Noncoding Variants as Genetic Contributors to Autoimmune Disease Pathogenesis

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Understanding the functions of disease-associated noncoding variants is essential for understanding the molecular mechanisms driving diseases with a genetic cause and for identifying therapeutic targets. Combined computational and experimental analyses have demonstrated that IRF5 is hyperactivated by a pathogenic allele of TNPO3 through long-distance chromatin looping. This finding identifies a molecular mechanism contributing to the polygenic autoimmune diseases of systemic lupus erythematosus and systemic sclerosis.

Genome-wide association studies allow us to pinpoint those genetic loci that increase risk of diseases. Robust statistical association that survives multiple testing corrections and replications of the association across numerous independent cohorts of patients and controls gives us confidence that there are real and important differences between the allele frequencies in people with a disease compared with those without the disease. Thynn et al. (2019) focus on the IRF5-TNPO3 risk locus, which is one example of these associated loci for systemic lupus erythematosus (SLE) and systemic sclerosis (SSc).

Notably, every published lupus study to date that has evaluated chromosome 7q32 for disease risk identified strong genetic association with variants in the IRF5 locus. Indeed, of the >85 genetic loci associated with SLE identified to date (Vaughn et al., 2012), the IRF5 locus ranks first for replication consistency (16 of 16 studies), high for magnitude (1.4 < odds ratio < 2.3), and very strong for genetic association (P < 10−50) (Kottyan et al., 2015). When evaluating SLE subphenotypes, the IRF5 locus is most closely associated with anti-Ro and anti-dsDNA autoantibodies, in which the odds ratio can be as high as 5.83 (Niewold et al., 2012).

Statistical analysis nominates single-nucleotide polymorphisms likely to be causal at IRF5-TNPO3

Bayesian fine mapping studies, such as the one performed by Thynn et al. (2019), are important because they nominate specific genetic polymorphisms as accounting for most of the posterior probability associated with a specific risk locus. In this study, the authors identified a variant in the promoter region of TNPO3 gene that was highly associated with disease risk and likely to be functionally relevant. As this risk variant is noncoding, the authors used existing functional genomic data to hypothesize that the nominated risk variant might change the transcriptional regulation of IRF5 (Thynn et al., 2019).

Noncoding risk variants alter transcriptional regulation of IRF5

In this age of big data and a growing tool box of molecular techniques, more effort should be devoted to determining molecular mechanisms of established genetic risk loci. Thynn et al. (2019) present a strong roadmap for this type of comprehensive study. In their study they:

1. Established that the DNA surrounding the nominated variant looped to the promoter of IRF5 through chromosome conformation capture (3C)
2. Measured genotype-dependent regulation of the IRF5 promoter using luciferase reporter assays
3. Performed genome editing to establish the necessity of the risk variant and surrounding DNA for enhanced IRF5 expression
4. Deleted the non-risk allele of variant and surrounding DNA to confirm that this did not affect IRF5 normal expression
5. Immunoprecipitated transcription factor EVI1 to detect genotype-dependent binding to the nominated variant
6. Used EVI1 shRNA to demonstrate the necessity for EVI1 in the genotype-dependent luciferase assays
7. Knocked down EVI1 to show decreased interaction between the nominated variant and the promoter of IRF5 through 3C
8. Silenced TNPO3 expression to show that the genotype-dependent expression of IRF5 was not dependent on TNPO3 expression

Altogether, Thynn et al. (2019) present data that clearly support a model in which EVI1 binds a disease risk variant in a genotype-dependent fashion, leading to chromatin looping and allelic regulation of the IRF5 promoter. The authors were careful to confirm various parts of this model through complementary assays. There is still room for improvement in this research map, and additional opportunities will emerge as the technology to perform genome editing in lymphocyte cell lines and chromatin capture with higher resolution improves.

A novel functional mechanism points to new therapeutic targets for SLE and SSc

Clinically, for numerous reasons, it is important to understand the genetic risk
of SLE and SSc. From a population perspective, genetic risk loci help us find cells that are etiologically critical. Thynn et al. (2019) identify EVI1 as a transcription factor that preferentially binds to the risk allele of the variant to drive pathogenic antibody secretion. They indicate that EVI1 is required in hematopoietic progenitor cells in the bone marrow, and a better understanding of the binding behavior of EVI1 and its expression patterns can nominate particular lymphoid cells and developmental lineages critical in disease etiology.

We can also use risk loci to identify common allelic mechanisms that span many disease loci. As the IRF5 loci is a shared genetic risk locus for SLE and SSc (Kottyan et al., 2015), EVI1 binding can affect chromatin looping and gene expression in both diseases, and will likely contribute to our understanding of gene regulation in the etiology of these diseases. This is applicable particularly for other IRF genes, which share similarities and common binding preferences but also have important differences in expression patterns and signaling pathways (Andrilenas et al., 2018).

At the individual level, much progress is being made to use a person’s cumulative burden of genetic variants to identify a polygenic risk score (PRS). The aspiration is to use these scores clinically to identify those individuals who have higher risk of a disease or symptom. The PRS can also be used to identify optimal Treatments. As exemplified by this study, understanding the mechanisms at genetic risk loci can help identify new molecular targets for patients.

This study is an example for future studies aimed at understanding the molecular mechanisms of genetic risk at noncoding loci (Thynn et al., 2019). The amount of work necessary to obtain this level of computational and experimental analysis for each risk locus for every disease with a polygenic component is daunting. The benefits of such efforts clearly support these ongoing efforts.

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**CONFLICT OF INTEREST**
The authors state no conflicts of interest.

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**Clinical Implications**
- Statistical analysis nominates single-nucleotide polymorphisms as likely to be functional at IRF5-TNPO3.
- Noncoding risk variants alter transcriptional regulation of IRF5.
- A novel functional mechanism points to new therapeutic targets for SLE and SSc.

**REFERENCES**

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**Spherical Nucleic Acids as Emerging Topical Therapeutics: A Focus on Psoriasis**

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Systemically delivered targeted biologics have revolutionized the treatment of moderate-to-severe psoriasis. For milder forms of psoriasis, topical therapies, primarily corticosteroids, remain the mainstay of treatment to reduce the risks and off-target side effects associated with systemic therapies. Most newly developed biologics, including monoclonal antibodies, are structurally complex and are unable to penetrate the skin barrier. Recently developed liposomal spherical nucleic acids overcome this barrier and enable topical delivery of antisense oligonucleotides capable of specifically targeting inflammatory pathways underlying psoriasis pathogenesis.


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