Genetic Factors Contributing to Systemic Lupus Erythematosus in Tunisian Patients

Hajer Fourati1*, Dorra Bouzid1, Olfa Abida1, Najla Kharrat2, Sameh Marzouk1, Samy Haddouk1, Constantin Fesel4, João Costa4, Mourad Ben Ayed1, Zouhair Bahloul2, Carlos Penha-Gonçalves4, Ahmed Rebai3 and Hatem Masmoudi1

1Immunology Department, Habib Bourguiba Hospital, University of Sfax, Sfax, Tunisia
2Department of Internal Medicine, Hedi Chaker, University of Sfax, Sfax, Tunisia
3Bioinformatics’ Unit, Biotechnology Center of Sfax, Sfax, Tunisia
4Instituto Gulbenkian de Ciência, Oeiras, Portugal

Abstract

Systemic lupus erythematosus (SLE) is a multi system autoimmune disease characterized by autoantibodies production, multi-organ damage and complex genetic inheritance. Multiple genetic and environmental factors contribute to the pathogenesis of this disease. Recent genome-wide studies, have added substantially to the number of genes associated with SLE. We performed a case control study using 138 SNPs in 93 Tunisian patients affected with lupus and 162 healthy controls. All SNPs were genotyped in a Sequenom platform. To confirm some associations, associated SNPs were analyzed using logistic regression which allows the test of association with a given SNP by adjusting for the effect of confounding variables. Association was especially reported with rs3733197 (P=0.0026, OR=2.04), rs17266594 (P=0.046, OR=1.56) in BANK1 gene, rs2070197 (P=0.0016, OR=2.31), rs2004640 (P=0.024, OR=1.54), rs10954213 (P=0.035, OR=1.53) in IRF5 gene and rs7574865 (P=0.017, OR=1.77) in STAT4 gene; previously confirmed SLE susceptibility genes. rs1800629 (P=0.00036, OR=2.26), rs417359 (P=0.026, OR=1.53) and rs11575812 (P=0.037, OR=1.55) of TNF-α, IR2RA and IL2 genes respectively were also associated with SLE. Haplotype analysis reported 2 susceptibility haplotypes: TGG (P=0.00421, OR=1.87) in BANK1 and TCA (P=0.00177, OR=2.34) in IRF5 genes. Our results show that numerous genes, some with known immune related function predispose to lupus.

Keywords: Systemic lupus erythematosus; Polymorphism; Genetic association

Introduction

Systemic lupus erythematosus (SLE) is an archetypal systemic autoimmune disease. It is characterized by a diverse array of clinical symptoms, indicative of widespread immune- mediated damage. It is also a heterogeneous disease with no single clinical or immunological features required to make a formal diagnosis [1]. The clinical manifestations of SLE, although different from one patient to another, are characterized by the production of various antibodies, immune complex deposition and chronic, intense inflammation. The prevalence of the SLE and the severity of its manifestations vary among different populations, but the disease is more common in Asians (46.7/100.00) than in Caucasians (20.7/100.00) [2]. The difference in the disease prevalence across different populations itself confirms the heterogeneity of the disease risk factors in different populations: these risk factors include environmental factors, but genetic components could be major determinants. Genetic factors are known to play an important role in the disease as shown by sibling recurrence risk ratio of 20 and 10-fold excess in SLE concordance between monozygotic twins over dizygotic twins [3,4].

Until 2008, only a handful of genetic loci affecting SLE susceptibility had been identified and reproduced via candidate gene studies [5]. The advent of the genome wide single nucleotide polymorphism (SNP) genotyping technology and the subsequent recent genome wide association studies (GWAS) have greatly expanded the number of established SLE risk alleles [6-8] to over twenty; most are located in immune-related pathways such as antigen presentation, B and T-cell receptor signaling and interferon signaling [5]. In less than four years time, STAT4 [9], BANK1 [10], IRF5 [11] and several other genes have been identified as associated with SLE [12-14].

Despite the varied disease prevalence and its severity across different populations, it is noteworthy that most previous studies were conducted on patients of European ancestry with under- representation of other ethnicities. In addition, evidence also suggests that in different ethnic groups, different genes may be involved; and for the same susceptibility gene, it may have different effect size in different ethnic groups. Genetic studies on Arab subjects and those in North Africans are nonexistent. Therefore, in the present study, we undertook a case-control association analysis attempting to replicate some previously identified associations and to investigate some other SNPs belonging to genes related to immune function in an independent Southern Tunisian population.

Subjects and Methods

Subjects

Blood samples for DNA extraction and genotyping were collected from 93 unrelated patients with SLE recruited from the department of Internal Medicine in Hedi Chaker hospital of Sfax, Tunisia. All the patients fulfilled the revised criteria of the ACR for classification of SLE [15]. Drug-induced SLE was excluded.

All the subjects were natives; they were born and live in the South of Tunisia. Female/Male ratio was 78/15.

*Corresponding author: Hajer Fourati, Immunology Department, Habib Bourguiba Hospital, University of Sfax, 3029 Sfax, Tunisia. Tel/Fax: +216 74 45 16 60; E-mail: hajerfourati@yahoo.fr

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The mean age of the diagnosis of our patients was 31 (range 14-90).

One hundred sixty two unrelated healthy controls were randomly collected from the South of Tunisia with a mean age of 42 years. There was no individual or family history of SLE or any other autoimmune disease.

In the SLE patients, autoantibodies, including anti-Sm, anti-Nuc, and anti-dsDNA, were determined either by indirect immunofluorescence or by Elisa methods. Clinical manifestations, such as lupus nephritis, arthritis, malar rash, were also recorded for each patient.

The study protocol has been approved by the local ethics committee (the ethics committee of the Hédi Chaker Sfax), and informed consent was obtained from all the healthy controls and patients.

SNP selection

We selected 138 SNPs belonging to the genes related to the regulation of immune response (Table 1). Some of the SNPs were selected because they were already shown to be strongly associated with SLE or other autoimmune diseases, or because they were involved in the autoimmune process.

All the information about the selected SNPs was extracted from the public database db SNP built 126 found at: http://www.ensemble.org (release 61). The SNPs were also typed using the HapMap database. All the identified SNPs are listed in Table 1.

| Gene          | RS     | Chromosome | Position (bp) | Type |
|---------------|--------|------------|---------------|------|
| CD3z (CD247)  | RS2995082 | 1          | 167406245     | T/C  |
| CD3z (CD247)  | RS2056626 | 1          | 167420425     | T/G  |
| CD3z (CD247)  | RS7523907 | 1          | 167427247     | C/T  |
| CD3z (CD247)  | RS1723015 | 1          | 167432894     | T/C  |
| CD3z (CD247)  | RS2995091 | 1          | 167434277     | T/C  |
| CD3z (CD247)  | RS12036775| 1          | 167437047     | C/T  |
| CD3z (CD247)  | RS1214611 | 1          | 167449104     | A/G  |
| CD3z (CD247)  | RS2949655 | 1          | 167451850     | G/A  |
| CD3z (CD247)  | RS16859085| 1          | 167453334     | A/G  |
| CD3z (CD247)  | RS585554  | 1          | 167454914     | A/G  |
| CD3z (CD247)  | RS5863455 | 1          | 167457624     | C/T  |
| CD3z (CD247)  | RS585545  | 1          | 167461391     | A/G  |
| CD3z (CD247)  | RS704848  | 1          | 167461873     | C/T  |
| CD3z (CD247)  | RS10918695| 1          | 167473975     | C/T  |
| CD3z (CD247)  | RS1214611 | 1          | 167449104     | A/G  |
| CD3z (CD247)  | RS2949655 | 1          | 167451850     | G/A  |
| CD3z (CD247)  | RS16859085| 1          | 167453334     | A/G  |
| CD3z (CD247)  | RS585554  | 1          | 167454914     | A/G  |
| CD3z (CD247)  | RS5863455 | 1          | 167457624     | C/T  |
| CD3z (CD247)  | RS585545  | 1          | 167461391     | A/G  |
| CD3z (CD247)  | RS704848  | 1          | 167461873     | C/T  |
| CD3z (CD247)  | RS10918706| 1          | 16746121      | C/T  |
| CD3z (CD247)  | RS585543  | 1          | 167467298     | A/G  |
| CD3z (CD247)  | RS1737506 | 1          | 167473425     | A/T  |
| CD3z (CD247)  | RS1799704 | 1          | 167475871     | A/C  |
| CD3z (CD247)  | RS2982481 | 1          | 167483306     | G/A  |
| CD3z (CD247)  | RS585535  | 1          | 167491485     | A/C  |
| CD3z (CD247)  | RS2143302 | 1          | 167492187     | T/C  |
| IL10          | RS3024498| 1          | 206641529     | T/C  |
| IL10          | RS3024495| 1          | 206642413     | C/T  |
| IL10          | RS1800871| 1          | 206646634     | A/G  |
| IL10          | RS1800896| 1          | 206646897     | T/C  |
| IL23R         | RS11805303| 1          | 67675516      | C/T  |
| JAK1          | RS2780816 | 1          | 65302413      | G/A  |
| JAK1          | RS3102247 | 1          | 65307409      | G/A  |
| JAK1          | RS310211  | 1          | 65319920      | C/T  |
| JAK1          | RS310229  | 1          | 65321388      | G/A  |
| JAK1          | RS2256298 | 1          | 65330682      | G/A  |
Genotyping was performed using the Sequenom MassARRAY platform at “the Instituto Gulbenkian de Ciência” according to manufacturer's instructions. Genomic sequence containing the SNP is amplified by multiplex PCR reactions. The amplified product is cleaned using shrimp alkaline phosphatase to neutralize any unincorporated dNTPs and used for an allele specific primer extension reaction. The reaction mixture is then spotted into a SpectroCHIP microarray and subjected to the MALDI-TOF mass spectrometry. SpectroTYPER software identifies the SNP-specific peaks according to their expected masses and automatically assigns the genotype calls. In our study, we excluded SNPs with call rate lower than 80% in cases or controls.

**Data analysis**

Case-control association analysis was performed for each SNP. Calculation of allelic and genotypic associations of SNPs with susceptibility to Lupus were performed using a home-made program written in R language (www.r-project.org) which computes standard chi-square tests automatically for a set of multiple markers. The association is declared statistically significant when the p-value is less than 0.05. Relative risks were calculated as odds ratio (OR) from 2×2 contingency tables. To validate association, a multivariate analysis was performed using logistic regression including all SNPs that were found associated in single marker tests. SNPs were tested for significant deviation from Hardy–Weinberg equilibrium in control samples prior to association testing. Those with (p<0.05) were removed from the analysis.

We also performed a case-only analysis (e.g. presence of renal disorder versus no renal disorder) to examine the risk that is conferred by associated SNPs on subtypes of SLE using logistic regression.

Deviation from HWE and logistic regression analysis were performed using the PLINK tool set. An analysis of haplotype diversity was performed for the associated SNPs that belong to the same chromosome using PLINK.

### Results

All the studied loci were in Hardy-Weinberg equilibrium in the control group.

#### Allelic and genotypic association using R

Full details of the association between SLE and alleles and genotypes of different markers were shown in Table 2.

Eighteen SNPs were found to be associated with SLE (p<0.05). Four of those 18 SNPs were located in CD3z, three in IRF5, two in each of BANK1, IL2RB and JAK1 and one in each of IL2, IL2RA, STAT4, TNF-α and PTPN6.

**Association study using logistic regression**

Some SNPs that are found significantly associated by a univariate chi-square test might lose their association when analyzed together with other SNP; this generally indicates that these SNPs have an indirect effect through their linkage disequilibrium with other SNPs.

In order to confirm those associations, those associated SNPs were further studied using logistic regression and association concerned especially SNPs belonging to IRF5, BANK1, TNF-α, STAT4, IL2 and IL2RA (Table 3).

For IRF5, the strongest signal of association (P=0.0016, OR=2.31) was observed for the SNP rs2070197. For the SNPs rs2004640 and

| IRF5   | RS10954213 | 7   | 128589427 | G/A |
|--------|------------|-----|-----------|-----|
| Terc2  | RS706      | 7   | 142499762 | A/G |
| CREM   | RS17583959 | 10  | 35422103  | G/A |
| CREM   | RS2343452  | 10  | 35492832  | A/G |
| CREM   | RS1148247  | 10  | 35496946  | G/A |
| IL2Rα  | RS12359875 | 10  | 6051107   | C/T |
| IL2Rα  | RS1244380  | 10  | 6053374   | A/G |
| IL2Rα  | RS663421   | 10  | 6055604   | C/T |
| IL2Rα  | RS2076846  | 10  | 6063253   | A/G |
| IL2Rα  | RS11256369 | 10  | 6066200   | C/G |
| IL2Rα  | RS707398   | 10  | 6079846   | A/G |
| IL2Rα  | RS7449924  | 10  | 6082396   | A/C |
| IL2Rα  | RS7076781  | 10  | 6086385   | C/T |
| IL2Rα  | rs11256497 | 10  | 6087794   | G/A |
| IL2Rα  | RS791587   | 10  | 6088699   | A/G |
| IL2Rα  | RS10905669 | 10  | 6092093   | C/T |
| IL2Rα  | RS706778   | 10  | 6098949   | C/T |
| IL2Rα  | RS214286   | 10  | 6099045   | T/C |
| IL2Rα  | RS7072793  | 10  | 6106266   | T/C |
| IL2Rα  | RS7073236  | 10  | 6106552   | T/C |
| IL2Rα  | RS11597367 | 10  | 6107534   | A/G |
| IL2Rα  | RS10795791 | 10  | 6108340   | A/G |
| IL2Rα  | RS4147359  | 10  | 6108439   | G/A |
| IL2Rα  | RS7090530  | 10  | 6110875   | C/A |
| RBM17  | RS41295061 | 10  | 6114660   | C/A |
| RBM17  | RS11594566 | 10  | 6122009   | T/A |
| RBM17  | RS12251307 | 10  | 6123495   | C/T |
| CD3e   | RS4606515  | 11  | 61817007  | C/T |
| CD3e   | RS3692150  | 11  | 61815346  | G/A |
| INS    | RS689      | 11  | 2182224   | A/T |
| CLEC2D | RS3764021  | 12  | 9833628   | C/T |
| NAA25  | RS17696736 | 12  | 112486818 | A/G |
| PTN1   | RS12423190 | 12  | 112909340 | T/C |
| PTN1   | RS11066323 | 12  | 112923361 | G/A |
| PTN1   | RS7952150  | 12  | 112941571 | G/A |
| PTN6   | RS7310161  | 12  | 7057134   | A/T |
| PTN6   | RS599052   | 12  | 7069620   | T/C |
| TCRA   | RS1263555  | 14  | 23018822  | G/A |
| TCRA   | RS438538   | 14  | 23019932  | G/A |
| TCRA   | RS9125     | 14  | 23021038  | G/A |
| IL2Rβ  | RS228957   | 22  | 37520971  | G/T |
| IL2Rβ  | RS84460    | 22  | 37525731  | A/G |
| IL2Rβ  | RS321832   | 22  | 37530332  | A/G |
| IL2Rβ  | RS321829   | 22  | 37533530  | G/C |
| IL2Rβ  | RS221094   | 22  | 37535471  | C/T |
| IL2Rβ  | RS28295    | 22  | 37542201  | G/A |
| IL2Rβ  | RS321825   | 22  | 37544245  | G/A |
| IL2Rβ  | RS2016771  | 22  | 37551310  | G/T |
| IL2Rβ  | RS743776   | 22  | 37551487  | T/C |

**Table 1:** Genes, positioning and alleles of selected SNPs.

Pb: Paire de base
rs10954213, the association was more modest (P=0.024, OR=1.54 and P=0.035, OR=1.53 respectively).

For BANK1, there was a significant association SNP rs3733197 (P=0.0026, OR=2.04). This association was weaker for the SNP rs17266594 (P=0.046, OR=1.56).

For STAT4 and TNF-α, significant association concerned SNPs rs7574865 and rs1800629 (P=0.017, OR=1.77 and P=0.00036, OR=2.26 respectively). This difference between patients and controls was less
found to be associated with SLE in our studied population, which is
intracellular calcium stores [16].

Discussion

The genetic architecture of SLE seems to be multigenic and/or highly heterogeneous.

The majority of the investigated SNPs have previously been shown to be strongly associated with SLE in European and non-European populations, and some are known to be functional.

BANK 1 encodes a B-cell-specific scaffold protein and its activation can affect B cell-receptor-induced calcium mobilization from intracellular calcium stores [16].

Two putative functional SNPs of BANK1, one in the ankyrin domain (rs3733197) and a branch point site (rs17266594), were found to be associated with SLE in our studied population, which is

Table 3: Associated SNPs after logistic regression.

| CHR | GENE | SNP OR P       |
|---|---|---|---|---|---|---|---|
| 2 | STAT4 | RS7574865 | 1.77 | 0.017 |
| 4 | BANK1 | RS3733197 | 2.04 | 0.0026 |
| 4 | BANK1 | RS17266594 | 1.56 | 0.046 |
| 6 | TNFA | RS1800629 | 2.26 | 0.00036 |
| 7 | IRF5 | RS2070197 | 2.31 | 0.0016 |
| 7 | IRF5 | RS2004640 | 1.54 | 0.024 |
| 7 | IRF5 | RS10954213 | 1.53 | 0.035 |
| 10 | IL2RA | RS147359 | 1.55 | 0.026 |

CHR: Chromosome; OR: Odds Ratio

Table 4: Haplotypic associations for IRF5 and BANK1 genes with SLE.

significant for the SNPs rs4147359 of IL2RA gene (P=0.026, OR=1.55) and rs11575812 of IL2 gene (P=0.037, OR=1.57).

Haplotyping associated SNPs belonging to IRF5 and BANK1 genes

Haplotypes were constructed using PLINK. For the IRF5 gene, several haplotypes were generated by the three associated SNPs rs2004640, rs2070197, and rs10954213 but only one TCA haplotype (P=0.00177, OR=2.31) was significantly associated with SLE (Table 4).

Association study between lupus subphenotypes and associated SNPs

A sub-phenotype analysis in our study did not produce any significant difference between cases and controls (data not shown).

in agreement with previous studies on different ethnicities [10,17-19] and populations.

Another functional polymorphism in BANK1, the non-synonymous SNP (rs10516487) which led to substitution of arginine to histidine at amino acid position 61 (R61H) was also reported to confer susceptibility in SLE [10,17,19,20] but in our study, we failed to find this association.

Kozyrev et al. [10] also provide evidence that several BANK1 variants affect regulatory sites and key functional domains and subsequently, they hypothesize that BANK1 variants may contribute to the sustained B cell-receptor signaling and B cell hyperactivity observed in SLE. They also showed that linkage disequilibrium between these SNPs has prevented dissection of their relationship to SLE susceptibility.

IFN regulatory factor 5 (IRF5) is a transcription factor that controls the transactivations of type I IFN system-related genes as well as inflammatory and immune response associated genes [21]. It is one of the strongest and most consistent genetic associations described for SLE, after HLA.

In addition, both SNPs rs2004640 and rs10954213 were suggested to be functional in SLE. In fact, the SNP rs2004640 is located in a splice junction of an alternative exon 1B of IRF5 and the major allele (T) of this SNP creates a splice donor site for exon 1B, which result in a detectable transcription in monocytes expressing exon 1B, and hence, an expression of multiple isoforms of IRF5 initiated at exon 1B [27,29]. The SNP rs10954213 is located in the 3’UTR of IRF5. It was shown that the major allele (A) of this SNP is correlated with a truncated IRF5 transcript with elevated expression levels in peripheral blood mononuclear cells (PBMC) [22,30].

We failed to find association of the SNP rs729302 and rs752637 in our study which is not in agreement with two other studies [20,23]. As for the haplotypic study, among the five haplotypes defined by the following SNPs rs2004640, rs2070197, and rs10954213, we have identified only one IRF5 haplotype associated with SLE. This association was already reported in a study on three sets of patients and controls from Spain, Germany and Argentina [29] and in a large family based case-control sample [22], which shows the important role that IRF5 gene plays in the susceptibility and the pathogenesis of SLE.

STAT4 encodes a transcription factor that transmits signals induced by several key cytokines, including interleukin-12, and type one interferon, as well as interleukin-23 in T cells and monocytes [31].

It has also been implicated in the optimal differentiation of the proinflammatory Th17 cells which play an important if not predominant role in chronic inflammatory disorders [32,33].

IL-12 induces the STAT4-dependent NK cell activation and differentiation of naïve CD4+ lymphocytes into Th1 effector cells and IFNγ production [34-36]. STAT4 also mediates the IL-23-dependent expansion of Th17 cells, contributing to autoimmune diseases [37].
Shah K et al. [38] demonstrated that patients with SLE have an increased frequency of circulating CD4+ T cells producing IL-17, which correlates with disease activity, compared with healthy subjects, whereas both groups maintain similar frequencies of Th1 cells. In addition, plasma levels of IL-6, a cytokine that promotes the development of Th17 cells, are higher in patients with SLE than in healthy subjects suggesting that the balance of Th17 and Th1 responses production is dysregulated.

It was shown that variation in STAT4 influences both innate and adaptive functions of immune responses in SLE. The rs7574865 risk variant has recently been shown to confer an increased sensitivity to IFNα signaling in peripheral blood mononuclear cells from SLE patients [39].

Our results reveal a significant association of the SNP rs7574865 with SLE. This association was already established in previous reports from other populations; in fact, this allelic association was first reported by Remmers et al. [9] in three lupus series of case control subjects of European ancestry. Subsequently, several studies have confirmed this association in different populations [20,40-45].

In addition, STAT4 has proved to play a crucial role in experimental models of autoimmunity. In fact, in a murine model of lupus, STAT4 deficiency is associated with accelerated nephritis and increased mortality [46] in contrast to the protective effects in the arthritis models [47].

The association of TNF-α SNPs with SLE is still not consistent. In fact, our report confirms a previous report from other authors showing an association of rs1800629 with SLE [48-52]. However, such association was not found in other populations [53-57]. In addition, allele-based comparisons of 21 studies [58], after stratification by ethnicity, detected a significant association of the A allele of this SNP in the European-derived groups, but not in Asian-derived or African-derived populations.

There is substantial evidence to show that TNF-α may play a proinflammatory role in human SLE. Serum levels of TNF-α in active SLE patients closely correlated with disease activity [59] and abundant TNF-α expression was demonstrated in lupus nephritis kidneys [60]. Besides, some studies have suggested that the regulation of TNF-α production by macrophages and T lymphocytes is influenced by SNPs in SLE suggesting a pathogenic role of the TNF-α in the mediation of the local inflammation and tissue damage. Nevertheless, data concerning the serum levels of TNF-α in patients with SLE are rather controversial. Ma et al. [61] observed a higher level of TNF-α in the plasma of patients during active disease. While other authors observed TNF-levels diminished as a function of disease activity [62,63].

In addition, the protective role of the TNF-α was reported in the first time in the mice model of SLE (NZB/W F1) which administration of TNF-α delay occurrence of SLE; then in lupus patients receiving anti-TNF-α therapy [64].

Interleukin-2 (IL-2) is a T lymphocyte produced molecule [65,66]. The T lymphocytes from SLE produce decreased amounts of IL-2 [67,68]. The Deficient production of IL-2 leads to an increased rate of infections and increased numbers of activated autoreactive cells [69,70]. Genetic variants in IL-2 have been discussed in the susceptibility SLE in only one study [71] and the association reported for SNP rs2096763 which not found to confer susceptibility to SLE in our study.

Our study has shown an association of SNP rs4147359 belonging to IL2RA gene with SLE. To the best of our knowledge, no study has focused on this polymorphism in SLE or any other autoimmune disease. In contrast, it was reported that susceptibility of IL2RA to SLE was conferred by SNP rs1594656 whose association was confirmed in three independent cohorts [72]. Weaker associations were also reported in another study with other SNPs [73].

On the other hand, it was already shown that significantly elevated serum soluble IL2RA has been described in various autoimmune diseases including SLE [74]. Those results and ours jointly provide evidence of the implication of IL2RA in the pathogenesis of SLE.

When we began the study, we were aware that the number of the patients with SLE was small and we were not likely to provide enough power to identify a genetic association with any of the polymorphisms. For this reason, we were not surprised when we failed to find an association with some genes that were already reported to be associated or involved in the pathogenesis of SLE such as CTLA4, PTPN22, CD28 and PCD1.

All this inconsistency in the different results may be due to the inadequate statistical power, racial and ethnic differences, and the publication bias.

**Conclusion**

In conclusion, the results of the present study confirm some SLE susceptibility loci.

However, in our cohort, only a few patients may carry the risk alleles and genotypes for those genes. Further studies involving a larger number of SLE patients should be performed before fetching definitive conclusions regarding the implications of the analyzed in SLE susceptibility in Tunisia.

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**Conflicts of Interest**

The authors had no conflicts of interest to declare in relation to this article.

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