# Mechanisms of Transcriptional Regulation in Trisomy 21 and Their Contribution to Elevated Immune Signaling and Inflammation

Isabella Perrin, Meaghan Courvan, Kira Cozzolino, Dylan Taatjes, Robin Dowell

## Importance and Overview

Down syndrome (DS), also known as Trisomy 21 (T21), is the most prevalent chromosomal disorder in human beings1. Caused by the triplication of chromosome 21, DS can be characterized by its significant range of co-occurring conditions, including congenital heart defects, neurodegenerative diseases like Alzheimer's Disease, sleep apnea, and autoimmune diseases2(Figure 1). While individuals with DS experience different co-occurring conditions with ranging severities, each of these comorbidities significantly impacts the quality of life for those with DS. Current DS research hypothesizes that increased inflammation drives presentation of co-occurring conditions, but particular mechanisms have yet to be fully understood.

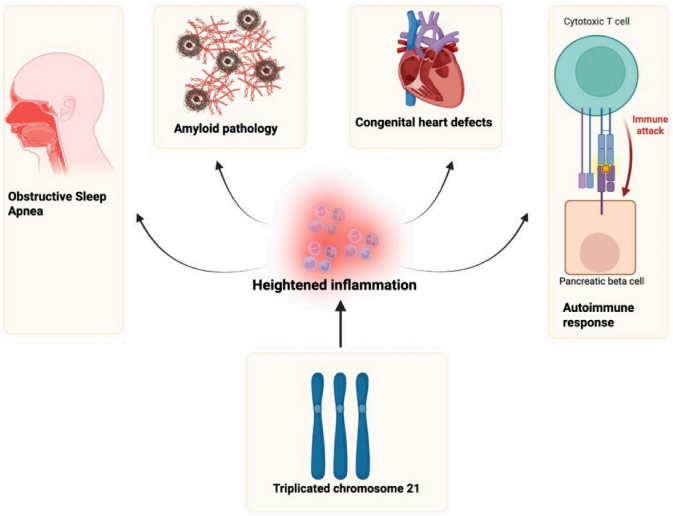


***Figure 1. DS comorbidity schematic.*** Graphical visualization of molecular interplay between Trisomy 21 and associated co-occurring conditions. Created in BioRender. Perrin, I. (2026) https://BioRender.com/np7y8c6

In attempts to further understand the interplay between heightened, chronic inflammation experienced by individuals with DS and the comorbidities they face, this research explores the role of interferon-gamma (IFNg), a pro-inflammatory cytokine, and the broader DS immune response. Specifically, by exploring transcriptional regulation in the face of IFNg, one can investigate how increased inflammation may be leading to the unique spectrum of co-occurring diseases seen in those with Trisomy 21.

Because IFNg is encoded on chromosome 21, when the triplication of the chromosome occurs,

individuals have three copies of the IFNg receptor. Current research highlights that when these cell surface receptors are increased in blood cells, more inflammation occurs in DS individuals3. Additionally, previous research has been done to better understand how gene expression regulation has underscored how DS cells differ in their responses when they are stimulated with IFNg4. However, there is little work done at the molecular level to investigate gene expression regulation differences. This project, therefore, focuses on exploring the molecular underpinnings of the complex immune processes that individuals with DS experience in order to contribute to research exploring clinical therapies.

As part of our immune response, when cells face a pathogenic threat, they release the inflammatory signaling molecule IFNg to begin pro-inflammatory responses throughout the body (Figure 2). To understand how this inflammation contributes to co-occurring diseases in DS, it's crucial to learn how DS transcriptional regulation differs compared to responses seen in those without DS.

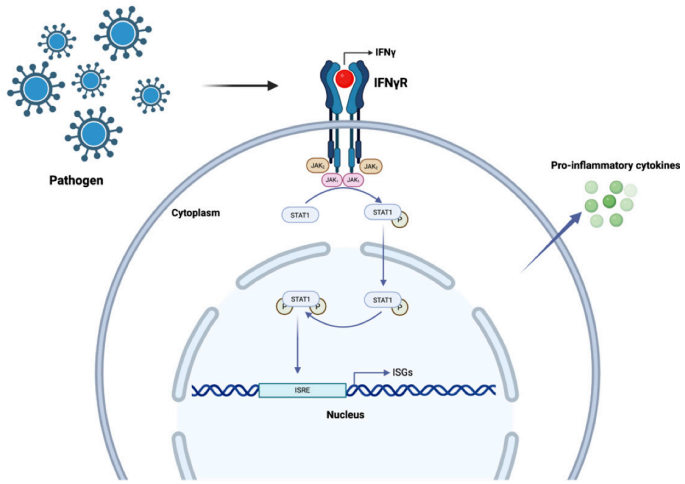


***Figure 2. IFNg-mediated signaling pathway.*** As part of pathogen recognition, Type II (IFNg) interferons bind at the cell surface, triggering a kinase cascade that promotes a pro-inflammatory response. Created in BioRender. Perrin, I. (2026) https://BioRender.com/s8g8743

## Results

Utilizing previously collected PRO-Seq (Precision Run-On Sequencing) data, which captures an intermediate transcriptional response and allows one to analyze the direct transcriptional outcomes of perturbations5, a comparison of the regulation of DS (T21) and non-DS (D21) cells treated with IFNg can be established. This is crucial for understanding both how DS and non-DS cells differentially alter their responses to IFNg, a proxy for observing how cells adapt to pathogen encounter, as well as the molecules that orchestrate this adaptation. PRO-Seq data was collected from D21 and T21 cells at 45 minutes following perturbation with and without IFNg. By collecting nascent RNA transcripts, those that are produced by RNA Polymerase II, which is actively transcribing along a portion of DNA, patterns of transcription can be established (Fig. 1A). PRO-Seq data highlights how many sequencing reads are aligned to a particular gene, which is directly related to how extensively that gene is being transcribed. The samples collected were consistently high quality, as indicated by distributions of read counts and reproducible signal across interferon genes (Fig. 1B). Additionally, these genes can indicate transcriptional differences between D21 and

T21 individuals and between conditions. IRF4, a transcription factor (TF) with a hallmark role in the IFN pathway, highlights the changing intensity of active transcription across samples: D21 (blue) and T21 (green) are both expressing this TF, but expression is higher in the T21 cells, allowing IRF4 to act as a positive control for the experiment (Fig. 3C). Lighter shades of both blue and green indicate control conditions while darker shades show IFNg perturbation. In the case of this TF, there does not seem to be a change in its transcriptional output in response to IFNg.

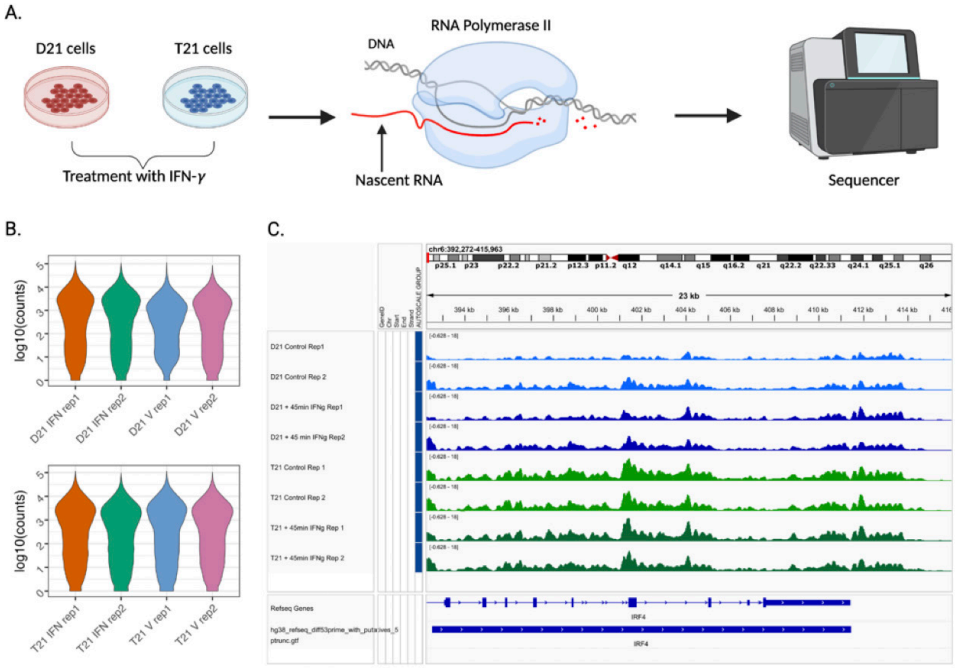


***Figure 3. PRO-Seq Collection and Visualization***. (A) Schematic of cell treatment and collection of PRO-Seq data. Created in BioRender. Perrin, I. (2026) https://BioRender.com/okmbb41 (B) Distribution of counts across samples (T21 vs. D21) and conditions (vehicle vs. IFNg treatment). (C) Annotations for TF IRF4 through Integrative Genomics Viewer (IGV) comparing samples (T21 vs. D21) and conditions (vehicle vs. IFNg treatment after 45 minutes).

DESeq2, which examines gene production changes across conditions, was then run to analyze the differential gene expression occurring between T21 and D21 individuals and between treatment groups. Hallmark IFN pathway genes, such as IRF1 and GBP5, act as controls, highlighting the IFN pathway's activation after IFNg treatment (Fig. 4A,B). Further labeling of the most significant genes (p-adjust < .05) indicates differences in changes in gene expression between T21 and D21 individuals (Fig. 4C,D). More differentially expressed genes in the IFNg pathway are also significant in T21 individuals, indicating that different genes are differentially expressed in T21 as compared to D21 individuals. GSEA (Gene Set Enrichment Analysis) and GO (Gene Ontology) pathway analysis were utilized to explore pathway-specific changes, allowing for the comparison between the T21 and D21 responses at the transcriptional level. Pathways involved in the interferon response were enriched in both T21 and D21 individuals, with important distinctions including Notch, Complement, and Apoptosis signaling pathways in T21 individuals (Figure 5).

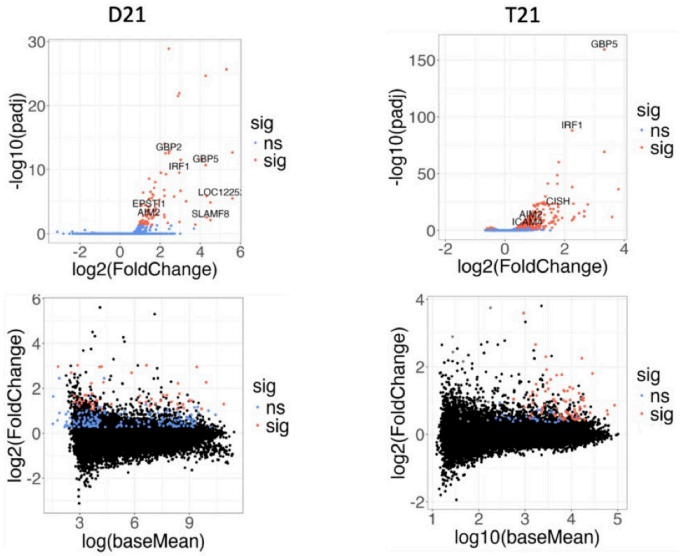


***Figure 4. DESeq2 reveals T21 and D21 differentially expressed genes following IFNg perturbation.*** (A, B) DESeq2 results show differentially expressed genes ( p-adjust < .05) in D21 and T21 individuals. (C, D) Differentially expressed genes (from DESeq2 results) in the IFNg pathway (from GSEA results) that are also significantly upregulated. Colored by significance (p-adjust < .05) in D21 and T21 individuals.

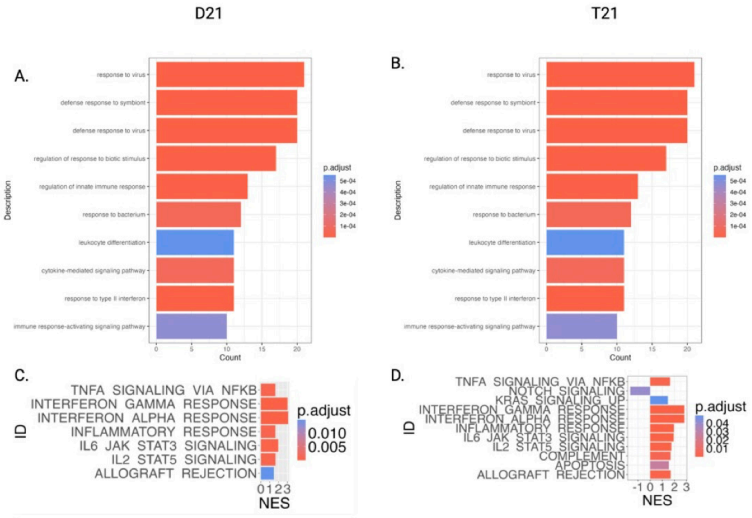


***Figure 5. GO and GSEA analyses underscore pathway-specific trends.*** (A, B) GO (Gene Ontology) pathway analysis results for D21 and T21 individuals. (C, D) GSEA results for D21 and T21 individuals, colored by p-adjust.

## Conclusions & Future Experiments

While individuals with DS and those without share many pathways in response to IFNg, such as those that involve inflammation, distinct genes were contributing to these differences. These genes were largely inflammatory genes, which supports the hypothesis that there is a change in transcriptional

regulation in response to IFNg that is producing the spectrum of phenotypic manifestations. Additionally, there were two pathways only heavily increased in DS: the complement pathway and the Notch pathway. The complement pathway is a key player in the immune system's defense: small proteins will coat a bacterium so that other immune cells like macrophages can engulf and destroy the bacteria6. The Notch pathway is an essential developmental pathway, which may mean that there is interplay between cell development pathways and this IFN pathway7.

While this research highlights the differential response T21 and D21 individuals have to IFNg perturbation at a single point in time, collecting PRO-Seq data at several time points would better allow for the analysis of the evolution of the IFN response over time. Additionally, TFEA (Transcription Factor Enrichment Analysis) can measure TF activity and will be crucial in further examining TF-dependent regulation that cells utilize. Finally, to observe downstream changes in transcription of IFNg-related genes, analysis of PRO-Seq data that involves T21 and D21 cells being perturbed by IFNg as well as Cortistatin A ( which interrupts the signaling cascade IFN begins in the cell) will be completed. By utilizing Cortistatin A to perturb this pathway, we seek to learn how a variety of transcriptional factors are involved in eliciting an immune response related to Down Syndrome, which may reveal the mechanisms that provide the unique array of comorbidities seen in those with Trisomy 21.

## References


1. Down Syndrome: Practice Essentials, Background, Pathophysiology. (2024). https://emedicine.medscape.com/article/943216-overview#a2?form=fpf
2. Chicoine, B., Rivelli, A., Fitzpatrick, V., Chicoine, L., Jia, G., & Rzhetsky, A. (2021). Prevalence of Common Disease Conditions in a Large Cohort of Individuals With Down Syndrome in the United States. Journal of Patient-Centered Research and Reviews, 8(2), 86–97. https://doi.org/10.17294/2330-0698.1824
3. Sullivan, K. D., Evans, D., Pandey, A., Hraha, T. H., Smith, K. P., Markham, N., Rachubinski, A. L., Wolter-Warmerdam, K., Hickey, F., Espinosa, J. M., & Blumenthal, T. (2017). Trisomy 21 causes changes in the circulating proteome indicative of chronic autoinflammation. Scientific Reports, 7(1), 14818. https://doi.org/10.1038/s41598-017-13858-3
4. Poss, Z. C., Ebmeier, C. C., Odell, A. T., Tangpeerachaikul, A., Lee, T., Pelish, H. E., Shair, M. D., Dowell, R. D., Old, W. M., & Taatjes, D. J. (2016). Identification of Mediator Kinase Substrates in Human Cells using Cortistatin A and Quantitative Phosphoproteomics. Cell Reports, 15(2), 436–450. https://doi.org/10.1016/j.celrep.2016.03.030
5. PRO-seq | Nascent Transcriptomics Core. (n.d.). Retrieved December 31, 2025, from https://ntc.hms.harvard.edu/pro-seq
6. Charles A Janeway, J., Travers, P., Walport, M., & Shlomchik, M. J. (2001). The complement system and innate immunity. In Immunobiology: The Immune System in Health and Disease. 5th edition. Garland Science. https://www.ncbi.nlm.nih.gov/books/NBK27100/
7. Notch signaling pathway: Architecture, disease, and therapeutics | Signal Transduction and Targeted Therapy. (n.d.). Retrieved November 20, 2025, from https://www.nature.com/articles/s41392-022-00934-y