LO-030 FUNCTIONAL CHARACTERIZATION OF 2P22.3 LOCUS IDENTIFIES PARP-1 AS AN ALLELE-SPECIFIC INTERACTOR AT RS13385731 WHICH MECHANISTICALLY REGULATES RASGRP3 EXPRESSION IN CONTRIBUTING SLE SUSCEPTIBILITY

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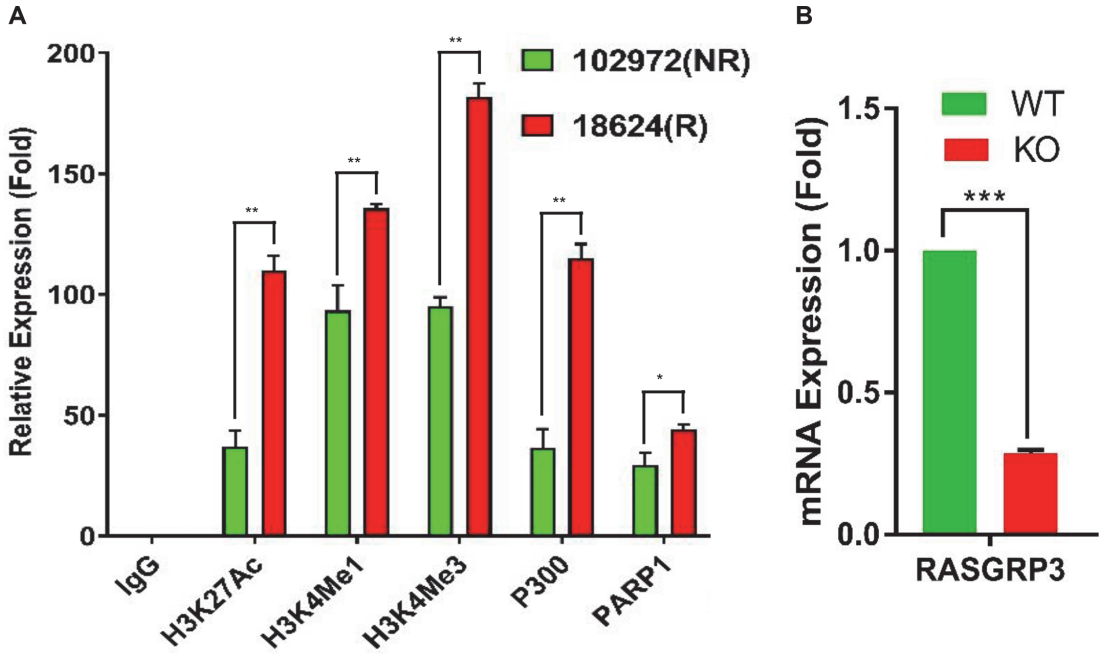
Background Systemic lupus erythematosus (SLE) is an inflammatory disease with complex genetic underpinnings. SLE association at 2p22.3 is one of the most consistently replicated SLE signals. We reported [Sun et al. 2016, Nat Genet] that multiple intronic variants near RasGrp3 (RAS Guanyl Releasing Protein 3) could explain this genetic signal in Asians. However, the underlying causal variant and molecular mechanisms for increasing SLE susceptibility are largely unknown.

Methods First, we used in silico bioinformatics to prioritize the potential regulatory candidate variants. Next, we applied a combination of molecular biology experiments to assess the allele-specific regulatory potential of the candidate variants in B-cell lines. Finally, we validated the effector gene function by CRISPR-based genome editing.

Results Comprehensive bioinformatics predicted that rs1338573, located in an active chromatin region, is a strong eQTL, and it has the potential to be a dual enhancer/promoter. Luciferase assays demonstrated significant ($p<0.005$) allele-specific enhancer and promoter activities with the risk allele (T) at rs13385731. DNA pulldown and EMSA suggested allele-specific bound protein ~100 kDa, which was identified as PARP1 protein by Mass Spectrometry and later confirmed by Super-shift and Western blot. We also verified the differential allele-specific binding to H3K27Ac, H3K3Me1, P300, PARP1, and IRF1 against rs13385731 using ChIP-qPCR (figure 1). Interestingly, while PARP1 binding affinity is higher with risk (TT) genotype, IRF1 binding affinity is higher with non-risk (CC) genotype of rs13385731. CRISPR-Cas9-based enhancer deletion as well as CRISPRa/i-based activation/silencing validated the significant difference in RasGrp3 transcript and protein levels. The risk allele dosage of rs13385731 correlates with RasGrp3 expression and ERK activity.

Conclusions Taken together, our data suggest that over-expression of RasGRP3 could dysregulate the ERK signaling pathway that might be associated with increased SLE risk. We provide mechanistic insights into how a non-coding functional variant, rs13385731, increases underlying SLE risk at the 2p22.3 locus.



Abstract LO-030 Figure 1 The significant allelic effect at rs13385731 and enhancer effect on RasGrp3 expression level. (A) ChIP-qPCR assays for determining allele-specific DNA-protein interactions with H3K27ac, H3K4me1, H3K4me3, p300, and poly(ADP-ribose) polymerase 1 (PARP-1) at R and NR homozygous cell lines. (B) Levels of RasGrp3 mRNA expression between WT and KO cells, based on 3 biological replicates. ** $p<0.005$, * $p<0.05$