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Lack of Association of the *TP53* Arg72Pro SNP and the *MDM2* SNP309 with systemic lupus erythematosus in Caucasian, African American, and Asian children and adults

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Abstract

The p53 tumour suppressor is the central regulator of apoptosis. Previously, the functional *TP53* Arg72Pro polymorphism was found to be associated with systemic lupus erythematosus (SLE) in Koreans but not Spaniards. MDM2 is the major negative regulator of p53. An intronic polymorphism in *MDM2*, the SNP309, attenuates p53 activity and is associated with accelerated tumour development in premenopausal women. Polymorphic variation in *MDM2* has never been studied in SLE. The aim of this study is to further assess the contribution of p53-pathway genetic variation to SLE by testing the association of the *TP53* Arg72Pro polymorphism and the *MDM2* SNP309 with SLE in a well-characterised and ethnically diverse cohort of patients with both childhood- and adult-onset SLE ($n = 314$). No association was found between the *TP53* Arg72Pro polymorphism and SLE in patients of European descent, Asian descent or in African Americans, nor was an association found between the *MDM2* SNP309 and SLE in patients of European descent or in African Americans. In addition, there was no correlation between either variant and early-onset disease or nephritis, an index of severe disease. It is concluded that neither the *TP53* Arg72Pro polymorphism nor the *MDM2* SNP309 contributes significantly to either susceptibility or disease severity in SLE.

Keywords

genetic polymorphism; nephritis; pediatrics; systemic lupus erythematosus

Introduction

The identification of single nucleotide polymorphisms (SNPs) that correlate with disease holds promise toward the goal of individualised disease risk assessment and treatment. In systemic lupus erythematosus (SLE), there are few predictors of susceptibility or progression to serious complications such as nephritis and central nervous system disease. Further, the toxicities associated with current therapies, such as infertility, osteoporosis, secondary malignancies and immuno-suppression, are significant. Hence, the need for biomarkers in SLE is an overriding translational priority.

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Although the aetiology of SLE remains unknown, many pathways have been implicated. A familial component to SLE susceptibility is suggested by data demonstrating familial clusters of SLE, concordance among twins, and a 4-fold increased risk for SLE in the first-degree relatives of women with SLE than first-degree relatives of women without SLE.[1] We reasoned that functional inherited genetic variation in pathways deranged in SLE may alter susceptibility or be associated with severe disease.

One pathway linked to SLE is apoptosis. It has been hypothesised that the impaired clearance of apoptotic material provides an important source of sensitising autoantigens and that defective apoptosis of autoreactive lymphocytes may drive disease progression.[2,3] The p53 tumour suppressor is a transcription factor, the central regulator of the apoptotic response to a variety of stresses.[4] In response to DNA damage-inducing stressors, signal transduction pathways are activated that lead to the post-translational modification of the p53 protein. These modifications stabilise and activate p53, whereupon it directs cellular response programs resulting in cell-cycle arrest or apoptosis.

MDM2 is an ubiquitin E3 ligase, the regulatory partner of p53.[5] It binds to and inactivates p53 and targets it for degradation. p53 positively regulates MDM2 levels thereby creating a negative feedback loop by which p53 is tightly regulated.

Here, we examined the role in SLE of two functional polymorphisms in the p53 pathway. First, there is a common C/G polymorphic variant in exon 4 of the *TP53* gene that results in the substitution of an arginine for a proline at codon 72 (p53 Arg72Pro). The Arg72 form is more effective at inducing apoptosis, whereas the Pro72 form is more effective at inducing cell-cycle arrest.[6] Second, a G/T SNP in the well-characterised intronic promoter region of *MDM2*, referred to as SNP309, has been described.[7] The G variant creates a putative binding site for the Sp1 transcriptional co-activator, resulting in increased levels of MDM2 and a subsequent decrease in p53 levels.

Previously, it was reported that the *TP53* Pro72 variant was associated with SLE in Koreans; however, this finding was not replicated in a subsequent study in Spain.[8,9] Despite its central role in the regulation of p53, polymorphic variation in MDM2 has never been studied in SLE. To shed further light on the contribution of functional p53 pathway variation to SLE in different populations, we tested the associations of the *TP53* Arg72Pro SNP with SLE in Caucasian, African American and Asian patients and of the *MDM2* SNP309 with SLE in Caucasian and African American patients.

Methods

Patients and controls

In this study, 98 cases were with childhood-onset SLE and 216 with adult-onset SLE. All the patients were followed at Hospital for Special Surgery (HSS) in New York city and met the American College of Rheumatology (ACR) criteria for SLE. Renal status was known for 249 of the total cohort. Demographic and clinical data are summarised in Table 1. Patients were recruited through the HSS Autoimmune Disease Registry (Age >18 years) and Pediatric Lupus Family Registry (Age <18 years at disease onset). All study subjects signed an informed consent form, approved by the HSS Institutional Review Board. Healthy unrelated controls for this study were local adult volunteers matched for gender and ethnicity without autoimmune disease or cancer, except for African American controls, which were both local controls and 100 healthy African American controls (17 men, 83 women; age range: 8 month–58 years) in the HD100AA Human Variation Collection (Coriell Institute).

DNA extraction

Genomic DNA was extracted from peripheral blood using Qiagen QIAamp DNA mini kit (Qiagen Inc., Valencia, California). The samples were then quantified using Pico Green dsDNA Quantitation kit per manufacturer's instructions (Molecular Probes, Eugene, Oregon).

SNP genotyping

Allele frequencies were determined by allele-specific polymerase chain reaction using the 5′ nuclease assay (Taqman, Applied Biosystems, Foster City, CA, USA). Primer and probe sequences were designed using Primer Express v.2 software (ABI PRISM, Applied Biosystems, Foster City, CA, USA). They were manufactured as Assays-by-Design (ABI) and performed according to the manufacturer's specifications. In brief, 10 μL reactions were set up in 96-well plates with 2 μL amplified template genomic DNA and cycled under standard conditions: 50 °C for 2 min, then a denaturation step at 95 °C for 10 min, followed by 60 cycles of 92 °C for 30 s, and 60 °C for 1 min. Endpoint reads were conducted on the ABI 7300 sequence detection system. Cluster analysis was conducted on the scatter plot of Allele A Rn versus Allele B Rn. Genotypic segregation was determined and displayed in the allelic plot with four clusters: no template control, Allele A, Allele B, and heterozygous. The data were then exported for further analysis.

For the *TP53* Arg72Pro SNP, the amplification primers used were 5′-ATGAAGCTCCCAGAATGC and 5′-GCCGGTGTAGGAGCT. The G allele-specific probe (encoding the Arg 72 variant) was 5′-FAM-CTGCTCCCCCCGTGGCCC-TAM. The C allele-specific probe (encoding the Pro72 variant) was 5′-VIC-CTGCTCCCCGCGTGGCCC-TAM.

For the *MDM2* SNP309, the amplification primers used were 5′-CGGGAGTTCAGGGTAAAGGT and 5′-GCGCAGCGTTCACACTAG. The T allele-specific probe was 5′-VIC-CTCCCGCGCCGAAG-TAM. The G allele-specific probe was 5′-FAM-TCCCGCGCCGCAG-TAM.

Statistical analysis

Hardy-Weinberg equilibrium (HWE) of *MDM2* SNP309 and *TP53* Arg72Pro loci was tested separately in the SLE cases and controls of each racial group using the chi-square test. Fisher's exact tests were used to compare genotype frequencies of *MDM2* SNP309 and *TP53* Arg72Pro between cases and controls. Odds ratios and 95% confidence intervals were calculated from logistic regressions, in which race was adjusted as the allele frequencies varied by race. Fisher's exact tests and logistic regression models were also used to compare childhood-onset with adult-onset SLE cases and to compare the cases with nephritis with the cases without nephritis. A two-sided P value < 0.05 was considered statistically significant.

Results

Patients and controls were divided by self-reported ethnicity into northern and western European (including non-Black Hispanics), African American and Asian. The distributions of genotypes for the *TP53* Arg72Pro SNP did not differ significantly from those predicted by HWE for any of these cohorts. Although there were significant differences among Europeans, African Americans and Asians, no significant differences were noted in the distribution of genotype frequencies for the *TP53* Arg72Pro SNP in patients with SLE compared to controls when stratified by ethnicity (Table 2). In addition, a race-adjusted analysis showed no association between risk of SLE and *TP53* Arg72Pro SNP.

We then considered the possibility that the clinical course of SLE might be modified by the *TP53* Arg72Pro SNP. Consequently, we examined the distribution of *TP53* genotypes stratified

by clinical characteristics. First, genotype distributions in patients with childhood-onset disease (presenting prior to their 18th birthday) were contrasted against those of patients with adult-onset disease with the analysis adjusted for race. As shown in Table 2, an association between *TP53* genotype and age of onset was not observed. To determine whether the *TP53* Arg72Pro SNP was associated with more severe disease, the SNP was tested for association with nephritis. Again, no statistically significant difference was observed (Table 2).

To determine whether the *MDM2* SNP309 is associated with risk of SLE, we compared the genotype frequencies of this SNP in 133 Caucasian cases and 248 healthy Caucasian controls and 47 African American cases and 122 healthy African American controls. The genotype frequencies of the *MDM2*SNP309 were not statistically different in cases versus controls in either Caucasians or African Americans (Table 3).

The *MDM2* SNP309 G allele has been associated with a younger age of onset in breast, colorectal and lung cancer, with the largest effect observed in GG homozygotes.[10] Consequently, we examined the association between *MDM2* genotype and age of onset of SLE in a combined analysis of all cases adjusted for race. We did not observe a significant difference in genotype distributions for patients who developed SLE less than the age of 18 (n = 80) compared to those who developed SLE over the age of 18 (n = 139; Table 3). Similarly, no association was observed between nephritis and *MDM2* SNP309 (Table 3).

Discussion

SLE is an idiopathic multisystem inflammatory disease characterised by polyclonal B cell activation and autoantibody production. Deregulated apoptosis may contribute to autoimmunity in SLE in several ways. First, elevated levels of apoptosis may increase the availability of immunogenic apoptotic fragments.[11] Second, phagocytosis of apoptotic cells appears to be impaired in SLE, and this aberrant clearance of apoptotic cells might expose otherwise inaccessible immunogens to the immune system.[3] Finally, decreased levels of apoptosis may contribute to autoimmunity by attenuating the deletion of autoreactive lymphocytes.[2]

The p53 tumour suppressor is a critical determinant of the apoptotic response. Considerable evidence suggests that p53 may also be associated with SLE. Its chromosomal locus, 17p13.1, has been linked to SLE in mapping studies.[12] Mononuclear cell p53 expression appears to be higher in patients with SLE with active disease as compared to controls and to patients with quiescent disease.[13] In addition, SLE patients with higher SLE disease activity index scores have elevated p53 protein levels in peripheral blood.

In light of these data, we hypothesised that genetic variation in the p53 pathway resulting in subtle alterations of p53 apoptotic function could be associated with altered susceptibility to SLE or disease severity. The *TP53* Arg72 variant has been shown to be more efficient at inducing apoptosis than the Pro72 variant,[6] and indeed, an association between SLE and the *TP53* Arg72Pro variant has been reported in a Korean population with SLE; this finding, however, could not be replicated in a Spanish population with SLE.[8,9]

In this study, we confirm that the *TP53* Arg72Pro polymorphism is not associated with SLE in Europeans; in contrast to previously reported results, we find no evidence for association between this SNP and SLE in Asians as well. We are also the first to report that this polymorphism is not associated with SLE in African Americans. We also find that this SNP is not associated with an early age of onset nor associated with worse disease.

MDM2 is the major negative regulator of p53 function. The *MDM2* SNP309 affects binding of the SP1 transcription factor.[7] Specifically, SP1 binds with higher affinity to the G allele

relative to the T allele at SNP309, increasing the steady-state levels of MDM2.[7] The GG genotype is associated with accelerated cancer development, especially in premenopausal women.[10,14] In light of the known clinical association between SLE and young women, we hypothesised that the *MDM2* SNP309 would be a modifier of SLE susceptibility or it would be associated with early disease presentation. We found that this SNP is not associated with SLE in either Caucasians or in African Americans nor associated with early-onset disease or worse disease.

Our findings that allelic variation in the functional *TP53* Arg72Pro SNP and the *MDM2* SNP309 are not associated with SLE susceptibility, age of onset or disease severity suggest that these well-described functional p53-pathway genetic variants are not associated with SLE. Of course, our data do not exclude the possibility that other SNPs in this pathway are associated with SLE. In addition, although the p53 tumour suppressor is the central regulator of cellular apoptosis, genetic variation in a variety of other pathways may be responsible for the observed increase in apoptosis in SLE.[15]

Taken together, our findings suggest that neither the *TP53* codon 72 polymorphism nor the *MDM2* SNP309 contributes to SLE susceptibility in Caucasians, African American or Asians. The fact that we did not observe the previously reported association between the p53 codon 72 variant and SLE in Asians underscores the importance of replication in association studies. Our data, however, do not rule out the possibility that genetic variation in the p53 pathway is associated with SLE or with specific clinical characteristics of SLE. Given the wealth of data implicating p53 in the aetiology of SLE, further investigation into the contribution of this critical apoptotic regulator to SLE is warranted.

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Table 1

Demographic and clinical characteristics of the SLE cases

	Childhood-onset[a] no (%)	Adult-onset no (%)
Number of subjects	98	216
Gender		
Male	17 (17)	9 (5)
Female	81 (83)	164 (95)
Race		
European	57 (58)	112 (65)
African American	22 (22)	36 (21)
Asian	19 (19)	25 (14)
Renal status[b]		
Nephritis	48 (51)	78 (51)
No nephritis	47 (49)	76 (49)

[a] Age at diagnosis <18 years.

[b] Renal status was available for 95/98 childhood-onset patients and 154/216 adult-onset cases. Nephritis was determined by the presence of active urinary sediment or renal biopsy.

Table 2

Tests for association between *TP53* Arg72Pro genotypes and SLE, age of disease onset and nephritis, stratified by ethnicity

	GG no (%)	GC no (%)	CC no (%)	P *value*
African American				
SLE (*n* = 57)	12 (21)	29 (51)	16 (28)	0.63
Control (*n* = 188)	40 (21)	83 (44)	65 (35)	
European				
SLE (*n* = 169)	84 (50)	69 (41)	16 (9)	0.09
Control (*n* = 285)	150 (53)	123 (43)	12 (4)	
Asian				
SLE (*n* = 44)	16 (36)	19 (43)	9 (20)	0.32
Control (*n* = 212)	70 (33)	114 (54)	28 (13)	
Adjusted odds ratio of SLE vs control (95% CI)[a]	1.0 (reference)	1.00 (0.72–1.37)	1.29 (0.81–2.05)	0.49
African American				
Childhood-onset (*n* = 21)[b]	7 (33)	7 (33)	7 (33)	0.10
Adult-onset (*n* = 36)	5 (14)	22 (61)	9 (25)	
European				
Childhood onset (*n* = 57)[b]	23 (40)	27 (47)	7 (12)	0.20
Adult onset (*n* = 112)	61 (54)	42 (38)	9 (8)	
Asian				
Childhood onset (*n* = 19)[b]	8 (42)	8 (42)	3 (16)	0.79
Adult onset (*n* = 25)	8 (32)	11 (44)	6 (24)	
Adjusted odds ratio of childhood onset vs adult onset SLE (95% CI)[a]	1.0 (reference)	1.07 (0.62–1.86)	1.31 (0.61–2.82)	0.78
African American				
Nephritis (*n* = 26)	4 (15)	19 (73)	3 (12)	0.01
No nephritis (*n* = 20)	6 (30)	6 (30)	8 (40)	
European				
Nephritis (*n* = 62)	32 (52)	23 (37)	7 (11)	0.90
No nephritis (*n* = 64)	32 (50)	26 (41)	6 (9)	
Asian				

	GG no (%)	GC no (%)	CC no (%)	P *value*
Nephritis (*n* = 23)	8 (35)	9 (39)	6 (26)	0.83
No nephritis (*n* = 14)	4 (29)	7 (50)	3 (21)	
Adjusted odds ratio of nephritis vs. non-nephritis SLE (95% CI)[a]	1.0 (reference)	1.17 (0.63–2.14)	0.78 (0.34–1.79)	0.61

[a] Odds ratio (95% confidence intervals) adjusted for race in logistic regression.

[b] Age at diagnosis <18 years old.

Table 3

Tests for association between *MDM2* SNP309 genotypes and SLE, age of disease onset, and nephritis, stratified by ethnicity

	TT no (%)	TG no (%)	GG no (%)	P *value*
African American				
SLE (*n* = 47)	33 (70)	12 (26)	2 (4)	0.90
Control (*n* = 122)	84 (69)	34 (28)	4 (3)	
European				
SLE (*n* = 133)	37 (28)	73 (55)	23 (17)	0.40
Control (*n* = 248)	86 (35)	123 (50)	39 (16)	
Adjusted odds ratio of SLE vs control (95% CI)[a]	1.0 (reference)	1.22 (0.82–1.83)	1.28 (0.71–2.31)	0.56
African American				
Childhood onset (*n* = 17)[b]	12 (71)	4 (24)	1 (6)	1.0
Adult onset (*n* = 30)	21 (70)	8 (27)	1 (3)	
European				
Childhood onset (*n* = 45)[b]	12 (27)	26 (58)	7 (16)	0.91
Adult onset (*n* = 88)	25 (28)	47 (53)	16 (18)	
Asian				
Childhood onset (*n* = 18)[b]	3 (17)	6 (33)	9 (50)	0.77
Adult onset (*n* = 21)	4 (19)	9 (43)	8 (38)	
Adjusted odds ratio of childhood-onset vs adult-onset SLE (95% CI)[a]	1.0 (reference)	1.05 (0.54–2.05)	1.13 (0.48–2.65)	0.96
African American				
Nephritis (*n* = 23)	14 (61)	8 (35)	1 (4)	0.33
No nephritis (*n* = 16)	13 (81)	2 (13)	1 (6)	
European				
Nephritis (*n* = 50)	12 (24)	28 (56)	10 (20)	1.00
No nephritis (*n* = 51)	13 (25)	29 (57)	9 (18)	
Asian				
Nephritis (*n* = 22)	1 (5)	9 (41)	12 (55)	0.20
No nephritis (*n* = 11)	3 (27)	4 (36)	4 (36)	
Adjusted odds ratio of nephritis	1.0 (reference)	1.65 (0.77–3.52)	1.88 (0.72–4.86)	0.34

	TT no (%)	TG no (%)	GG no (%)	P *value*
vs non-nephritis SLE (95% CI)[a]				

[a] Odds ratio (95% CI) adjusted for race in logistic regression.

[b] Age at diagnosis <18 years old.