Understanding Down syndrome as an Interferonopathy

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Individuals with Trisomy 21 have a different ‘disease spectrum’

Heart Disease
Cancer
Stroke
Coronary Artery Disease
Atherosclerosis
Hypertension
Angiopathies (e.g. diabetic retinopathies)

Alzheimer’s
Diabetes
Leukemias
Autism
Epilepsy
Congenital Heart Defects
Autoimmune Disorders (e.g. T1D, Celiac Disease, Thyroid Dysfunction, Vitiligo) and more...

The >400,000 Americans with trisomy 21 may hold solutions to major medical conditions
Each one of them is dealing with trisomy 21 in their own unique, personal way.

They are more awesome than different, yet they are **ALL** different.
Project Goal
To identify the **consistent** molecular events activated by trisomy 21

Gene >20,000

Cell | Genome (DNA) | DNA | Chromosome

transcription | RNA | translation | Protein
Project Goal
To identify key signaling pathways \textbf{consistently} activated by trisomy 21

What's the blue circle?
The ideal scenario

In the ideal scenario, the ill effects of trisomy 21 are caused by one, or a few, molecular pathways that can be blocked with FDA-approved drugs.
Approach

Functional genomics approaches to elucidate the molecular events activated by trisomy 21

Measurements performed:
- >16,000 RNAs
- 600 metabolites
- 654 kinases (important enzymes)
- >4,000 proteins

Cell types employed:
- Skin Fibroblasts Cell Lines
- Lymphoblastoids Cell Lines (B cells)
- Circulating Monocytes
- Circulating T cells
First experiment
To measure thousands of RNAs from skin cells

Cell → Genome (DNA) → Chromosome → DNA

Gene >20,000 → transcription → RNA → translation → Protein
Employing highly diverse pools of skin fibroblasts to discover **consistent** effects caused by trisomy 21

**Disomic Cell Lines (typicals, D21)**

- GM08447
- GM05659
- GM00969
- GM02036
- GM03377
- GM03440

**Trisomic Cell Lines (T21)**

- GM04616
- AG05397
- AG06922
- GM02767
- AG08941
- AG08942

Different ages, gender and biopsy sites

**Protocol:** grow cells in the lab, extract their RNA, then measure >16,000 RNAs with *RNA-seq* technology
The power of inter-individual variation

Two Disomic Males

John 20 years old → Pete 19 years old

Control Grouping

Group 1
Group 2

Up in John

MA plots

Significant, FDR < 10%
Trisomy 21 causes consistent changes in RNA abundance

T21 vs. typical

MA plots

Significant, FDR<10%
Trisomy 21 causes consistent changes in RNA expression that withstand variations in age, gender and site of biopsy.

T21 vs. typical

Only 12% can be accounted by increased gene dosage due to the trisomy.

What drives the remaining 88%?
Trisomy 21 causes a consistent gene expression signature (even outside of chr21) that withstands age, gender and site of biopsy…

Signal amplification across the genome

T21 vs. typical

Manhattan plot
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 plots**

Significance: -  —  —  —  —  +  —  Significant FDR<10%
What is the signal amplifier?

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

Advanced bioinformatics analysis (i.e. computational biology)
Upstream regulator analysis identifies the Interferon pathway (IFN) as the key mediator of the gene expression changes caused by trisomy 21
Interferon

From Wikipedia, the free encyclopedia

Interferons (IFNs) are a group of signaling proteins[^1] made and released by host cells in response to the presence of several pathogens, such as viruses, bacteria, parasites, and also tumor cells. In a typical scenario, a virus-infected cell will release interferons causing nearby cells to heighten their anti-viral defenses.

IFNs belong to the large class of proteins known as cytokines, molecules used for communication between cells to trigger the protective defenses of the immune system that help eradicate pathogens.[^2] Interferons are named for their ability to "interfere" with viral replication[^2] by protecting cells from virus infections. IFNs also have various other functions: they activate immune cells, such as natural killer cells and macrophages; they increase host defenses by up-regulating antigen presentation by virtue of increasing the expression of major histocompatibility complex (MHC) antigens. Certain symptoms of infections, such as fever, muscle pain and "flu-like symptoms", are also caused by the production of IFNs and other cytokines.

[^1]: [1]
[^2]: [2]
Interferons are key components of the innate immune system

Interferon signals neighboring cells to destroy RNA and reduce protein synthesis

Interferon signals neighboring infected cells to commit ‘suicide’ (apoptosis)

Interferon activates immune cells
Three types of Interferon

Ligands
- Type-I IFNB1/A1/A2
- Type-II IFNG
- Type-III IFNLI

Receptors
- IFNAR1
- IFNAR2
- IFNGR1
- IFNGR2
- IFNL1R1
- IL10RB

Protein Kinases
- JAK1, JAK2, TYK2

Transcription Factors
- IRFs and STATs

Interferon Stimulated Genes (ISGs)
- APP
- TMEM50B
- MX1
- MX2
- IFIT2
- CMPK2
- IL1A
- MYH6
- IDO1
- IFI27
- IFI44L
- RSAD2
- OAS1
- IFITM1
- TNFSF10
- HERC6
- OAS2
- BST2
- IFI6
- ISG15

These genes are **consistently** activated in trisomy 21 cells

Why are **Interferon Stimulated Genes** activated in trisomy 21 cells?
4 out of 6 IFN receptors are encoded on chr21!

Ligands
Type-I IFNB1/A1/A2
Type-II IFNG
Type-III IFNLI

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IFNAR2
IFNGR1
IFNGR2
IFNLR1
IL10RB

Protein Kinases
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Transcription Factors
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Genes encoded on chromosome 21
4 out of 6 Interferon receptors are encoded on chr21 and ‘induced’

Type-I
IFNB1/A1/A2

Type-II
IFNG

Type-III
IFNLI

IFNAR1
IFNAR2
IFNGR1
IFNGR2
IFNLR1
IL10RB

Upstream Regulators
T21 vs D21 DEG
chr21 Encoded DEG

IFNAR1
p: 5.03-04

IFNAR2
p: 4.17e-07

IFNGR1
p: 0.01

IFNGR2
p: 6.24e-10

IFNLR1
p: 6.6e-03

IL10RB
p: 8.23e-05

mRNA expression (RPKM)

D21 T21
D21 T21
D21 T21
D21 T21
D21 T21
D21 T21
Trisomy 21 cells show massive induction of Interferon Inducible Genes (ISGs)

IFN receptors

JAK1, JAK2, TYK2

IRFs and STATs

Interferon Stimulated Genes

TBX1: p: 4.3e-10
TNFSF10: p: 0.001
CMPK2: p: 4e-04
IDO1: p: 3.22e-07
IFI27: p: 4.8e-11
APP: p: 6.73e-10
Significant inter-individual variation, even for Interferon Stimulated Genes encoded on chr21

RNA
MX1
p: 4.6e-03

Protein
Western Blot

D21  T21

MX1: Myxovirus Resistance Protein 1
An Interferon Stimulated Gene involved in the antiviral response encoded on chr21
This protein is key to fight off the flu virus
Additional results:

- Trisomy 21 causes **consistent** activation of the Interferon pathway in lymphoblastoid B cells, monocytes and T cells (all immune cells).

- Activation of Interferon Inducible Genes is also observed at the **protein** level

- Activation of Interferon Inducible Genes is also observed in ‘Down syndrome’ mice

- Activation of Interferon Inducible Genes is also observed in **brain samples** from both individuals with Down syndrome and Down syndrome mice
Implications

Down Syndrome could be classified as an Interferonopathy, along with other genetic conditions caused by hyper-activation the Interferon pathway.

The ill effects of Down Syndrome could be ameliorated, even perhaps eliminated, with available inhibitors of the IFN pathway.
• 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
• Hypocomplementemia
• Common skin lesions (e.g. acrosyanosis)
Aicardi-Goutieres Syndrome (AGS)

AGS is a ‘Type I Interferonopathy’

What is an Interferonopathy?

Interferonopathies are a group of genetic disorders characterized by upregulation of the Interferon response.
What if Down syndrome is also an Interferonopathy?

To what degree the increased dosage of 4 Interferon receptors contributes to the various aspects of Down syndrome?
Five lines of evidence from the literature

1. Cells from people with trisomy 21 are hyper-sensitive to Interferons (Tan, Epstein and Ruddle, 1970’s)

2. In mouse models of Down syndrome, treatment with anti-Interferons, or reduction of the copy number for IFN receptors, decreases the ill effects of the trisomy (Dr. Maroun’s work in the 1980’s and 1990’s)

3. If you give typical people Interferons, they start showing symptoms associated with Down syndrome (20 decades of research on the side effects of Interferon treatment).
Five lines of evidence from the literature

4. If a person is born with an extra copy of interferon receptors, but without trisomy 21, they develop Down syndrome (segmental duplication of a short fragment of chr21 reported by Wensfeld-Adams et al, Genomics Medicine 2016)

5. Drugs that block Interferon cure some of the conditions associated with Down syndrome!
Blocking the Interferon pathway with available drugs

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- BST2
- IFI6
- ISG15

Monoclonal Antibodies (e.g. Sifalimumab)
- Decoy Receptors
- Viral IFN-binding Proteins (e.g. B18)

JAK inhibitors
- (e.g. ruxolitinib, taxocitinib)

IDO1 inhibitors
- and a lot more...

Genes encoded on chromosome 21
Alopecia areata, treated with Interferon antagonists

Alopecia Areata (autoimmune hair loss) is one of the many autoimmune conditions more prevalent in people with trisomy 21.

Ruxolitinib: An FDA-approved JAK inhibitor

Clynes et al, Nature Medicine 2014
Future Directions

To fully dissect the role of hyperactive Interferon signaling in the development of Down syndrome.

To define the therapeutic potential of Interferon antagonists to ameliorate, or even perhaps eliminate, the ill effects of trisomy 21.
Credits

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