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A common SNP in the *CD40* region is associated with systemic lupus erythematosus and correlates with altered *CD40* expression: implications for the pathogenesis

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ABSTRACT

Background In systemic lupus erythematosus (SLE) sustained CD40L expression by T cells and platelets activates a variety of cells via its receptor CD40 contributing to disease pathogenesis. Although *CD40* has recently been identified in genome-wide association study as a novel rheumatoid arthritis susceptibility gene such an association has not been documented for SLE.

Objective To investigate whether the rs4810485 *CD40* single nucleotide polymorphism (SNP) is associated with increased risk for SLE and its impact on *CD40* expression.

Materials and methods The primary sample set consisted of 351 patients with SLE and 670 matched healthy controls of Greek origin. 158 patients with SLE and 155 controls from Turkey were used as a replication sample. Genotyping of rs4810485 was performed by restriction fragment length polymorphism and the Sequenom MassArray technology. The expression of *CD40* mRNA and protein was assessed in unstimulated and lipopolysaccharide-stimulated peripheral blood mononuclear cells by quantitative real time PCR and flow cytometry, respectively.

Results The minor allele T of *CD40* rs4810485 SNP was significantly under-represented in Greek patients with SLE compared with healthy controls (OR=0.65, 95% CI 0.54 to 0.79). The association was replicated in the Turkish cohort (OR=0.57, 95% CI 0.41 to 0.80; meta-analysis of 509 patients with SLE and 825 healthy controls: OR=0.63, 95% CI 0.53 to 0.74, $p = 2\times10^{-8}$). In both cases and controls, the rs4810485 G/T and T/T genotypes were associated with significantly reduced *CD40* mRNA and protein expression in peripheral blood CD14+ monocytes and CD19+ B cells compared with G/G genotype, both under basal conditions and following stimulation.

Conclusions *CD40* has been identified as a new susceptibility locus in Greek and Turkish patients with SLE. The rs4810485 minor allele T is under-represented in SLE and correlates with reduced *CD40* expression in peripheral blood monocytes and B cells, with potential implications for the regulation of aberrant immune responses in the disease.

INTRODUCTION

There is increasing evidence that different autoimmune diseases may share common pathogenic pathways. Systemic lupus erythematosus (SLE) is a multifactorial, systemic autoimmune disease characterised by production of autoantibodies directed against cell surface and nuclear components. The aetiology of the disease remains elusive even though it has been intensively studied.[1 2] A number of genetic susceptibility loci, conferring low-to-moderate risk for SLE, have been recently identified through genome-wide association studies.[3–5] The most important genes that confer susceptibility are located in the *HLA* locus,[6] but non-HLA genes also operate such as *IRF5*, *PTPN22*, *STAT4*, *CDKN1A* and *BLK*.[7]

Recent genome-wide searches for susceptibility loci have provided new insights into autoimmune disease pathogenesis and have identified several loci showing significant linkage to the diseases, some of which have been confirmed by independent studies.[8–10] Among them, the *CD40* locus has been identified as a genetic risk factor for rheumatoid arthritis (RA).[11] *CD40* spans 11 kb, has nine exons,[12] is constitutively and inducibly expressed on the surface of various immune and non-immune cell types, such as B cells, monocytes, dendritic cells, keratinocytes, epithelia, microglia and endothelial cells[13] and is implicated in immune and non-immune responses.[14] CD40-mediated cellular functions include T cell-dependent B-cell humoral responses,[15] secretion of growth factors and cytokines from monocytes[16 17] and expression of adhesion molecules on endothelial cells. CD40 signalling has been linked to pathogenic processes of chronic inflammatory and autoimmune diseases.[18] A pathogenic role for CD40–CD40L interactions has been well established in lupus (reviewed by Koshy *et al*[19]) with sustained expression of CD40L on activated T cells[19] and platelets[20] in patients with active SLE. Importantly, interruption of this pathway results in disease attenuation both in animals[21] and in humans.[22]

Our group has focused on the overlap between the genetic determinants for SLE, RA, type-1 diabetes mellitus (T1D), psoriasis and other autoimmune diseases. Through analysis of well-characterised case–control studies, we have previously documented significant associations between T1D and psoriasis with two SLE and RA susceptibility genes—namely, *TRAF1/C5* and *STAT4*, respectively.[23–26] Assuming that genes involved in

regulation of inflammatory responses may confer risk for different immune-mediated diseases, and based on the pathogenic role of the CD40–CD40L pathway in lupus, herein we demonstrate, for the first time, that the *CD40* single nucleotide polymorphism (SNP) rs4810485 is associated with SLE in individuals of Greek and Turkish origin. Importantly, our in vitro studies demonstrate that rs4810485 may affect *CD40* expression in primary mononuclear cells, with potential implications for the disease pathogenesis.

MATERIALS AND METHODS

Study population

The primary sample set included 351 patients with SLE from Greece who were followed up at the Department of Rheumatology, Clinical Immunology, and Allergy, University of Crete. All patients met the 1982 American College of Rheumatology revised classification criteria[27] (online supplementary table S1). Six hundred and seventy age- and gender-matched healthy individuals were recruited from the Department of Transfusion Medicine. The study was approved by the ethics committee of the University Hospital of Crete. The replication sample set consisted of 158 patients with SLE and 155 age- and gender-matched healthy controls from Turkey (Marmara University Medical School). Sixteen patients with SLE and 24 controls were selected for the *CD40* expression experiments presented in detail elsewhere (supplementary text).

Analysis of the rs4810485 *CD40* polymorphism

Whole blood was collected in EDTA-containing tubes and genomic DNA was extracted using the Qiamp DNA Blood Mini kit (QIAGEN Inc, Valencia, California, USA). Genotyping for *CD40* rs4810485 was performed by PCR-restriction fragment length polymorphism or the Sequenom MassArray technology according to the manufacturer's instructions (supplementary text).

Preparation of mononuclear cells, cell culture and stimulation

Peripheral blood mononuclear cells (PBMCs) from patients with SLE and healthy controls were isolated, cultured and stimulated as described in the supplementary text.

RNA extraction and quantitative real-time RT-PCR

Total RNA was extracted from fresh or cultured PBMCs using the RNeasy RNA Isolation kit (Qiagen) and incubated with RNase-free DNase I (Qiagen) according to the manufacturer's protocol. Reverse transcription reactions were performed with 500 ng total RNA using random hexamers and the Thermoscript RT system (Invitrogen, Carlsbad, California) and stored at −20°C until analysed. PCR conditions and the quantification of the mRNAs levels of *CD40* and *GAPDH* are described in the supplementary text.

Immunophenotyping of PBMCs

A direct immunofluorescence technique was used to evaluate CD40 protein expression within subsets of PBMCs (supplementary text). Normalised mean fluorescence intensity (MFI) was expressed against the IgG isotypic control according to the following equation: normalised MFI = $(MFI_{CD40} - MFI_{IgG})/MFI_{IgG}$.

Statistical analysis

Statistical analysis was performed with GraphPad Prism statistical program (GraphPad Software, San Diego, California, USA). In the case–control comparisons, only unrelated samples were used (supplementary text).

RESULTS

The *CD40* rs4810485 SNP is associated with SLE in two independent ethnic cohorts

Allele and genotype frequencies for rs4810485 in the primary cohort of Greek patients with SLE and healthy controls are

Table 1 Genotypes and allele frequencies of the *CD40* rs4810485 polymorphism analysed in 351 patients with SLE and 670 controls from Greece and 158 patients with SLE and 155 controls from Turkey

	rs4810485				
	Genotype			Allele	
	GG	GT	TT	G	T
Greek cohort, N (%)					
Patients with SLE (n=351)	139 (39.6)	190 (54.1)	22 (6.3)	468 (66.7)	234 (33.3)
Healthy controls (n=670)	216 (32.2)	326 (48.7)	128 (19.1)	758 (56.6)	582 (43.4)
*OR (95% CI)	1.00	0.91 (0.69 to 1.20)	0.27 (0.16 to 0.44)	1.00	0.65 (0.54 to 0.79)
*p Value (df 1)	–	5.0×10^{-1}	7.0×10^{-8}	–	9.6×10^{-6}
†p Value (df 2)			2.1×10^{-7}		
Turkish cohort, N (%)					
Patients with SLE (n=158)	69 (43.7)	82 (51.9)	7 (4.4)	220 (69.6)	96 (30.4)
Healthy controls (n=155)	52 (33.5)	72 (46.5)	31 (20.0)	176 (56.8)	134 (43.2)
*OR (95% CI)	1.00	0.86 (0.53 to 1.39)	0.17 (0.07 to 0.42)	1.00	0.57 (0.41 to 0.80)
*p Value (df 1)	–	5.0×10^{-1}	3.2×10^{-5}	–	9.0×10^{-4}
†p Value (df 2)			1.0×10^{-4}		
Combined cohort, N (%)					
Patients with SLE (n=509)	208 (40.9)	272 (53.4)	29 (5.7)	688 (67.6)	330 (32.4)
Healthy controls (n=825)	268 (32.5)	398 (48.2)	159 (19.3)	934 (56.6)	716 (43.4)
*OR (95% CI)	1.00	0.88 (0.69 to 1.12)	0.24 (0.15 to 0.36)	1.00	0.63 (0.53 to 0.74)
*p Value (df 1)	–	3.0×10^{-1}	7.4×10^{-12}	–	2.0×10^{-8}
†p Value (df 2)			2.2×10^{-11}		

A meta-analysis of the two cohorts is also presented. Reduced frequency of the rs4810485 minor allele T and the T/T genotype in patients with SLE compared with healthy controls.
*p Values with df 1 and OR (95%CI) were calculated taking as reference the G/G genotype or the major (G) allele.
†p Values with df 2 were calculated with a 2×3 χ^2 test of independence and account for the overall difference between the three genotypes.
df, degrees of freedom; SLE, systemic lupus erythematosus.

shown in table 1. Analysis by a 2×3 χ^2 test of independence showed a significant difference in the genotype distribution between cases and controls ($p = 2.1\times10^{-7}$, df 2). Post hoc analysis by 2×2 χ^2 test revealed that rs4810485 T/T homozygosity was less common in patients with SLE than in healthy controls (6.3% vs 19.1%, $p = 7.0\times10^{-8}$, df 1). The presence of minor allele T was under-represented in SLE (OR=0.65, 95% CI 0.54 to 0.79, $p = 9.6\times10^{-6}$) (table 1). Genotyping of one-third of the control samples was replicated by using Sequenom MassArray technology and the results were 100% identical to those obtained by restriction fragment length polymorphism (data not shown).

We then proceeded to replicate our findings in a case-control population from neighbouring Turkey. The study group consisted of 158 Turkish patients with SLE (5 men, 153 women) with a mean (± SD) aged 41 ± 11 years. The control group consisted of 155 unrelated healthy subjects from Turkey (5 men, 150 women) aged 39 ± 7 years. In accordance with the findings in the Greek cohort, the rs4810485 T/T genotype was less frequent in SLE than in healthy controls (4.4% vs 20.0%, $p = 3.2\times10^{-5}$). Minor T allele had significantly lower frequency in SLE (OR=0.57, 95% CI 0.41 to 0.80, $p = 9.0\times10^{-4}$) (table 1). In the meta-analysis of the two cohorts, the T/T genotype was significantly ($p = 7.4\times10^{-12}$) less common and the T allele was significantly under-represented (OR=0.63, 95% CI 0.53 to 0.74, $p = 2.0\times10^{-8}$) in patients with SLE compared with controls. Observed frequencies of the *CD40* rs4810485 genotypes were in Hardy–Weinberg equilibrium (data not shown).

Of note, a preliminary association study performed in our laboratory in patients with Wegener's granulomatosis showed that the T allele of the rs4810485 SNP of the *CD40* gene was less common in patients with the disease (14.3%) than in control individuals (39.9%) (unpublished data). Furthermore, we found no evidence for association between *CD40* and T1D; no statistically significant difference in T-allele frequencies was observed between patients with T1D and controls either in the first population examined (OR=1.00, 95% CI 0.63 to 1.6) or in the replication group (data not shown).

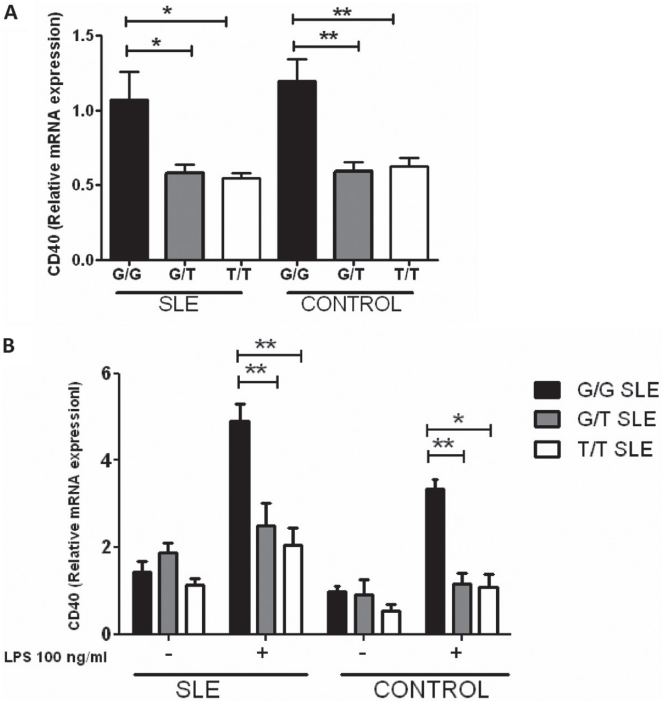

Figure 1 Basal and induced *CD40* mRNA expression in peripheral blood mononuclear cells (PBMCs) from patients with systemic lupus erythematosus (SLE) and healthy controls according to rs4810485 genotype. (A) Expression of *CD40* mRNA was assessed by quantitative real-time PCR in freshly isolated PBMCs. rs4810485 T/T and G/T genotypes were associated with almost twofold lower *CD40* mRNA levels than the G/G genotype. Similar results were obtained in PBMCs from healthy controls (Mann–Whitney U test, *p<0.05, **p<0.01). (B) PBMCs from patients with SLE or healthy controls were cultured for 24 h in the presence or absence of lipopolysaccharide (LPS) 100 ng/ml, and *CD40* mRNA expression was determined. In both groups, there was significantly lower expression in G/T and T/T than in the G/G genotype in the presence of LPS (Mann–Whitney U test, *p<0.05, **p<0.01). Bars represent the mean ± SEM of the normalised expression of *CD40* mRNA relative to *GAPDH* mRNA.

The rs4810485 T allele is associated with reduced *CD40* mRNA in freshly isolated and lipopolysaccharide-stimulated PBMCs from patients with SLE and healthy controls

Expression of *CD40* was evaluated by real-time PCR in freshly isolated PBMCs from patients with SLE and healthy controls with different rs4810485 genotypes. *CD40* mRNA levels were almost twofold lower in patients with SLE who had the T/T or G/T genotype than those with the G/G genotype (figure 1A). To further verify our results, we examined the *CD40* mRNA in freshly isolated PBMCs from healthy controls, and we also found significantly reduced expression in individuals with T/T and G/T compared with the G/G genotype (figure 1A).

We next examined *CD40* mRNA expression after stimulation with lipopolysaccharide (LPS) 100 ng/ml for 24 h. As shown in figure 1B,LPS treatment induced *CD40* expression both in patients with SLE and in healthy controls. In both groups, stimulated PBMCs with the G/T and T/T genotype had significantly reduced *CD40* mRNA compared with those with the G/G genotype. PBMCs from patients with SLE with the G/G genotype showed the strongest induction of CD40 after LPS stimulation (figure 1B). Collectively, these results indicate that the rs4810485 T allele is associated with reduced *CD40* mRNA expression both in the basal, non-stimulated and the stimulated state.

Basal membrane protein expression of CD40 in PBMCs is reduced in patients with SLE and controls with the rs4810485 T allele

We next used flow cytometry to examine the effect of rs4810485 on CD40 membrane protein expression (measured by CD40 MFI or the percentage of CD40+ cells) on freshly isolated CD19+ B cells and CD14+ monocytes. To minimise any possible interfering factors, we included patients with inactive disease and without significant B-cell lymphopenia (<3% of total lymphocytes). Figure 2A shows representative flow cytometry histograms for CD40 expression on unstimulated B cells from patients with SLE (upper panel) and healthy controls (lower panel) with different rs4810485 genotypes. In both patients and controls, rs4810485 T/T and G/T were associated with significantly reduced CD40 MFI on CD19+ B cells compared with G/G (p<0.05 for T/T vs G/G and G/T vs G/G pairwise comparisons) (figure 2B). The rs4810485 T allele also correlated with decreased proportion of peripheral blood CD40+ CD19+ B cells both in patients with SLE and in healthy controls (supplementary figure S1). We performed a similar analysis in CD14+ monocytes, and found that rs4810485 T/T and G/T patients and controls had reduced CD40 MFI in comparison with their G/G counterparts (figure 2C,D). In contrast, we found no significant variation in the proportion of

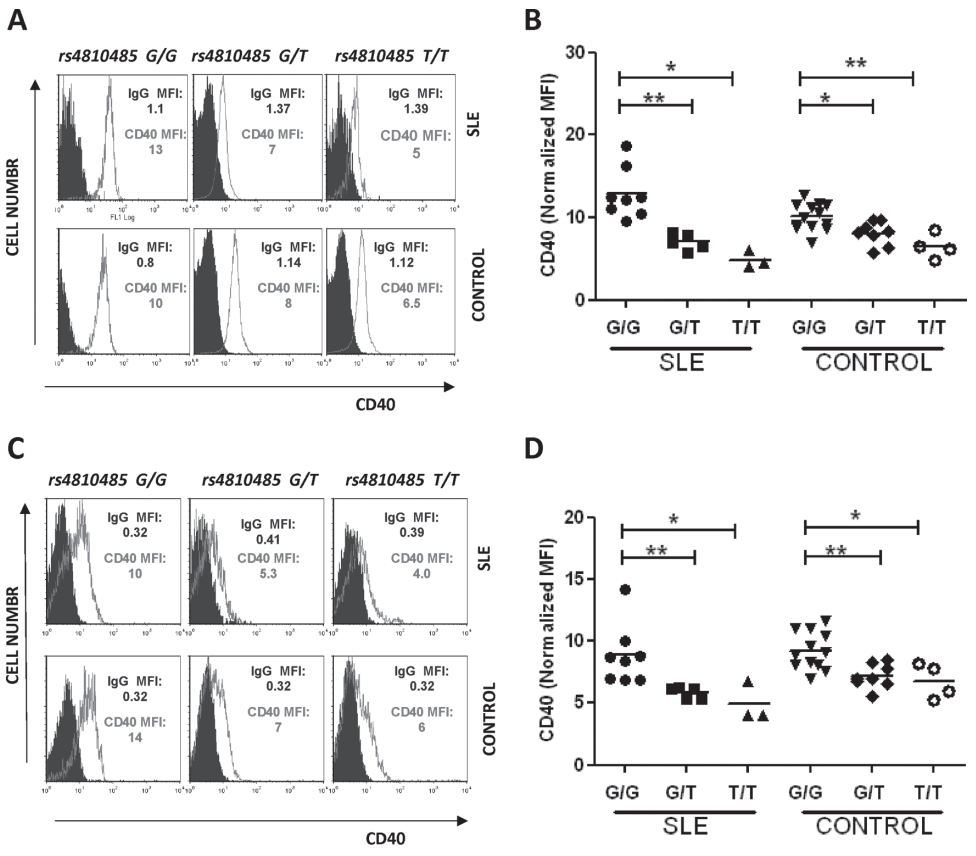

Figure 2 Differential basal membrane expression of CD40 on B cells and monocytes in patients with SLE and healthy controls according to rs4810485 genotype. (A) Representative flow cytometry histograms for CD40 expression on CD19+ B cells from patients with SLE (upper panel) and healthy controls (lower panel) with the rs4810485 G/G, G/T and T/T genotypes. (B) Patients with SLE and healthy controls with the T/T or G/T genotype have significantly lower CD40 mean fluorescence intensity (MFI) on freshly isolated CD19+ B cells than individuals with the G/G genotype (Mann–Whitney U test, *p<0.05, **p<0.01). (C) Representative flow cytometry histograms for CD40 expression on CD14+ monocytes from patients with SLE (upper panel) and healthy controls (lower panel) with the rs4810485 G/G, G/T and T/T genotypes. (D) Patients with SLE and healthy controls with the T/T or G/T genotype have significantly lower CD40 MFI on CD14+ monocytes than individuals with the G/G genotype (Mann–Whitney U test, *p<0.05, **p<0.01). Scatter plots represent individual cases.

CD40+ monocytes according to rs4810485 genotype (data not shown). Overall, these results suggest that under basal, non-induced, conditions, the rs4810485 allele T is associated with lower CD40 surface expression on B cells and monocytes from patients with SLE and healthy controls.

Stimulation of PBMCs induces lower membrane expression of CD40 in patients with SLE and controls with the rs4810485 T allele

Although CD40 is constitutively expressed on the surface of mononuclear cells, its expression is further upregulated upon cell stimulation. To examine the effect of rs4810485 SNP on inducible CD40 expression, PBMCs were cultured for 24 h in the presence or absence of LPS 100 ng/ml, and CD40 expression was evaluated by flow cytometry. In LPS-stimulated CD19+ B cells, CD40 membrane expression (MFI) was significantly lower in patients with the T/T or G/T genotype than in those with the G/G genotype (figure 3A,B); the effect was more pronounced in patients with SLE than in healthy controls (figure 3B). Similar results were obtained with the percentage of CD40+ CD19+ B cells (supplementary figures S2A,B).

With regard to CD14+ monocytes, LPS-induced CD40 MFI levels were lower in both patients and controls with the rs4810485 T/T and G/T rather than the G/G genotype (figure 3C,D). The proportion of CD40+ monocytes also correlated inversely with presence of the minor T allele (supplementary figure S2C,D). Together, our results indicate that the rs4810485 SNP is accompanied by genotype-specific differences on induced CD40 membrane expression in healthy controls and more profoundly in patients with SLE, with the T/T genotype associated with lower induction than the G/G genotype.

DISCUSSION

Identification of shared genetic determinants for clinically distinct disorders is the emerging premise underlying the results of recent genome-wide scans.[28 29] In this study, we explored one such example of overlap between disease susceptibility loci by investigating the association of the RA-associated SNP rs4810485 of the *CD40* gene with SLE. We report for the first time that rs4810485 is associated with SLE in the Greek and Turkish Mediterranean population, and correlates with differential *CD40* expression in peripheral blood B cells and monocytes.

In view of the role of *CD40,* a tumour necrosis factor superfamily membrane member, in generating effective immune responses through interaction with its ligand CD40L, we reasoned that it may represent an excellent candidate susceptibility gene for various autoimmune diseases.[30] The rs4810485 SNP has recently been associated with RA in individuals of

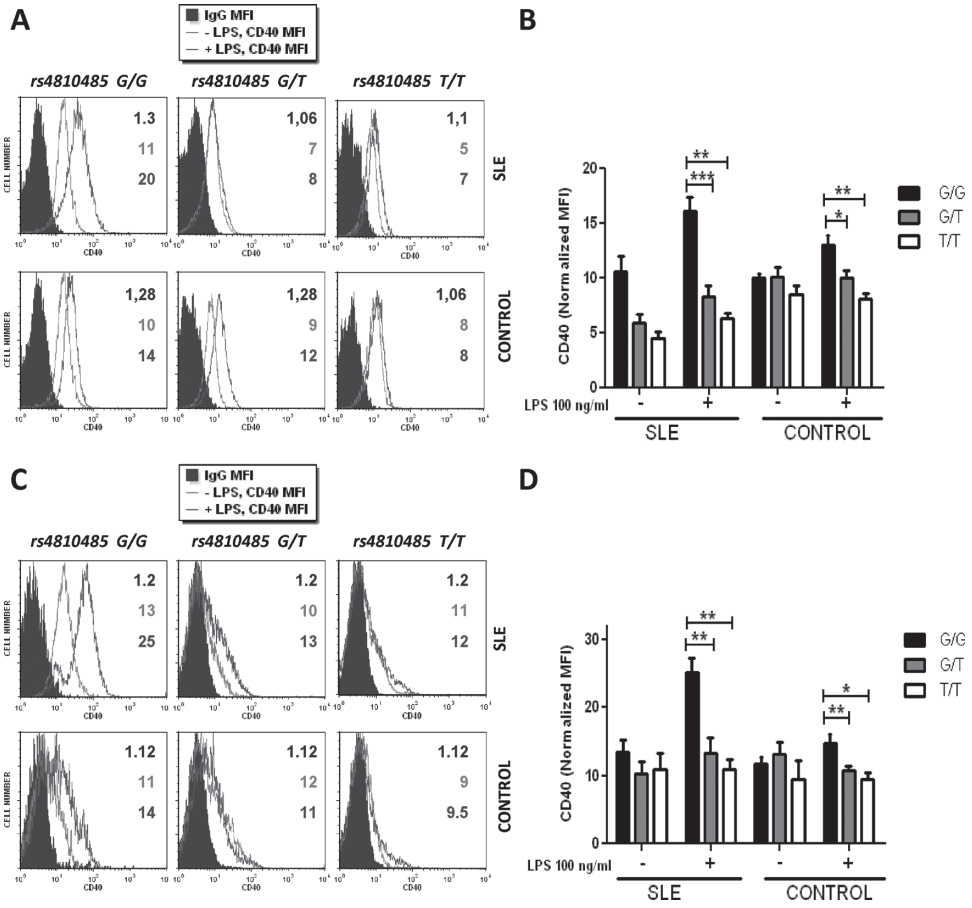

Figure 3 Effect of rs4810485 on lipopolysaccharide (LPS)-induced CD40 membrane expression on B cells and monocytes from patients with SLE and healthy controls. (A) Representative flow cytometry histograms for CD40 membrane expression on LPS-stimulated CD19+ B cells from patients with SLE (upper panel) and healthy controls (lower panel) with the rs4810485 G/G, G/T and T/T genotypes. (B) Expression of CD40 on LPS-stimulated B cells with the T/T and G/T genotypes was significantly lower than the G/G genotype both in patients with SLE and controls (Mann–Whitney U test, *p<0.05, **p<0.01, ***p<0.001). Patients with SLE with the G/G genotype demonstrated the most profound CD40 upregulation. Bars represent mean ± SEM. (C) Representative flow cytometry histograms for CD40 expression on LPS-stimulated CD14+ monocytes from patients with SLE (upper panel) and healthy controls (lower panel) with the rs4810485 G/G, G/T and T/T genotypes. (D) LPS-induced CD40 mean fluorescence intensity (MFI) on CD14+ monocytes is significantly lower in patients with SLE and healthy controls with the T/T and G/T genotype than in those with the G/G genotype (Mann–Whitney U test, *p<0.05, **p<0.01). The induction was more prominent in patients with SLE with the GG genotype. Values are the mean ± SEM.

European[11] but not Korean ancestry.[31] A replication study in a Greek population confirmed the association of rs4810485 with RA.[32] Importantly, the *CD40* locus resides in a region (20q11–13) that has been previously linked with SLE in European-Caucasians, Mexican-Americans and African-Americans.[33–35] This suggests an underlying pathogenic mechanism involving a risk allele of a shared disease locus that may account for increased susceptibility in two distinct diseases. Other studies have also suggested the *CD40–CD40L* pair as strong SLE candidate genes because of their ability to induce T-cell mediated humoral responses. However, in a previous family-based genetic study of CD40 in SLE[36] no association was found; probably, the SNP data available at the time of the previous study did not allow for detection of the effect from rs4810485. Notably, an association between *CD40* polymorphisms and genetic susceptibility to Grave's disease[37] and Kawasaki disease,[38] but not to T1D, has been reported, an observation that may help to delineate pathogenic mechanisms in the aforementioned autoimmune diseases. Of note, several other SNPs in genes involved in the CD40 signalling pathway have been identified, including TNFAIP3 (A20, an E3-ubiquitin ligase) and TRAF1-C5.[18] TRAF1 is an adaptor protein that cooperates with TRAF2 to enhance CD40 signals.[39]

The rs4810485 SNP resides in the second intron of the *CD40* gene and its functional consequence remains to be identified. As a first attempt to study this issue, we looked for a possible association between rs4810485 and *CD40* expression in peripheral blood B cells and monocytes. In both patients with SLE and healthy controls, individuals with the T/T or G/T genotype had significantly reduced basal and induced CD40 mRNA and protein levels compared with those with the G/G genotype, suggesting that rs4810485 could be a functional polymorphism. This result is biologically plausible since the minor allele T associated with reduced *CD40* expression was found to be underrepresented in patients with SLE (OR=0.63 in the meta-analysis of the Greek and Turkish cohorts). Of interest, in the presence of the major allele G, both basal and induced *CD40* levels were higher in patients with SLE than in healthy controls, indicating that rs4810485 might also influence *CD40* expression in a disease-specific manner.

To explore whether rs4810485 might modify any transcription factor-binding site, we performed sequence analysis

using the Genomatix Software (Genomatix Software GmbH, "Gene2Promoter" program, Muenchen, Germany). Allele G disrupted the putative binding of the transcription factor Pax6, which was predicted to bind to allele T. Although no information exists about the expression and function of Pax6 in SLE immune cells, another member of the Pax family transcription factors, Pax5 is implicated in mature B-cell programming and its repression is required for plasma cell differentiation.[40] Moreover, rs4810485 allele G is predicted to enhance the DNA binding of a member of the ETS1 transcription factors family. ETS1 is a negative regulator of B- and Th17-cell differentiation that has been associated with the development of SLE.[41] Of interest, lower levels of ETS1 mRNA have been detected in PBMCs of patients with SLE.[42]

Increased *CD40* expression in antigen presenting cells might contribute to enhanced signalling upon interaction with CD40L,[43] which is upregulated on activated T cells in patients with SLE and RA. Interactions between CD40 and CD40L might also influence the function of non-immune cells such as endothelial cells, leading to endothelial dysfunction[44] and vascular inflammation,[45] which are common in these patients.

In conclusion, we have demonstrated that the rs4810485 SNP of the *CD40* locus is associated with SLE in a genetically homogeneous population. We also provide evidence that rs4810485 may affect CD40 mRNA and protein expression on B cells and monocytes, with potential implications for regulation of the inflammatory responses in the disease. The CD40/CD40L pathway has an established pathogenic role and represents a valid therapeutic target in lupus. Our data corroborate these observations and offer a plausible explanation for the increased susceptibility to SLE conferred by this genetic variant of *CD40*. Sustained expression of CD40L coupled with increased expression of CD40 observed in over half of lupus patients amplify cellular and humoral responses in a variety of immune and non-immune cellular targets, thus contributing to disease pathogenesis. Intriguingly, polymorphisms in the *CD40* gene may affect expression and identify a subgroup of patients with SLE with a higher efficacy of anti-CD40 therapeutic intervention.

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Competing interests None.

Ethics approval The study was approved by the ethics committee of University Hospital of Crete (Greece) and the University of Marmara (Instanbul, Turkey).

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REFERENCES

1. **Rahman A,** Isenberg DA. Systemic lupus erythematosus. *N Engl J Med* 2008;**358**:929–39.
2. **Bertsias GK,** Salmon JE, Boumpas DT. Therapeutic opportunities in systemic lupus erythematosus: state of the art and prospects for the new decade. *Ann Rheum Dis* 2010;**69**:1603–11.
3. **Barton A,** Thomson W, Ke X, *et al.* Re-evaluation of putative rheumatoid arthritis susceptibility genes in the post-genome wide association study era and hypothesis of a key pathway underlying susceptibility. *Hum Mol Genet* 2008;**17**:2274–9.
4. **Barton A,** Worthington J. Genetic susceptibility to rheumatoid arthritis: an emerging picture. *Arthritis Rheum* 2009;**61**:1441–6.
5. **Gregersen PK,** Olsson LM. Recent advances in the genetics of autoimmune disease. *Annu Rev Immunol* 2009;**27**:363–91.
6. **Martens HA,** Nolte IM, van der Steege G, *et al.* An extensive screen of the HLA region reveals an independent association of HLA class I and class II with susceptibility for systemic lupus erythematosus. *Scand J Rheumatol* 2009;**38**:256–62.
7. **Yang W,** Ng P, Zhao M, *et al.* Population differences in SLE susceptibility genes: STAT4 and BLK, but not PXK, are associated with systemic lupus erythematosus in Hong Kong Chinese. *Genes Immun* 2009;**10**:219–26.
8. **Rhodes B,** Vyse TJ. The genetics of SLE: an update in the light of genome-wide association studies. *Rheumatology (Oxford)* 2008;**47**:1603–11.
9. **Harley IT,** Kaufman KM, Langefeld CD, *et al.* Genetic susceptibility to SLE: new insights from fine mapping and genome-wide association studies. *Nat Rev Genet* 2009;**10**:285–90.
10. **Moser KL,** Kelly JA, Lessard CJ, *et al.* Recent insights into the genetic basis of systemic lupus erythematosus. *Genes Immun* 2009;**10**:373–9.
11. **Raychaudhuri S,** Remmers EF, Lee AT, *et al.* Common variants at CD40 and other loci confer risk of rheumatoid arthritis. *Nat Genet* 2008;**40**:1216–23.
12. **Cookson WO,** Ubhi B, Lawrence R, *et al.* Genetic linkage of childhood atopic dermatitis to psoriasis susceptibility loci. *Nat Genet* 2001;**27**:372–3.
13. **Foy TM,** Aruffo A, Bajorath J, *et al.* Immune regulation by CD40 and its ligand GP39. *Annu Rev Immunol* 1996;**14**:591–617.
14. **Kroczek R,** Hamelmann E. T-cell costimulatory molecules: optimal targets for the treatment of allergic airway disease with monoclonal antibodies. *J Allergy Clin Immunol* 2005;**116**:906–9.
15. **van Kooten C,** Banchereau J. CD40-CD40 ligand. *J Leukoc Biol* 2000;**67**:2–17.
16. **Suttles J,** Stout RD. Macrophage CD40 signaling: a pivotal regulator of disease protection and pathogenesis. *Semin Immunol* 2009;**21**:257–64.
17. **Melter M,** Reinders ME, Sho M, *et al.* Ligation of CD40 induces the expression of vascular endothelial growth factor by endothelial cells and monocytes and promotes angiogenesis in vivo. *Blood* 2000;**96**:3801–8.
18. **Peters AL,** Stunz LL, Bishop GA. CD40 and autoimmunity: the dark side of a great activator. *Semin Immunol* 2009;**21**:293–300.
19. **Koshy M,** Berger D, Crow MK. Increased expression of CD40 ligand on systemic lupus erythematosus lymphocytes. *J Clin Invest* 1996;**98**:826–37.
20. **Duffau P,** Seneschal J, Nicco C, *et al.* Platelet CD154 potentiates interferon-alpha secretion by plasmacytoid dendritic cells in systemic lupus erythematosus. *Sci Transl Med* 2010;**2**:47ra63.
21. **Wang X,** Huang W, Mihara M, *et al.* Mechanism of action of combined short-term CTLA4Ig and anti-CD40 ligand in murine systemic lupus erythematosus. *J Immunol* 2002;**168**:2046–53.
22. **Boumpas DT,** Furie R, Manzi S, *et al.* A short course of BG9588 (anti-CD40 ligand antibody) improves serologic activity and decreases hematuria in patients with proliferative lupus glomerulonephritis. *Arthritis Rheum* 2003;**48**:719–27.
23. **Zervou MI,** Sidiropoulos P, Petraki E, *et al.* Association of a TRAF1 and a STAT4 gene polymorphism with increased risk for rheumatoid arthritis in a genetically homogeneous population. *Hum Immunol* 2008;**69**:567–71.
24. **Zervou MI,** Mamoulakis D, Panierakis C, *et al.* STAT4: a risk factor for type 1 diabetes? *Hum Immunol* 2008;**69**:647–50.
25. **Zervou MI,** Goulielmos GN, Castro-Giner F, *et al.* STAT4 gene polymorphism is associated with psoriasis in the genetically homogeneous population of Crete, Greece. *Hum Immunol* 2009;**70**:738–41.
26. **Kurreeman FA,** Goulielmos GN, Alizadeh BZ, *et al.* The TRAF1-C5 region on chromosome 9q33 is associated with multiple autoimmune diseases. *Ann Rheum Dis* 2010;**69**:696–9.
27. **Tan EM,** Cohen AS, Fries JF, *et al.* The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982;**25**:1271–7.
28. **Seldin MF,** Amos CI. Shared susceptibility variations in autoimmune diseases: a brief perspective on common issues. *Genes Immun* 2009;**10**:1–4.
29. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 2007;**447**:661–78.
30. **Peters AL,** Plenge RM, Graham RR, *et al.* A novel polymorphism of the human CD40 receptor with enhanced function. *Blood* 2008;**112**:1863–71.
31. **Lee HS,** Korman BD, Le JM, *et al.* Genetic risk factors for rheumatoid arthritis differ in Caucasian and Korean populations. *Arthritis Rheum* 2009;**60**:364–71.
32. **Plant D,** Flynn E, Mbarek H, *et al.* Investigation of potential non-HLA rheumatoid arthritis susceptibility loci in a European cohort increases the evidence for nine markers. *Ann Rheum Dis* 2010;**69**:1548–53.
33. **Moser KL,** Neas BR, Salmon JE, *et al.* Genome scan of human systemic lupus erythematosus: evidence for linkage on chromosome 1q in African-American pedigrees. *Proc Natl Acad Sci USA* 1998;**95**:14869–74.
34. **Shai R,** Quismorio FP, Jr , Li L, *et al.* Genome-wide screen for systemic lupus erythematosus susceptibility genes in multiplex families. *Hum Mol Genet* 1999;**8**:639–44.
35. **Gaffney PM,** Ortmann WA, Selby SA, *et al.* Genome screening in human systemic lupus erythematosus: results from a second Minnesota cohort and combined analyses of 187 sib-pair families. *Am J Hum Genet* 2000;**66**:547–56.
36. **Chadha S,** Miller K, Farwell L, *et al.* Haplotype structure of TNFRSF5-TNFSF5 (CD40-CD40L) and association analysis in systemic lupus erythematosus. *Eur J Hum Genet* 2005;**13**:669–76.
37. **Kurylowicz A,** Kula D, Ploski R, *et al.* Association of CD40 gene polymorphism (C-1T) with susceptibility and phenotype of Graves' disease. *Thyroid* 2005;**15**:1119–24.

38. **Huang FY,** Chang TY, Chen MR, *et al.* Genetic polymorphisms in the CD40 ligand gene and Kawasaki disease. *J Clin Immunol* 2008;**28**:405–10.
39. **Bishop GA,** Moore CR, Xie P, *et al.* TRAF proteins in CD40 signaling. *Adv Exp Med Biol* 2007;**597**:131–51.
40. **Lin KI,** Duclos CA, Kuo TC, *et al.* Blimp-1 dependent repression of Pax-5 is required for differentiation of B cells to immunoglobulin M-secreting plasma cells. *Mol Cell Biol* 2002;**22**:4771–80.
41. **Yang W,** Shen N, Ye DQ, *et al.* Genome-wide association study in Asian populations identifies variants in ETS1 and WDFY4 associated with systemic lupus erythematosus. *PLoS Genet* 2010;**6**:e1000841.
42. **Yun L,** Liang-Dan S, Wen-Sheng L, *et al.* Expression analysis of ETS1 gene in peripheral blood mononuclear cells with systemic lupus erythematosus by real-time reverse transcription PCR. *Chin Med J* 2010;**123**:2287–8.
43. **Iezzi G,** Sonderegger I, Ampenberger F, *et al.* CD40-CD40L cross-talk integrates strong antigenic signals and microbial stimuli to induce development of IL-17-producing CD4+ T cells. *Proc Natl Acad Sci USA* 2009;**106**:876–81.
44. **Chen C,** Chai H, Wang X, *et al.* Soluble CD40 ligand induces endothelial dysfunction in human and porcine coronary artery endothelial cells. *Blood* 2008;**112**:3205–16.
45. **Chakrabarti S,** Blair P, Freedman JE. CD40-40L signaling in vascular inflammation. *J Biol Chem* 2007;**282**:18307–17.

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