

Acknowledgements Andrew D. Paterson, Genetics and Genome Biology, Research Institute, the Hospital for Sick Children, Toronto, Canada

John Harley, Centre for Autoimmune Genomics and Aetiology, Cincinnati Children's Hospital Medical Centre, Cincinnati, USA

GG-12 ALTERED EXPRESSION OF LONG NONCODING RNA IS ASSOCIATED WITH A LUPUS-ASSOCIATED VARIANT IN COMPLEMENT RECEPTOR 2

Brendan M Giles, Bryan T Nycz, Susan A Boackle*. *Division of Rheumatology, University of Colorado School of Medicine, USA*

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Background Systemic lupus erythematosus is a multisystemic autoimmune disease characterised by the production of autoantibodies to nuclear antigens. We have identified a variant in intron 1 of complement receptor 2 (*CR2/CD21*) that is associated with decreased risk of lupus (rs1876453; $P_{meta} = 4.2 \times 10^{-4}$, OR = 0.85). Its effect is strongest in subjects with anti-dsDNA antibodies (case-control $P_{meta} = 7.6 \times 10^{-7}$, OR = 0.71; case-only $P_{meta} = 1.9 \times 10^{-4}$, OR = 0.75), suggesting a preferential association with this endophenotype. rs1876453, located 97 nucleotides from the 5' end of *CR2* intron 1, alters the binding of multiple protein complexes, including one containing CTCF, and is associated with increased B cell-specific expression of the adjacent gene, complement receptor 1 (*CR1/CD35*). The transcriptional mechanism connecting these observations remains unclear, and we hypothesised that long noncoding RNA (lncRNA) play a role.

Materials and methods cDNA was generated by reverse transcription from RNA purified from the Raji B cell line as well as from human tonsil and spleen, peripheral blood mononuclear cells, and purified primary B cells. PCR was performed using 5' and 3' primers that targeted spliced exons from known lncRNA sequences in the intergenic region 5' of *CR2*, in the *CR2* gene, and in *CR1* intron 1. Quantitative PCR of primary B cell transcripts was performed using cDNA transcribed using random primers and MultiScribe reverse transcriptase (Applied Biosystems), customised lncRNA primers and probe that targeted spliced exons, Taqman assays for *U6 snRNA* and *b-actin* mRNA, and the Applied Biosystems 7500 Real-Time PCR System. Relative expression levels of lncRNA, normalised to either *U6 snRNA* (A) or *b-actin* (B), were calculated using the comparative C_T method. P values were determined using a two-tailed Student t test and a p value of <0.05 was considered significant.

Results We confirmed the presence of annotated lncRNAs in the *CR2-CR1* genomic region in various cell types. One annotated lncRNA located downstream of rs1876453 in *CR2* intron 1 was readily detected in B cells. We determined the allele-specific expression of this lncRNA by quantitative RT-PCR and found that it was ~3-fold increase in individuals with the minor protective allele at rs1876453 ($p = 0.0025$ normalised to *U6 snRNA* and $p = 0.0054$ normalised to *beta-actin*).

Conclusions Our data suggest that the generation of pathogenic autoantibodies associated with early, active, and severe lupus is modified by expression of a *CR2* lncRNA that appears to have long-range effects. Examination of its mechanism and effects may therefore reveal a novel target for the treatment of lupus.

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GG-13 PATHOGENESIS OF LUPUS PROLIFERATIVE GLOMERULONEPHRITIS (LPGN): PODOCYTES AS TARGETS AND RESPONDERS PROVIDE EVIDENCE FOR THE IMPORTANCE OF LOCAL FACTORS CONTROLLING END ORGAN DAMAGE

¹Shu Man Fu*, ¹Hongyang Wang, ¹Chao Dai, ¹Sun-sang J Sung, ²Felicia Gaskin. ¹Division of Rheumatology and Immunology, the Centre of Inflammation, Immunity and Regenerative Medicine and Division of Nephrology, Department of Medicine, University of Virginia, Charlottesville, VA, USA; ²Department of Psychiatry and Neurobehavioral Sciences University of Virginia, Charlottesville, VA, USA

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Background LPGN is the prototypic immune-complex (IC) mediated disease. The current dogma that IC deposition with complement (C') activation inevitably leads to renal damage is neither sufficient nor comprehensive to account for the pathogenesis of LPGN. Our genetic studies support the thesis that acute GN (aGN) with IC deposition with C' activation and cell infiltration and mesangial proliferation is a distinct phenotype from chronic GN (cGN) that is characterised by glomerulosclerosis, tubular dilatation and interstitial fibrosis with severe proteinuria and premature mortality. Furthermore, circulating ANA and anti-dsDNA Abs are not required for LPGN.

Materials and methods Female mice of NZM2328 and its intrachromosomal recombinant congenic line NZM2328.Lc1R27 (R27) were used. Anti-GBM induced GN was used as a model for IC mediated LPGN. Immunofluorescence was used to identify cell populations that made cytokines and complement components.

Results R27 developed aGN and mild proteinuria without progression to cGN, end stage renal failure and early mortality. The kidneys of aged R27 had IC deposition and cellular infiltration, not distinguishable from that of aGN in NZM2328. Multiple approaches showed that the lack of progression from aGN to cGN in R27 was due to podocyte resistance to IC-mediated damage, a phenotype controlled by the allelic *Cgms1* gene.

With a novel method to study intra-glomerular cytokine production, NZM podocytes were shown to be the major cell population that makes IL-1 β in cGN, infiltrating CD11b+ macrophages make TNF α and the mesangial cells make IL-6. R27 mice do not show this compartmentalization of cytokine production. Preliminary data showed that the podocytes in Class III and IV lupus GN make IL-1 β .

Podocytes at the early and late cGN were shown to make C1q and C3. The expression of these complement components is less evident in R27 kidneys. C1q and C3 were present in some podocytes in biopsies of class III and IV lupus nephritis. Urinary podocytes making C1q were detected by us in of four patients with LPGN but not in normal individuals.

Conclusions Our studies of lupus GN in both mouse and in man have provided significant information and insight regarding the role of podocytes as targets and as responders to IC mediated injuries. Our results suggest that the pathogenesis of LPGN should be revisited with focus on the local factors that may be of