Toll-like receptors revisited; a possible role for TLR1 in lupus nephritis

Several studies in systemic lupus erythematosus (SLE) have shown a possible role of endosomal toll-like receptors (TLRs) in lupus nephritis (LN), but the role of those interacting with ligands in the plasma membrane remains unclear.[1] Herein, we revisit the genetic contribution of TLRs in SLE inspired by a patient with LN who carries a rare TLR1 variant.

We analysed coding and regulatory regions of the TLR1–10 genes in 855 patients with SLE (online supplemental data 1). Six variants (rs142003616, rs76600635, rs72493538, rs41305843, rs113706342, rs41311400) within TLR1, one (rs10006364) within TLR2, one (rs79088436) within TLR5 and two (rs55695972, rs117985012) within TLR6 were significantly enriched in LN but only rs142003616 (TLR1) remained significant after Bonferroni correction ($p<0.039$, online supplemental table 1). To assess its biological significance, we employed in-silico functional annotation. The calculated deleteriousness score, CADD PHRED, for rs142003616 (5.56) points at the variant's potential functional importance. The rare risk allele is predicted to create a strong binding site for the core binding factor (CBF).[2] CBF, also known as runt-related transcription factors (RUNX), are also associated with SLE, psoriasis and rheumatoid arthritis.[3] To evaluate rs142003616 functional potential, we lastly performed a reporter assay that demonstrated a significantly higher expression of the reporter with the G allele in Jurkat ($p<0.0001$) and Daudi cells ($p<0.001$), and a strong enhancer potential without allelic difference in THP-1 (figure 1).

Of interest, despite the higher prevalence of proliferative LN overall (215 of 292 LN, 75%), it was less associated with rs142003616 in comparison to membranous LN ($p=0.047$, Fisher's exact test). Table 1 summarises the characteristics of patients with LN carrying the minor allele of rs142003616. One of them was a 39-year-old woman (#6 table 1) admitted

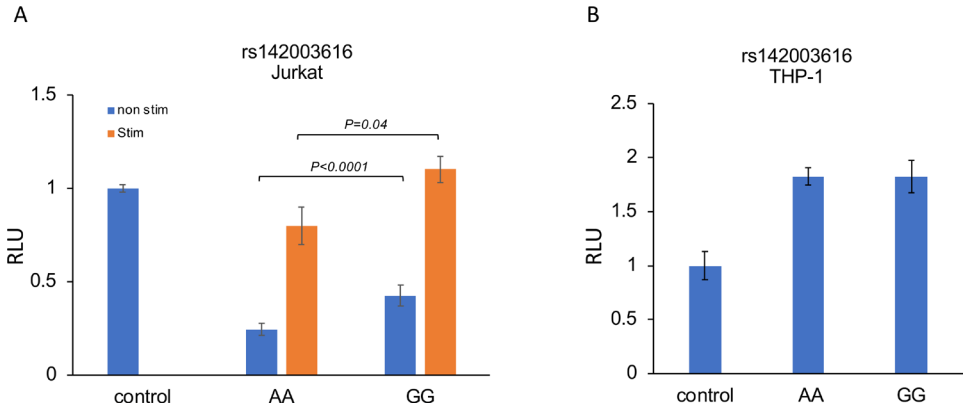

Figure 1 DNA fragments of 160 bp length with the variant in the middle were synthesised and cloned into pGL4.26 vector. Jurkat cells were transfected with reporter constructs containing different alleles of rs142003616 (A). Cells were left unstimulated for 48 hours or stimulated (stim) with PMA and ionomycin for 16 hours before harvesting. Protein lysates were assayed by the dual-reporter assay (Promega). Unpaired t-test was used for analysis of allelic difference. Similar low transcriptional levels and statistically significant allelic difference was detected upon transfection of Daudi cells (not shown). In THP-1 cells (B), the constructs show enhancer potential without allelic difference. Bars represent mean values +/-SEM. Non-stim, non-stimulated; RLU, relative light units.

to the hospital with prolonged fever (39°C), headache, non-productive cough, rash, leucopenia, high CRP (78 mg/L), microscopic haematuria and pyuria. Because of sinusitis and interstitial pneumonitis confirmed by chest tomography, the patient received doxycycline, and the fever and cough slowly disappeared, as did rash. Due to positive ANA (1/400), and persistence of haematuria, she was referred to the rheumatology department. The laboratory results showed dsDNA (1/40), decreased classical complement function (23% of normal; (N: 80%–120%)), low C4, anaemia, proteinuria (0.4gr/d) and haematuria. Her diagnosis was confirmed with SLE after immunofluorescent staining of her renal biopsy resulted in WHO Class Vb LN, although light microscopy result demonstrated postinfectious glomerulonephritis. She went into remission with hydroxychloroquine, prednisone and enalapril for 7 years. Due to uprising proteinuria (up to 1gr/d), rebiopsy was performed, which demonstrated WHO Class IIb. We calculated her polygenic risk score (PRS), which was normal, 8.27.[4] Our patient's history commencing with symptomatic infection, low PRS, which was against the general observation in LN, besides recovering to WHO class IIb without immunosuppressive therapy intrigued us and led to hypothesise that variants within TLR genes might contribute to the development or progression of LN.

Growing evidence highlights the role of podocytes in LN, not only as an integral part of kidney filtration barrier, but also their active involvement in immune-mediated kidney injury.[5] Podocytes constitutively express TLR1–6 and TLR8, respond to TLR ligands with proinflammatory cytokine release, activation of type I IFN signalling, and, ultimately, podocyte injury with proteinuria.[6] While innate immune responses play a central role in podocyte injury, evidence suggests that podocyte injury can initiate kidney damage in LN.[5] We identified a rare variant associated with LN, which affects TLR1 gene expression and might exert its effect via podocytes and immune cells. In conclusion, exogenous TLR ligands might contribute to the development of LN, rare polymorphisms in this locus might be considered when treating patients with LN triggered by exogenous agents.

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Table 1 Clinical characteristics of patients with lupus nephritis carrying TLR1 minor allele (rs142003616)

Patient	Gender	Age at diagnosis	European descent	ACR 1	ACR 2	ACR 3	ACR 4	ACR 5	ACR 6	ACR 7	ACR 8	ACR 9	ACR 10	ACR 11	AutoAbs*	Biopsy ever	WHO-class†
1	F	16	Yes	Yes	No	No	No	Yes	No	Yes	No	No	Yes	Yes	Y:N:N	No	–
2	F	26	Yes	Yes	No	Yes	No	Yes	No	Yes	No	Yes	Yes	Yes	Y:Y:N	No	–
3	F	16	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	N:N:N	Yes	Unknown
4	F	53	Yes	Yes	Yes	Yes	No	Yes	No	Yes	Yes	No	No	Yes	N:N:Y	Yes	V
5	F	15	Yes	Yes	No	No	No	Yes	No	Yes	No	Yes	Yes	Yes	Y:N:N	Yes	IV d
6‡	F	39	Yes	No	No	No	No	No	No	Yes	No	Yes	Yes	Yes	Y:N:Y	Yes	V b

ACR classification criteria,[7 8] ACR 1: malar rash, ACR 2: discoid rash, ACR 3: photosensitivity, ACR 4: oral ulcer, ACR 5: non-erosive arthritis, ACR 6: pleuritis or pericarditis, ACR 7: renal disorder, ACR 8: neurological disorder, ACR 9: haematological disorder, ACR 10: immunologic disorder, ACR 11: positive ANA.
*Autoantibodies, anti-ds-DNA: anti-Sm: anti-phospholipid antibodies, respectively.
†WHO-classification of lupus nephritis.
‡The case represented in the text. The detailed clinical characteristics of all patients with SLE (855 with SLE, of whom 292 had LN) can be found in Bolin K *et al*.[9]
AutoAbs, autoantibodies; LN, lupus nephritis; N, no; SLE, systemic lupus erythematosus; Y, yes.

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