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RESEARCH ARTICLE

C1q rs292001 polymorphism association with Rheumatoid arthritis and Behçet's disease

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Abstract

Objective: To analyze whether *C1q* rs292001 polymorphism is associated with rheumatoid arthritis (RA) and Behçet's disease susceptibility and disease phenotype. ***Methods:*** Typing of *C1q* rs292001 polymorphism using Restriction Fragment Length Polymorphism was done for 118 RA and 51 Behcet's patients and 208 healthy controls. ***Results:*** Non-significant associations were found between *C1q* rs292001 polymorphism and RA as well as disease phenotype except for the association between the heterozygous *C1q AG* genotype and the higher VAS score (i.e. greater pain intensity) (P=0.049) and the protective effect of the homozygous *C1q GG* genotype against the development of erosion in crude or adjusted models (P=0.045 and 0.023 respectively). Significant increase in the distribution of *C1q* rs292001 *A* allele in Behçet's disease patients and *G* allele, *GG* genotype in controls were found (P=0.033 and 0.025 respectively). The heterozygous AG genotype was associated with more ulcers especially oral ulcers (P<0.001 and 0.02 respectively) and with less ocular involvement (P=0.025). ***Conclusion:*** The *A* allele of *C1q* rs292001 may be risk factor for BD**.** The *GG* genotype and *G* allele may be protective factors in BD and against development of erosion in RA**.**

INTRODUCTION

Rheumatoid arthritis (RA) is one of the most common autoimmune diseases characterized by chronic inflammation of systemic joints (Alamanos , Drosos 2005) and there is now a general consensus that RA has a spectrum of disease stages that can begin many years before the onset of clinical symptoms (Yarwood et al 2014, Mosaad et al 2015). RA is a multifactorial disease due to a combination of genetic and environmental factors (Dieudéa, Cornélisa 2005, Mosaad et al 2014).

It is widely thought that understanding the complex interplay between genetics and environment, and their role in pathogenesis, is essential in gaining further insight into the mechanisms that drive disease development and progression. More than 100 genetic susceptibility loci have now been identified for RA through studies that have focused on patients with established RA compared with healthy controls (Yarwood et al 2014, Mosaad et al 2015). Identification of the genetic factors involved in the pathogenesis of RA should open up avenues for developing radical treatment strategies directed at the cause of the disease (Dieudéa, Cornélisa 2005, Mosaad et al 2014).

Behçet's disease (BD) is a chronic, relapsing, multisystem inflammatory disorder of unknown etiology (Perra et al 2012). However, an autoimmune reaction triggered by an infectious agent in a genetically predisposed individual has been suggested (Kimura et al 2015). Recurrent oral ulcers in combination with genital ulcers, ocular disease, cutaneous lesions, arthritis, and less frequently, involvement of the gastrointestinal (GI) tract, central nervous system, and vascular beds have been typically observed (Perra et al 2012). The disease can affect both sexes, and although it has a worldwide distribution, it is more prevalent in the Mediterranean and Far East (Kimura et al 2015).

Complete genetic deficiency of C1q is associated strongly with the development of systemic lupus erythematosus (SLE) (Botto , Walport 2002). Several studies have implicated the C1q in the emergence of SLE and several genetic variants located in the *C1q* region seem to associate with adult and juvenile SLE (Martens et al 2009, Namjou et al 2009, Racila et al 2003, Rafiq et al 2010, Zervou et al 2013, Mosaad et al 2015). In addition, genetic variants of *C1q* had an effect on the progression of cancer and the efficacy of rituximab treatment for lymphoma (Racila et al 2006, Racila et al 2008).

It was observed that the complement deposits were found in the synovium of RA patients (Konttinen et al 1996), also systemic complement activation via the classical pathway in patients with RA correlated with disease activity and the C1q-C4 complexes may be used as a biomarker for RA (Wouters et al 2006). Therefore, in the present study, we analyzed whether *C1q* rs292001 gene polymorphism is associated with RA and BD in a cohort of Egyptian population and investigated the effect of this SNP on disease phenotype.

Subjects and methods:

Patients and Controls

This case control study on a cohort of Egyptian population included two groups of patients, 118 patients with RA and 51 patients with BD in addition to 208 healthy individuals as a control group. RA patients included 95 females and 23 males with mean age of 40.2± 11.9 years. The patients were diagnosed according to the American College of Rheumatology (ACR) / European League against Rheumatism (EULAR) collaborative initiative for RA (Aletaha et al 2010) and were consecutively recruited into the study between February 2012 and January 2013 with disease duration of (Median, range) 9.0 (1.0 – 30) years. Patients were recruited from the outpatient's clinic of Rheumatology and Rehabilitation Department, Mansoura University Hospitals, Egypt. All patients underwent clinical evaluation by a rheumatologist and data about demographics and disease parameters were collected.

Disease activity was evaluated using disease activity score 28 (DAS28-CRP) that measures 28 tender and swollen joints, degree of pain by visual analogue scales (VAS), patient's and doctor's global assessment of disease activity using VAS and inflammatory markers such as C-reactive protein (CRP) (van Riel , Schumacher 2001). DAS-28 as described by Prevoo et al. 1995 defines the level of RA activity as follows; ≥ 5.1 indicate high disease activity; between 5.1 and 3.2 indicates moderate disease activity; between 3.2 and 2.6 indicates low disease activity; and < 2.6 indicates clinical remission. Assessment of severity was performed using rheumatoid arthritis medical records based index of severity (RARBIS) based on potential indicators including radiological and laboratory results; surgeries; extra-articular manifestations; clinical and functional status; and medications (Cabral et al 2005, Ting et al 2008). All patients underwent radiographs of the hands and feet. X-rays were scored by an expert radiologist using the Steinbrocker method (Steinbrocker et al 1949). Antero Posterior radiographs of hand (including wrist) and feet were taken to assess the erosive changes.

The modified Health assessment questionnaire (mHAQ) is a questionnaire for the assessment of disease-related disability, discomfort, and quality of life in patients with RA. It was developed to include questions concerning perceived patient satisfaction regarding the same activities of daily living, along with perceived change in degree of difficulty. The eight activities measured by the mHAQ are: dressing and grooming, arising, eating, walking, hygiene, reach, grip, common daily activities. Patients are asked to rate these daily activities on a scale ranging from 1 to 4 with: without difficulty, with some difficulty, with much difficulty, unable to do. The mHAQ is calculated as the average of the single scores. To do that the following scoring is applied: without difficulty = 0, with some difficulty = 1, with much difficulty = 2, unable to do = 3, Values <0.3 are considered normal (Pincus et al 1983).

The Behçet's disease patients were recruited from the outpatient's clinic of Rheumatology and Rehabilitation Department, Mansoura University Hospitals, Egypt, between October 2013 and August 2014. There

were 21 males and 30 females with mean age of 34.4±10.8 years. All 51 BD patients met the criteria established by the International Study Group for Behcet's Disease.

The healthy control group consisted of 208 unrelated healthy subjects without RA or BD and was recruited from blood bank donors. Written informed consent was obtained from patients and controls after approving the study protocol by Local Ethical Committee.

Immunoturbidimetric assay was used to quantify Rheumatoid factor (RF) and CRP using Turbox-plus analyzer (Orion Diagnostica, Espoo, Finland). Anti-Cyclic Citrullinated Peptide (Anti-CCP) antibodies were analyzed by 3rd generation ELISA (CCP3 IgG, INOVA QUANTA LiteTM, Sandiego, USA). According to manufacturer, serum is considered positive when the titer is ≥10 mg/ L for CRP. RF <25 IU/ml=negative, 25-50 IU/ml = borderline positive, > 50= postive, >100 IU/ml= strongly elevated. Anti-CCP antibodies <20 = negative; 20-39 =weak positive; 40-59 =moderate positive and ≥60 =strongly positive.

Typing of *C1q* rs292001 gene polymorphism

Genomic DNA was extracted from whole venous EDTA blood using the GeneJET Whole Blood Genomic DNA Purification Mini Kits (Thermo Scientific, K0781, lot 00147705, Lithuania, EU) and stored at −20°C until use. The genotypes of *C1q* rs 292001 single nucleotide polymorphism (SNP) was analyzed by the polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP) method as described by Zervou et al.

Genomic DNA from the cases and controls was subjected to PCR analysis of the *C1q* gene using the following primers; forward 5'- GTC CAA AGC AGA CCA GAA GGA TCA CAT AGA CAT TTA -3', reverse 5'- GGC ACT TGG GAA AGT GTC AG -3'. Reaction volume was 25 µl: 5 µl DNA at 100 ng/µl, 15.0 µl DreamTaq Green PCR master mix (Fermentas, Germany) , 0.5 µl of each primer (25 pmol/ µl) , and 4.0 µl H2O.

Reaction conditions were carried out in thermocycler PTC-100 (Biorad, USA) with the following cycling parameters. The PCR conditions included an initial 94°C for 5 min followed by 35 cycles of 94°C for 30 s, 63°C for 30 s, and 72°C for 30 s and a final extension at 72°C for 5 min. 10 µl of PCR products were resolved in 2% agarose gel to check the PCR products at 197 bp.

Restriction fragment length polymorphism (RFLP) analysis was done using FastDigest HpyCH4III (ThermoScientific) restriction enzyme in 30 µl total volume by mixing: 10 µl of PCR products + 1.0 µl of restriction enzyme + 2.0 µl 10X FastDigest green buffer + 17 µl nuclease- free water. The mixture was incubated at 37 °C for 10 minutes followed by heating at 65°C for 10 minutes. Genotyping for the *C1q* rs292001 SNP was as follow : the *A* allele showed one band 197 bp (no enzyme cut) and the allele *G* showed two bands 159-bp and 38-bp fragments (enzyme cut). Therefore, *AA* homozygous genotype= one band at 197 bp, *GG* homozygous genotype= two bands at 159 bp and 38 bp, and *AG* heterozygous genotype= 3 bands at 197 bp, 159 bp and 38 bp.

Statistical Analysis

The statistical analysis of data was done using excel program (Microsoft Office 2013) and SPSS (statistical package for social science) program (SPSS, Inc, Chicago, IL) version 20. Qualitative data were presented as number and percentage. Chi square and Fisher's exact tests were used to compare groups. Quantitative data were presented by mean, SD or median and range. For comparison between two groups; student t-test, and Mann-whitney test (for non parametric) were used; for comparison between more than two groups; ANOVA or Kruskal Wallis tests (for non-parametric). Deviations from Hardy–Weinberg equilibrium expectations were determined using the chi-squared test. Odds ratio and 95% confidence interval were calculated. Erosion prediction in RA patients was assessed by logistic regression; while activity, severity and functional disability prediction were assessed by ordinal regression. p is considered significant if <0.05 at confidence interval 95%.

Results:

Demographics and baseline characteristics of RA and BD patients are summarized in Table 1 and 2. This sample of individuals was selected randomly from population in lower Delta, Egypt. Applying Hardy Weinberg equation (HWE) revealed that *C1q* rs292001 SNP in both cases and controls were independent (i.e., they are in HW equilibrium). There is no evidence to reject the assumption of HWE in the sample (p=0.155 for RA patients, 0.068 for BD patients and 0.065 for controls).

Analysis of the distribution of *C1q* rs292001 polymorphism in RA patients and controls showed non-significant differences in the frequencies of *C1q* alleles or genotypes between RA patients and healthy controls. Analysis of the distribution of *C1q* rs292001 polymorphism in BD patients and controls demonstrated that the *A* allele was higher in patients and *G* allele and homozygous *GG* genotype were higher in healthy controls and the differences were statistically significantly (P=0.033, 0.025 respectively). Table 3

The frequency of *C1q* rs292001 genotypes was analyzed in relation to the patient's characteristics, Clinical manifestation, VAS score, DAS28-CRP for disease activity, RARBIS for severity and mHAQ for disease-related disability and was presented in table 4. No significant associations were found except for the association between the *C1q* heterozygous *AG* genotype and the higher VAS score (i.e. greater pain intensity) (P=0.049).

In the same time, the frequency of *C1q* rs292001 genotypes was analyzed in relation to the patient's characteristics and clinical manifestation of BD. The heterozygous AG genotype was associated with more ulcers especially oral ulcers (P<0.001 and 0.02 respectively) and with less ocular involvement (P=0.025). Table 5

Prediction of erosion, disease activity, disease severity and functional activity using *C1q* rs292001 genotypes in RA patients in either crude or adjusted models (adjusted for age, gender, disease duration and steroid therapy requirement) were done and presented in table 6. The *GG* genotype was protective against erosion development in either crude or adjusted models in RA patients (P=0.045 and 0.023 respectively).

Table 1: Demographics, and baseline characteristics of RA patients

	Value
Age (years); mean± SD	40.2±11.9
Gender: Male / Female; N (%)	23/95 (19.5/80.5)
Disease duration (years); median (range)	9.0 (1-30)
Disease onset (years); median (range)	35 (4-71)
VAS; median (range)	5.0 (0-9)
Erosion; N (%)	26 (22)
mHAQ; median (range)	1.5 (0-3)
RARBIS; median (range)	7.0 (2-12)
Family history; N (%)	40 (33.9)
Rheumatoid factor IU/ML; median (range)	108 (1-369)
Positive /Negative Rheumatoid factor; N (%)	100 (84.7)
RF grade (number of RA patients); N (%)	
Negative	18 (15.3)
Borderline positive	17 (14.4)
Positive	22 (18.6)
Strongly elevated	61 (51.7)
Anti-CCP IU/ML; median (range)	240 (2-377)
Positive / Negative Anti-CCP; N (%)	99 (83.9)
Anti-CCP grade (number of RA patients); N (%)	
Negative	19 (61.1)
Weak positive	4.0 (3.4)
Moderate Positive	4.0 (3.4)
Strongly elevated	91 (77.1)
CRP mg/L; median (range)	19 (3-189)
DAS CRP; median (range)	5.4 (2-8)
DAS CRP grade (number of RA patients); N (%)	

High activity	65 (55.1)
Moderate	47 (39.8)
Low	3.0 (2.5)
Remission	3.0 (2.5)
Consanguinity; N (%)	36 (30.5)

Table 2: Demographics, and baseline characteristics of Behçet's disease patients

	Value
Age years (M ± SD)	34.4±10.8
Gender : Male / Female	21/30
Ulcers; N (%)	
Yes/ No	35/16 (58.6/31.4)
Oral ulcers	35 (68.6)
Genital ulcers	25 (49)
Joint affection; N (%)	
Yes/ No	35/16 (68.6/31.4)
Arthralgia	14 (27.5)
Arthritis	33 (64.7)
Knee	19 (37.3)
Ankle	14 (27.5)
Wrist	8 (15.7)
Ocular involvement; N (%)	
Yes/ No	35/16 (68.6/31.4)
Uveitis	22 (43.1)
Hypopion	7.0 (13.7)
Retinal vasculitis	6.0 (11.8)
Skin lesions; N (%)	
Yes/ No	29/22 (56.9/43.1)
Erythema nodosum	4.0 (7.8)
Pustules	16 (31.4)
Pyoderma	4.0 (7.8)
Positive pathergic reaction	5.0 (9.8)
Vascular involvement; N (%)	
Yes/ No	22/29 (43.1/56.9)
Deep venous thrombosis	13 (25.5)
Superficial thrombophilibitis	9.0 (17.6)
Diarrhea; N (%)	9.0 (17.6)

Table: 3 Distribution of *C1qA* rs292001 alleles and genotypes in RA and Behçet's disease patients versus healthy controls.

***C1q* Polymorphism**	**RA Patient (N=118) N (%)**	**Controls (N= 208) N (%)**	**OR (95% CI)**	***p* value**
***C1q* alleles**				
A	99 (42)	156 (37.5)	1.20 (0.86-1.66)	0.26
G	137 (58)	260 (62.5)	0.8 (0.59 – 1.15)	
***C1q* genotypes**				
AA	17 (14.4)	23 (11.1)	1.35 (0.69-2.65)	0.37
AG	65 (55.1)	110 (52.9)	1.09 (0.69-1.72)	0.70
GG	36 (30.5)	75 (36.1)	0.78 (0.48-1.26)	0.31
***p*HW**	0.155	0.065		
	Behçet's patients (N=51) N (%)	**Healthy controls (N=208) N (%)**	**OR (95% CI)**	***p* value**
***C1q* alleles**				
A	50 (49)	156 (37.5)	1.60 (1.04-2.48)	**0.033**
G	52 (51)	260 (62.5)	0.62 (0.40-0.97)	
***C1q* genotypes**				
AA	9 (17.6)	23 (11.1)	1.72 (0.74-3.99)	0.20
AG	32 (62.7)	110 (52.9)	1.50 (0.80-2.82)	0.21
GG	10 (19.6)	75 (36.1)	0.43 (0.21-0.91)	**0.025**
***p*HW**	0.068	0.065		

***p*HW**, probability for Hardy Weinberg equilibrium.

Table 4: Relation between genotype frequency of *C1qA* rs292001 and demographics / baseline characteristics of RA patients

	***AA* N=17**		***AG* N=65**		***GG* N=36**		***P***
Age (years); M± SD	38.9	(9.0)	40	(12.5)	41.1	(12.3)	0.81
Gender: Male / Female; N (%)	4/13	(23.5/76.5)	14/51	(21.5/78.5)	5/31	(13.9/86.1)	0.58
Disease duration (years); median (range)	7	(1-30)	10	(1-30)	8.5	(1-29)	0.56
Disease onset (years); median (range)	35	(17-55)	37	(4-61)	33.3	(15-71)	0.79
VAS; median (range)	4	(2-9)	5	(0-9)	4.5	(1-8)	**0.049**
Erosion; N (%)	6	(35.3)	16	(24.6)	4	(11.1)	0.08
mHAQ; median (range)	0.88	(0-3)	1.63	(0-3)	1.56	(0-3)	0.21
RARBIS; median (range)	7	(4-12)	8	(4-12)	7	(2-11)	0.83
Family history; N (%)	6	(35.3)	22	(33.8)	12	(33.3)	1.0
Rheumatoid factor IU/ML; median (range)	81	(5-289)	118	(1-369)	90.5	(6-309)	0.95

Positive /Negative Rheumatoid factor; N (%)	15	(88.2)	54	(83.1)	31	(86.1)	0.94
RF grade (RA patients number)							
Negative	2	(11.8)	11	(16.9)	5	(13.9)	
Borderline positive	3	(17.6)	9	(13.8)	5	(13.9)	
Positive	4	(23.5)	9	(13.8)	9	(25.0)	0.84
Strongly elevated	8	(47.1)	36	(55.4)	17	(47.2)	
Anti-CCP (IU/ML); median (range)	261	(2-344)	245	(3-377)	236	(3-365)	0.88
Positive / Negative Anti-CCP; N (%)	13	(76.5)	57	(87.7)	29	(80.6)	0.35
Anti-CCP grade (number of RA patients)							
Negative	4	(23.5)	8	(12.3)	7	(19.4)	
Weak positive	0	(0)	3	(4.6)	1	(2.8)	0.31
Moderate Positive	2	(11.8)	1	(1.5)	1	(2.8)	
Strongly elevated	11	(64.7)	53	(81.5)	27	(75.0)	
CRP (mg/L);median (range)	15	(3-97)	20	(3-185)	18.5	(4-189)	0.95
DAS-CRP; median (range)	5.27	(2-7)	5.57	(3-8)	5.11	(2-7)	0.43
DAS-CRP grade (number of RA patients)							
High activity	9	(52.9)	38	(58.5)	18	(50.0)	
Moderate	7	(41.2)	25	(38.5)	15	(41.7)	
Low	0	(0)	2	(3.1)	1	(2.8)	0.46
Remission	1	(5.9)	0	(0)	2	(5.6)	
Consanguinity; N (%)	7	(41.2	18	(27.7)	11	(30.6)	0.56

Table 5: Relation between genotype frequency of *C1qA* rs292001 and demographics / baseline characteristics of Behcet's disease patients

	AA **N=9**		***AG*** **N=32**		***GG*** **N=10**		***P***
Age years (M ± SD)	36.6±11.4		33.9±11.5		34.1±8.3		0.81
Gender : Male / Female	4/5	(44.4/55.6)	12/20	37.5/62.5)	5/5	(50/50)	0.78
Ulcers; N (%)							
Total	3	(33.3)	30	(93.8)	6	(60)	**<0.001**
Oral ulcers	3	(33.3)	26	(81.3)	6	(60)	**0.02**
Genital ulcers	3	(33.3)	18	(56.3)	4	(40)	0.41
Joint affection; N (%)							
Total	5	(55.6)	23	(71.9)	7	(70)	0.62
Arthralgia	3	(33.3)	6	(18.8)	5	(50)	0.15
Arthritis	4	(44.4)	22	(68.8)	7	(70)	0.40
Knee	2	(22.2)	12	(37.5)	5	(50)	0.59
Ankle	2	(22.2)	10	(31.3)	2	(20)	0.74
Wrist	0	(0)	6	(18.8)	2	(20)	0.53

Ocular involvement; N (%)							
Total	7	(77.8)	18	(56.3)	10	(100)	**0.025**
Uveitis	4	(44.4)	12	(37.5)	6	(60)	0.43
Hypopion	3	(33.3)	2	(6.3)	2	(20)	0.05
Retinal vasculitis	0	(0)	4	(12.5)	2	(20)	0.39
Skin lesions; N (%)							
Total	7	(77.8)	16	(50.0)	6	(60)	0.37
Erythema nodosum	2	(22.2)	2	(6.3)	0	(0)	0.21
Pustules	2	(22.2)	10	(31.3)	4	(40)	0.76
Pyoderma	2	(22.2)	2	(6.3)	0	(0)	0.21
+ve pathergic reaction	1	(11.1)	2	(6.3)	2	(20)	0.33
Vascular involvement; N (%)							
Total	5	(77.8)	14	(43.8)	3	(30)	0.56
Deep venous thrombosis	2	(22.2)	10	(31.3)	1	(10)	0.43
Superficial thrombophilibitis	3	(22.2)	4	(12.5)	2	(20)	0.33
Diarrhea; N (%)	2	(22.2)	4	(12.5)	3	(30)	0.44

Table 6: Prediction of erosion, activity, severity and functional activity using *C1q* rs292001 genotypes in RA patients.

Erosion						
	Crude model			**Adjusted model***		
	p	**OR**	**95% CI**	***p***	**OR**	**95% CI**
AA	0.161	2.209	0.729-6.694	0.072	3.027	0.905-10.124
AG	0.455	1.185	0.759-1.849	0.628	1.124	0.701-1.802
GG	**0.045**	0.229	0.054-0.966	**0.023**	0.163	0.034-0.777
Disease activity assessed by DAS28 CRP grade						
	Crude model			**Adjusted model***		
	P	**β**	**SE**	***P***	**β**	**SE**
AA	0.822	-0.116	0.516	0.734	-0.178	0.524
AG	0.320	0.364	0.367	0.370	0.375	0.323
GG	0.364	-0.358	0.395	0.416	-0.329	0.405
Disease severity assessed by RARBIS						
	Crude model			**Adjusted model***		
	P	**β**	**SE**	***P***	**β**	**SE**
AA	0.832	-0.102	0.481	0.879	-0.074	0.487
AG	0.540	0.202	0.329	0.566	0.191	0.333
GG	0.613	-0.180	0.356	0.610	-0.184	0.362
Functional disability assessed by mHAQ						
	Crude model			**Adjusted model***		
	P	**β**	**SE**	***P***	**β**	**SE**
AA	0.095	-0.781	0.469	0.138	-0.705	0.475
AG	0.153	0.462	0.323	0.122	0.513	0.331
GG	0.748	-0.109	0.339	0.571	-0.196	0.346

*Adjusted for age, gender, disease duration and steroid therapy requirement , β, regression coefficient; SE, standard error.

Discussion:

Immune system undergoes dysregulation (due to certain genetic and epigenetic variations) which leads to the loss of self-tolerance and autoimmune diseases (Invernizzi and Gershwin 2009). Autoimmune diseases are usually multifactorial and thus various genetic and environmental factors contribute to the disease onset and progression (Boscolo et al. 2008; Lleo et al. 2008). Rheumatoid arthritis is a chronic, systemic autoimmune inflammatory disorder that primarily manifests as arthritis that clinically represents as joint pain, stiffness, and swelling. While about 60 % of the genetic contribution to RA pathogenesis seems to be mediated by human leukocytes antigen (HLA) variants, the non-HLA genes also contribute to disease manifestations (de Vries 2011, Kiani et al 2015).

Behçet's disease is defined as a multisystemic inflammatory disease. Although the precise pathogenesis and etiology is still a mystery, accumulating evidence shows that genetic variants of immune-related genes have a profound influence on the development of BD (Hou et al 2012). Family studies suggested the importance of non-HLA genes to the genetic susceptibility for BD (Gul et al 2001). Identified non-HLA variations may implicate defects in the sensing and processing of microbial and endogenous danger signals as well as in the regulation of innate and adaptive immune responses in BD pathogenesis (Gul 2014).

The results of the present study on a cohort of Egyptian population revealed non-significant association between *C1q* rs292001 polymorphism and RA susceptibility, disease activity , disease severity and erosion except for the association between the heterozygous *C1q AG* genotype and the higher VAS score (i.e. greater pain intensity) (P=0.049) and the protective effect of the homozygous *C1q GG* genotype against the development of erosion in crude or adjusted models (P=0.045 and 0.023 respectively). Tables 3 and 6

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Regarding the Behçet's disease, the distribution of *C1q* rs292001 alleles and genotypes showed significant increase in the frequency of *A* allele in patients, *G* allele and *GG* genotype in controls (P=0.033 and 0.025 respectively). These findings implicate that the *A* allele may be susceptibility risk factor (OR=1.6) and the *G* allele (OR=0.62) and the *GG* genotype (OR=0.43) may be protective against development of BD. Table 3 In the same time, The heterozygous *AG* genotype was associated with more ulcers especially oral ulcers (P<0.001 and 0.02 respectively) and with less ocular involvement (P=0.025). Table 5

Trouw et al 2013 analyzed the relation between genetic variants of *C1q* (*C1qA, C1qB* and *C1qC)* and RA in a cohort of Dutch population (845 cases /1046 controls). They performed replication in population from North America (868 cases/1193 controls) and also performed meta-analysis for combined samples from European descent (8000 cases/23,262 controls). They reported significant association between *C1q* rs292001 and RA (P=0.0006).

Mosaad et al 2015 on their work on a cohort of Egyptian children with SLE reported the *A* allele and *AA* genotype of *C1q* rs292001 as susceptibility risk factor and the *GG* genotype as protective for juvenile SLE and lupus nephritis (LN). Also, the rs292001 *A* allele was associated with a susceptibility to SLE in a Turkish cohort (Zervou et al 2011) and with increased risk for type 2 diabetes (T2D) in the Greek population (Goulielmos et al 2013). These studies suggested a possible key role of rs292001 in different autoimmune diseases. On the contrary, the *C1q* rs292001 SNP was not associated with the risk of SLE in polish population (Korczowska et al 2012), LN risk in the Bulgarian population (Radanova et al 2015) and risk to schizophrenia in Armenian population (Zakharyan et al 2011). Martins et al 2009 reported association between *C1qA* rs292001 and more severe SLE on Caucasian patients with SLE and their family members from Netherlands.

The controversial results observed between various reports may be due to different studied diseases (RA, SLE, T2D, Schizophrenia and BD), different disease phenotypes, different age groups, extensive geographic variations between different studied populations, different sample size, different technique of typing and difference in ethnic background.

Neutrophil hyperactivation plays a pivotal role in the inflammatory vasculitis characterizing the main pathologic lesion of BD. Neutrophil hyperactivation is due to the priming cytokines derived from antigen presenting cells (APCs) and T-cells (Eksioglu-Demiralp et al 2001). The activated neutrophils produce cytokines that cause Th1 stimulation and the crosstalk between APCs / Th1 and neutrophils has been proposed as a critical parameter of the immune-mediated mechanism in BD (Pay et al 2007). Overproduction of activated monocyte-derived pro-inflammatory cytokines (e.g., IL-1, IL-6, IL-8, and TNF-α) has been implicated in the BD pathogenetic process, and their increased levels may represent a disease activity marker (Azizlerli et al 2003). Th1 immune response polarization is a main characteristic of BD immunopathogenesis (Kapsimali et al 2011).

Besides its role as an initiator of the classical complement activation pathway C1q has several other possible functions. It can bind to early apoptotic cells and facilitate phagocytosis and may also participate in clearance of late apoptotic cells via IgM/C1q mediated classical pathway activation. It can induce dendritic cell maturation via C1q receptors on the immature dendritic cells, it may modulate T lymphocytes, which have been shown to express C1q receptors, and C1q is suspected to participate in the negative selection of autoreactive B-cells (Schejbel et al 2011, Nayak et al 2010, Mosaad et al 2015).

C1q binds directly to ligands on apoptotic cells and participates in their opsonization. These ligands include cell-derived molecules (e.g. DNA) or blood-borne factors, (e.g. CRP and natural IgM) and are recognized by the globular domain of C1q. Defect in C1q function due to mutation will lead to failure to opsonize apoptotic cells and hence defective phagocytosis by monocytes and decreased threshold for activation of dendritic cells. The consequences will be active immune response and generation of autoantibodies (Roumenina et al 2011, Mosaad et al 2015).

Conclusion*:*

The *A* allele of *C1q* rs292001 may be risk factor for BD**.** The *GG* genotype and *G* allele may be protective factor in BD and against development of erosion in RA**.**

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