ALTERED EXPRESSION OF LONG NONCODING RNA IS ASSOCIATED WITH A Lupus-Associated Variant in Complement Receptor 2

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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 from known lncRNA 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 \Delta \Delta 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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paramount importance. In addition to enhance our basic knowledge of podocyte biology, our results may provide novel targets for intervention and new urinary biomarkers to monitor therapeutic responses.

Interferons

I-01 NOVEL MECHANISM OF ACTION OF ANTI-MALARIAL DRUGS IN THE INHIBITION OF TYPE I INTERFERON PRODUCTION

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Background Anti-malarial drugs (AMD) such as Hydroxychloroquine (HCQ) and Quinacrine (QC) are effective in the treatment of skin rash and arthritis in systemic lupus erythematosus (SLE). However, which mechanism(s) are responsible for their beneficial action is uncertain. Type I interferon, (IFN-I) is strongly implicated in the pathogenesis of SLE and ‘interferonopathies’ such as Aicardi-Goutieres Syndrome (AGS) A new DNA activated IFN-I pathway, cyclic GMP-AMP (cGAMP) synthase (cGAS), was recently discovered and linked to AGS and mouse models of Lupus. Preliminary data indicate that a subset of SLE patients also have elevated cGAS and cGAMP (the cyclic dinucleotide responsible for activation of STING and IFN-I).

Materials and methods In silico structure-based drug screening were provided by the CANDO docking algorithm. Predictions made by CANDO were confirmed by Autodock Vina and analysed via PyMOL. cGAS activity/cGAMP production was analysed by Thin Layer Chromatography (TLC). DNA-binding to cGAS in the presence or absence of AMD was determined by an Electrophoretic Mobility Shift Assay (EMSA). Following DNA cell transfections, cytokines were quantified by qPCR, ELISA or an ISRE-luciferase reporter assay. cGAMP in patient samples was quantified by mass spectrometry.

Results In silico screening of drug libraries identified several antimalarial drugs (AMD) which could potentially inhibit cGAS activity by interacting with cGAS/DNA dimer complex. Electrophoretic Mobility Shift Assay revealed that AMD disrupted the double stranded DNA-cGAS complex in a dose dependent manner. These AMD also inhibited IFN-I expression in THP1 cells transfected with dsDNA and in 293 T cells transfected with cGAS/STING plasmids validating that cGAS is a target of AMD. We synthesised new AMD like drugs that are also able to inhibit cGAS as well as Toll pathways. These drugs could be beneficial for the treatment of AGS and/or Lupus.