



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

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Adding years of healthy life

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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 populations¹. 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 rs1143679². 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 types³.

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 Immuchip 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 LN⁴, 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 types³, these may have an impact on SLE subphenotypes.

Table 1: Functional effects associated with *ITGAM* SNPs

| LD (r^2) | SNP | Reference Allele | Alternate Allele | African frequency | American frequency | Asian frequency | European frequency | Promoter | Enhancer | DNase | Proteins | | Motifs | | GENCODE genes | RefSeq genes | dbSNP functional annotation | |
|--------------|------------|------------------|------------------|-------------------|--------------------|-----------------|--------------------|-------------------------------|--|--|----------|--|--------------------------|--------------------------|---------------|--------------|-----------------------------|----------|
| | | | | | | | | | | | bound | changed | genes | genes | | | | |
| 1 | rs4561481 | G | A | 0.64 | 0.64 | 0.75 | 0.68 | | breast, skin, blood | breast, skin | | Arid3a, Bsx, Cdx2, DMRT3, DMRT5, Dix2, Dix3, E | 12kb 5' of <i>ITGAM</i> | 12kb 5' of <i>ITGAM</i> | | | | |
| 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 <i>ITGAM</i> | 11kb 5' of <i>ITGAM</i> | | | | |
| 1 | rs889551 | A | G | 0.93 | 0.67 | 0.75 | 0.68 | | breast, skin, blood, lung | | | EWSR1-FLI1, Ets, HDAC2, Zfp410 | 11kb 5' of <i>ITGAM</i> | 11kb 5' of <i>ITGAM</i> | | | | |
| 1 | rs4889640 | C | A | 0.91 | 0.67 | 0.75 | 0.68 | BLD | blood, GIT, lung | blood | USF1 | STAT, TATA | 7kb 5' of <i>ITGAM</i> | 7kb 5' of <i>ITGAM</i> | | | | |
| 1 | rs889549 | C | T | 0.62 | 0.65 | 0.75 | 0.7 | | blood | | | Hand1, Smad3, Smad, TCF12 | 4.4kb 5' of <i>ITGAM</i> | 4.3kb 5' of <i>ITGAM</i> | | | | |
| 0.98 | rs11645526 | A | G | 0.49 | 0.64 | 0.74 | 0.7 | | blood | | | GATA, Irf, Pax-5, Znf143 | 3.1kb 5' of <i>ITGAM</i> | 3kb 5' of <i>ITGAM</i> | | | | |
| 0.98 | rs8057320 | C | T | 0.5 | 0.64 | 0.74 | 0.7 | | blood | | | Arid5b, Maf, Mef2, Pou2f2 | 1.8kb 5' of <i>ITGAM</i> | 1.7kb 5' of <i>ITGAM</i> | | | | |
| 0.98 | rs7193943 | G | A | 0.76 | 0.66 | 0.74 | 0.7 | blood | blood, thymus, spleen | blood, thymus | | | 247bp 5' of <i>ITGAM</i> | 223bp 5' of <i>ITGAM</i> | | | | |
| 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 | <i>ITGAM</i> | <i>ITGAM</i> | | | intronic | |
| 0.98 | rs3764327 | T | C | 0.78 | 0.66 | 0.74 | 0.7 | blood | blood, fat | | | DEC, HEN1 | <i>ITGAM</i> | <i>ITGAM</i> | | | intronic | |
| 0.97 | rs7196256 | T | A | 0.78 | 0.66 | 0.74 | 0.7 | blood | blood | | | CTCF, E2A, PU.1 | <i>ITGAM</i> | <i>ITGAM</i> | | | 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 | <i>ITGAM</i> | <i>ITGAM</i> | | | intronic | |
| 0.84 | rs2359661 | A | G | 0.36 | 0.48 | 0.73 | 0.54 | | skin, lung, blood | skin | | | MF1, RFX5, RREB-1 | <i>ITGAM</i> | <i>ITGAM</i> | | | intronic |

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