Functional Characterization of *TNIP1* Causal Variants associated with Systemic Lupus Erythematosus

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Introduction

- Genome-wide association studies (GWAS) have identified more than 80 susceptibility or risk genes/loci associated with SLE and other autoimmune diseases.
- One of these genes is *TNIP1* which encodes for ABIN1 protein. ABIN1 functions in the immune system by restricting the NF-kB signaling.
- *TNIP1* is expressed ubiquitously in human tissue, and strong expression has been observed in various hematopoietic immune cell lines, including Molt-4, Jurkat, and HL-60
- Genetic fine-mapping study by Adrianto et al., in 5 ethnically diverse SLE case-control populations identified multiple *TNIP1* variants that were associated with SLE.
- The study identified two independent SLE risk haplotypes within *TNIP1.* Both the risk haplotypes demonstrated reduced expression of *TNIP1* mRNA and ABIN1 protein.

Objective

Characterize the SLE associated SNPs in *TNIP1* locus and identify candidate causal variants that influence hypomorphic expression of *TNIP1*.

Methods

We selected 11 SLE-associated *TNIP1* SNPs with low binding scores (RegulomeDB) for EMSAs to evaluate whether SLE risk alleles affect binding of nuclear protein complexes extracted from different immune cells treated with and without PMA/Ionomycin (P/I). Affinity DNA pull-down assays and Western blotting (WB) were performed to identify proteins bound to the rs10036748 probe. Enhancer activity of rs10036748 was measured by luciferase assay.

Results

Figure 1. SNPs carried on *TNIP1* SLE risk haplotypes demonstrate complex binding activity of nuclear factors analyzed by EMSA.

Figure 2. Majority of the SNPs carried on *TNIP1* SLE risk haplotypes exhibit allelic dependent enhancer activity.

Results

Figure 3. Risk allele of *TNIP1* variant rs10036748 (T) shows reduced binding of nuclear proteins in different cell types compared to Non-risk allele (C).

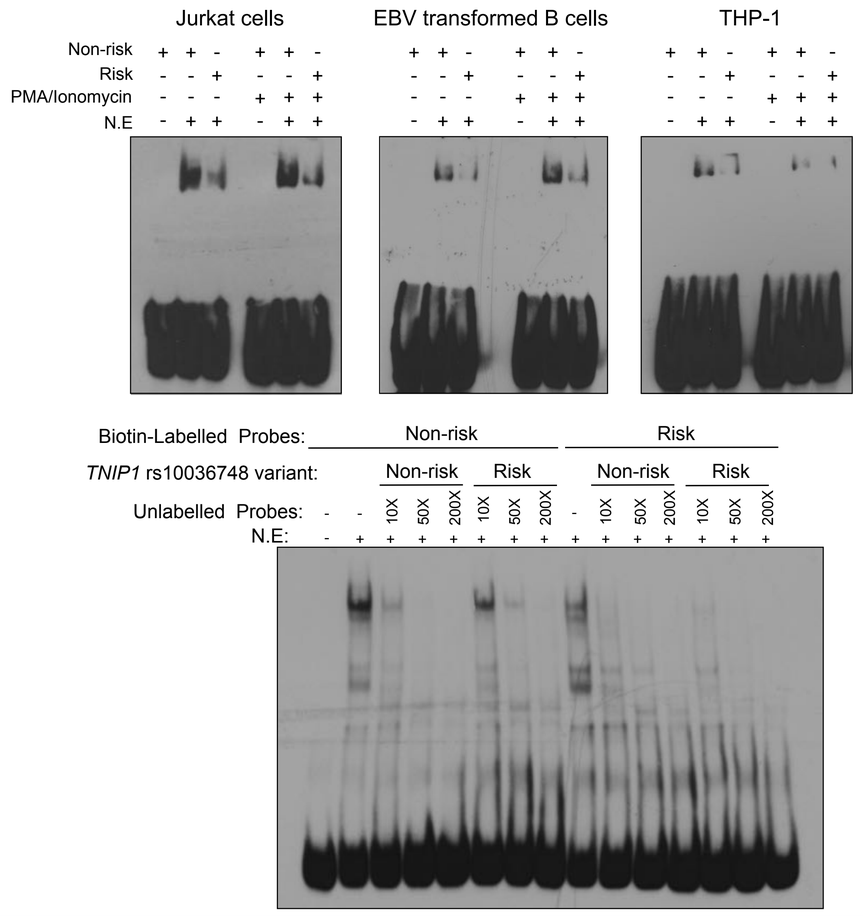


Figure 4. Risk allele of *TNIP1* variant rs10036748 shows increased enhancer activity in HEK293T and EBV transformed B cells.

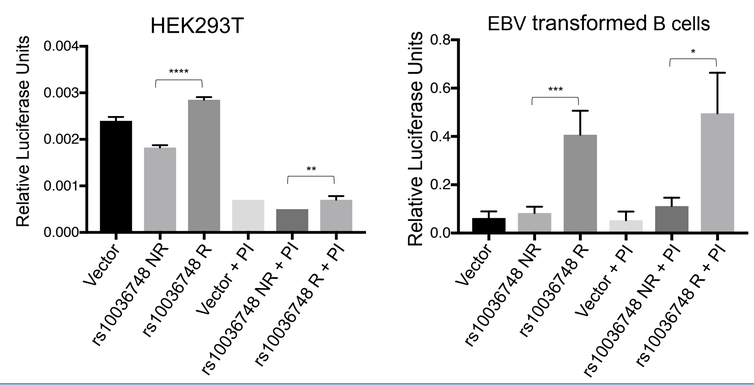
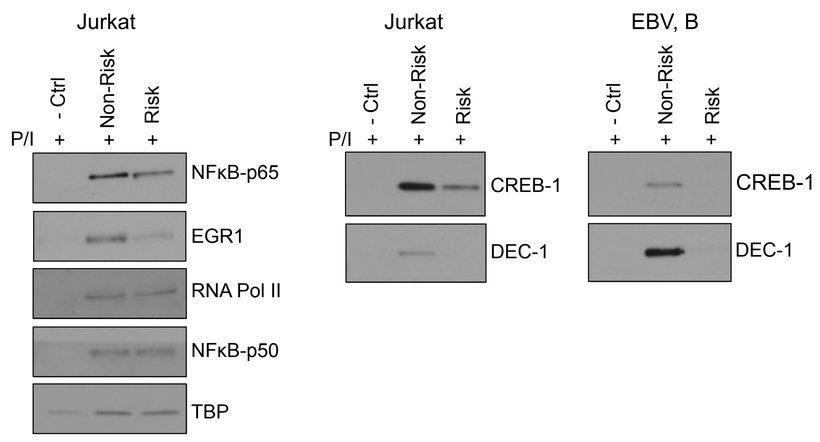


Figure 5. CREB-1 and DEC-1 binding to risk allele of *TNIP1* rs10036748 variant is significantly reduced.



Summary and Conclusion

- The SNPs carried on both *TNIP1* SLE risk haplotypes demonstrated complex binding activity; Jurkat T cells exhibited the most activity with 8 of 11 SNPs showing differential binding
- The rs10036748 variant demonstrated reduced binding of nuclear proteins to the risk allele in all cell types.
- The rs10036748 non-risk allele bound early growth response protein 1 (EGR-1), cyclic AMP-responsive element binding protein 1 (CREB-1), and class E basic helix-loop-helix protein 40 (bHLHe40) with more affinity than the risk allele.
- ➢ Functional analyses of SNPs in *TNIP1* SLE risk haplotypes suggest a complex regulation at *TNIP1* locus. Further, these SNPs exhibit cell type specific, stimulation dependent and allele specific binding of transcriptional protein complexes. Regulatory insights gained will better direct future characterization of individual SLE-associated *TNIP1* variants *in-vivo* to decipher molecular mechanisms and cell states that contribute to SLE pathogenesis. The binding of nuclear protein complexes to SLE associated *TNIP1* variants is highly regulated showing cell type specificity, stimulation dependency and allele specific binding.

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