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RESEARCH ARTICLE

Analysis of two susceptibility SNPs in HLA region and evidence of interaction between rs6457617 in *HLA-DQB1* and *HLA-DRB1**04 locus on Tunisian rheumatoid arthritis

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Abstract. Previous genomewide association studies (GWAS) and meta-analyses have enumerated several genes/loci in major histocompatibility complex region, which are consistently associated with rheumatoid arthritis (RA) in different ethnic populations. Given the genetic heterogeneity of the disease, it is necessary to replicate these susceptibility loci in other populations. In this case, we investigate the analysis of two SNPs, rs13192471 and rs6457617, from the human leukocyte antigen (HLA) region with the risk of RA in Tunisian population. These SNPs were previously identified to have a strong RA association signal in several GWAS studies. A case–control sample composed of 142 RA patients and 123 healthy controls was analysed. Genotyping of rs13192471 and rs6457617 was carried out using real-time PCR methods by TaqMan allelic discrimination assay. A trend of significant association was found in rs6457617 TT genotype with susceptibility to RA ($P = 0.04$, $p_c = 0.08$, OR $= 1.73$). Moreover, using multivariable analysis, the combination of rs6457617*TT–*HLA-DRB1**04$^+$ increased risk of RA (OR $= 2.38$), which suggest a gene–gene interaction event between rs6457617 located within the *HLA-DQB1* and *HLA-DRB1*. Additionally, haplotypic analysis highlighted a significant association of rs6457617*T–*HLA-DRB1**04$^+$ haplotype with susceptibility to RA ($P = 0.018$, $p_c = 0.036$, OR $= 1.72$). An evidence of association was shown subsequently in antiCCP$^+$ subgroup with rs6457617 both in T allele and TT genotype ($P = 0.01$, $p_c = 0.03$, OR $= 1.66$ and $P = 0.008$, $p_c = 0.024$, OR $= 1.28$, respectively). However, no association was shown for rs13192471 polymorphism with susceptibility and severity to RA. This study suggests the involvement of rs6457617 locus as risk variant for susceptibility/severity to RA in Tunisian population. Secondly, it highlights the gene–gene interaction between *HLA-DQB1* and *HLA-DRB1*.

Keywords. association study; gene–gene interaction; haplotypic analysis; human leukocyte antigen region; rheumatoid arthritis.

Introduction

Rheumatoid arthritis (RA, OMIM# 180300) is relatively the most common autoimmune inflammatory arthritis with poorly understood genetic aetiology, which like other autoimmune disorders remains elusive and still an open question. This disease is characterized by synovial joint inflammation and cartilage destruction, which result in joint deformities, leading to functional disability and is associated with the presence of anticitrullinated protein antibodies (ACPA) (Firestein 2003). The prevalence of the disease is ~about 0.4% in African population (Dowman *et al.* 2012), and 1% in several European and North American populations (Riise *et al.* 2000; Gabriel 2001; Carmona *et al.* 2002). However, a high prevalence was shown in some native American–Indian populations such as the Pima Indians (5.3%) (Del Puente *et al.* 1989) and Chippewa Indians (6.8%) (Harvey *et al.* 1981). Although, the pathophysiology of the disease is well studied, there are only a limited number of risk factors with low–moderate

effects, which are described by Klareskog *et al.* (2006). The strongest genetic risk factor for RA was described with about 60% of heritability (MacGregor *et al.* 2000). Moreover, the polymorphic human leukocyte antigen (HLA) region still confers a strong risk for susceptibility to RA in which the genetic association of the disease has commonly been attributed to *HLA-DRB1* alleles (WTCCC 2007).

Single-nucleotide polymorphisms (SNPs), the current form of DNA variation are often associated with susceptibility to RA. Genomewide association studies (GWAS) are previously considered to be an important tool for identifying a broad number of novel genetic loci underlying susceptibility to autoimmune diseases (Manolio *et al.* 2008), which can lead to the comprehension of the causal effect and pathways of diseases and subsequently providing a sign for therapeutic targets (Hirschhorn 2009).

In the last few years, an extensive list of additional genetic risk loci with relatively high statistical power from GWAS was found associated with RA in different ethnic populations (WTCCC 2007). In fact, there are about 100 loci in nonhuman leukocyte antigen (non-HLA) which are systematically identified associated with RA susceptibility (Okada *et al.* 2014). However, the genetic contribution for these loci remains poor and account only for 4.7–5.5% of RA heritability (Okada *et al.* 2014). Several studies in GWAS and meta-analyses have confirmed the largest predisposing genetic risk for *HLA-DRB1* locus and identified and combined a number of SNPs related to RA, both in HLA and non-HLA genes (Chatzikyriakidou *et al.* 2013). In fact, GWAS studies including the Wellcome Trust Case Control Consortium (WTCCC), the North-American Rheumatoid Arthritis Consortium (NARAC) and the Swedish Epidemiological Investigation of Rheumatoid Arthritis (EIRA) showed considerable significant associations with various SNPs near the *HLA-DRB1* gene such as rs660895, rs6457617, rs13192471 and rs7765379 with RA in European population and replicated in Korean and Japanese GWAS RA (WTCCC 2007; Kochi *et al.* 2010; Freudenberg *et al.* 2011). In addition, several other HLA class II-region SNPs, particularly rs6457617 and rs9275390, situated across *HLA-DQB1* gene showed a strong association with RA in the global analyses ($P < 10^{-9}$) in a Spanish replication GWAS study (Julià *et al.* 2008). Further, this was confirmed for rs6457617, which is identified as a susceptibility locus to RA in Han Chinese population (Li *et al.* 2013).

Replication analysis of association of different susceptibility loci identified in GWAS is important because of the genetic heterogeneity of RA. Despite that the major histocompatibility complex (MHC) loci are the most genetically variable loci in the human population; SNPs within the MHC region were less investigated among other populations. More examination of the contribution of other causal variants or candidate genes/loci will eventually facilitate to understand the genetic architecture of RA.

This study looks at two SNPs from the HLA region, rs13192471 and rs6457617, identified previously in GWAS studies (WTCCC 2007; Julià *et al.* 2008; Li *et al.* 2013) to see whether they are associated with RA and to investigate the possible gene–gene interaction with *HLA-DRB1* in the Tunisian population.

Materials and methods

RA subjects and controls

In this case–control study, we enrolled 142 Tunisian patients with RA (37 men and 105 women). All patients fulfilled the 1987 American College of Rheumatology criteria for RA (Arnett *et al.* 1988). A rheumatology university fellow reviewed all clinical data. The mean age at disease onset was 39.5 years ± 20 standard deviation (SD). Among our RA patients, 64% were RF^+; 57% were $antiCCP^+$; 74% had radiographically visible hand erosions and 47% had another autoimmune disease (AID+; Sjögren's syndrome, autoimmune thyroid diseases, systemic lupus erythematosus and type 1 diabetes). Control group consisted of 123 healthy unrelated subjects, originating from the same area as the RA samples were recruited (40 men and 83 women). The mean age at analysis was 42.8 years. None of them suffered from any autoimmune or inflammatory diseases. All patients and healthy controls gave informed consent for participation in the study. This study was approved by the Ethical Committee of the University Hospital Hedi Chaker of Sfax, Tunisia.

Autoantibody analysis

Patient's sera were obtained from 127 samples at the time of diagnosis and examined for rheumatoid factor by nephelometry and from 122 samples for antiCCP by enzyme-linked immunosorbent assay (ELISA) (second-generation test; Euro-Diagnostica, Arnhem, the Netherlands).

SNP selection and genotyping methods

Two SNPs in the MHC region near the *HLA-DRB1* gene with strong association signal in European population were used in this study: rs6457617 with a minor allele frequency (MAF) of 0.465 and rs13192471 with a MAF of 0.181 (1000 genome population). Genomic DNA from whole blood was extracted from peripheral blood leukocytes samples taken from patients and controls by standard methods (Gustincich *et al.* 1991). The rs6457617 and rs13192471 were genotyped with a TaqMan 5′ allelic discrimination assay on an Applied Biosystems StepOne Instrument real-time polymerase chain reaction (PCR) machine (assay for rs6457617: C_30051540_30; assay for rs13192471: C_31859106_10).

Statistical analysis

Hardy–Weinberg equilibrium was examined in the control group using a χ^2 test. Case–control association analyses were performed by χ^2 or Fisher's exact test, as appropriate. P values, odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated. Multiple logistic regression was conducted to estimate the relative risk of each SNP adjusted for age and gender using the Statistical Package for the Social Sciences (SPSS) ver. 17.0 for Windows (SPSS, Chicago, USA).

The genotype relative risk (GRR) method was used to compare the genotype distribution in controls and patients. The GRR test adjusts the genotype frequencies in the controls to the expected Hardy–Weinberg proportions and yields more accurate risk estimates (Lathrop 1983). Significant P values were corrected (p_c) by the number of alleles tested or subgroups analysed according to Bonferroni's corrections.

A pairwise linkage disequilibrium (LD) analysis and haplotype association between the two SNPs and results of typing *HLA-DRB1**04 alleles as described by Ben Hamad *et al.* (2012) were performed using an online software SHEsis (http://analysis.bio-x.cn/myAnalysis.php) (Shi and He 2005) and the Haploview program (v.4.2) (http://www.broad.mit.edu/mpg/haploview/), respectively. The pairwise SNP LD analysis was applied for measuring the LD around the candidate region in healthy controls. Two LD coefficients, Lewontin's D' and Hill's r^2 were evaluated and high values of LD were defined as $r^2 > 0.33$ (Ardlie *et al.* 2002) and $D' > 0.7$ (Gabriel *et al.* 2002).

Haplotype blocks were defined by solid spine rule and corresponding frequencies were compared between cases and controls using Fisher's exact test. For all the analyses, differences were considered statistically significant when the P value was <0.05. Further, we calculated the empirical power of the study by bootstrapping from the dataset of patients and controls (random sampling with replacement of the same number of patients and controls from the original dataset). For each bootstrap, a GRR test was calculated. Bootstraps 100,000 were used and the empirical power was estimated as the number of times the GRR test exceeded the χ^2 threshold (when a true association declared) at a 5% significance level (3.84) divided by the total number of bootstraps. Calculations were performed using a program written in R language (www.r-project.org) (de Bakker *et al.* 2005). Moreover, using the same program we performed interaction test between SNPs analysed and *HLA-DRB1**04 alleles.

Results

We genotyped two SNPs, which have been well defined in previous GWAS studies, across the HLA region in 142 Tunisian RA patients and 123 control individuals. For rs6457617, two patients and one control with missing data were excluded. Both SNPs do not deviate from Hardy–Weinberg equilibrium ($P = 0.60$ for rs13192471 and $P = 0.57$ for rs6457617) in healthy controls.

Association analysis of rs13192471 and rs6457617 polymorphisms with RA

In the allele and genotype frequency analyses shown in table 1, we did not find statistical differences between patients with RA and controls for rs13192471 polymorphism. For the rs6457617, the frequency of the 'T' allele was slightly higher in RA patients compared to healthy controls (58.4% of the patients versus 51.2% of the controls) with a trend towards association ($P = 0.07$, OR = 1.36, 95% CI: 0.96–1.92). Further, the genotypic distribution revealed a higher frequency in RA patients of the homozygous wild-type genotype 'TT' compared to the minor and the heterozygous genotype 'CC and TC' with a trend of association with RA ($P = 0.04$, $p_c = 0.08$, OR = 1.73, 95% CI: 1.02–2.95) (table 1).

This result was confirmed by the assessment of the empirical power of our case–control sample to detect association when it exists. Power calculations indicated that our sample sizes provided 30% power for the genotypic association of rs6457617 polymorphism.

Multivariable analysis and gene–gene interaction

The stratification of rs6457617 genotypes according to *HLA-DRB1**04 status (carriers versus noncarriers) shows no significant association ($P = 0.27$ and 0.23, respectively). Here, we suggest that the loss of significance in our results may be explained either by the decrease in frequency of the two groups during the stratification or by an interaction event between the two loci. In fact, we have conducted analysis of interaction between rs6457617 and *HLA-DRB1* locus using a program written in R language and we found a significant interaction ($P = 0.04$). In fact, data analysis of gene–gene interaction by logistic regression analysis revealed a significant global association of the four combinations ($P = 0.016$, OR = 1.32, 95% CI: 1.05–1.66) (data not shown). Further, as shown in table 2, the combination of rs6457617 (TT) and *HLA-DRB1**04$^+$ has 2.34-fold increased risk of RA ($P = 0.02$, OR = 2.34, 95% CI: 1.14–4.82) compared to individuals with other genotypes (table 2).

LD and haplotype analysis

LD analysis was performed within the tested SNPs and HLA-DRB1 locus. Pairwise D' values between all SNPs were calculated to determine the extent of LD. In healthy controls, our results show a strong LD between rs13192471/rs6457617 with a value of $D' = 0.99$ and

Table 1. Allele and genotype frequencies of SNPs analysed in Tunisian cohort.

SNP	Allele	Allelic analysis				Genotypic analysis					Lathrop *P*
		Case number (%)	Control number (%)	OR (95% CI)	*P*	Genotype	Case number (%)	Control number (%)	*P*	OR (95% CI)	
rs13192471	T	229 (81)	201 (81.5)	0.9 (0.6–1.45)	0.75	TT	92 (65)	83 (67.5)	0.84	0.89 (0.53–1.48)	0.71
	C	55 (19.4)	45 (18.4)			TC	45 (32)	35 (28.5)		1.17 (0.69–1.98)	0.69
						CC	5 (3.5)	5 (4.1)		0.86 (0.24–3.04)	0.77
rs6457617	T	166 (58.4)	126 (51.2)	1.36 (0.96–1.92)	0.07	TT	52 (36.6)	31 (25.2)	0.12	**1.73 (1.02–2.95)**	**0.04***, $p_c = 0.08$
	C	114 (40.1)	118 (47.9)			TC	62 (43.7)	64 (52)		0.72 (0.44–1.17)	0.18
						CC	26 (18.3)	27 (22)		0.80 (0.44–1.46)	0.47

Data shown in *n* (%); OR, odds ratio; 95% CI, 95% confidence interval; allele analyses for association were used by Pearson χ^2 test, and genotype analyses for association were used by GRR Lathrop: each genotype was analysed versus others. For e.g., *: TT versus TC + CC. Significant associations are indicated in bold.

Table 2. RA risk associated with genotypic combinations of rs6457617 and *HLA-DRB1* polymorphisms in cases and controls.

Gene–gene	Controls, *n*	Cases, *n*	OR (95% CI)	*P*
rs6457617–*HLA-DRB1*				
TC/CC–*04$^-$	70	46	Reference	–
TC/CC–*04$^+$	21	20	1.44 (0.71–2.94)	0.26
TT–*04$^-$	15	16	1.61 (0.73–3.54)	0.42
TT–*04$^+$	16	25	**2.34 (1.14–4.82)**	**0.02**

OR, odds ratio; CI, confidence interval. Significant associations are indicated in bold.

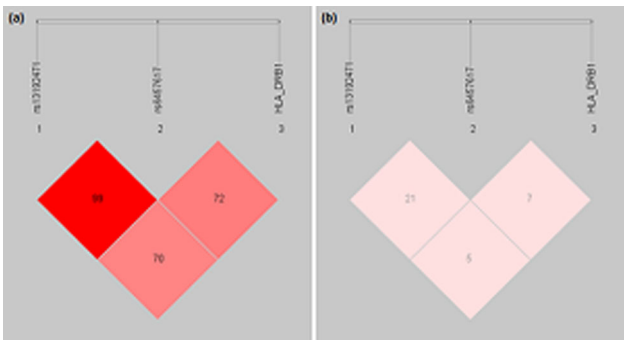

Figure 1. Pairwise LD analysis of RA susceptibility polymorphisms for healthy controls. Corresponds to the (a) Lewontin correlation (D') and (b) Pearson correlation (r^2).

rs6457617/*HLA-DRB1* ($D' = 0.72$). However, the r^2 values between them were poor ($r^2 = 0.21$ and 0.07, respectively). This was due to one SNP being much rare than the other. Thus, the SNPs could not substitute each other. Similarly, a weak LD was revealed between rs13192471 and *HLA-DRB1* locus ($D' = 0.70$, $r^2 = 0.05$) (figure 1).

As shown in table 3, haplotypic analysis for rs13192471/rs6457617 and rs6457617/*HLA-DRB1* was defined as a block with three haplotypes. Infact, the rs13192471/rs6457617 haplotype revealed no significant association with RA ($P > 0.05$). Moreover, three other haplotypes were defined between rs6457617 and *HLA-DRB1*. A significant evidence of association with RA was revealed in rs6457617*T/*HLA-DRB1**04$^+$ haplotype ($P = 0.018$, $p_c = 0.036$, OR = 1.72, 95% CI: 1.08–2.73) (table 3).

Stratification analysis of rs13192471 and rs6457617 polymorphisms by immunological and clinical data

Distribution analysis of alleles and genotypes of rs6457617 SNP according to immunological data showed a significant association of T allele and TT genotype with anti-CCP$^+$ RA subsample ($P = 0.01$, $p_c = 0.03$ and $P = 0.008$, $p_c = 0.024$; respectively). Likewise, a modest increase in frequency was observed among RF$^+$ subgroup compared to healthy controls with a trend association of

Table 3. Haplotype frequencies in case–control cohort of patients with RA.

Haplotype	Freq. cases	Freq. controls	χ^2	P	OR (95% CI)
Block 1 (rs13192417/rs6457617)					
T/C	0.40	0.48	3.40	0.06	0.70 (0.51–1.02)
T/T	0.40	0.33	2.8	0.09	1.36 (0.95–1.94)
C/T	0.19	0.18	0.07	0.78	1.05 (0.68–1.63)
Block 2 (rs6457617/*HLA-DRB1*)					
C/*04$^-$	0.40	0.47	3.18	0.05	0.70 (0.47–1.00)
T/*04$^-$	0.36	0.35	0.005	0.95	1.00 (0.68–0.47)
T/*04$^+$	0.27	0.16	4.90	**0.018** (p_c = **0.036**)	**1.72 (1.08–2.73)**

Blocks were constructed according to the value of D' generating from our own data. Haplotypes were defined as the method of solid spine of LD. Haplotype data was analysed using Haploview 4.2. *HLA-DRB1**04$^{+/-}$: carrier/noncarrier *04 allele. Significant associations are indicated in bold.

the T allele and TT genotype, which did not reach significance after Bonferroni's correction ($P = 0.03$, $p_c = 0.09$ and $P = 0.02$, $p_c = 0.06$; respectively). However, no significant differences were observed either in anti-CCP$^-$ or RF$^-$ subsamples. Compared to the controls, we observe a slight increase in the TT genotype in the RA subgroup positive for erosion with $P = 0.04$ which loses significance after the Bonferroni's correction ($p_c = 0.12$) (table 4). Regarding rs13162471, no association was detected for none of the subgroups ($P > 0.05$) (data not shown).

Discussion

The development of several GWAS studies in autoimmune diseases has suggested a number of associated SNP polymorphisms and replicated analysis of association in different populations which could indicate the importance of a variety of susceptibility loci in disease aetiology, independently of environmental factors. In the present study, we investigated the replication of two SNPs rs13192471 and rs6457617 in the Tunisian RA population, identified previously in GWAS studies with a powerful genetic implication in RA (WTCCC 2007; Kochi *et al.* 2010; Freudenberg *et al.* 2011). These SNPs are located in MHC region near the *HLA-DRB1* gene. Association of SNPs in the HLA region was previously shown and clearly demonstrated that the immune genes still represent the major and the common players in RA among different ethnicities (Prasad *et al.* 2012). The data presented here provides trend of an association between the rs6457617 TT wild-type genotype and susceptibility to RA in Tunisian population (OR = 1.73, $p_c = 0.08$).

Previous replication GWAS studies in Spanish population (410 patients with RA and 394 control subjects) and Indian population (983 RA patients and 1007 healthy controls) have identified a statistically significant association of rs6457617 with RA ($P = 10^{-9}$ and 1.6×10^{-9}, respectively), but these reports did not show more details (Julià *et al.* 2008; Prasad *et al.* 2012). Moreover, other replication studies in various ethnic populations have yielded conflicting results about the risk allele to RA between the minor C and the wild-type T allele of rs6457617, our result is consistent with previous studies in Han Chinese population (1894 RA patients and healthy individuals) and Korean population (1316 unrelated RA patients and 1006 controls) (Han *et al.* 2009; Li *et al.* 2013), which have found that TT wild-type genotype of rs6457617 had increased risk to RA compared to the CC/CT genotype. Nevertheless, in the WTCCC British samples, the minor allele of rs6457617 was T and individuals bearing the TT genotype showed a strong susceptibility effect with RA (OR = 5.21) (WTCCC 2007). Additionally, a protective effect of the minor allele C was found in the Han Chinese population ($P = 0.001$, OR = 0.59) (Li *et al.* 2013).

These different findings can be explained by the important role of rs6457617 variant which play a causal susceptibility locus to RA despite the heterogeneity in allele frequencies and ethnicity among populations.

It is worth noting that rs6457617 SNP is located in *HLA-DQ* gene on 6p21.3 between the *HLA-DQB1* encoded β chain of MHC-II protein and *HLA-DQA2* encoded α chain of MHC-II protein, interestingly nearby 5′-*HLA-DRB1* gene. Recently, a study conducted in north-Tunisians revealed a strong association of *HLA-DRB1**04-*DQB1**03 haplotype with susceptibility to RA (OR = 3.95, $p_c = 16.8 \times 10^{-5}$) (Lagha *et al.* 2016). Our finding showed a strong LD between rs6457617 and results of *HLA-DRB1* gene described by Ben Hamad *et al.* (2012) ($D' = 0.72$ in healthy controls) and related haplotypic analysis showed a significant association between (rs6457617*T/*HLA-DRB1**04$^+$) haplotype and susceptibility to RA in Tunisian population ($p_c = 0.034$; OR = 1.72; 95% CI: 1.08–2.73).

Otherwise, gene–gene interaction analysis in our study reflected a model of susceptibility almost identical to independent gene effects. We highlighted that the presence of a variant either at rs6457617 (TT) or *HLA-DRB1* (04$^+$) locus increased risk by 2.38-fold in RA (OR = 2.38). This

Table 4. Allele and genotype frequencies of the MHC rs6457617 SNP in Tunisian RA patients and Tunisian controls, stratified by clinical and immunological data.

Subgroups	Allelic association				Genotypic association				
	T (%)	C (%)	*P*	Allelic OR	TT (%)	TC (%)	CC (%)	TT versus (TC + CC) *P*	Genotypic OR
Anti-CCP$^+$ ($n = 79$)	101 (64)	57 (35)	**0.01**; **p_c=0.03**	1.66 (1.1–2.5)	34 (43)	33 (41.8)	12 (15.2)	**0.008*; p_c=0.024**	1.28 (1.00–2.25)
RF$^+$ ($n = 88$)	109 (61.2)	67 (37.6)	0.03; $p_c = 0.09$	1.52 (1.02–2.26)	35 (39.8)	39 (44.3)	14 (16)	0.02; $p_c = 0.06$	1.12 (0.65–1.94)
Erosive$^+$ ($n = 101$)	40 (57.1)	30 (42.8)	0.09	1.25 (0.73–2.14)	39 (38.6)	43 (42.6)	19 (18.8)	0.04; $p_c = 0.12$	1.06 (0.63–1.8)
Controls ($n = 122$)	126 (51.2)	118 (47.9)			31 (25.2)	64 (52)	27 (22)		

Anti-CCP, anticitrullinated peptide antibodies; RF, rheumatoid factor; (%), frequencies of alleles and genotypes. A significant *P* value using a Bonferroni correction at 5% (<0.05); OR, odds ratio; 95% CI, confidence interval. Significant associations are indicated in bold.

interaction was more explained by the combined genetic effect of the two markers which may additively or synergistically contribute to the increased risk of RA.

However, by simple binary logistic regression, single markers suggested no significant association between these markers and risk to RA. In fact, the genotype TT of rs6457617 is linked to alleles *04 from *HLA-DRB1*, thus we suggest that the influence of rs6457617 on susceptibility can be partially determined by interaction with *HLA-DRB1* gene and vice versa. Given that the rs6457617 SNP located at *HLA-DQB1*, it is previously considered that there can be a tag SNP for *HLA-DQA1**03 which encodes MHC-II α chain (de Bakker *et al.* 2006; Nakanishi and Shima 2010). *HLA-DQA1**03 is reported to be associated with several autoimmune and infectious diseases including type 1 diabetes (Nakanishi and Inoko 2006) and chronic HCV infection (Tibbs *et al.* 1996). Additionally, it was suggested that it may be a shared epitope for RA (Li *et al.* 2013). According to these data and our findings, we can suggest that there is a gene–gene interaction (epistasis) between *HLA-DRB1* and *HLA-DQB1* genes with risk to RA. There is increasing evidence that this epistatic event could be of major pertinence in susceptibility to various common diseases (Evans *et al.* 2006; Julia *et al.* 2007).

Further, several lines of evidence support the association of rs6457617 of *HLA-DQB1* with infectious and autoimmune diseases such as cervical carcinoma and multiple sclerosis (Radstake *et al.* 2010; Allanore *et al.* 2011; Dutta *et al.* 2015). These findings can lead to the functional role of rs6457617 SNP and its influence in the expression of β chain within MHC II molecules (Li *et al.* 2013) and to confirm this, future functional studies were needed to be focussed.

Regarding rs13192471 SNP, the present study suggests that there is no evidence of an association in alleles and genotypes with susceptibility to RA in our population ($P > 0.05$). This SNP is located within the mitochondrially encoded cytochrome C oxidase 3 pseudogene 1 (MT-CO3 pseudogene 1). There are a few GWAS studies, which have revealed a powerful signal of association of this SNP with RA in the Korean and Japanese populations ($P = 1.1 \times 10^{-20}$, OR = 2.1; $P = 1.9 \times 10^{-58}$, OR = 1.97, respectively) (Kochi *et al.* 2010; Freudenberg *et al.* 2011).

Stratification of patients according to autoantibodies status reveals a significant increase of rs6457617 T allele and rs6457617 TT genotype in anti-CCP positive subgroup compared to healthy controls in our cohort ($p_c = 0.03$ and 0.024, respectively). Similarly, we report a tendency of association in RF positive subsample (T allele: $P = 0.03$, $p_c = 0.09$; TT genotype: $P = 0.02$, $p_c = 0.06$). These results suggest an epistatic effect of rs6457617 and immunoglobulin genes in the susceptibility to RA but do not exclude an artifact effect due to the small number of subgroups in our sample.

The discrepancy in results between various populations for SNP polymorphisms identified in different GWAS

studies and replicated in our study may reflect a genetic heterogeneity in the genetic basis of susceptibility and/or severity to RA and other autoimmune disorders. On the other hand, the small number of samples in this study in comparison with GWAS studies could also contribute to differences in statistical power to detect the genetic effect of the different polymorphisms studied.

In conclusion, our results reveal a trend of a positive association of rs6457617*TT genotype with susceptibility to RA. This result was enhanced by the presence of significant interaction between this SNP of *HLA-DQB1* and *HLA-DRB1**04 alleles. The rs6457617 may be an important RA susceptibility locus and could be a good predictor of RA risk. There was no significant association in rs13192471 and susceptibility/severity to RA in Tunisian sample. More studies among other populations are necessary to prove the general relevance of these SNPs from HLA region with RA.

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