GENETICS

Potential causal variants for RA identified

Raychaudhuri and colleagues narrowed down the possible genetic associations to six potentially causal variants for RA

Genome-wide association studies (GWASs) have previously revealed 101 non-MHC loci associated with rheumatoid arthritis (RA), but the causal genetic variants had not been defined. By combining fine-mapping techniques with functional studies, the authors of a new study published in *Nature Genetics* have identified strong candidate causal genetic variants for RA and for type 1 diabetes mellitus (T1DM).

“In this study we used statistical genetic strategies, dense genotyping data, functional investigation of non-coding variants and a combined analysis of data from patients with RA and patients with T1DM,” explains corresponding author Soumya Raychaudhuri. “Statistical data for non-coding variation is important but often insufficient for identifying functional alleles, so using the statistical data, and combining it with non-coding functional assays to demonstrate that the candidate alleles had the potential to alter enhancer function, was key to defining causal variation.”

Starting with a fine-mapping approach, the researchers investigated 76 non-MHC risk-associated loci for RA (11,475 cases and 15,870 controls) and T1DM (9,334 cases

and 11,111 controls). The resulting list was refined to include only loci with ≤ 10 variants in their credible sets, and these loci were taken forward for functional studies (electrophoretic mobility shift assays and allele-specific luciferase assays) using immortalized T cell and monocyte lines.

Using this integrated approach, Raychaudhuri and colleagues narrowed down the possible genetic associations to six potentially causal variants for RA. These variants included missense mutations in *TYK2* (rs35018800) and *DNASE1L3* (rs35677470), an indel in *TNFAIP3* (rs35926684) and a non-coding mutation in the *CD28-CTLA4* locus (rs117701653). Shared missense mutations with T1DM in *PTPN22* and *TYK2* that had been previously described in GWASs were also confirmed.

“Amongst the most exciting results are a loss-of-function allele in *DNASE1L3* and non-coding causal alleles in *TNFAIP3* and *CD28-CTLA4*,” says Raychaudhuri. “These results point to key pathways and genes that are driving RA risk.”

The loss-of-function missense mutation in *DNASE1L3* produced a nuclease that could no longer cleave DNA during apoptosis. Importantly, similar missense mutations in *DNASE1L3* have previously been reported for systemic sclerosis and systemic lupus erythematosus (SLE).

Similarly, variants of *TNFAIP3* are associated with several autoimmune diseases, including SLE and Sjögren syndrome. A20, the protein coded for by *TNFAIP3*, reduces signalling via the transcription factor NF- κ B and thereby inhibits apoptosis.

Interestingly, the results of Hi-C promoter-capture analysis suggested that the rs35926684 region interacts

with the promoters for *TNFAIP3*, *IL22RA* (involved in inflammation, apoptosis and angiogenesis) and *IFNGR1* (involved in interferon responses), indicating that several genes could be influenced by this one variant.

The rs117701653 variant of *CD28-CTLA4* (located in the CD28 region) also showed interactions with multiple promoters via Hi-C analysis, suggesting that this variant might affect *CTLA4* (involved in T cell co-stimulation) and *RAPH1* (involved in cell adhesion).

“The success of this study is to identify primary disease-associated variants,” says Gerry Wilson, an expert in the genetics that underlie RA who was not involved in this study. “It’s a great example of integration of state-of-the-art statistical analysis and straightforward biological experiments. Moving forward, it will be important to determine if the functional effects observed are specific to T cells or potentially occur in other relevant cell types such as synovial fibroblasts or B cells.”

“There remain questions as to what context these alleles are working in, both in terms of cell-type and cell-state, what the downstream genes are that are regulated by the RA risk alleles and what the upstream regulatory machineries are that are driving RA risk,” concludes Raychaudhuri. “Additional cellular investigations including CRISPR strategies and single cell investigation of inflamed tissues in RA will be critical to define the set of candidate genes driving cellular phenotypes.”

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