Single Nucleotide Polymorphisms of Integrin-α-M (*ITGAM*) are Associated with Lupus Nephritis in an Asian Systemic Lupus Erythematosus Cohort

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Background:

Lupus nephritis (LN) is a major cause of morbidity and mortality in systemic lupus erythematosus (SLE). The integrin-α-M (ITGAM; CD11b) is a component of the macrophage-1 antigen complex which mediates leukocyte adhesion, migration and phagocytosis of complement-coated particles. The *ITGAM* single nucleotide polymorphism (SNP) rs1143679 on chromosome 16 has been associated with susceptibility to SLE and LN in several ethnic groups, including oriental Chinese and Thai populations[1]. We previously identified 13 *ITGAM* SNPs that were associated with susceptibility to SLE in our cohort of patients with SLE, however, we found no association with rs1143679[2]. The strongest association was with rs4561481. These SNPs were in strong linkage disequilibrium (LD) (r^2=0.92-1.0) and located in a region spanning from 5' upstream of *ITGAM* to intron 5 of *integrin-α-X* (*ITGAX*). Most of the associated SNPs were in the regulatory region of *ITGAM*-bearing promoter/enhancer histone marks (Table 1) and have been associated with different levels of RNA and protein expression in several cell types[3].

Aim:

To determine associations of *ITGAM* SNPs with SLE subphenotypes and autoantibodies.

Methods:

We studied 248 patients fulfilling the 1997 ACR revised classification criteria for SLE. Custom-designed Immunochip arrays were employed to study 147 SNPs covering approximately 140kb of the *ITGAM-ITGAX* region. Significant differences in allelic frequencies for each SNP was examined by gPLINK 1.062 software, with Bonferroni corrections for multiple comparisons. *ITGAM* SNPs significantly associated with SLE disease susceptibility in our cohort were identified. Using chi-square and Fisher's tests and binary logistic regression, associations were tested between these SNPs as well as rs1143679 with SLE subphenotypes (malar or discoid rash, serositis, mouth ulcers, arthritis, haematological, renal or neurological involvement) and the presence of anti-dsDNA, anti-Ro, anti-RNP and anti-Sm.

Table 2: Associations of *ITGAM* SNPs with SLE subphenotypes & auto-antibodies

SNP	Allele	Lupus nephritis OR (95% CI)	*p*	Discoid rash OR (95% CI)	*p*	Anti-Sm OR (95% CI)	*p*
rs4561481	G	1.39 (1.08-1.78)	0.009	0.58 (0.36-0.91)	0.03	1.54 (0.96-2.47)	0.07
rs8051304	C	1.39 (1.08-1.78)	0.009	0.58 (0.36-0.91)	0.03	1.54 (0.96-2.47)	0.07
rs889551	A	1.39 (1.09-1.77)	0.008	0.57 (0.36-0.91)	0.03	1.65 (1.02-2.65)	0.04
rs4889640	C	1.39 (1.08-1.78)	0.009	0.58 (0.36-0.91)	0.03	1.54 (0.96-2.47)	0.07
rs889549	C	1.39 (1.08-1.78)	0.009	0.58 (0.36-0.91)	0.03	1.54 (0.96-2.47)	0.07
rs11645526	A	1.39 (1.08-1.78)	0.009	0.58 (0.36-0.91)	0.03	1.54 (0.96-2.47)	0.07
rs8057320	C	1.39 (1.08-1.78)	0.009	0.58 (0.36-0.91)	0.03	1.54 (0.96-2.47)	0.07
rs7193943	G	1.39 (1.08-1.78)	0.009	0.58 (0.36-0.91)	0.03	1.54 (0.96-2.47)	0.07
rs11865830	G	1.39 (1.08-1.78)	0.009	0.58 (0.36-0.91)	0.03	1.54 (0.96-2.47)	0.07
rs3764327	T	1.39 (1.08-1.78)	0.009	0.58 (0.36-0.91)	0.03	1.54 (0.96-2.47)	0.07
rs7196256	T	1.41 (1.10-1.81)	0.006	0.57 (0.36-0.90)	0.02	1.51 (0.94-2.43)	0.10
rs3815801	C	1.39 (1.08-1.78)	0.009	0.58 (0.36-0.91)	0.03	1.43 (0.90-2.28)	0.14
rs2359661	A	1.46 (1.13-1.89)	0.002	0.59 (0.37-0.93)	0.03	1.36 (0.85-2.16)	0.23
rs1143679	A	1.67 (1.34-2.08)	0.03	0.39 (0.06-2.58)	0.46	0.35 (0.05-2.28)	0.30

(uncorrected p-values shown)

Results:

All 13 SLE-related *ITGAM* SNPs were associated with LN (Table 2). The strongest association was with rs2359661 (p = 0.002, uncorrected). The rs1143679 SNP was also associated with LN (p = 0.03, uncorrected), but was uncommon in our cohort (n=11, 4%). Subjects with these SNPs were less likely to have discoid rash. There was a trend towards an association with anti-Sm. Logistic regression models for the most frequent 11 SNPs retained the factors LN, discoid rash and anti-Sm, suggesting strong LD for these SNPs, consistent with our previous findings.

Conclusions:

This study demonstrated novel *ITGAM* SNP associations with LN and confirmed the association of rs1143679 with LN[4], as well as its rarity in Asian populations[1,5]. Most associated SNPs were in the regulatory region of ITGAM bearing promoter/enhancer histone marks. As these SNPs have been associated with modulation of levels of *ITGAM* RNA and protein expression in various cell types[3], these may have an impact on SLE subphenotypes.

Table 1: Functional effects associated with *ITGAM* SNPs

LD	SNP	Reference Allele	Alternate Allele	African	American	Asian	European	Promoter	Enhancer	DNAse	Proteins	Motifs	GENCODE	RefSeq	dbSNP
(r^2)				frequency				histone marks			bound	changed	genes	genes	functional annotation
1	rs4561481	G	A	0.64	0.64	0.75	0.68		breast, skin, blood	breast, skin		Arid3a,Bsx,Cdx2,DMRT3,DMRT5,Dlx2,Dlx3,E-1,Hoxa10,Hoxa3,Hoxa5,Hoxa7,Hoxa9,Hoxc6,Hoxd10,Isl2,Lhx3,Lhx4,Nkx2,Nkx3	12kb 5' of ITGAM	12kb 5' of ITGAM	
1	rs8051304	C	A	0.63	0.64	0.75	0.68		breast, skin, blood, GIT	breast, skin, blood	YY1	HNF6,Pax-2,Sox	11kb 5' of ITGAM	11kb 5' of ITGAM	
1	rs889551	A	G	0.93	0.67	0.75	0.68		breast, skin, blood, lung			EWSR1-FLI1,Ets,HDAC2,Zfp410	11kb 5' of ITGAM	11kb 5' of ITGAM	
1	rs4889640	C	A	0.91	0.67	0.75	0.68	BLD	blood, GIT, lung	blood	USF1	STAT,TATA	7kb 5' of ITGAM	7kb 5' of ITGAM	
1	rs889549	C	T	0.62	0.65	0.75	0.7		blood			Hand1,Smad3,Smad,TCF12	4.4kb 5' of ITGAM	4.3kb 5' of ITGAM	
0.98	rs11645526	A	G	0.49	0.64	0.74	0.7		blood			GATA,Irf,Pax-5,Znf143	3.1kb 5' of ITGAM	3kb 5' of ITGAM	
0.98	rs8057320	C	T	0.5	0.64	0.74	0.7		blood			Arid5b,Maf,Mef2,Pou2f2	1.8kb 5' of ITGAM	1.7kb 5' of ITGAM	
0.98	rs7193943	G	A	0.76	0.66	0.74	0.7	blood	blood, thymus, spleen	blood, thymus			247bp 5' of ITGAM	223bp 5' of ITGAM	
0.98	rs11865830	G	A	0.66	0.65	0.74	0.7	blood, fat, brain, heart, GIT	blood, spleen	blood, placenta		Nanog,RXRA,VDR	ITGAM	ITGAM	intronic
0.98	rs3764327	T	C	0.78	0.66	0.74	0.7	blood	blood, fat			DEC,HEN1	ITGAM	ITGAM	intronic
0.97	rs7196256	T	A	0.78	0.66	0.74	0.7	blood	blood			CTCF,E2A,PU.1	ITGAM	ITGAM	intronic
0.96	rs3815801	C	T	0.65	0.64	0.74	0.68	skin, lung, blood	breast, blood, skin, GIT, muscle, placenta, thymus, lung, pancreas, spleen	breast, blood, skin, muscle, thymus, lung, blood	GR,NFKB,BCL11A,EBF1,PAX5C20,PAX5N19,POL2,POL24H8,TCF12	BDP1,RXRA	ITGAM	ITGAM	intronic
0.84	rs2359661	A	G	0.36	0.48	0.73	0.54		skin, lung, blood	skin		MIF-1,RFX5,RREB-1	ITGAM	ITGAM	intronic

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