The Interferonopathy of Down Syndrome

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DSMIG 2025

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Boettcher Investigator
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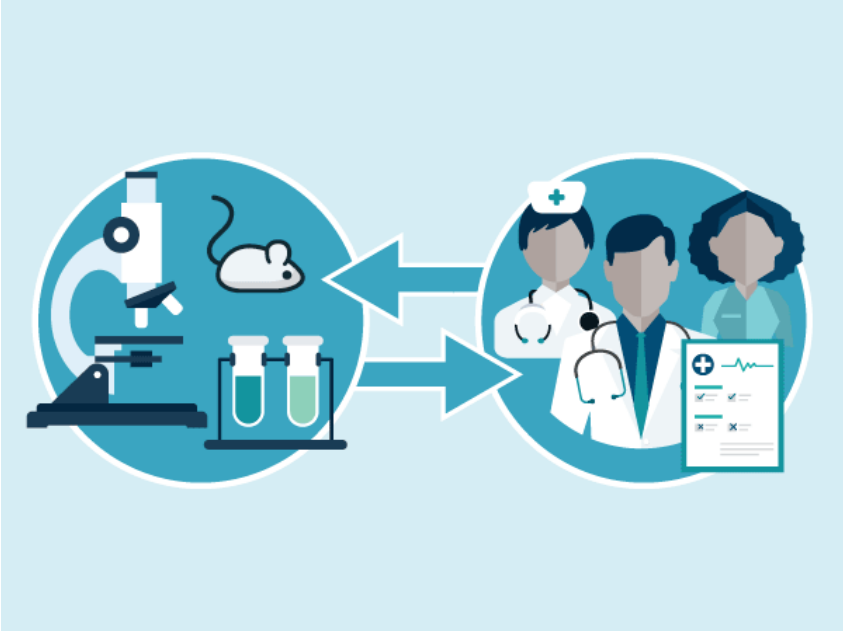
University of Colorado Anschutz Medical Campus

No disclosures

The Interferonopathy Down syndrome:

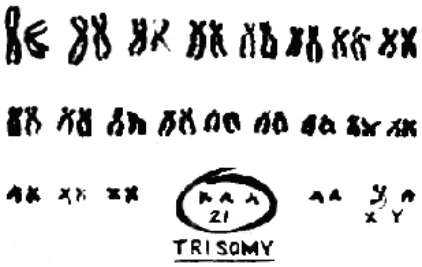
From bench to bedside

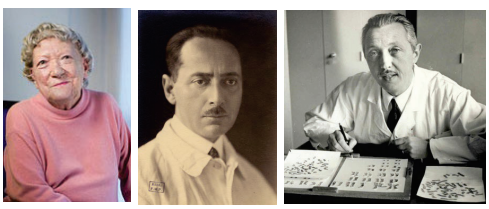


A journey at the Linda Crnic Institute for Down Syndrome 2015-2025 (and beyond).

Down syndrome:

The ultimate challenge in precision personalized medicine





Gautier Turpin Lejeune

The chromosomal abnormality causing Down syndrome (i.e., trisomy 21) has been known since the late 1950's.

Chromosome 21 was sequenced back in 2000, leading to the identification of ~225 genes.

No mutations, simply 1.5x gene dosage.

How does an extra chromosome 21 cause the various hallmarks of Down syndrome?

People with Down syndrome have a unique disease spectrum

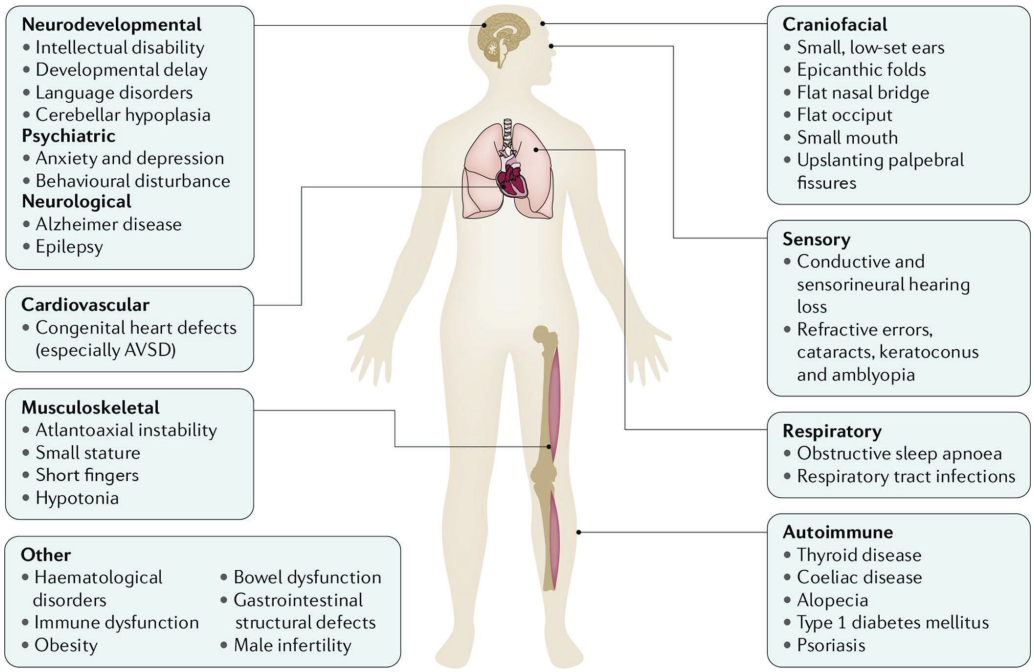
INCREASED incidence compared to typicals

- **100%** — **Alzheimer's** brain pathology by age 40. **Cognitive deficits.**
- **~10x** — **Autism.** **Epilepsy.**
- **Up to 400x** — Pediatric **hematological proliferative disorders:** TMD, AMKL, AML, ALL.
- **2-25x** — **Autoimmune disorders:** Alopecia, arthritis, celiac, Hashimoto's, T1D, vitiligo.
- **50%** — **Obstructive sleep apnea.**
- **37x** — **Pulmonary hypertension.**

DECREASED incidence compared to typicals

- **~5x** — **Coronary artery disease.** **High blood pressure.**
- **Over 10x** — **Solid Malignancies** (except testicular cancer).

T21 adversely affects virtually every tissue and organ system in the body



Antonarakis et al. Nat Rev Dis Primers 2020

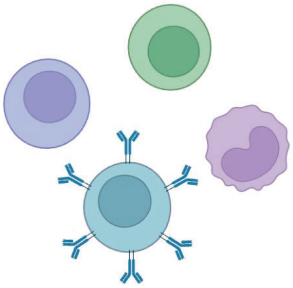
How does Trisomy 21 cause Down syndrome?

Trisomy 21

Down syndrome

How can we study the complex cause-effect relationships between T21 and pathophysiology?

1. primary human samples



2. animal models of DS

Integration of data from a variety of sources and experimental models can accelerate discovery of DS-related conditions and identify mechanisms of pathophysiology

The Human Trisome Project (HTP)

A large **cohort study** with deep clinical data,
a multidimensional biobank, and -omics datasets

>1,400 participants
recruited since 2016

LINDA CRNIC INSTITUTE
HUMAN TRISOME PROJECT
GLOBAL DOWN SYNDROME FOUNDATION

GLOBAL
DOWN SYNDROME FOUNDATION

trisome.org

Thousands of biospecimens collected
and 'omics datasets generated

- Clinical histories
- Genomes
- Transcriptomes
- Immune maps
- Metabolomes
- Microbiomes

Data shared through the
INCLUDE Data Hub

INCLUDE Data Hub

includedcc.org

50+ research projects supported

30+ publications

Funded by:

NIH *Eunice Kennedy Shriver* National Institute of Child Health and Human Development

NIH National Heart, Lung, and Blood Institute

NIH National Institute on Aging

NIH National Institute of Allergy and Infectious Diseases

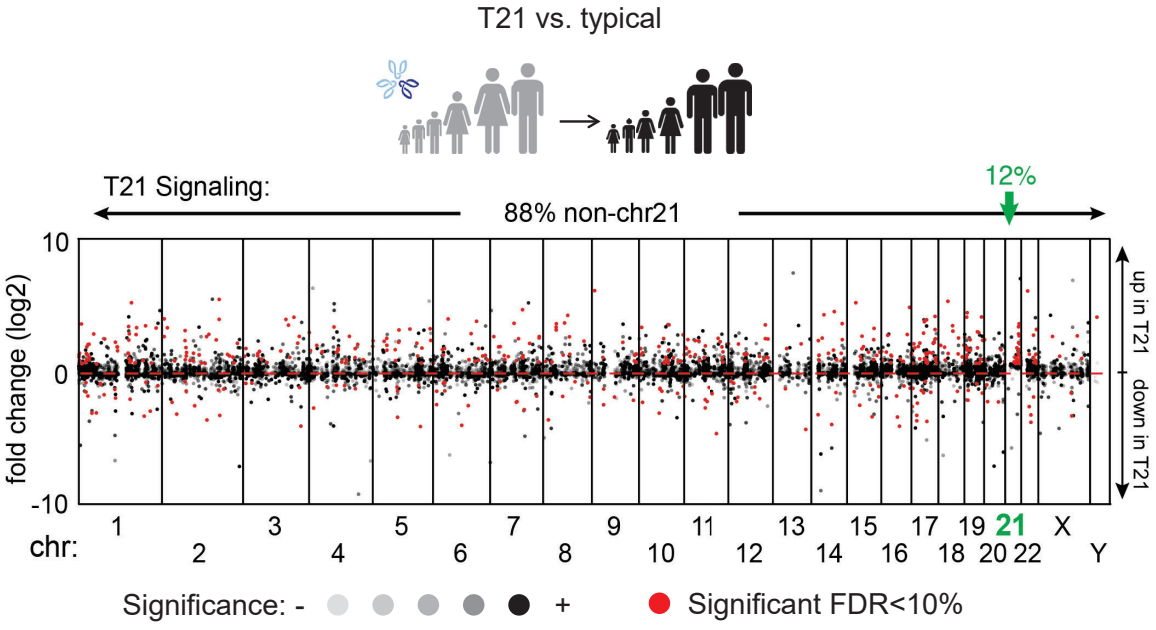
NIH National Institute on Deafness and Other Communication Disorders

NIH National Center for Advancing Translational Sciences

A simple question: what is the impact of Trisomy 21 on the transcriptome?

Signal amplification across the genome

Trisomy 21 causes a **consistent** gene expression signature (even **outside of chr21**) that withstands age, gender and site of biopsy…

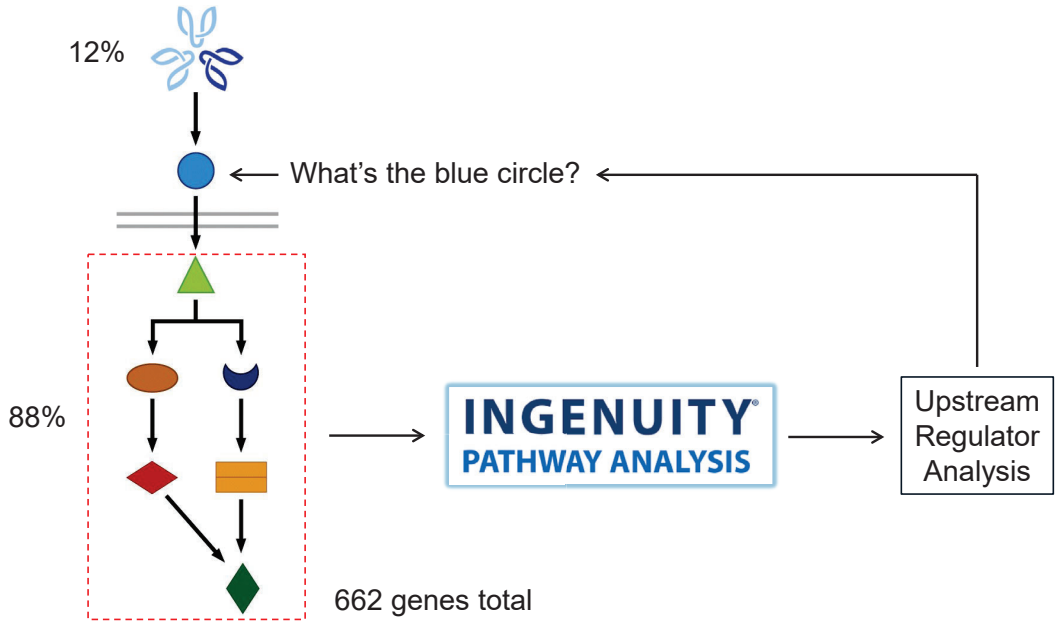


Manhattan plot

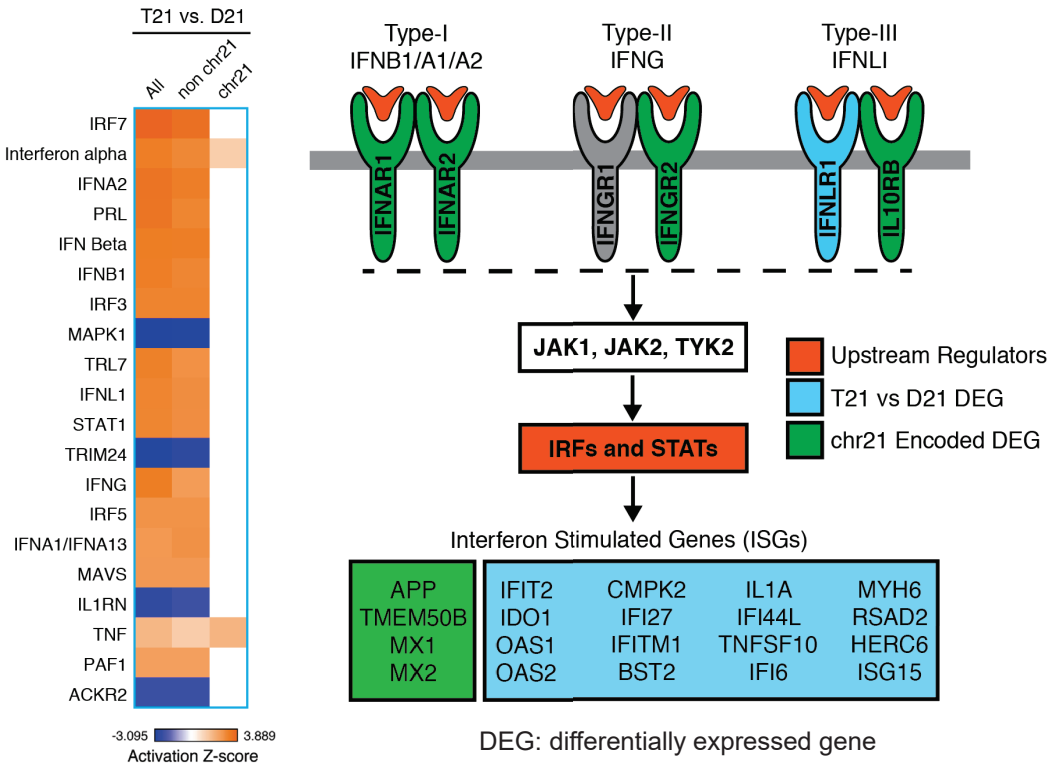
Sullivan et al., eLIFE, 2016

What is the signal amplifier?

Upstream Regulator Analysis of the **consistent** gene expression signature activated by trisomy 21

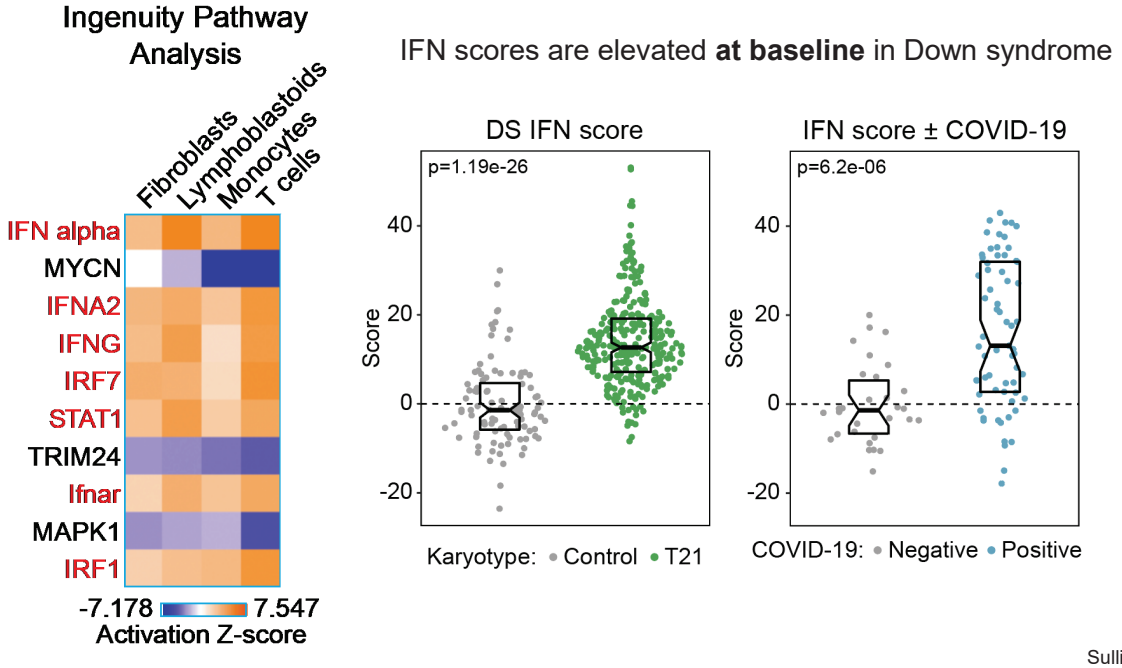


Interferon, Interferon, Interferon



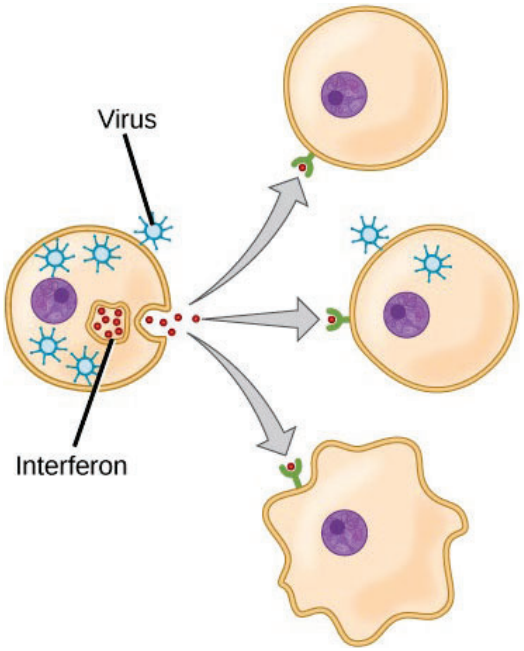
Sullivan et al., eLIFE, 2016

A transcriptional signature of hyperactive interferon signaling is observed in multiple cell types



Sullivan et al., *eLIFE*, 2016
Galbraith et al., *Science Advances*, 2023

People with Down syndrome have hyperactive interferon signaling



Interferon (IFN) signaling is an important part of the immune system involved in the anti-viral defense.

Interferons are ‘cytokines’ that activate many different types of immune cells.

Interferon hyperactivity is a known risk factor for autoimmunity.

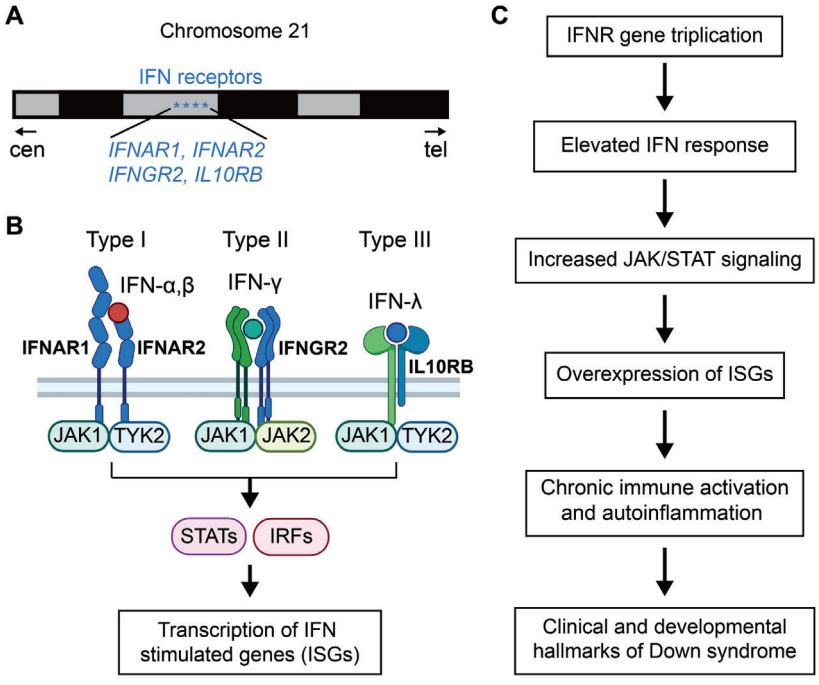
Trisomy 21 consistently activates the interferon response

Kelly D Sullivan[1,2,3,4*], Hannah C Lewis[1,2], Amanda A Hill[1,2], Ahwan Pandey[1,2,3,4], Leisa P Jackson[1,3,4], Joseph M Cabral[1,3,4], Keith P Smith[1], L Alexander Liggett[1,5], Eliana B Gomez[1,3,4], Matthew D Galbraith[1,2,3,4], James DeGregori[1,5,6,7,8,9], Joaquín M Espinosa[1,2,3,4*]

eLIFE elifesciences.org

Sullivan et al, eLIFE 2016

Why do people with Down syndrome have hyperactive interferon signaling?



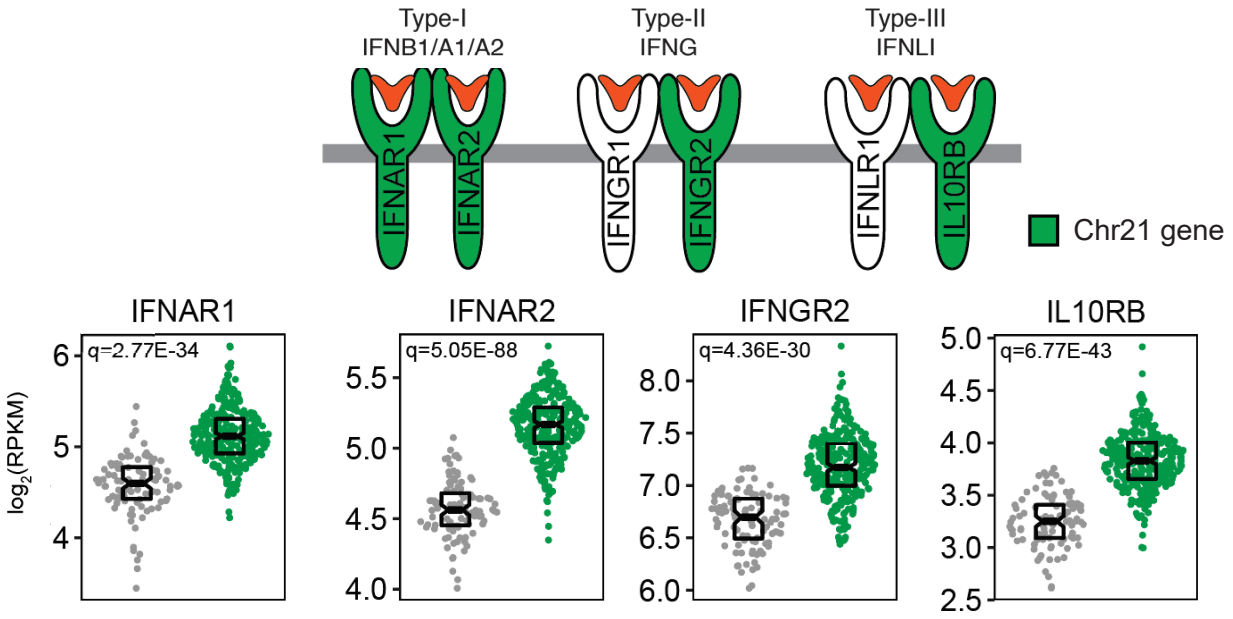
Four interferon receptors (IFNRs) are encoded on chromosome 21!!!

Hypotheses:

IFNR triplication causes elevated interferon responses, increased JAK/STAT signaling, and autoinflammation.

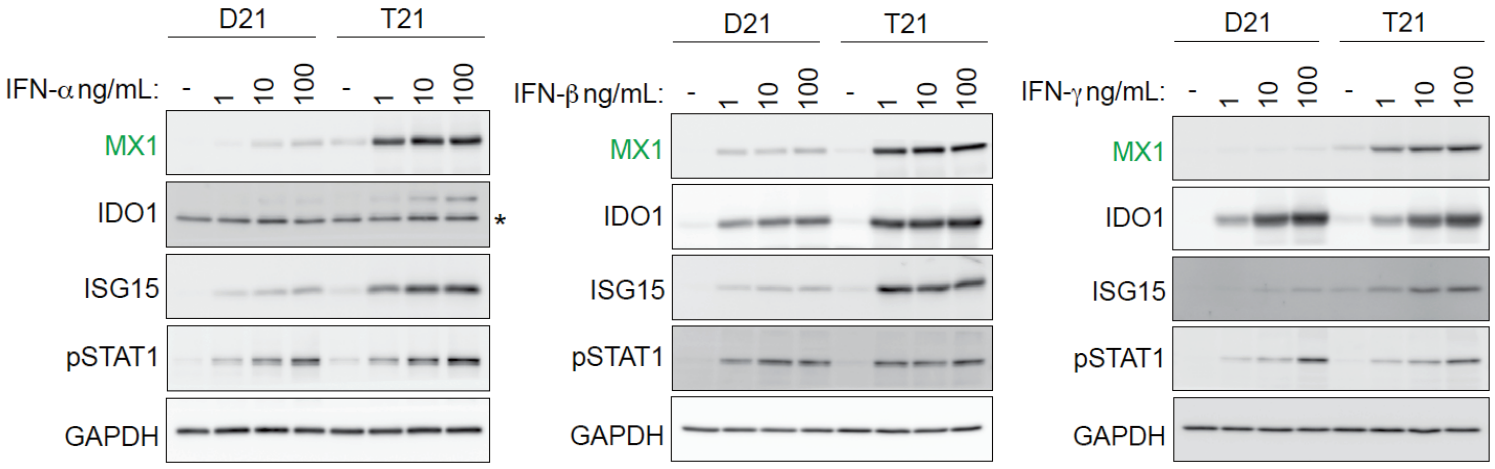
Interferon hyperactivity is a driver of pathology in Down syndrome.

Why is IFN signaling activated?
IFNRs are overexpressed in T21 cells!



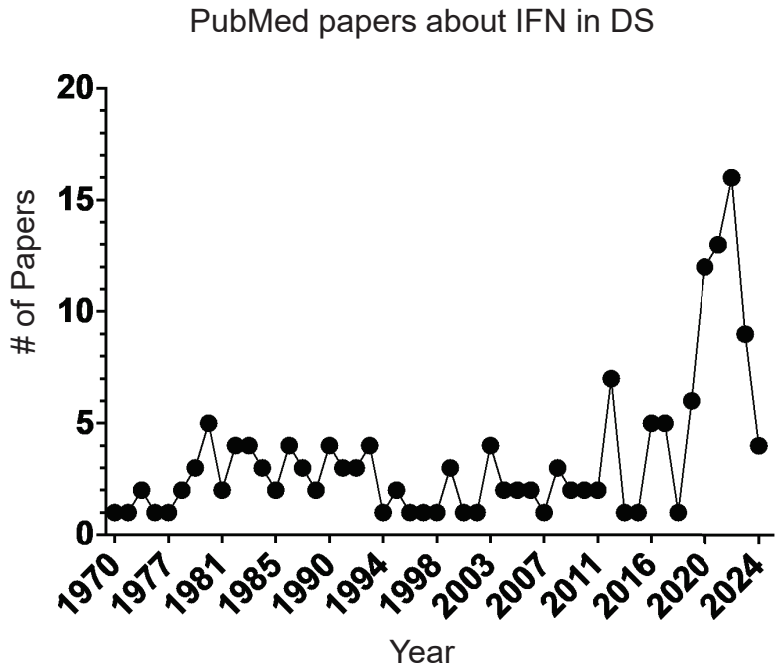
Sullivan et al., *eLIFE*, 2016
Galbraith et al., *Science Advances*, 2023

T21 cells are hypersensitive to IFN stimulation



Sullivan et al., *eLIFE*, 2016

Interferon dysregulation in T21 is not an entirely new concept, just a long dormant one…



Interferon dysregulation in T21 is not an entirely new concept, just a long dormant one…

> Science. 1974 Oct 4;186(4158):61-3. doi: 10.1126/science.186.4158.61.

Human chromosome 21 dosage: effect on the expression of the interferon induced antiviral state

Y H Tan, E L Schneider, J Tischfield, C J Epstein, F H Ruddle

PMID: 4371269 DOI: 10.1126/science.186.4158.61

> J Theor Biol. 1980 Oct 7;86(3):603-6. doi: 10.1016/0022-5193(80)90356-2.

Interferon action and chromosome 21 trisomy

L E Maroun

PMID: 6163931 DOI: 10.1016/0022-5193(80)90356-2

> Cytokine. 2012 Dec;60(3):875-81. doi: 10.1016/j.cyto.2012.08.020. Epub 2012 Sep 18.

Expression of interferon-γ, interferon-α and related genes in individuals with Down syndrome and periodontitis

Marcia H Tanaka [1], Elisa M A Giro, Lícia B Cavalcante, Juliana R Pires, Luciano H Apponi, Sandro R Valentini, Denise M P Spolidório, Marisa V Capela, Carlos Rossa Jr, Raquel M Scarel-Caminaga

Down Syndrome is an Interferonopathy

Discussion

We report here that T21 leads to consistent activation of the IFN pathway. As discussed below, IFN hyperactivation could explain many of the developmental and clinical impacts of T21. In fact, we posit that Down syndrome can be understood largely as an interferonopathy, and that the variable clinical manifestations of T21 could be explained by inter-individual differences in adaptation to chronic IFN hyperactivity.

Sullivan et al., eLIFE, 2016

Down Syndrome is an Interferonopathy

Journal of Clinical Immunology (2020) 40:807–819
https://doi.org/10.1007/s10875-020-00803-9

ORIGINAL ARTICLE

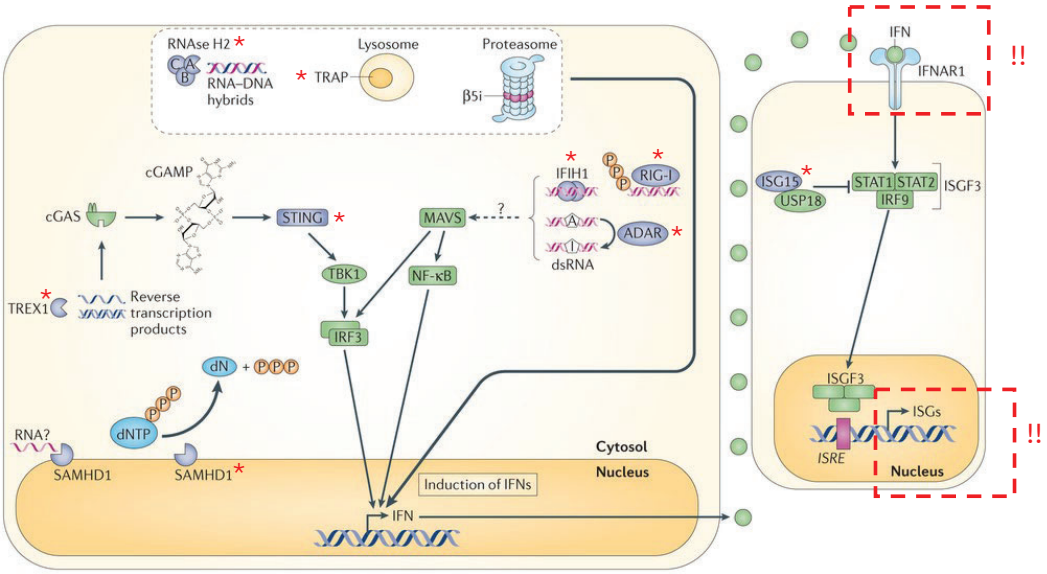
Check for updates

Three Copies of Four Interferon Receptor Genes Underlie a Mild Type I Interferonopathy in Down Syndrome

Xiao-Fei Kong[1,2] · Lisa Worley[3,4] · Darawan Rinchai[5] · Vincent Bondet[6,7] · Puthen Veettil Jithesh[5] · Marie Goulet[8] · Emilie Nonnotte[8] · Anne Sophie Rebillat[8] · Martine Conte[8] · Clotilde Mircher[8] · Nicolas Gürtler[9] · Luyan Liu[10] · Mélanie Migaud[10] · Mohammed Elanbari[5] · Tanwir Habib[5] · Cindy S. Ma[3,4] · Jacinta Bustamante[10,12] · Laurent Abel[1,10] · Aimé Ravel[8] · Stanislas Lyonnet[13] · Arnold Munnich[14] · Darragh Duffy[6,7] · Damien Chaussabel[5] · Jean-Laurent Casanova[1,10,11,12,15] · Stuart G Tangye[3,4] · Stéphanie Boisson-Dupuis[1] · Anne Puel[10]

Type I Interferonopathies are caused by constitutive Interferon signaling

Mutations in *TREX1*, *RNASEH2A-C*, *SAMHD1*, *ADAR*, *IFIH1*, *RIG-I*, *TRAP*, *STING* and *ISG15* lead - through various mechanisms - to increased IFN signaling



Crow and Manel, Nature Reviews Immunology 2015

Interferonopathies are a group of genetic disorders characterized by upregulation of the Interferon response:

Aicardi-Goutieres Syndrome, SAVI, CANDLE, Singleton–Merten syndrome, spondyloenchondrodysplasia, dyschromatosis symmetrica hereditaria, familial chilblain lupus, Nakajo-Nishimura syndrome, spondylochondromatosis, etc.

Many features shared with Down syndrome:

- Severe neurological dysfunction
- Severe developmental delay
- Less white matter in the brain
- Seizures
- Cerebellar atrophy
- Spastic diplegia, a form of cerebral palsy (CP), a chronic neuromuscular condition of hypertonia and spasticity
- Dystonic posturing
- Hyper- or hypotonia
- Profound psychomotor difficulties
- Thrombocytopenia (deficiency of platelets)
- CSF lymphocytosis (too many white blood cells in the spinal fluid)
- Systemic immune abnormalities, strong predisposition to autoimmunity
- Hypocomplementia
- Common skin lesions (e.g. acrosyanosis)

What is the impact of Trisomy 21 on other biosignatures?

What is the impact of trisomy 21 on the circulating proteome?

Trisomy 21 causes changes in the circulating proteome indicative of chronic autoinflammation

Kelly D. Sullivan[1,2], Donald Evans[1], Ahwan Pandey[1,2], Thomas H. Hraha[3], Keith P. Smith[1], Neil Markham[1], Angela L. Rachubinski[4], Kristine Wolter-Warmerdam[5], Francis Hickey[5], Joaquin M. Espinosa[1,2,6] & Thomas Blumenthal[1,6,7]

SCIENTIFIC REPORTS
2017

Evans Smith Rachubinski Hickey Blumenthal





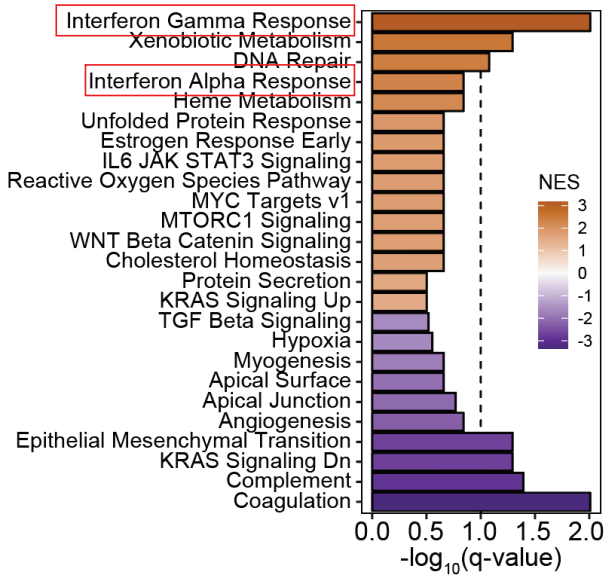
SomaLogic Tom Hraha, Larry Gold

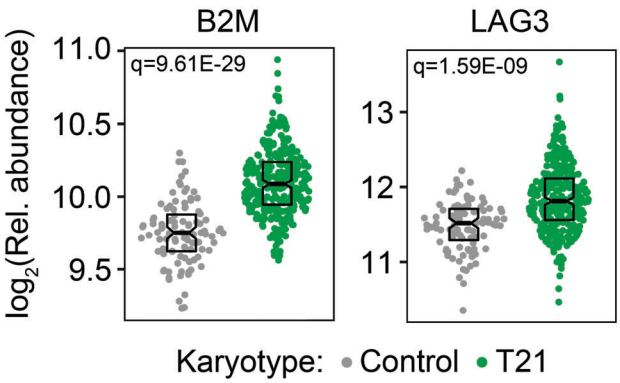
SOMAscan® proteomics

Strong signs of elevated IFN signaling in the proteome of people with Down syndrome

Plasma proteomics analysis of 419 research participants (316 with trisomy 21) in the Human Trisome Project

GSEA: proteome changes in trisomy 21 versus euploid controls



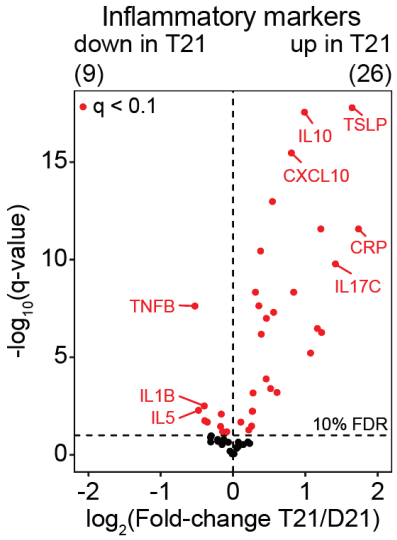


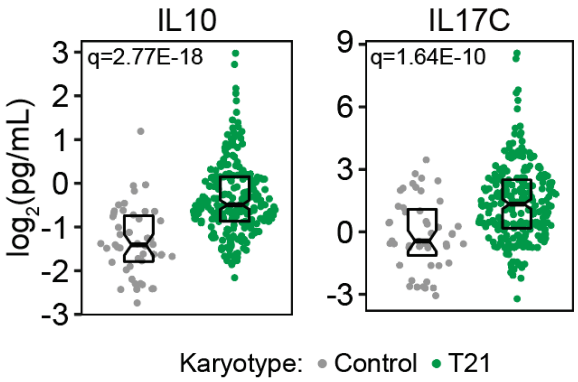
B2M: beta 2 microglobulin
LAG3: lymphocyte activating 3

Galbraith et al, *Sciences Advances* 2023

How much of the inflammatory profile of DS is associated with the interferonopathy?

Persons with Down syndrome show elevated levels of many inflammatory markers



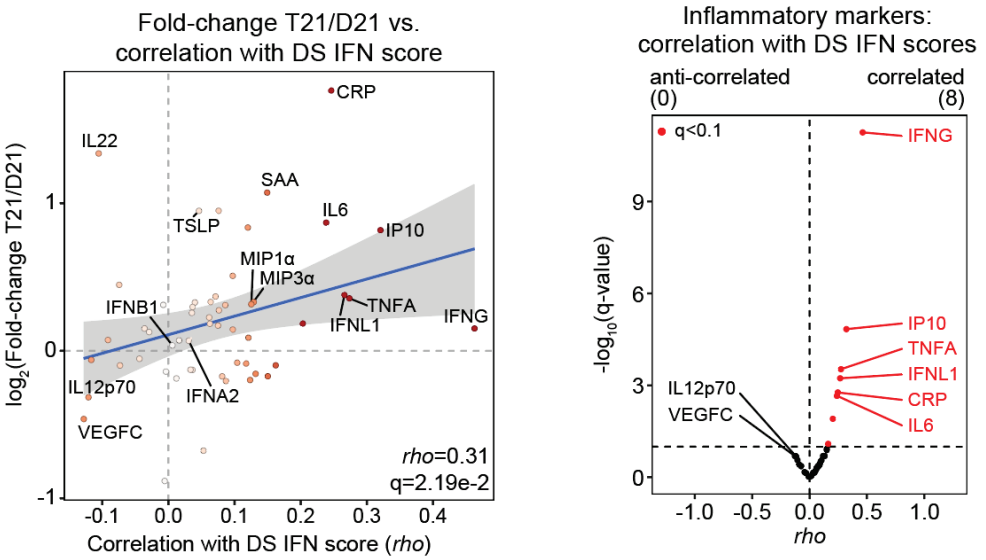


Quantitative targeted proteomics using Meso Scale Discovery (MSD assays) for 54 inflammatory markers

Galbraith et al, *Sciences Advances* 2023

Much (but certainly not all) of the inflammatory profile of Down syndrome can be linked to IFN hyperactivity

Associations between IFN scores and changes in inflammatory markers



Quantitative targeted proteomics using Meso Scale Discovery (MSD assays) for 54 inflammatory markers

Galbraith et al, *Sciences Advances* 2023

What are the impacts of trisomy 21 on the metabolome?

Employing mass-spectrometry approaches to map the metabolic impacts of trisomy 21

Trisomy 21 activates the kynurenine pathway via increased dosage of interferon receptors

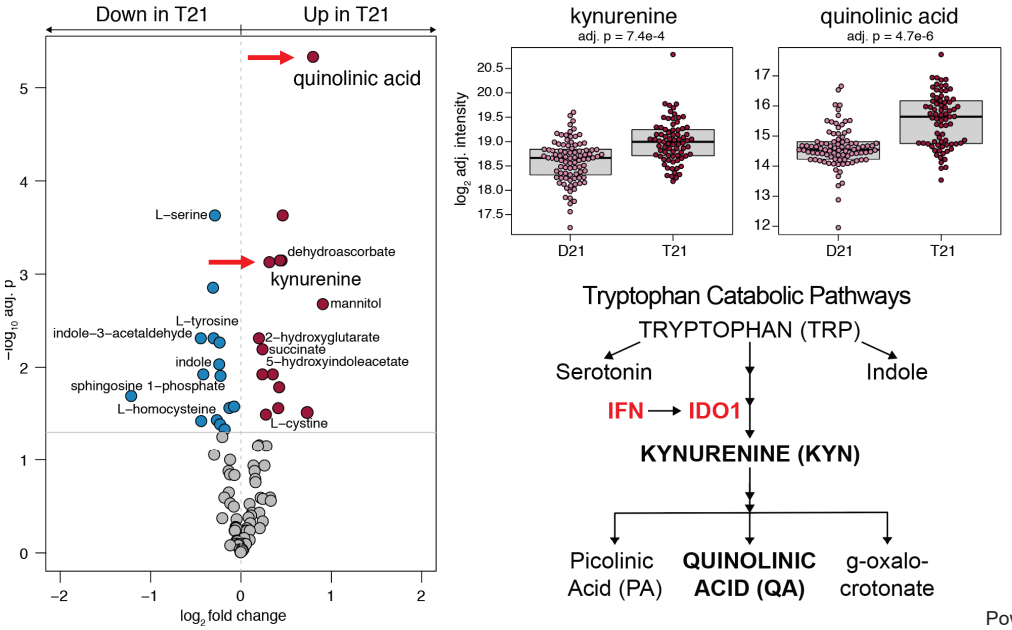
Rani K. Powers[1,2,3], Rachel Culp-Hill[4], Michael P. Ludwig[1,3], Keith P. Smith[1], Katherine A. Waugh[1], Ross Minter[1], Kathryn D. Tuttle[1], Hannah C. Lewis[1], Angela L. Rachubinski[1,5], Ross E. Granrath[1], María Carmona-Iragui[6,7], Rebecca B. Wilkerson[4], Darcy E. Kahn[1], Molishree Joshi[8], Alberto Lleó[6], Rafael Blesa[6], Juan Fortea[6,7], Angelo D'Alessandro[1,4], James C. Costello[2,3], Kelly D. Sullivan[1,3,5,8*] & Joaquin M. Espinosa[1,3,8,9*]

November 2019 nature COMMUNICATIONS

Powers Ludwig Smith D'Alessandro Costello

People with Down syndrome display activation of the kynurenine pathway

Plasma metabolomics measuring 91 metabolites
120 participants, 72 with trisomy 21



Powers et al, *Nature Comms* 2019

Quinolinic acid, the inescapable neurotoxin

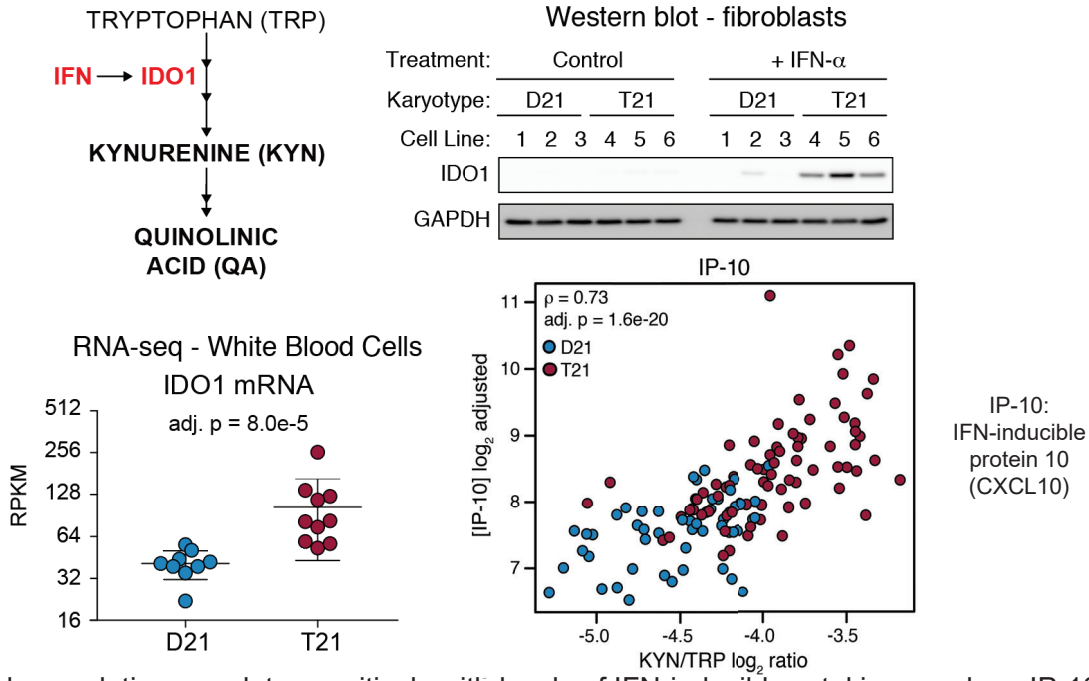
- Quinolinic acid (QA) is super-agonist of NMDA receptors
- QA induces excitatory toxicity
- Memantine (an NMDR *antagonist*) protects from QA-mediated neurotoxicity in mice
- Circulating levels of QA were **associated with lower cognition in older adults with AD** in the typical population
- QA is a potent **convulsant** involved in the etiology of **epilepsy** and **seizures**, which are more common in Down syndrome

Quinolinic acid, the inescapable neurotoxin

Gilles J. Guillemin[1,2]

the FEBS Journal

People with Down syndrome overexpress IDO1, the rate-limiting enzyme in the kynurenine pathway

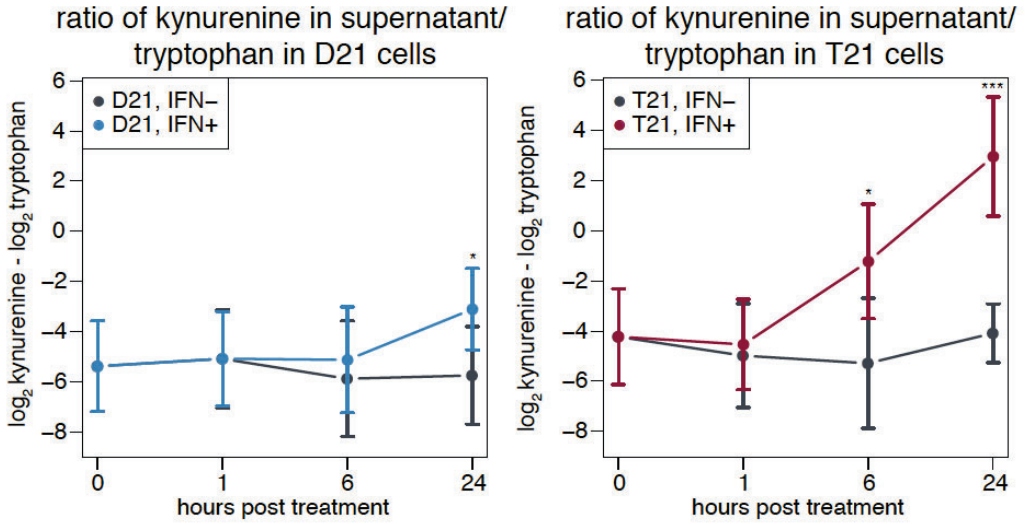


IP-10: IFN-inducible protein 10 (CXCL10)

Kynurenine dysregulation correlates positively with levels of IFN-inducible cytokines such as IP-10

T21 cells show super-induction of IDO1 and KYN over-production upon IFN stimulation

IDO1, the key enzyme required to shunt tryptophan into the kynurenine > quinolinic acid pathway,
is a well known Interferon Stimulated Gene!



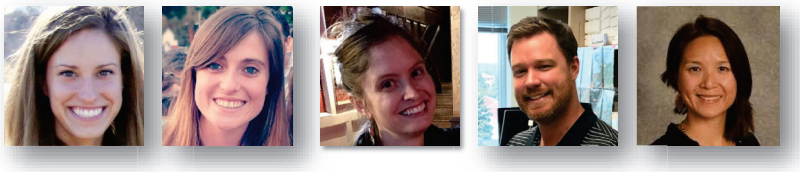
Powers et al., *Nature Communications*, 2019

What is the impact of trisomy 21 on the immune cell repertoire?

High resolution mapping of the immune system in Down syndrome

Employing CyTOF technology to map the immune system of people with Down syndrome

Waugh Araya Tuttle Smith Hsieh



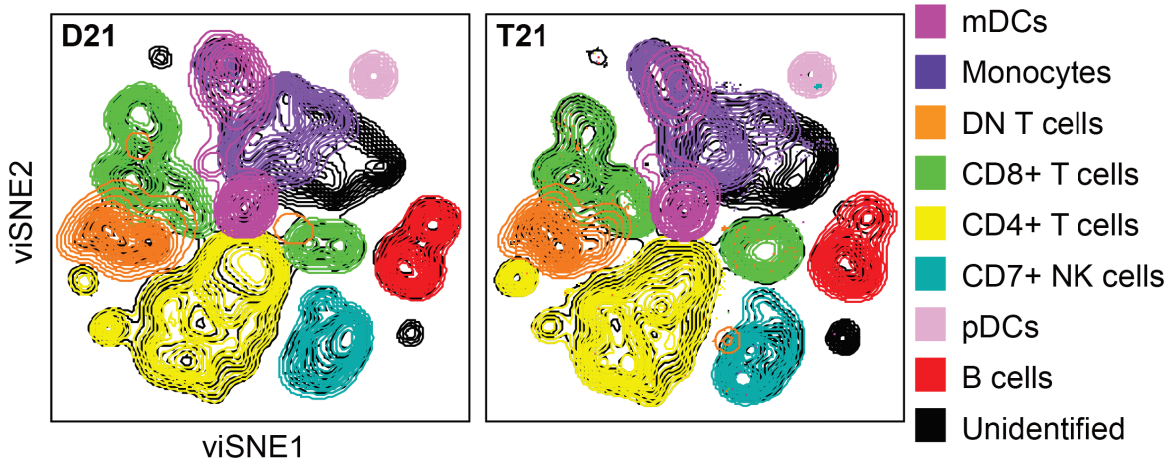
Mass Cytometry Reveals Global Immune Remodeling with Multi-lineage Hypersensitivity to Type I Interferon in Down Syndrome

Katherine A. Waugh,[1] Paula Araya,[1] Ahwan Pandey,[1,2,3] Kimberly R. Jordan,[4] Keith P. Smith,[1] Ross E. Granrath,[1] Santosh Khanal,[2] Eric T. Butcher,[1] Belinda Enriquez Estrada,[1] Angela L. Rachubinski,[1,5] Jennifer A. McWilliams,[4] Ross Minter,[1] Tiana Dimasi,[1] Kelley L. Colvin,[1,5,6] Dmitry Baturin,[7] Andrew T. Pham,[1] Matthew D. Galbraith,[2] Kyle W. Bartsch,[1] Michael E. Yeager,[1,5,6] Christopher C. Porter,[8] Kelly D. Sullivan,[1,2,5] Elena W. Hsieh,[1,4,5] and Joaquin M. Espinosa[1,2,3,9,*]

November 2019 Cell Reports

Bulk immune subsets are preserved in Trisomy 21

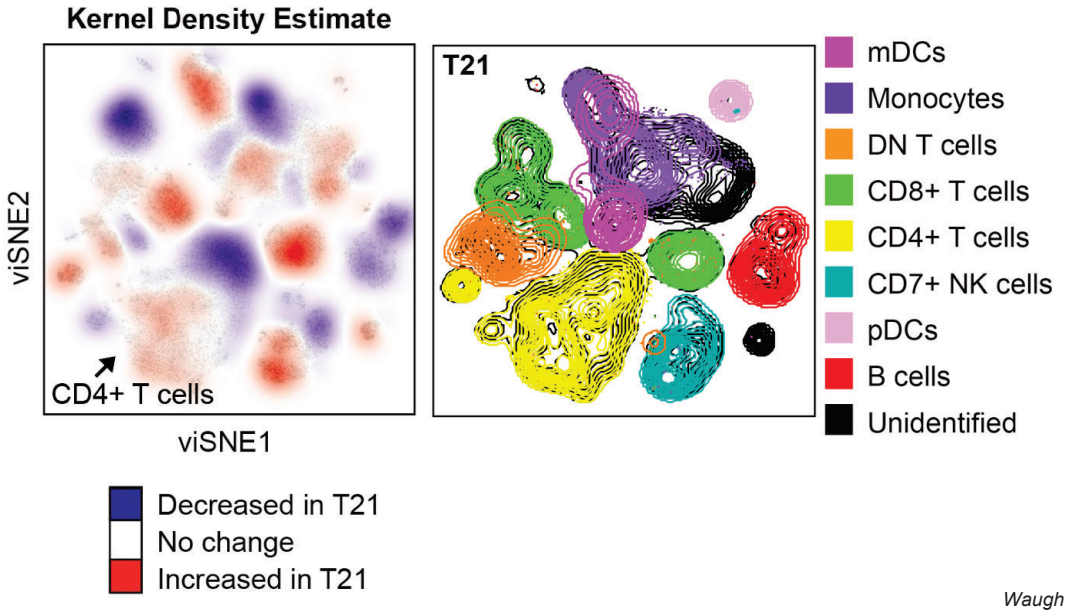
Overlay of manually gated cell types



Waugh et al, Cell Reports, 2019

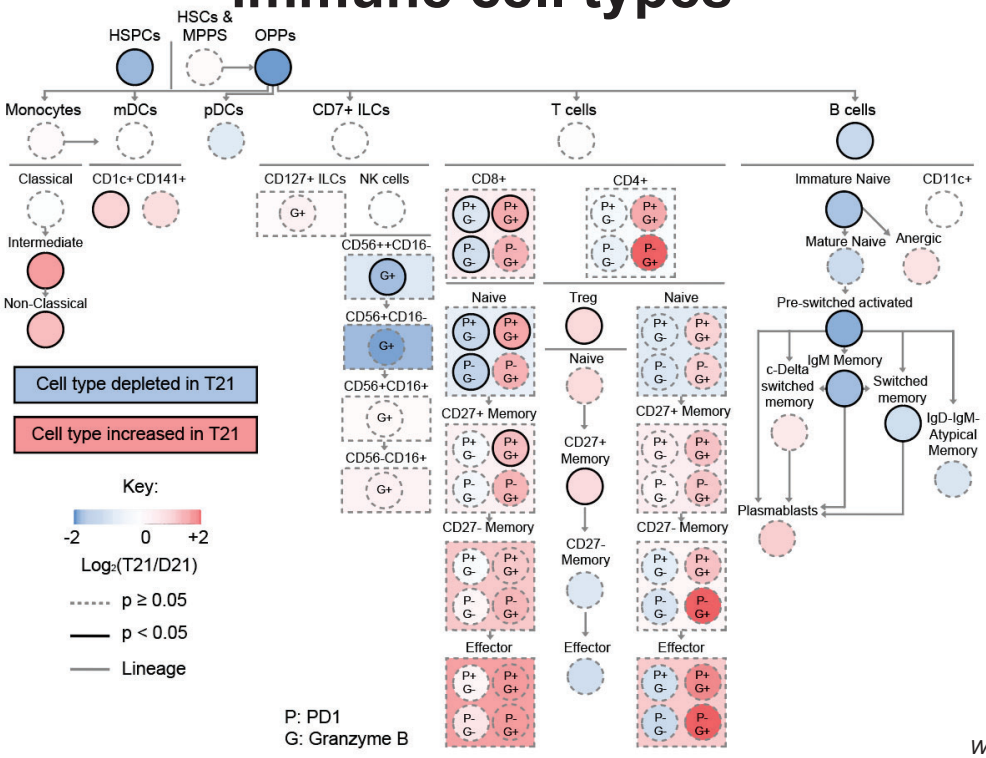
Topographic analysis highlights global immune dysregulation among individuals with Trisomy 21

Kernal Density Estimate (KDE) of viSNE plots to quantitatively compare densities:



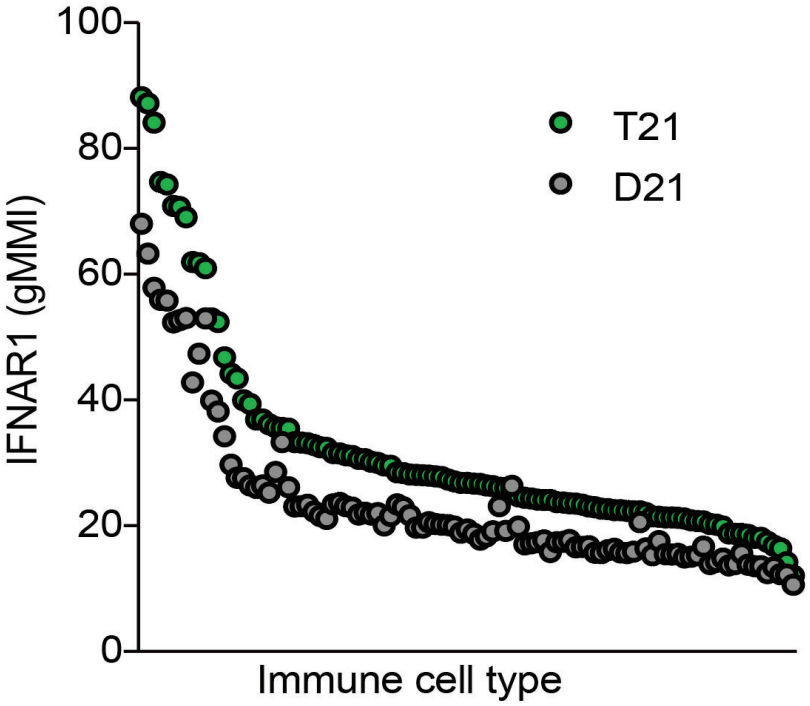
Waugh et al, Cell Reports, 2019

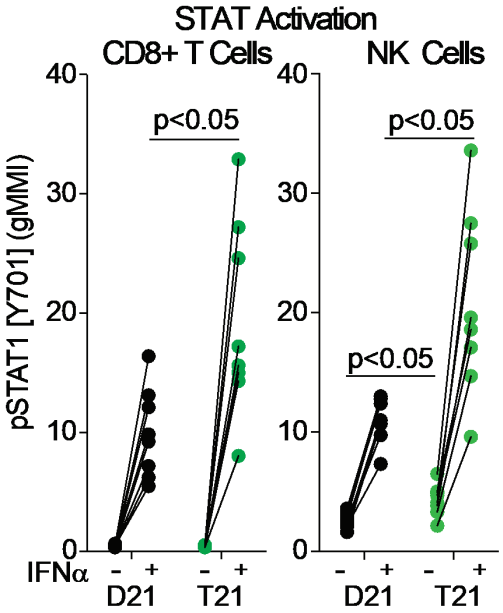
Trisomy 21 impacts abundance of numerous immune cell types



Waugh et al, Cell Reports, 2019

Why is IFN signaling activated?
IFNRs are overexpressed in T21 cells!

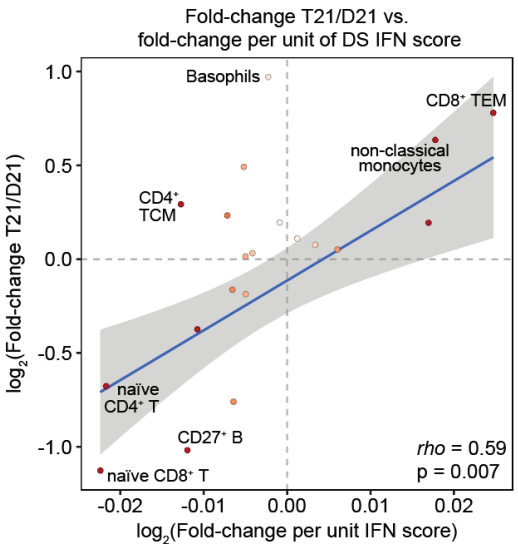


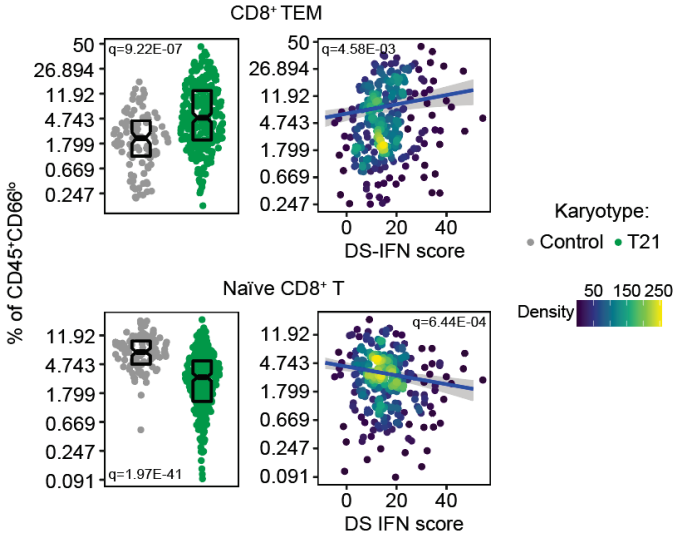


Waugh et al., *Cell Reports*, 2019

IFN hyperactivity strongly associates with the extent of immune remodeling

The higher the IFN score, the more dysregulated the immune system





High IFN scores = more cytotoxic T cells, fewer B cells, more inflammatory monocytes

Galbraith et al, *Sciences Advances* 2023

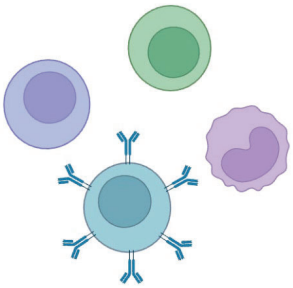
Summary I

- IFN signaling is constitutively activated in T21 cells
- T21 cells are hypersensitive to IFN stimulation
- Signs of IFN activation are present in other ‘omic’ biosignatures

How does IFNR triplication impact the immune response *in vivo*?

How can we study the complex cause-effect relationships between T21 and pathophysiology?

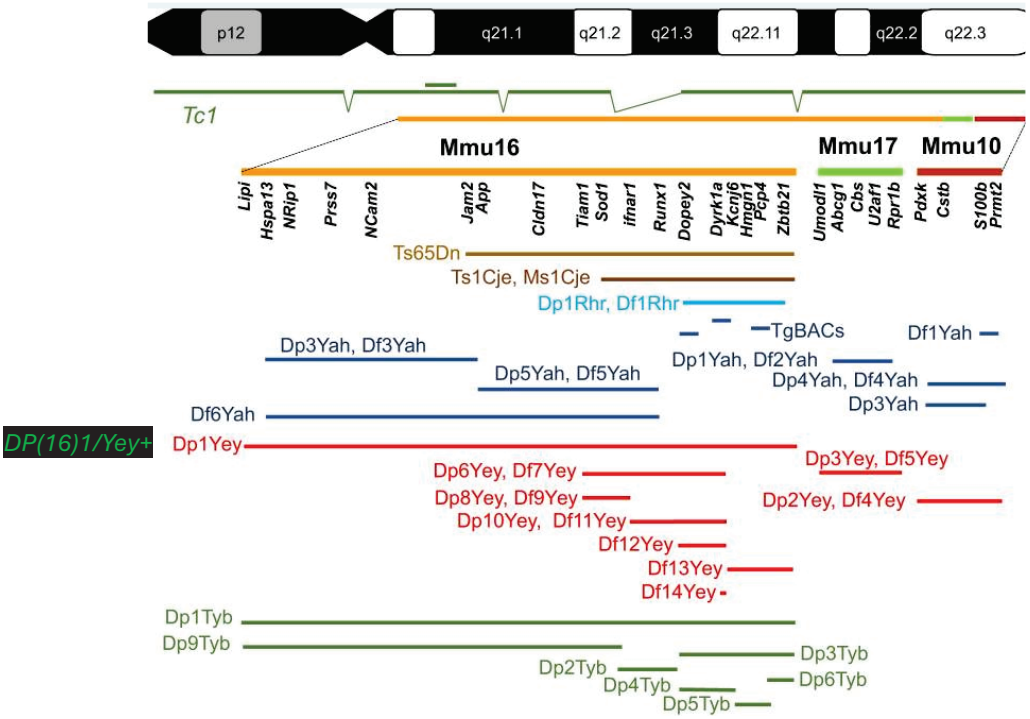
1. primary human samples



2. animal models of DS

Integration of data from a variety of sources and experimental models can accelerate discovery of DS-related conditions and identify mechanisms of pathophysiology

We can effectively study Down syndrome using mouse models



Dp16 mice exhibit hallmarks of DS:

Congenital heart defects

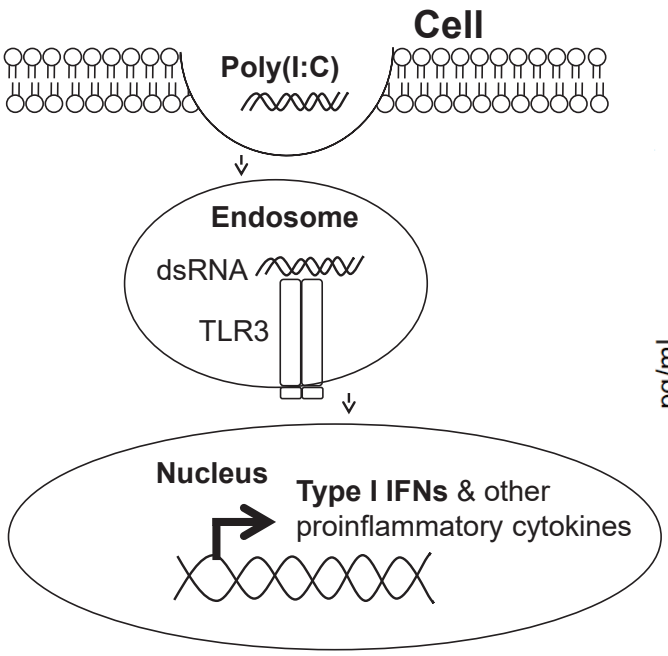
Developmental delays

Cognitive deficits

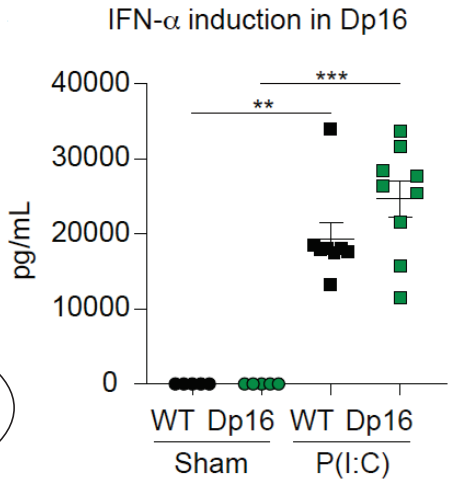
Craniofacial anomalies

Herault et al., *Dis. Model Mech*, 2017

What if we stimulate IFN signaling systemically?

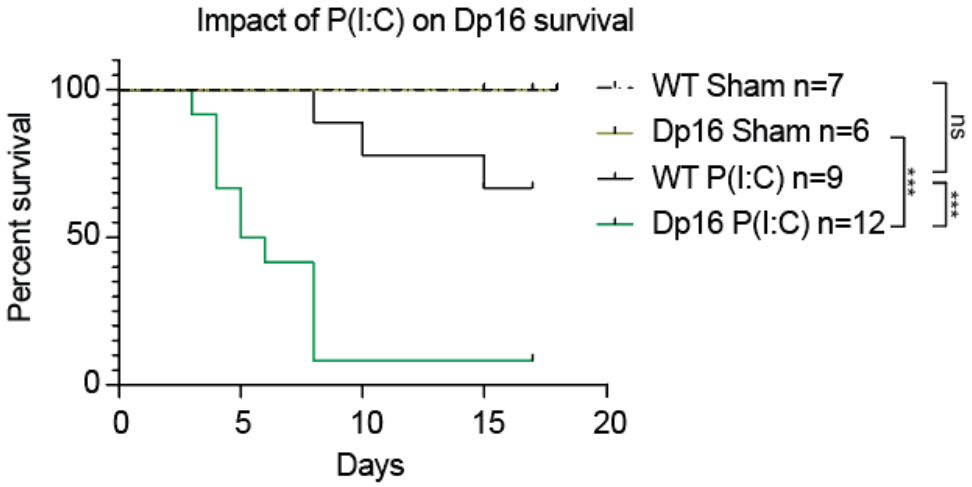


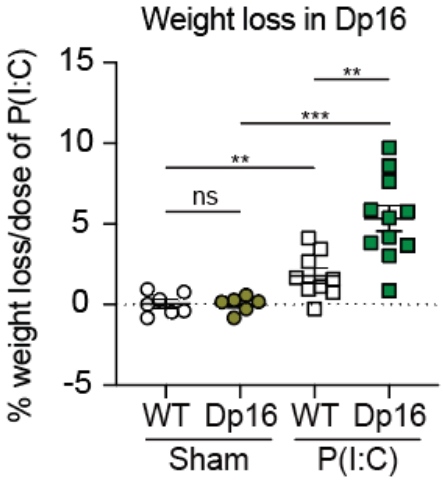
Poly(I:C) is a synthetic dsRNA that serves as viral mimetic



Tuttle et al., *Cell Reports*, 2022

Dp16 Mice are hypersensitive to chronic treatment with the IFN inducer, poly(I:C)





Tuttle et al, *Cell Reports*, 2022

An early prediction of high risk for severe COVID-19 in Down syndrome

Perspective

Down Syndrome and COVID-19: A Perfect Storm?

Joaquin M. Espinosa[1,2,*]
[1]Linda Crnic Institute for Down Syndrome, University of Colorado Anschutz Medical Campus, Aurora, CO 80045, USA
[2]Department of Pharmacology, School of Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO 80045, USA
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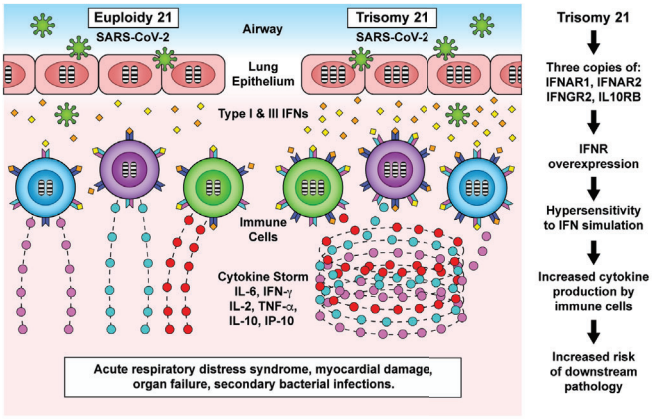
https://doi.org/10.1016/j.xcrm.2020.100019

Cell Reports Medicine

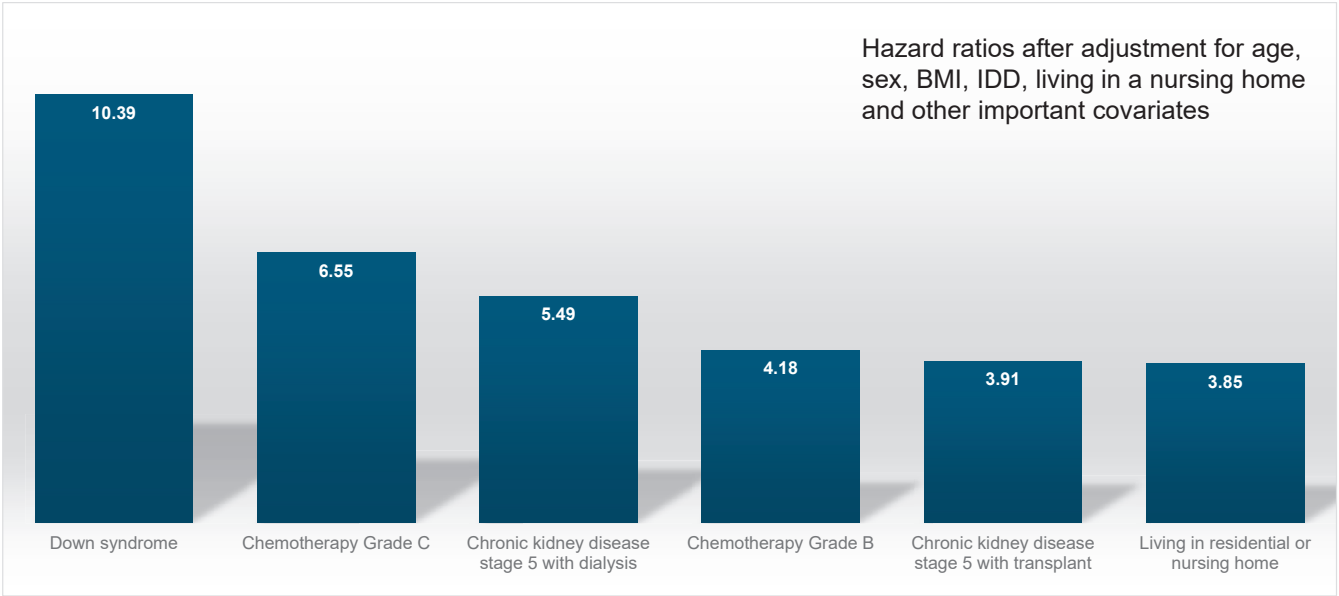
'Despite the clear limitations imposed by the lack of available data, I provide evidence that individuals with trisomy 21 should be considered at high risk of developing more severe symptoms and increased rates of hospitalization, intensive care, secondary bacterial infections, and mortality from SARS-CoV-2 infections relative to the general population, thus justifying increased monitoring and specialized care for those with COVID-19 and Down syndrome.'



Published May 1 2020

Trisomy 21 is a major risk factor for severe COVID-19

After reviews of >8 million medical records in England, people with Down syndrome were found to be 10 times more likely to die of COVID-19

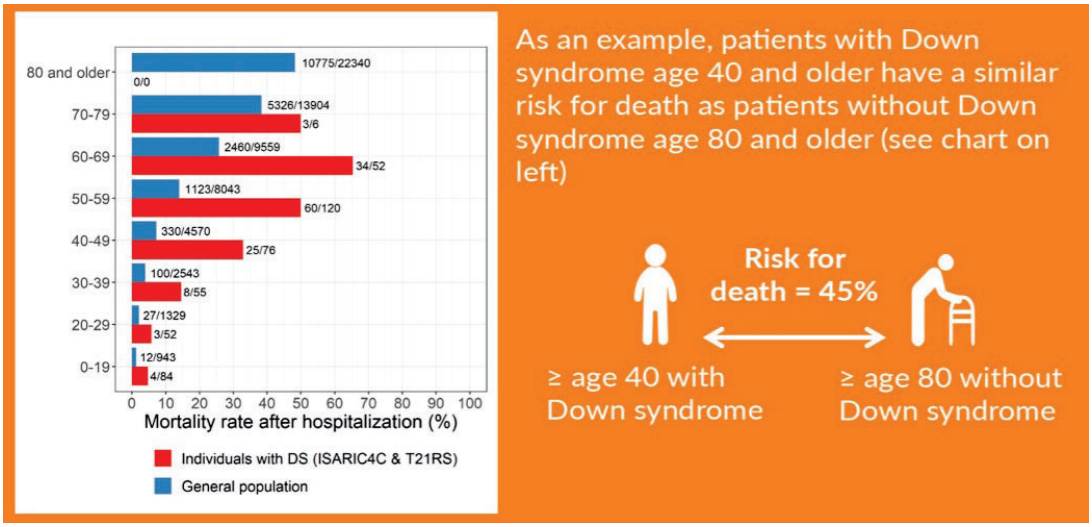


Clift et al, Annals of Internal Medicine, October 21 2020

Trisomy 21 is a major risk factor for severe COVID-19

Having trisomy 21 adds ~40 years to your birth certificate

T21 Research Society Survey

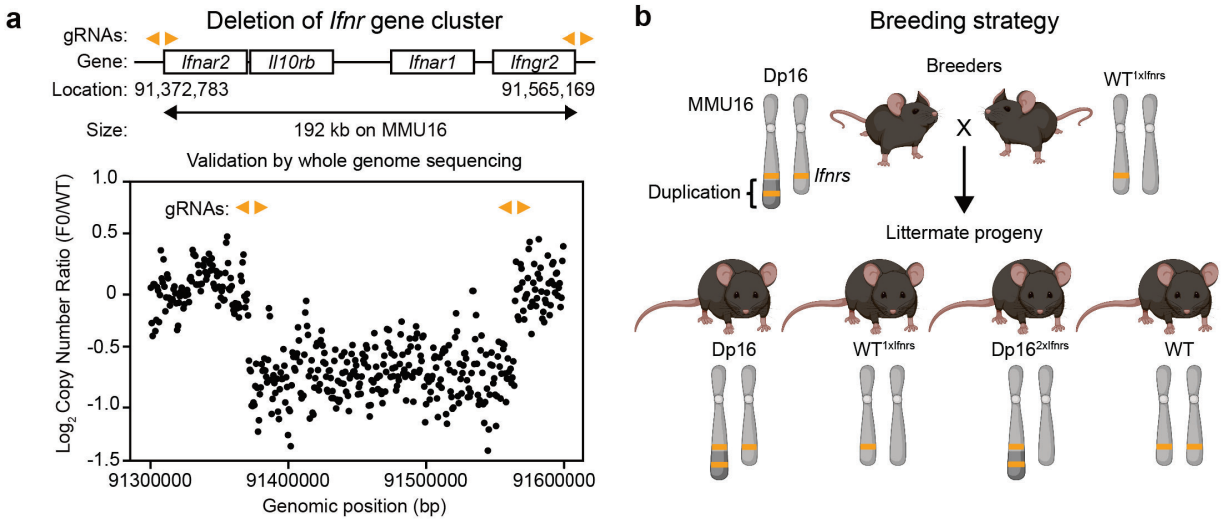


Huls et al, Medical vulnerability of individuals with Down syndrome to severe COVID-19: data from the Trisomy 21 Research Society and the UK ISARIC4C survey.
EclinicalMedicine. 2021 Mar;33:100769. doi: 10.1016/j.eclinm.2021.100769. Epub 2021 February 2021.

What if we normalize *Ifnr* copy number?

Normalizing interferon receptor gene dosage in mice

Clean genomic deletion of the four interferon receptors
while preserving triplication of ~120 other genes in this mouse model



Through various crosses in the vivarium, we can now monitor the impact of reducing interferon receptor copy number from 3 copies (Down syndrome) to 2 copies (the correct number)

Normalizing interferon receptor gene dosage in mice

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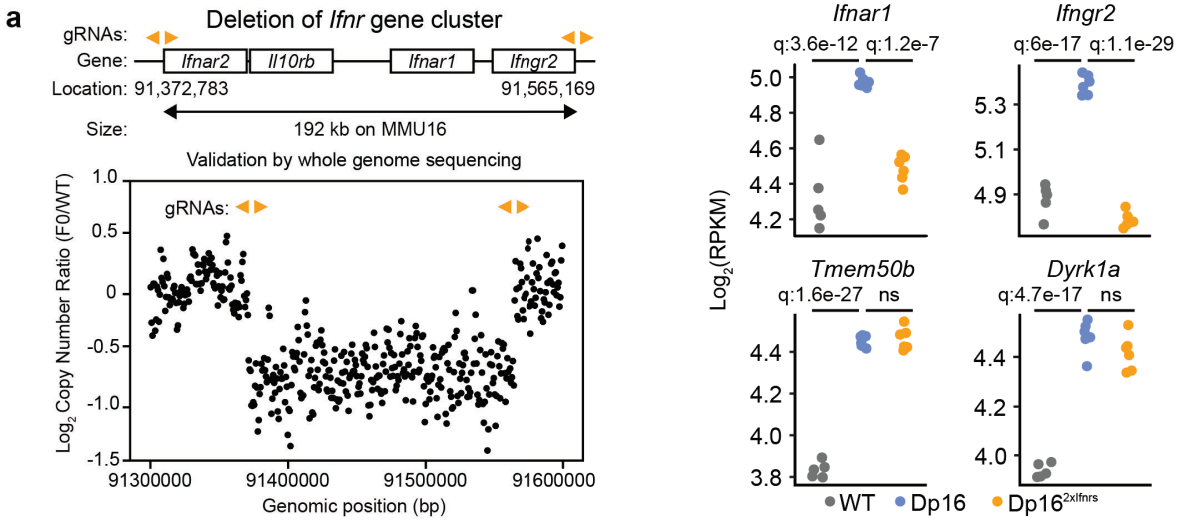
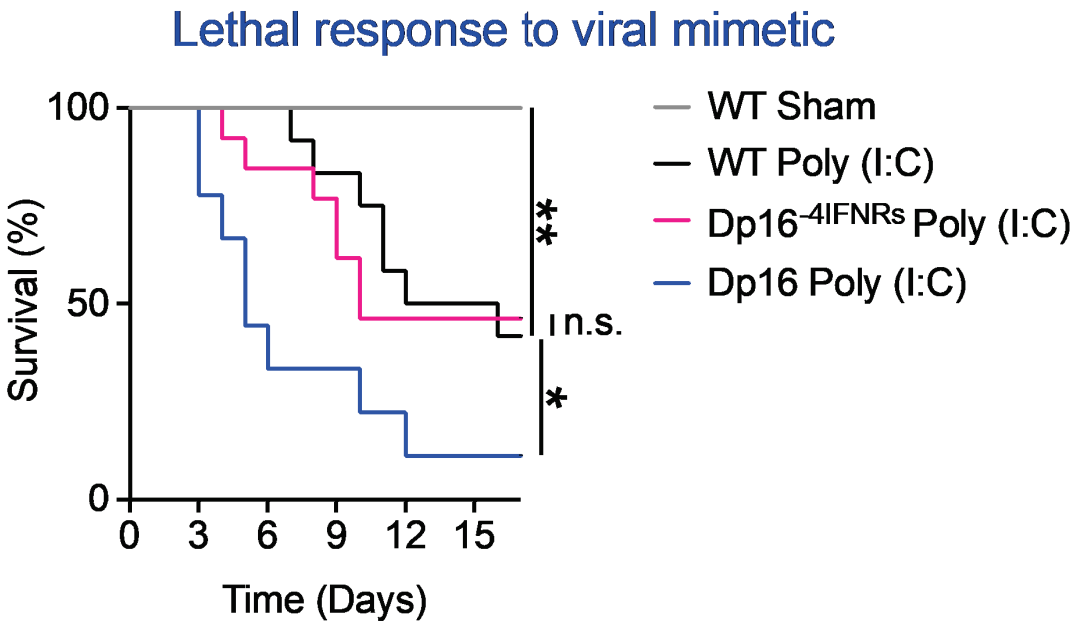


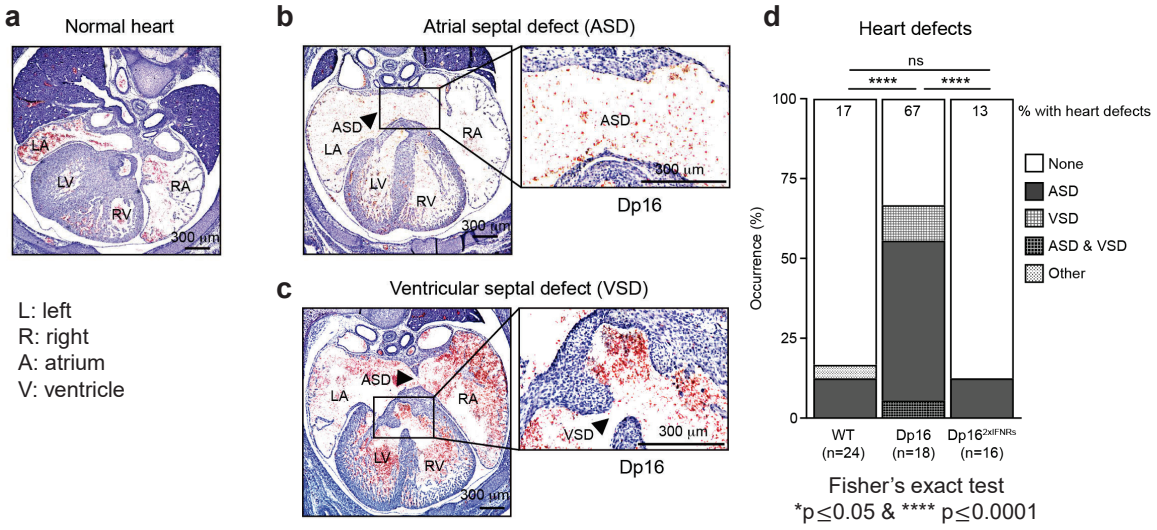
Through various crosses in the vivarium, we can now monitor the impact of reducing interferon receptor copy number from 3 copies (Down syndrome) to 2 copies (the correct number)

Normalization of IFNR copy number rescues poly(I:C) hypersensitivity



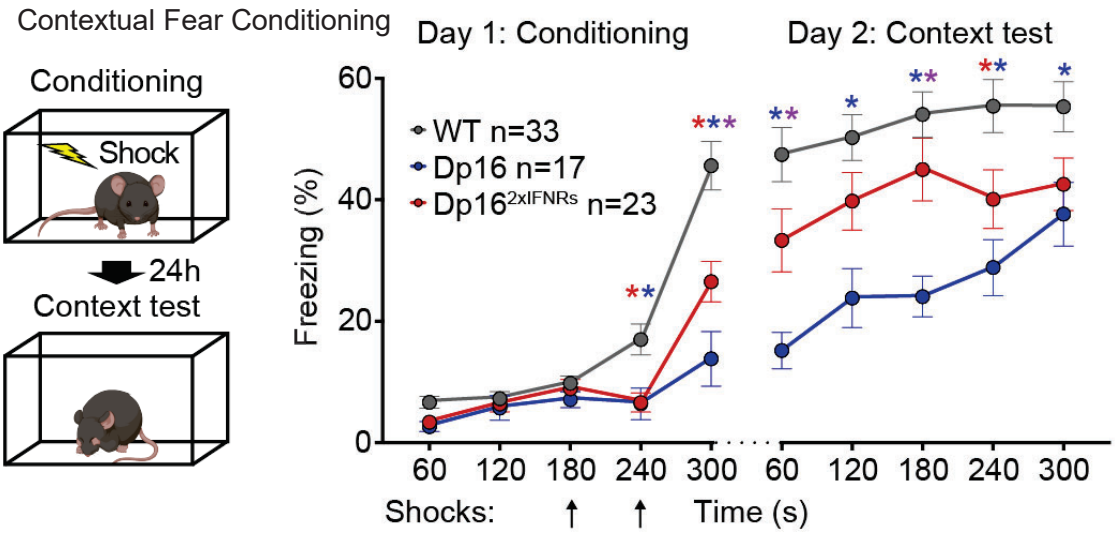
Waugh et al., *Nature Genetics*, 2023

Reduction of *Ifnr* gene dose normalizes prevalence of heart malformations in Dp16 E15.5 embryos



Waugh et al., *Nature Genetics*, 2023

Normalization of deficits in learning and memory in adult Dp16 mice with corrected *Ifnr* gene dose

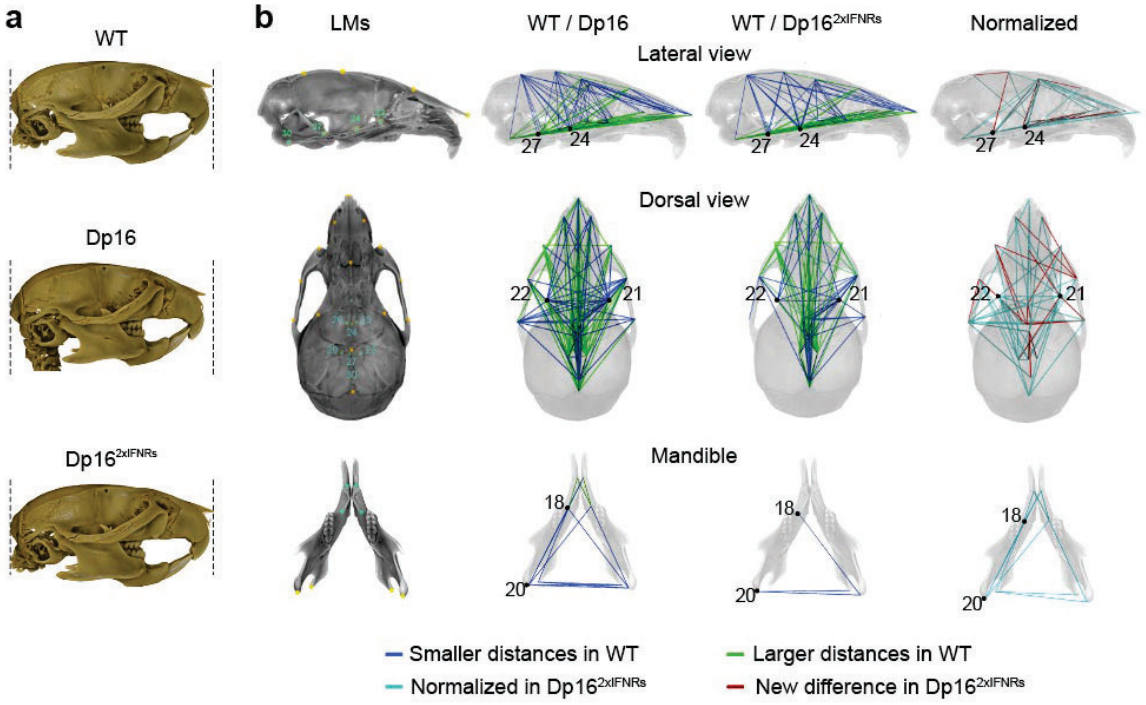


Repeated measures two-way ANOVA with post-hoc Tukey's HSD

*p≤0.05, Dp16 v WT *p≤0.05, Dp16 2xIFNRs v WT *p≤0.05, Dp16 v Dp16 2xIFNRs

Waugh et al., *Nature Genetics*, 2023

Normalization of *Ifnr* gene dose ameliorates craniofacial abnormalities in Dp16 mice



Waugh et al., *Nature Genetics*, 2023

Summary II

Normalization of IFN receptor copy number from 3 back to 2 rescues in whole or in part:

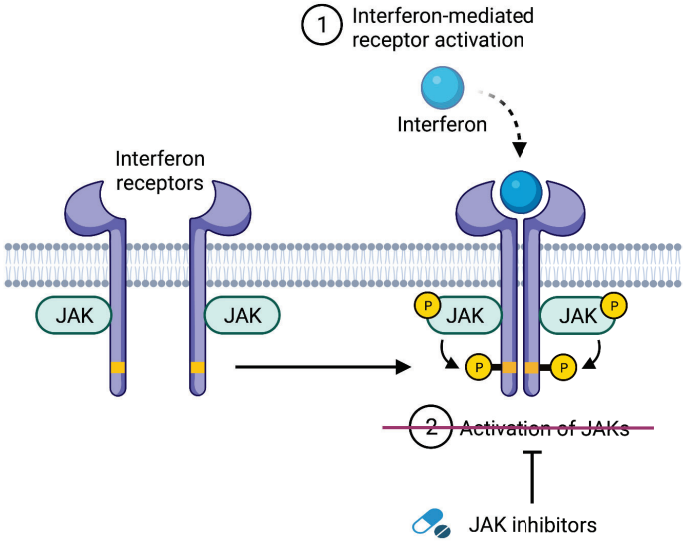
- changes in gene expression across tissues
- dysregulated antiviral response
- congenital heart defects
- developmental delays
- cognitive deficits
- craniofacial patterning

so…

Waugh et al., *Nature Genetics*, 2023

Is there a way to intervene pharmacologically?

JAK inhibitors could attenuate the ill effects of interferon receptor triplication

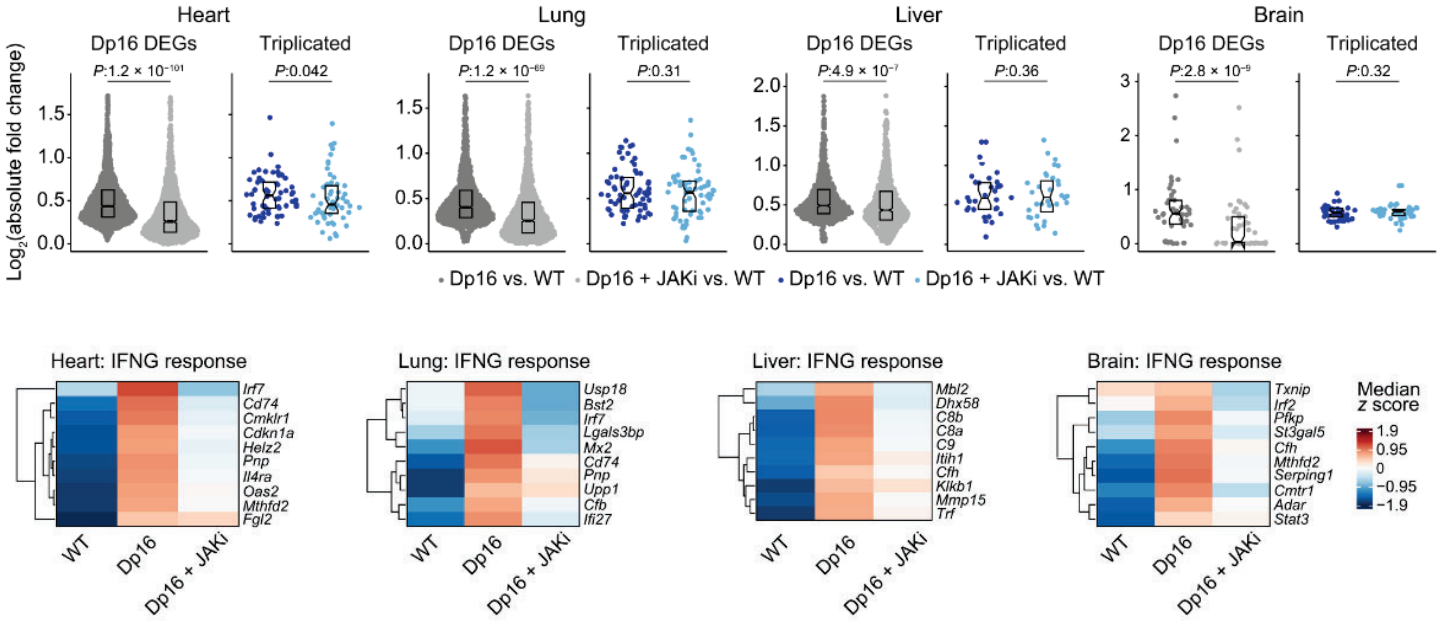


JAK inhibitors are small molecules designed to inhibit the JAK enzymes acting ‘downstream’ of the interferon receptors.

JAK inhibitors are taken daily orally as pills and have a short ‘half-life’ in the body.

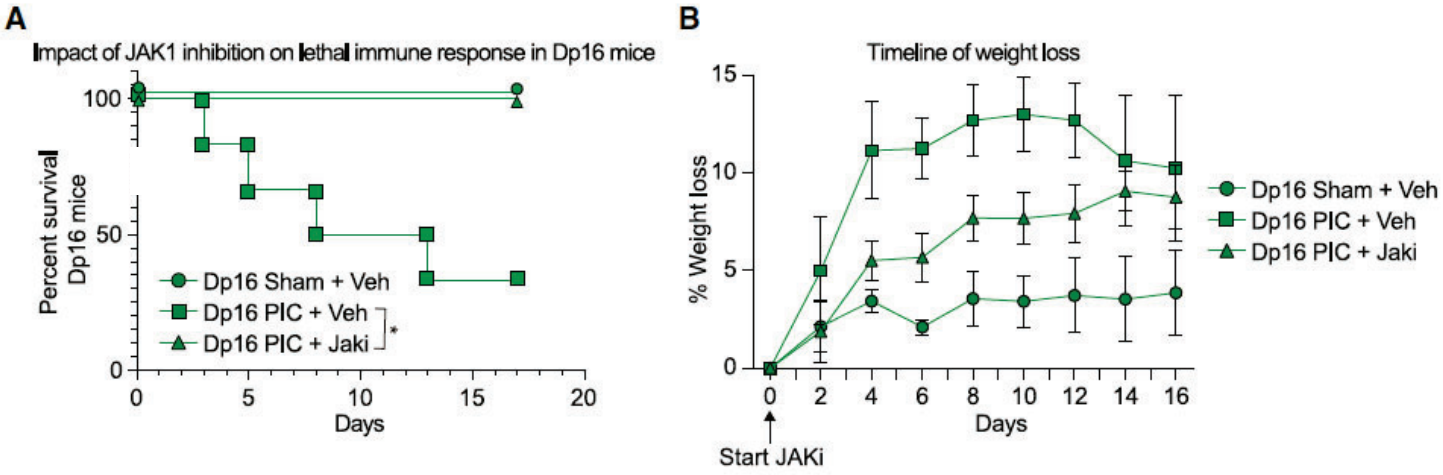
The action of JAK inhibitors is fully reversible, as they are rapidly cleared from the human body within hours.

JAK inhibition ameliorates gene expression changes across tissues in Dp16 mice



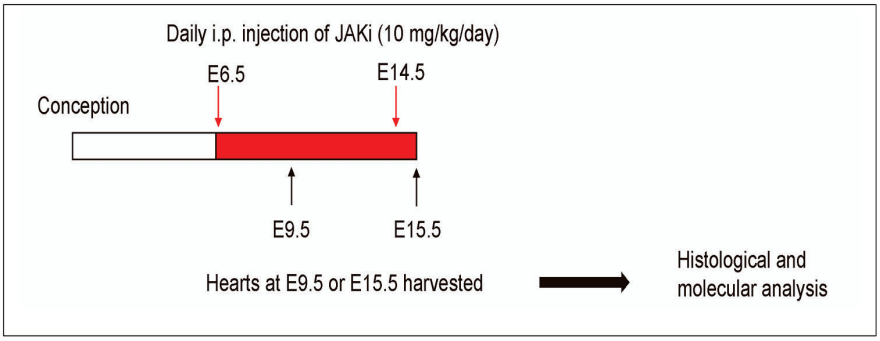
Galbraith et al., *Science Advances*, 2023

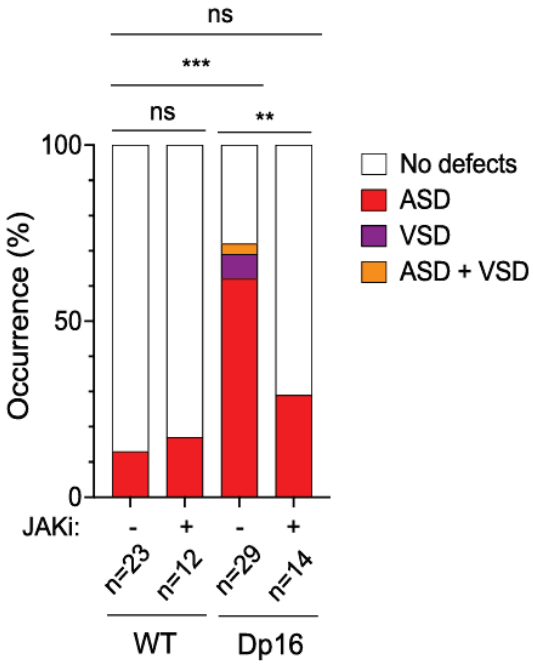
JAK inhibition blocks lethal immune hypersensitivity



Tuttle et al., *Cell Reports*, 2020

In utero JAK inhibition rescues congenital heart defects





iScience

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Article

Interferon hyperactivity impairs cardiogenesis in Down syndrome via downregulation of canonical Wnt signaling

Congwu Chi,[1,2,3] Walter E. Knight,[1,4] Andrew S. Riching,[1,4] Zhen Zhang,[1] Roubina Tatavosian,[2,5] Yonghua Zhuang,[6] Radu Moldovan,[5] Angela L. Rachubinski,[2,6] Dexiang Gao,[6] Hongyan Xu,[7] Joaquin M. Espinosa,[2,5] and Kunhua Song[1,2,3,4,8,*]

Would drugs that decrease the interferon response improve the health of persons with Down syndrome?

Approved therapies that decrease the interferon response: JAK inhibitors

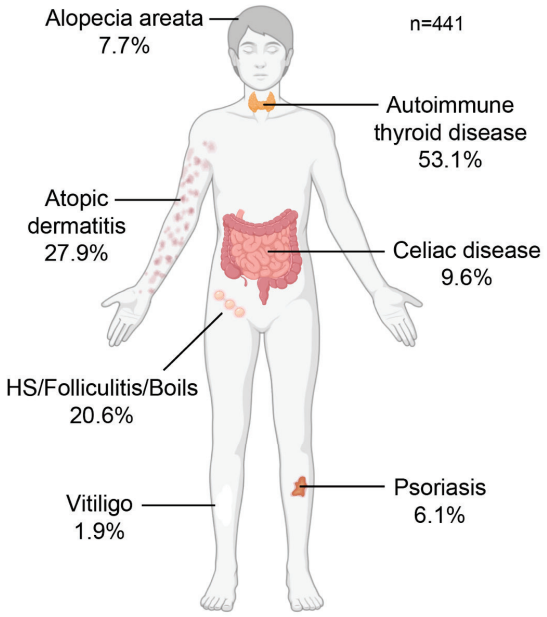
Target	JAK1/3	JAK1/2	JAK1	JAK1/2	JAK1
Rheumatoid arthritis	+	+	+		
Psoriatic arthritis	+		+		
Polyarticular course JIA	+				
Ulcerative colitis	+		+		
Atopic dermatitis			+		+
COVID-19		+			
Alopecia areata		+			
Chron's disease			+		
Polycythemia vera				+	
Ankylosing spondylitis			+		
Myelofibrosis				+	
GVHD				+	
Axial spondylarthritis			+		

There are many JAK inhibitors approved for many different indications.

These medicines are used by rheumatologists, dermatologists, gastroenterologists, hematologists and more!

Could JAK inhibitors 'normalize' the immune system in Down syndrome?

Key observation: widespread autoimmunity in Down syndrome



Rachubinski et al, eLIFE 2024

~75% of adults with Down syndrome have been diagnosed with at least one autoimmune condition

>50% of people with Down syndrome have autoimmune thyroid disease (AITD), leading to **hypo**thyroidism or **hyper**thyroidism

>35% adults with Down syndrome have been diagnosed with one or more autoimmune skin conditions

~10% of adults with Down syndrome have been diagnosed with celiac disease

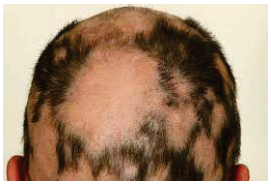
Type I diabetes, 'Down syndrome arthropathy', and other, more rare autoimmune conditions, are also more common

>80% of these autoimmune conditions are diagnosed during pediatric age.

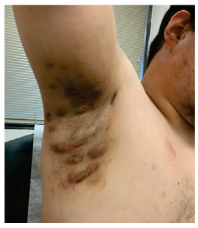
First clinical trial for JAK inhibition in Down syndrome

Treating five immune skin conditions in one trial

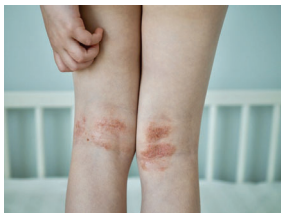
Alopecia areata (patchy hair loss)



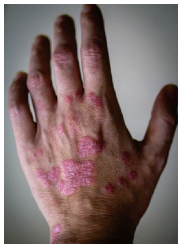
Hidradenitis suppurativa (boils)



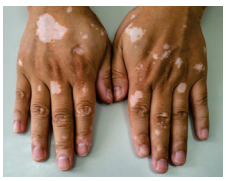
Atopic dermatitis (eczema)



Psoriasis



Vitiligo



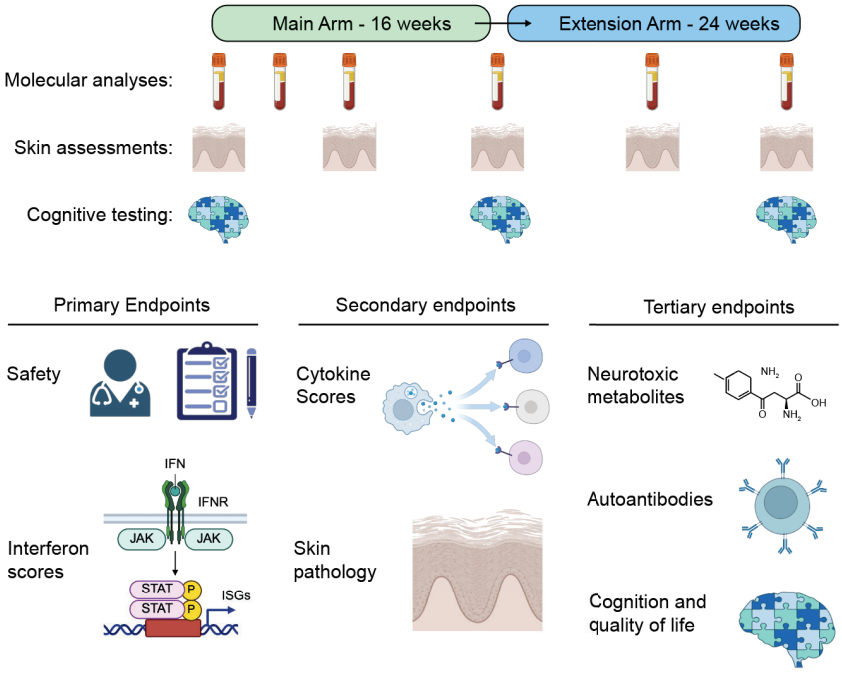
All five conditions are more common in people with Down syndrome

More than 35% of adults with Down syndrome have been affected by one of these conditions

4-9 months of treatment with the FDA-approved JAK inhibitor Tofacitinib (Xeljanz)

Funded by: NIH THE INCLUDE PROJECT NIH National Institute of Arthritis and Musculoskeletal and Skin Diseases

First clinical trial for JAK inhibition in Down syndrome



- ✓ Phase II, open label
- ✓ Ages 12-50
- ✓ 40 participants completing 16 weeks of treatment
- ✓ JAK inhibitor: tofacitinib

Key endpoints:

- Safety
- Immune markers
- Skin pathology
- Cognition

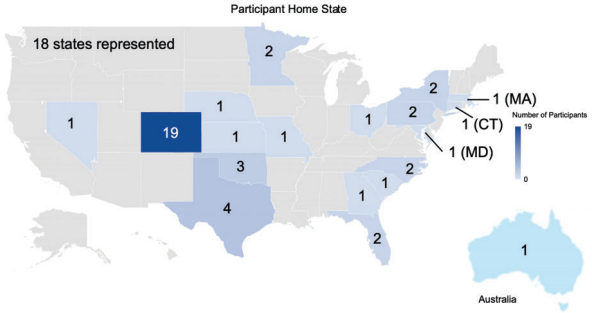
First clinical trial for JAK inhibition in Down syndrome

Top metrics:

Half males, half females

25% Hispanics | 15% Black or biracial

60% from outside of Colorado



Most common qualifying conditions:

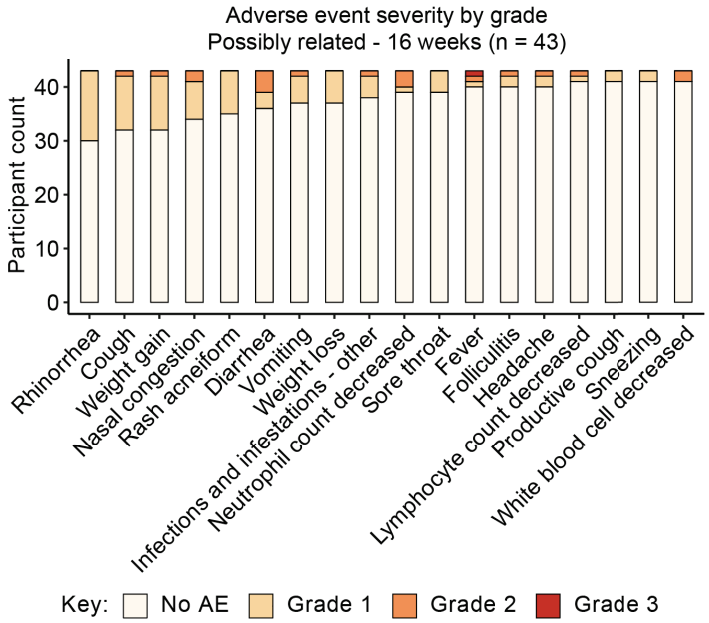
1. Hidradenitis suppurativa
2. Alopecia areata
3. Psoriasis

THE INCLUDE PROJECT

NIH National Institute of Arthritis and Musculoskeletal and Skin Diseases

JAK inhibition is safe in Down syndrome

Safety endpoint was met!



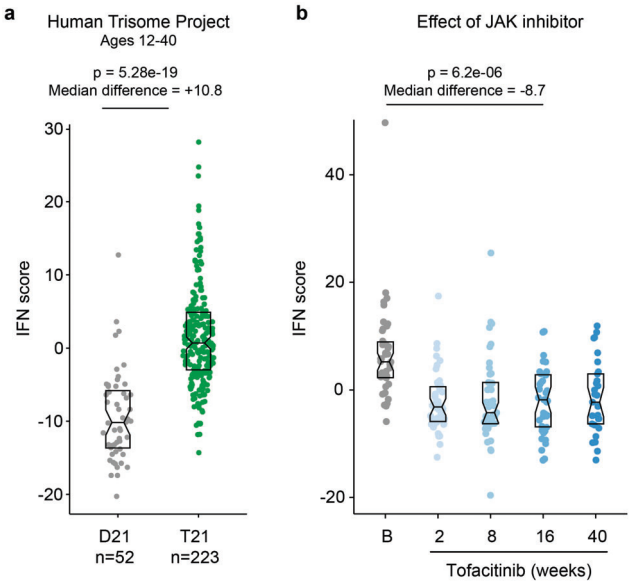
Safety profile reminiscent of that observed in the general population:

- Symptoms of upper respiratory infection
- Weight change
- Skin rash
- Mild clinical lab abnormalities

A single serious adverse event documented over 27.7 years of observation: an episode of thromboembolism in a participant taking oral contraceptive pills, which are known to increase risk of thromboembolism. Participant recovered favorably.

JAK inhibition reduces interferon scores and other biomarkers of autoinflammation

Normalization of IFN scores without overt immune suppression



D21: euploid controls T21: trisomy 21 B: baseline

The JAK inhibitor reduces IFN scores down to the range observed in the general population, not any lower.

The JAK inhibitor also reduces cytokine scores and kynurenine pathway metabolites.

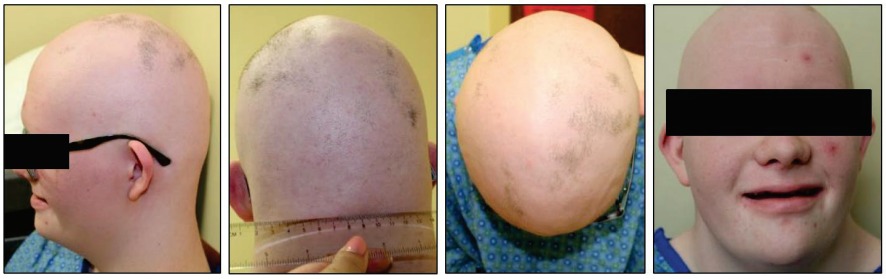
Endpoints met!

Galbraith et al, *Science Advances* 2023
Rachubinski et al, *eLIFE* 2024

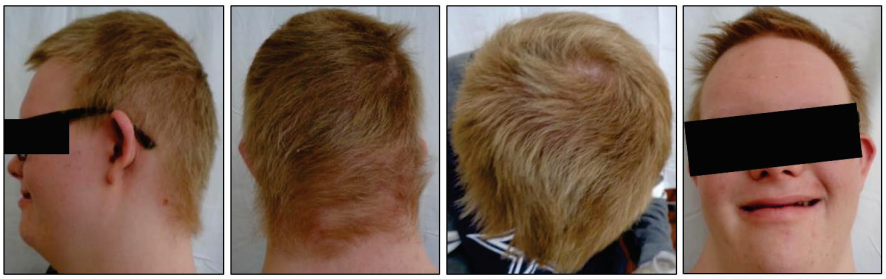
JAK inhibition can be safely used for alopecia areata

When a picture is worth a thousand words

Baseline
SALT = 86



Week 16
SALT = 4

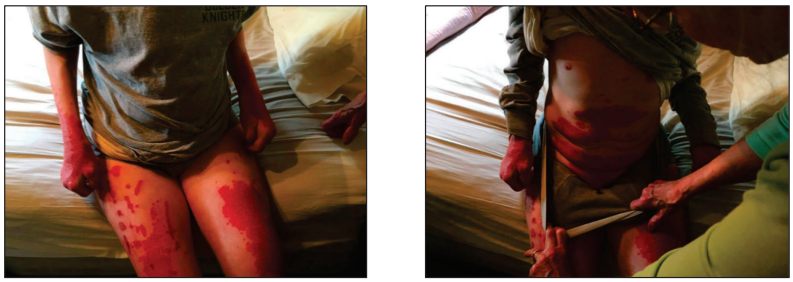


Participant referred known as ‘Ed Sheeran’ to the research team

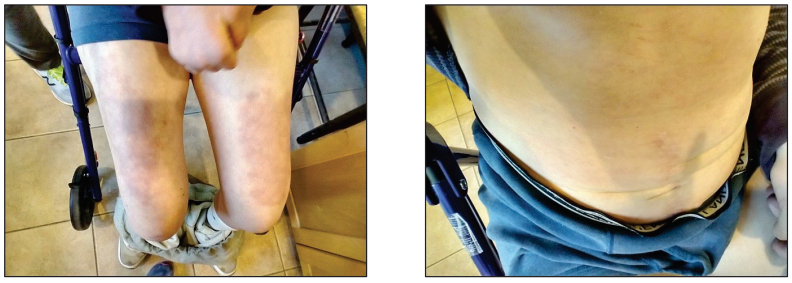
JAK inhibition can be safely used for psoriasis

When a picture is worth a thousand words

Before



After



Participant monitored outside of the trial at the University of Vermont Medical Center, pictures courtesy of Dr. Ralph Budd

Summary III

- DS can be understood, in part, as an interferonopathy
- Genetic normalization of IFNR copy number moderates hallmarks of DS
- JAK inhibition improves autoimmune conditions in DS

Outstanding questions

- What is the long-term safety profile of JAK inhibition in people with Down syndrome?
- What are all the possible benefits of immunomodulation in Down syndrome?
- How early could treatment start? Is pre-natal treatment even possible?
- Should everyone with Down syndrome be treated or only those with clinically evident autoimmunity?

Acknowledgements

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Ross Minter, MS
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Hannah Lewis, PhD
Joe Cabral, PhD
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Ahwan Pandey, MS
Santosh Khanal, MS

THE INCLUDE PROJECT

NIH National Institute on Deafness and Other Communication Disorders

NIH National Institute of Arthritis and Musculoskeletal and Skin Diseases

NIH National Institute of Neurological Disorders and Stroke

NIH National Institute of Allergy and Infectious Diseases

boettcher FOUNDATION

GLOBAL DOWN SYNDROME FOUNDATION

ANNA & JOHN J. SIE FOUNDATION

All of the individuals with Down syndrome that share their perspectives, time, energy, and samples with us!

School of Medicine
UNIVERSITY OF COLORADO ANSCHUTZ MEDICAL CAMPUS