *Annals of the New York Academy of Sciences* 580: 575–580. https://doi.org/10.1111/j.1749-6632.2009.04873.x.

Wadi, W., N. E. Elhefny, E. H. Mahgoub, et al. 2014. "Relation Between HLA Typing and Clinical Presentations in Systemic Lupus Erythematosus Patients in Al-Qassim Region, Saudi Arabia." *International Journal of Health Sciences* 8, no. 2: 159–165. https://doi.org/10.12816/0006082.

Wang, T., H. Wang, L. Qiu, et al. 2022. "Association of HLA-DR1, HLA-DR13, and HLA-DR16 Polymorphisms With Systemic Lupus Erythematosus: A Meta-Analysis." *Journal of Immunology Research* 2022: 8140982. https://doi.org/10.1155/2022/8140982.

Webb, K., H. Peckham, A. Radziszewska, et al. 2018. "Sex and Pubertal Differences in the Type 1 Interferon Pathway Associate With Both X Chromosome Number and Serum Sex Hormone Concentration." *Frontiers in Immunology* 9: 3167. https://doi.org/10.3389/fimmu.2018.03167.

Yao, Z., H. P. Seelig, H. Ehrfeld, et al. 1994. "HLA Class II Genes and Antibodies Against Recombinant U1-nRNP Proteins in Patients With Systemic Lupus Erythematosus." *Rheumatology International* 14: 63–69. https://doi.org/10.1007/BF00300249.

Zen, M., L. Salmaso, C. Barbiellini Amidei, et al. 2023. "Systemic Lupus Erythematosus Incidence and Prevalence in a Large Population-Based Study in Northeastern Italy." *Rheumatology* 62, no. 8: 2773–2779. https://doi.org/10.1093/rheumatology/keac685.