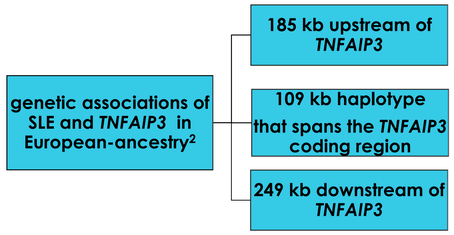
The SLE risk variant, rs10499197, upstream of *TNFAIP3* modulates enhancer function and *TNFAIP3* gene expression

Ajay Nair, Satish Pasula, Mandi Wiley, Yao Fu, Jaanam Gopalakrishnan, Kandice L. Tessneer, and Patrick M. Gaffney

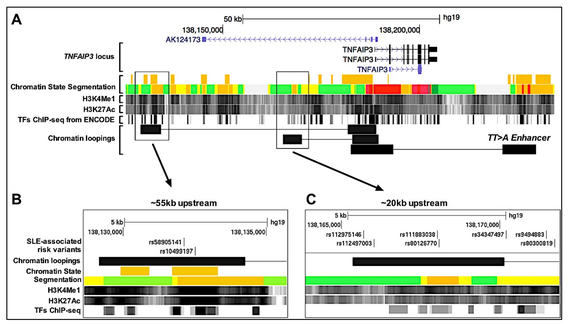
Division of Genomics & Data Science, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma, USA

MOTIVATION

- Estimated 90% of GWAS signals reside in non-coding DNA elements, with ~60% mapping to immune-cell enhancers [1].



- **Data from ENCODE ChIA-PET suggest a long-range interaction with a putative enhancer upstream of *TNFAIP3*. Variant, rs10499197 carried on the SLE risk haplotype is situated near this enhancer and could affect its function.**



Location of SNP in putative enhancer region. A. The relative locations of three strong DNA loopings mediated by Pol2 in K562 cell line (chronic myeloid leukemia). **B, C**. A zoom in view of two upstream interaction regions. We used the table browser tool in the UCSC Genome browser to cross reference the two upstream interaction regions with the ENCODE Integrated Regulation super-track, which contains Chromatin State Segmentation, H3K4Me1 Marks, H3K27Ac Marks, and transcription factor ChIP-seq data. SLE-associated risk variants (rs58905141, rs10499197) overlap with H3K4Me1 and H3K27Ac Marks and are very close to strong ChIP-seq signals (the darkness of the segment is proportional to the signal strength).

METHODS

Functional characterization of rs10499197

Q.1. Are there allelic differences in the biological function of the variant?
- EMSA Assay
- Affinity purification of nuclear factors
- ChIP-qPCR assay

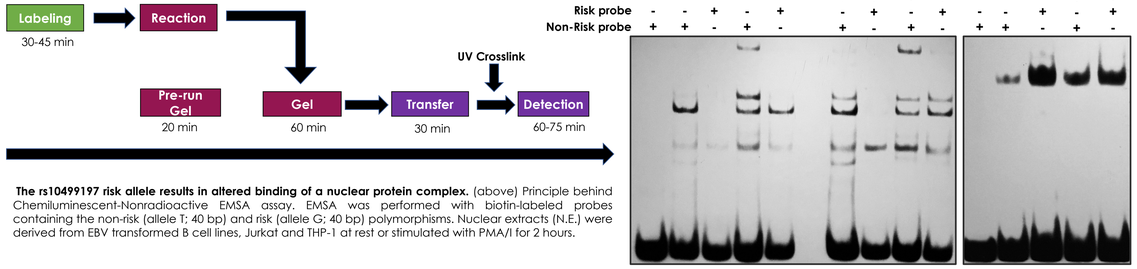
Q.2. Is the variant near an enhancer?
- Dual Luciferase Assay

Q.3. Does the upstream DNA element interact with *TNFAIP3*?
- Hi-ChIP
- 3C-qPCR Assay

RESULTS

1. Risk allele rs10499197 altered nuclear protein affinity.

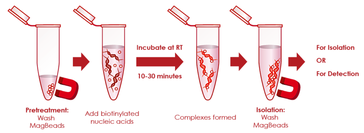
Electrophoretic Mobility Shift (EMSA) assay

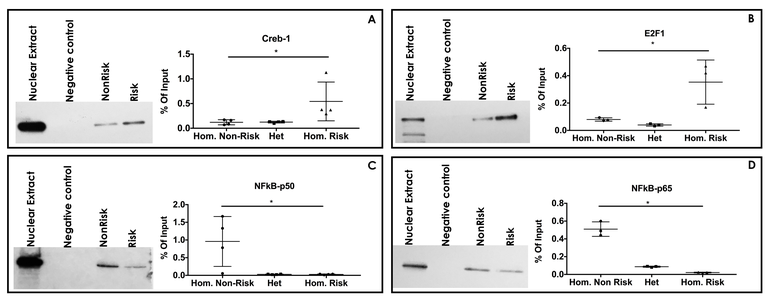


The rs10499197 risk allele results in altered binding of a nuclear protein complex. (above) Principle behind Chemiluminescent-Nonradioactive EMSA assay. EMSA was performed with biotin-labeled probes containing the non-risk (allele T; 40 bp) and risk (allele G; 40 bp) polymorphisms. Nuclear extracts (N.E.) were derived from EBV transformed B cell lines, Jurkat and THP-1 at rest or stimulated with PMA/I for 2 hours.

2. DNA sequence around risk allele affected TF-binding sites.

Affinity Purification and ChIP-qPCR assay

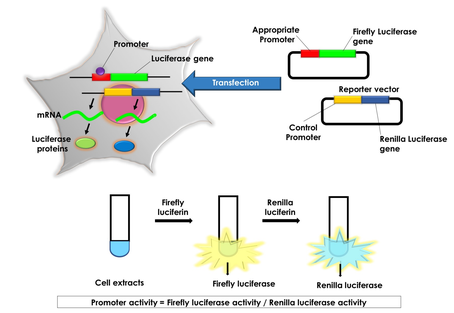


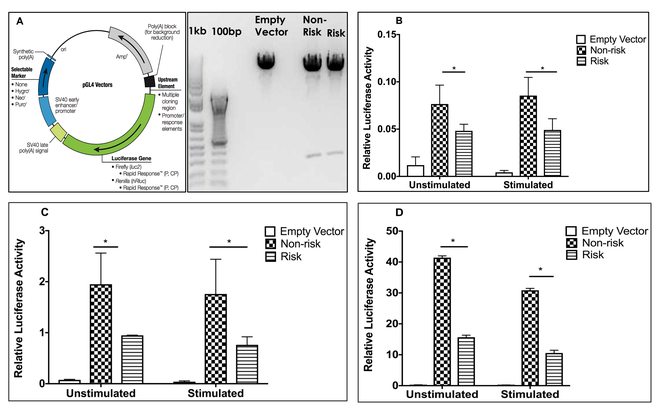


Risk allele rs10499197 alters transcription factor binding to neighboring upstream DNA sequence. (above) Principle behind Affinity purification assay. (A-D) Nuclear extracts from PMA/I stimulated EBV cells were incubated with biotinylated oligonucleotides (40bp) bound to streptavidin beads. The bound proteins were eluted and analyzed by Western blot. EBVcell lines (G/G; T/G; T/T) stimulated with PMA/I. ChIP was performed with antibodies specific against, Creb-1 (A), E2F1(B), NF-κB p50 (C) and p65 (D). This is followed by qPCR with primers neighboring T>G polymorphic region. Anti-H3K4Me1 and Rabbit IgG were used as positive and negative controls respectively. Statistical comparisons were made using one-way ANOVA; * indicates p<0.05.

3. rs10499197 variant is located in an enhancer element.

Dual Luciferase Assay



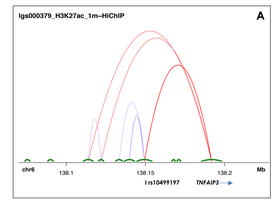


Risk allele rs10499197 identified an upstream DNA element as an enhancer. (above) Principle behind Dual Luciferase assay. **(A)** Clone verification by restriction digest. Relative Luciferase activity from HEK293T (**B**),Jurkat (**C**) and THP-1 (**D**) cells containing either empty vector, vector with non-risk insert or vector with risk insert. Statistical comparisons were performed using a Student's t-test of replicates from two independent experiments, * Indicates p<0.05.

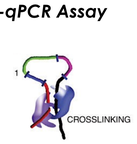
RESULTS *(contd.)*

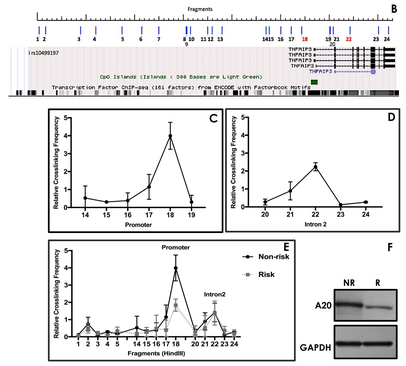
4. rs10499197 enhancer interacted with *TNFAIP3* by DNA looping.

Hi-ChIP



3C-qPCR Assay





Hi-ChIP and 3C analyses showed long-range interaction between rs10499197 enhancer and the *TNFAIP3* gene region. (left) Principle behind 3C-qPCR assay. (A) Hi-ChIP data generated from EBV cells. Red-loops show enhancer-promoter interactions, purple-loops show enhancer-enhancer interactions and green-loops show H3k27ac anchors. (B) Upper track shows location of the *TNFAIP3* primers used to identify potential amplified interaction fragments tested by 3C across the highlighted region. Primers 18 and 22 produced the highest signals and are shown in red. The middle and bottom track show the genomic region of *TNFAIP3* with the location of the promoter CpG island and ENCODE defined transcription factor binding sites. (C--E) Quantification of Ligation products obtained from 3C samples. For each fragment, performed duplicate quantification and calculated the mean Ct. The final value was calculated using the parameters of the standard curve (b: intercept; a: slope) as follows: value=10(Ct-b)/a. Values were normalized to *GAPDH* (loading control). (E) 3C-qPCR assays were performed in multiple EBV-transformed B cells homozygous for either risk or non-risk alleles in the upstream regulatory element; Statistical significance was obtained by performing paired t tests of replicates. (E) Blot representing A20 expression in cell lines homozygous for non-risk and risk alleles.

CONCLUSIONS

- **Following our work on the TT>A enhancer, this is the second likely causal variant to impact *TNFAIP3* expression, and perhaps SLE pathophysiology.**
- **rs10499197 may disrupt function of a putative enhancer upstream of *TNFAIP3*, which may impair *TNFAIP3* expression, enhance NF-κB signaling, and heighten immune responses in a cell type specific manner.**

REFERENCES

1. Farh K.K. et. Al. (2015). Genetic and epigenetic fine mapping of causal autoimmune disease variants. *Nature*. 518(7539):337-43.
2. Adrianto et. al. (2011). Association of a functional variant downstream of *TNFAIP3* with systemic lupus erythematosus. *Nature Genetics*. 43(3):253-8.
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