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PAPER

Association of the nitric oxide synthase (*eNOS*) gene polymorphism with increased risk for both lupus glomerulonephritis and rheumatoid arthritis in a single genetically homogeneous population

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Nitric oxide (NO), a short-lived gaseous free radical, synthesized from L-arginine by NO synthases (NOS), is a potent mediator of biologic responses involved in the pathogenesis of autoimmune rheumatic diseases, such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). Most biological necessary NO is produced by the family of three NOS. To date, several functionally relevant genetic polymorphisms in the *eNOS* gene have been associated with various vascular, infectious and autoimmune diseases. To our knowledge, no study has explored these polymorphisms for both SLE and RA in the same population. The objective of this study was to investigate the influence of the *eNOS* gene intron 4 a/b VNTR polymorphism (a 27-base-pair tandem repeat-based polymorphism) on susceptibility to SLE and RA in patients living in the island of Crete, a genetically homogeneous population. A group of 145 healthy subjects and 190 SLE patients were included in this study. Similarly, a second group of 235 healthy controls and 202 RA patients were analysed. In both cases, patients and controls were sex- and age-matched. Herein we report that the presence of a/b genotype of the *eNOS* gene may act as a risk factor not for the presence of SLE but for the development of glomerulonephritis (OR 2.71, 95% CI: 1.4–5.2), while it may be a susceptibility gene for RA (OR: 2.005, 95% CI: 1.31–3.07). Thus, in our population, the a/b genotype of the *eNOS* gene represents a severity rather than a susceptibility genotype for SLE. *Lupus* (2007) 16, 867–874.

Key words: endothelial nitric oxide synthase gene; *eNOS* polymorphism; nitric oxide (NO); rheumatoid arthritis (RA); systemic lupus erythematosus (SLE)

Introduction

Nitric oxide (NO), a highly potent endogenous vasodilator, is one of the most important biological molecules, which has a role in many biological systems. It acts as a trigger, mediator or effector to a variety of biological reactions and signal transduction pathways.¹ It participates in inflammatory and autoimmune responses as well as in host defence against microbes and tumour cells. Its effects are exerted

either directly from reactions between NO and specific biomolecules or indirectly from reactive nitrogen oxide species through oxidation.² NO synthesis is tightly regulated by nitric oxide synthases (NOS), which appear in three isoforms: neuronal (nNOS), inducible (iNOS) and endothelial (*eNOS*). All three isoforms of NOS function as homodimers, in which the oxygenase amino-terminal domain of two NOS molecules binds to each other.³ Endothelial NOS (*eNOS*) is a 135-Kda protein, encoded on chromosome 7q35–36, consisting of 26 exons and spanning a genomic region of 21 kb.⁴ It is expressed primarily in endothelial cells and at low levels in platelets,⁵ where it produces NO constitutively. NO produced by *eNOS* is considered to prevent smooth muscle cell proliferation, platelet adherence and neutrophil activation and

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adhesion.⁶ Through its anti-inflammatory and vasodilatory properties, it may protect subjects against cardiovascular and renal complications commonly accompanying the progression of autoimmune diseases. Loss of *eNOS* activity confers to intravascular thrombosis, neutrophil activation and diapedesis.⁷

Systemic lupus erythematosus (SLE) is a multiorgan systemic autoimmune disorder of unknown aetiology, although several genetic factors affected by environmental agents affect the development of the disease. Studies have shown that the levels of NO are significantly elevated in SLE patients in comparison with healthy controls and, in addition, a correlation has been found out between serum NO and SLE disease activity.^{8,9} However, these findings could not be confirmed in a prospective study, thus indicating that the role of NO in the pathogenesis of SLE is not yet fully established.¹⁰ Lupus nephritis represents a predominant reason of morbidity and mortality in patients with SLE; it has been suggested that an abnormal regulation of the complex system consisting of the renin/angiotensin (RAS) and NO system, both regulating vascular tone and inflammation, may have a profound effect in the pathogenesis and progression of SLE and the development of lupus nephritis. The *eNOS* gene locus has been found, by linkage analysis studies, to be associated with the disease. Glomerular *eNOS* expression is decreased in individuals with lupus nephritis and inversely correlates with glomerular injury. Nevertheless, it is not clear whether the observed decrease in *eNOS* production in SLE patients is a primary reason or the result of the disease.¹¹

Similarly, several lines of evidence indicate that NO may be important in the pathogenesis of rheumatoid arthritis (RA).¹² Peripheral blood mononuclear cells isolated from RA patients exhibited increased expression of iNOS and enhanced formation of NO that is correlated with the activity of the disease.¹³ Moreover, NO has been considered a key mediator of apoptosis within RA joints.¹⁴ Considering the preferential susceptibility of Th1 cells to induction of apoptosis, it seems probable that NO regulates the Th1/Th2 balance by promoting or suppressing apoptosis at high/low doses. The cytoprotective properties of low/intermediate levels of NO might limit tissue damage during inflammation, independent of attenuating Th1 responses. NO is also a key regulator of the T helper 1 (Th1)/Th2 balance in autoimmune diseases.¹⁵

In the past, it was proposed that NO contributes to tissue destruction in inflammatory/autoimmune diseases.¹⁶ Recent reports offer sufficient evidence for an extension of this concept, by including the immunoregulatory effects of NO in autoimmune disease as it is a cytotoxic effector molecule. The impact of NO on immune cell function is not merely

damaging (i.e., leading to non-specific suppression of various functions). Rather, immune cells appear to exhibit a specific response when exposed to NO, with substantial consequences for cytokine- and apoptosis-dependent immunoregulatory balance.¹⁵ Thus, depending upon the disease state, the local tissue involved and the genetic background, damaging or immunoregulatory Th1-limiting roles of NO will prevail. Finally, patients with RA has been suggested that they have a certain abnormality in NO-dependent vascular function due to NO overproduction, a finding reminiscent of that of sepsis, during which NO is markedly increased.^{17,18,19}

Several functionally relevant genetic polymorphisms in the *eNOS* gene have been associated so far with different vascular, infectious and autoimmune disease such as SLE and RA in some populations. These polymorphisms may alter the levels of *eNOS* expression, thus being of certain relevance either to the pathogenesis of SLE and RA or the progression of specific manifestations of these diseases – that is, atherosclerosis, renal complications and so on. Intron 4 a/b polymorphism is based on a variable 27-base-pair tandem repeat in intron 4 with four repeats (allele a), five repeats (allele b) or six repeats (allele c). It had been suggested formerly that this polymorphism may be responsible for the levels of NO in plasma and, therefore, investigation of the polymorphism revealed correlation between NO and various diseases such as high-altitude pulmonary oedema, coronary lesions, asthma and lupus nephritis.^{20,21} In particular, the four-repeat allele (a) has been associated with coronary artery disease, lipid abnormalities, idiopathic recurrent miscarriages, advanced diabetic nephropathy, end-stage nephropathy in non-diabetics and acute myocardial infarction in some populations.²² One of the major reasons to investigate this *eNOS* polymorphism in patients with SLE was the reported link between intron 4 a/b polymorphism and cardiorenal disease.

Genetic studies in the Cretan population (0.65 million), which shares the same genetic and cultural background and a common environment, look intriguing enough since, in such a 'geographically isolated' gene pool, certain alterations in alleles frequencies due to founder effect may be detected.²³ The definitive advantages of population isolates, such as the Cretan population, refer to a potential higher prevalence for some diseases, good genealogical records and more uniform environment.²⁴ In addition, natives of Crete show low migration rates, a fact that confers in the genetic homogeneity of this population. Given the expected lower environmental variation and the less complex genetic make-up of this population, possibly owing to founder effects, it is not necessary to examine a large sample size.

The aim of the present study is to investigate whether *eNOS* gene intron 4 a/b polymorphism is associated with the development of SLE and RA in subjects from island of Crete and determine its role as a predisposing factor in pathogenetic process of SLE and RA.

Materials and methods

Study population

The first study group comprises of 145 healthy subjects and 190 SLE patients from unrelated families living in Crete. Similarly, a second group of 235 healthy controls and 202 RA patients were analysed. Since the two groups of patients differed significantly in demographics, two groups of controls were used. In both cases, age- and sex-matched healthy volunteers from the Department of Transfusion Medicine of the University Hospital of Crete served as controls. All SLE patients met the 1982 American College of Rheumatology (ACR) revised criteria for the classification of SLE,²⁵ while RA patients met the American Rheumatism Association 1987 revised criteria.²⁶ Patients were studied following written informed consent. Ethnic bias within the population studied was minimized by excluding patients who were not of Cretan origin. Cretan origin was defined as having the four grandparents of each individual possessing Cretan ancestry. The study was performed in the laboratory of Internal Medicine, Faculty of Medicine, University of Crete, after obtaining the approval of the research committee and the informed consent of the patients.

*Analysis of 27-bp repeat polymorphism on intron 4 of *eNOS* gene*

Upon obtaining the informed consent, we proceeded in collecting whole blood in EDTA-containing tubes as anticoagulant. Genomic DNA was isolated from peripheral blood leucocytes of each patient by using the commercial kit PUREGENE (Gentra SYSTEMS, MN, USA). The extracted DNA was stored at -20°C until analysed. Genotypes were scored blindly and the analysis of all ambiguous samples was repeated. The cDNA of the human *eNOS* sequence (GenBank Accession Number NM 000603) was used to design primers for PCR amplification of the corresponding fragment. The upstream primer 5'-AGGCCCTATG-GTAGTGCCTTT-3' and the downstream primer 5'-TCTCTTAGTGCTGTGGTAC-3' were used to generate the region of 27-bp repeat in intron 4 of the *eNOS* gene. The amplification was carried out

using a Taq polymerase provided by Minotech (IMBB-FORTH, Hellas). A hot start was used with initial heating at 94°C for 5 min, followed by the addition of the polymerase and then 35 cycles of denaturing (at 94°C for 30 s), annealing (at 63°C for 30 s) and chain extension (at 72°C for 1 min), followed with a final extension step at 72°C for 5 min. PCR products were analysed through electrophoresis on 2% agarose gel and ethidium bromide fluorescence in reference to a molecular weight marker. The 420-bp band indicated five repeats of the 27-bp sequence and the 393-bp band represented four repeats, corresponding to b and a alleles, respectively.²⁷ About 10% of the samples were amplified twice for checking the accuracy of the results.

Confirmation of polymorphism by direct sequencing

Selected PCR-amplified segments, corresponding to a/a and a/b genotypes, were completely sequenced on both strands in a LiCor 4200L sequencer of the laboratory of Microchemistry (IMBB-FORTH, Crete, Greece) in order to confirm that the amplified products represent genuine *eNOS* intron 4 regions.

Statistical analysis

Descriptive statistics were reported as means and standard deviations (SD) for quantitative varieties and in absolute frequencies and percentages for qualitative variables. In the case-control comparisons, only unrelated cases and controls were used. The *eNOS* gene variant investigated was evaluated for deviation from Hardy-Weinberg equilibrium by comparing observed and expected genotype frequencies by means of Fisher's exact test, separately in cases and controls. The statistical difference in genotype distribution and allele frequencies in both control and case subjects was assessed by using standard $2 \times 2 \chi^2$ test or Fischer's exact test when appropriate. Odds ratios (ORs) and their confidence intervals (CIs) were calculated according to Rothman.²⁸ As one polymorphism was being investigated for *eNOS* gene, a *P*-value of 0.05 was defined as significant.

Results

Demographic characteristics of SLE and RA patients

Characteristics of the SLE study group ($n = 190$) are summarized in Table 1. Mean (\pm SD) age in patients with SLE was 39.82 ± 13.27 years, 178 women and 12 men. From the total of 190 SLE patients, 66 (35%)

Table 1 Demographic characteristics of SLE patients

Age (years)	39.82 ± 13.27 ^a
Sex	
Female	178 (94%) ^b
Male	12 (6%) ^b
Ethnicity	
Cretan/other	190/0

^aMean ± S.D.

^b*n* (%).

Table 2 Demographic characteristics of RA patients

Age (years)	60.57 ± 16.61 ^a
Sex	
Female	141 (70%) ^b
Male	61 (30%) ^b
Ethnicity	
Cretan/other	202/0

^aMean ± S.D.

^b*n* (%).

had lupus nephritis diagnosed by ACR criteria (persistent urine protein excretion >0.5 gr/day or cellular casts). Unrelated healthy control subjects (*N* = 145) were of similar age, sex and, apparently, geographical origin.

Characteristics of the RA study group (*n* = 202) are summarized in Table 2. Mean (±SD) age in patients with RA was 60.57 ± 16.61 years. There were 141 women and 61 men, while unrelated healthy controls (*N* = 235) were of similar age and sex.

A case-control study for SLE

Since former studies had assumed that the intron 4 b→a polymorphism reduced eNOS activity and linked to renal disease, we attempted to determine whether allelic differences may enhance the risk of developing SLE and lupus nephritis. After PCR amplification of the target DNA, we analysed electrophoretically (by

agarose gel) the amplified fragments without revealing any significant association neither between a allele nor between a/b or a/a genotype and SLE (Table 3). Our results showed that the b/b genotype was the most frequent in both, cases and controls (72 and 72.4%, respectively). The distribution of genotypes showed no deviation from Hardy-Weinberg equilibrium both in patients and controls (Fisher's exact test: *P* = 0.5 for patients, *P* = 0.5 for controls). The mutated genotype (a/b) was observed in 26% of patients and 25.51% of healthy controls. The observed differences were not statistically significant when they evaluated with χ^2 test of independence (*P* < 1). The same result was obtained for the homozygous mutated genotype (a/a). This genotype was observed in 2 and 2.08% in SLE patients and healthy controls, respectively, without statistical significant difference between them. Similar findings were observed and for the alleles frequencies (a and b) in patients and controls. The b allele was most frequently observed in patients (85.17%) and controls (84.13%), whereas the mutated a allele was observed in 15.27% of patients and 14.83% of controls. Again, the difference was not statistically significant (*P* < 1).

Endothelial NOS polymorphisms have been exhaustively studied from the viewpoint of their putative association with renal diseases, including glomerulonephritis,²⁹ end-stage renal disease³⁰ and diabetic nephropathy.³¹ Most reports for the Japanese population showed that the intron 4 polymorphism was positively associated with different forms of renal diseases.³² Moreover, Zanchi *et al.*³¹ found that this polymorphism was predictive of nephropathy in a white population. Therefore, it was of great importance to explore any putative association between the intron 4 eNOS polymorphism and lupus glomerulonephritis. Interestingly, when we compared the genotype or allele frequencies in SLE patients with glomerulonephritis versus SLE patients without glomerulonephritis, the results were quite different (Table 4). In particular, the heterozygous mutated genotype a/b

Table 3 Alleles and genotype frequencies in SLE patients and controls

	SLE patients <i>N</i> = 190	Control group <i>N</i> = 145	<i>P</i> -value ^a	OR (95% CI) ^a
Genotype				
Wild type b/b	136 (72%)	105 (72.4%)		
Mutated a/b	50 (26%)	37 (25.51%)	Not significant	1.04 (0.64–1.67)
Mutated a/a	4 (2%)	3 (2.08%)	Not significant	1.02 (0.22–4.6)
Alleles				
Wild type b	322 (84.33%)	247 (85.17%)		
Mutated a	58 (15.27%)	41 (14.83%)	Not significant	1.00 (0.7–1.7)

^aFor the presence of mutated genotype (a/b or a/a) or mutated allele (a) in SLE patients when compared with control group.

Table 4 Association between eNOS intron 4 VNTR polymorphism and lupus glomerulonephritis

	SLE patients with GN N = 66 (100%)	SLE patients without GN N = 124 (100%)	P-value ^a	OR (95% CI) ^a
Genotype				
Wild type b/b	39 (60%)	97 (78.22%)		
Mutated a/b	26 (39.3%)	24 (19.35%)	<0.01	2.71 (1.4–5.2)
Mutated a/a	1 (0.7%)	3 (2.43%)	NS	0.6205 (0.06–6.09)
Alleles				
Wild type b	104 (78.78%)	218 (88%)	<0.025	1.96 (1.11–3.44)
Mutated a	28 (21.22%)	30 (12.0%)		

^aFor the presence of mutated genotype (a/b or a/a) or mutated allele (a) in SLE patients with glomerulonephritis when compared with SLE patients without glomerulonephritis. GN, glomerulonephritis; NS, non-significant.

was observed in 39.3% of SLE patients with glomerulonephritis and in 19.35% of SLE patients without glomerulonephritis. When the observed differences were compared with a 2×2 χ^2 test, they were statistically significant ($P < 0.01$), thus suggesting an association between the mutated genotype a/b and lupus glomerulonephritis. This result was confirmed by the calculation of the OR (2.71, 95% C.I: 1.4–5.2), indicating an approximately three-fold increase in relative risk for developing lupus nephritis in SLE patients with the a/b genotype. Similarly, the same result was observed with the a allele, which was found more frequent in patients with nephritis than patients without nephritis. A statistical significant difference between the two groups was detected ($P < 0.025$) and an OR counted to 1.96 (95% C.I: 1.11–3.44). No difference was observed when the a/a genotype was assessed in relation to lupus glomerulonephritis but we have to point out the limited number of patients with this genotype found in our cohort.

It is worthwhile noting that the third allele in intron 4 of eNOS gene, which has been observed in other ethnic populations (allele c), was not found neither in patients nor in controls. Altogether, our current data

suggest that the 27-bp tandem repeats of intron-4 of the eNOS gene are not associated with susceptibility to SLE but it has a strong association with lupus nephritis.

A case-control study for RA

All data are summarised in Table 5. Our results show that the b/b genotype was the most frequent in both, cases and controls (63.36 and 77%, respectively). As in SLE, no deviation from Hardy–Weinberg equilibrium was detected when we compared the distribution of genotypes, neither in patients nor in controls (Fisher's exact test: $P = 0.2$ for patients, $P = 0.2$ for controls). In contrast, the mutated genotype (a/b) was observed in 35.14% of RA patients and 21% of healthy controls. The observed difference was statistically significant when it was evaluated with a 2×2 χ^2 test of independence ($P < 0.01$). Thus, it can be assumed that there is an apparent correlation between mutant genotype (a/b) and RA in patients of Cretan origin. OR rate was used for the evaluation of relative risk (OR: 2.005, 95% CI: 1.31–3.07), indicating that the presence of the aforementioned genotype contributes to the increase of

Table 5 Genotype and allele frequencies of eNOS intron 4 VNTR in RA patients and healthy controls

	RA patients N = 202	Control group N = 235	P-value ^a	OR (95% CI) ^a
Genotype				
Wild type b/b	128 (63.36%)	180 (77%)		
Mutated a/b	71 (35.14%)	50 (21%)	<0.01	2.005 (1.31–3.07)
Mutated a/a	3 (2.5%)	5 (2%)	NS	0.6935 (0.16–3.0)
Alleles				
Wild type b	327 (81%)	410 (87.28%)	<0.025	1.609 (1.11–2.32)
Mutated a	77 (19%)	60 (12.72%)		

^aFor the presence of mutated genotype (a/b or a/a) or mutated allele (a) in RA patients as compared to control group. NS, non-significant.

the disease risk. The mutated genotype a/a was observed in 2.5 and 2% in patients and controls, respectively, without statistical significant difference between them, but again there were little number of patients and controls with this genotype in our cohort. Similar findings were observed and for the alleles frequencies (a and b) in patients and controls. The b allele was most frequently observed in patients (81%) and controls (87.28%) whereas the mutated a allele was observed in 19% of patients and 12.72% of controls. Moreover, based on the χ^2 test of independence, the frequency of a allele appears higher in RA patients and this difference was statistically significant ($P < 0.025$). The presence of this allele increased the disease risk by ~2 times (OR: 1.609, 95% CI: 1.11–2.32). These findings clearly support the implication of intron 4 a/b polymorphism in the development of RA in Crete.

Discussion

In recent years, numerous studies have tested the association between various candidate genes and the development or progression of SLE and RA and/or specific clinical manifestations of both diseases, in patients of different ethnic origin. Given the variety of NO physiological roles and the speed of its reaction and inactivation in various cellular systems, strict control of NO production/destruction is crucial for its selective actions. Thus, in this framework, the existence of *eNOS* gene variants may influence disease phenotypes and outcomes such as autoimmune diseases, insulin dependent diabetes mellitus (IDDM), cardiovascular diseases and so on. In the field of SLE and RA, as in numerous other research fields attempting to link genetic polymorphisms and different diseases, the large number of conflicting studies has led to a confusing picture. The discrepancies could represent differences in the genetic background between populations studied, which result in the multigene interactions with varying environmental factors and, in parallel, could be caused by problems such as small sample size or inadequate definition of phenotypes.

Nevertheless, some specific strengths of our study deserve further discussion. Ethnic variation and genetic admixture have been considered in any evaluation of the genetic background of multifactorial diseases as SLE and RA. In our study, a special advantage, particularly with respect to other studies that attempted to address the same issue, was the remarkable attention paid regarding the selection of a genetically and ethnically homogeneous patient's cohort and a proper control group. Thus, we reduced any possible error in the interpretation of our results by only considering Cretan patients as well as controls and excluding those of non-

Cretan origin. A possible weakness of our study deals with the limited sample size. Nevertheless, the genetic homogeneity of this population, in combination with the expected lower environmental variation, justifies why it is not necessary to examine a large sample size that, apparently, is not possible to be collected in a restricted geographical area.

In this study, we investigated whether the *eNOS* gene intron 4 a/b polymorphism is implicated in susceptibility to SLE and RA in natives of Crete. We demonstrated here that there was a trend of increase of a/b genotypic and a allelic frequencies of the *eNOS* gene intron 4, respectively, in RA patients, when compared with healthy controls group, but not in SLE patients. Among many polymorphisms of the *eNOS* gene, it has become clear that functional polymorphisms referred to exonic regions have a major contribution to the development of certain clinical manifestations of SLE and/or RA. However, the a allele of *eNOS* gene intron 4 has been related to low NO metabolite levels, and subjects homozygous for the a allele were found exhibiting a rate of NO metabolites levels 20% lower than that appearing in b/b homozygous subjects.¹⁶ Similarly, the clear relevance of *eNOS* gene intron 4 a/b polymorphism to diabetic nephropathy in IDDM has been reported. Thus, it has been suggested that the deletion of one of the five nucleotide repeats in intron 4 may affect the rates of *eNOS* transcription and processing rate, thus resulting in an altered *eNOS* enzymatic activity and, apparently, affecting plasma NO concentrations in patients.³³ Another possible explanation for the association of the intron 4 a/b polymorphism with the risk for RA is that the allele a is in linkage disequilibrium with other functional variants within the *eNOS* or another gene.

The allele frequencies of the 27-bp intron 4 a/b polymorphism are similar between Japanese and whites, but they are much lower than in African Americans.³⁴ While vascular changes could be a possible origin of the appearing renal diseases, this hypothesis cannot be interpreted easily since NO in the kidney participates in various processes, apart from its involvement in vascular wall changes. The role of NO in the regulation of the glomerular microcirculation and the relaxation of mesangial cells has been postulated so far, thus indicating that *eNOS* may be involved in the progression of renal diseases by a different way in comparison with other vascular diseases.¹ Of note, NO dysfunction may be observed in glomerular disease, but also systemic diseases indirectly caused by changes in kidney, such as glomerular hypertension.

Recently, Lee *et al.*²¹ have reported an association between intron 4 *eNOS* polymorphism and lupus

nephritis in a Korean population, concluding that a/b genotype represented a significant risk factor for the development of lupus nephritis due to its contribution to the initiation or maintenance of an increase in intraglomerular pressure.³⁵ In the Cretan cohort under study, a significant association of the intron 4 a/b polymorphism and glomerulonephritis but not SLE *per se* was detected, thus suggesting that this polymorphism and its consequences are implicated in the development of the specific clinical manifestation examined. Upon now, contradictory results have been accumulated dealing with a possible association of a allele with SLE, mainly depending on the ethnic origin and the genetic background and homogeneity of the populations studied. However, the validity of our results is strengthened by the definite genetic homogeneity of the Cretan cohort used.

Previous findings referred to a Spanish cohort of RA patients did not support the implication of another eNOS polymorphism, the exon 7 298Glu/Asp one, in susceptibility to RA.³⁴ However, when we examined the association between eNOS mutated genotype (a/b or a/a) or allele (a) in RA patients and the presence of rheumatoid factor, no correlation was found, as the differences were not statistically significant (data not shown).

The present study constitutes the first attempt to access the implication of eNOS gene intron 4 a/b polymorphism in susceptibility to SLE and RA in natives of Crete, a population sharing a common genetic and cultural background and showing low migration rates.³⁶ We found that the presence of a/b genotype or a allele of the eNOS gene intron 4 a/b VNTR polymorphism may act as a risk factor for the development of RA in the island of Crete. In contrast, the same eNOS polymorphism is not associated with SLE susceptibility. Prospective longitudinal studies are needed to determine how predictive the eNOS variants are for the new onset of lupus glomerulonephritis and RA. New technologies using microarray analysis may provide some insights regarding new potential links between the pathogenesis of SLE and RA and other complex autoimmune diseases.

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