

# Therapeutic target identification and validation using gene expression networks for autoimmune diseases

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## Introduction

Drug discovery have been traditionally focused in identifying single target molecules without considering molecular interactions or role of the target molecule in its biological network. A systems approach to therapy in complex diseases includes targeting molecules with critical roles in the system with the aim to modulate the system to a healthier state. Here, we analyze the case of an autoimmune disease, such as Multiple Sclerosis (MS) by using a gene expression network analysis approach. We developed a functional analysis of the network topology and a quantitative analysis of the interaction weight differences for identifying new therapeutic targets. Indeed, we used *in vitro* systems for testing new therapies and assessing its functional impact and synergies for the development of combination therapies.

## Methods

We reversed engineered a gene expression network of 20 genes controlling the T cell activation process by using gene co-expression databases from Ingenuity database and experimental data from 104 individuals by quantifying gene expression of PBMCs by real time PCR and applying a bayesian algorithm (BayesianLab software). We analyzed the functional topological properties of the resulting network by building the dependency matrix and performing the minimal cut sets analysis using CAN software. We compared differences in gene interaction weights using the Kublar-Leibler divergence and statistical analysis between groups (controls, patients, and patients treated with immunomodulatory therapy).

## Results and conclusion

The topology of the resulting network was strongly associated with the biological function since it was able to capture the opposing role of genes involved in the Th1-Th2-Tr differentiation as well as in identifying the pleiotropic nature of other modules. The quantitative analysis revealed significant differences between patients and control networks involving several subnetworks related with the T cell activation control, as well as being able to identify the effect of interferon beta therapy in the network. The subnetwork centred in JAG1 gene behave significantly different in MS and it was not returned to the normal state by therapy, becoming a therapeutic target. We validated JAG1 as a therapeutic target by analyzing the effect in the network of treating *in vitro* PBMCs with an agonistic peptide of Jagged1 (the protein of JAG1). After 24h therapy, the gene interaction weights in the JAG1 sub-network were significantly different compared before therapy and closer to the healthier state. Indeed, combination therapy with jagged1 agonistic peptide and interferon beta showed significant effects in different subnetworks, suggesting a synergistic effect.

Finally, we validated *in vivo* JAG1 target by treating animals suffering experimental autoimmune encephalomyelitis with Jagged1 agonistic protein. Jagged1 treated animals displayed a significant milder disease. Indeed, we found that the therapy decreased Th1 function and increased Th2 and Treg function, explaining the beneficial effects and in agreement with the topological analysis of the network.