An Unprecedented and Exciting Down Syndrome Research Discovery Engine – The Crnic Institute Human Trisome Project

Global Down Syndrome Foundation’s Webinar Series

Presenters: Michelle Sie Whitten, President & CEO & Dr. Joaquin Espinosa, PhD

Wednesday, February 7th, 2018
Global Down Syndrome Foundation
A Unique Affiliate Model!

The Global Down Syndrome Foundation is part of a network of affiliate organizations that work closely together on a daily basis to deliver on our mission, vision, values, and goals:

**Global & Affiliates**

- **Global**: was established as a 501(c)3 in 2009 and is “Dedicated to significantly improving the lives of people with Down syndrome through Research, Medical Care, Education, and Advocacy”

- **Affiliates are:**
  - Established with a lead gift from Anna & John J. Sie Foundation
  - Must work closely together to benefit people with Down syndrome
  - Must be self-sustaining financially
The Human Trisome Project

Joaquín M. Espinosa, PhD
Linda Crnic Institute for Down Syndrome
University of Colorado School of Medicine
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.

Our motto:

*Nothing in the study of Down syndrome makes sense except in the light of Personalized Medicine.*
People with Down syndrome have a different ‘disease spectrum’

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

Alzheimer’s
Autoimmunity
Leukemia
Autism, Seizures,
Congenital Heart
Defects
Autoimmune Disorders
(e.g. T1D, Celiac
Disease, Hashimoto’s,
Vitiligo, Rheumatoid
Arthritis) and more...

The ~6 million human beings alive today with trisomy 21 may hold solutions to many major medical conditions
The largest and most comprehensive study of its kind, The Human Trisome Project will help us understand why individuals with Down syndrome (trisomy 21) are protected from some medical conditions, such as cancer, while highly predisposed to others, such as Alzheimer's disease.

This research will serve first and foremost the population with Down syndrome, but also the millions of individuals without Down syndrome who are affected by the many medical conditions modulated by trisomy 21.
Project goals

1. To enable a Precision Medicine approach to Down syndrome.
2. To define how trisomy 21 causes a novel disease spectrum.
3. To develop novel diagnostic and therapeutic tools that will benefit those with trisomy 21, and also millions of typical individuals.
Project goals – short term

1. To massively accelerate the pace of Down syndrome research.
2. To complete the most comprehensive cohort study of a population of individuals with trisomy 21 to date.
3. To create the largest public database for Down syndrome research to date.
4. To create the most comprehensive biobank of biological samples for Down syndrome research.
The Human Trisome Project
Unleashing the Power of Three

Opening the Black Box: The Ten Layers

Digital Phenotypes

Stem Cells and Immortalized Cells

Genome

Chromosome

Epigenome

DNA

Transcription

RNA

Transcriptome

Gene

Translation

Protein

Proteome
Metabolites (e.g. sugars, lipids, aminoacids, neurotransmitters)

Our ‘other genome’
Bloodworks and Immune Phenotype

Characterizing the blood and the immune system with exquisite detail

Functional Genomes

Using molecular scissors (CRISPR-Cas9) to find the genes that matter

Opening the Black Box: The Ten Layers

The Human Trisome Project

Unleashing the Power of Three
A large cohort study with multi-omics datasets, deep clinical data and a matching multi-dimensional biobank

Datasets

Biobank samples

Intermediates

Blood Extraction

Plasma

Monocytes

White blood cells

RBCs

B cells

LCLs

Urine Sample

Kidney Epithelial Progenitor Cells

iPSCs

Saliva and stool

Microbiomes

Digital Phenotypes

Proteomes, Exosomes, Metabolomes, Antibody Profiling

Genomes, Transcriptomes, Epigenomes

Differentiated Cell Types

Functional Genomes

Bloodwork

Metabolomes

LCLs: lymphoblast cell lines, iPSCs: induced pluripotent stem cells, RBCs: red blood cells
One of the largest datasets ever produced for any medical condition

Rosalind
Central Data Repository
5 petabytes 700+ CPUs

Discoveries!
The Power of Multidimensional Datasets

Turning data into discoveries

Digital Phenotypes
Genomes
Transcriptomes
Proteomes
Epigenomes
Functional Genomes
Metabolomes
Microbiomes
Bloodwork
Immune Phenotypes

Digital Phenotypes

The Power of Multidimensional Datasets

Going beyond the blueprint

Barnes  Kahn  Costello  Kechris  Dowell  Stolovitzky

SCHOOL OF MEDICINE
Biomedical Informatics & Personalized Medicine
UNIVERSITY OF COLORADO ANSCHUTZ MEDICAL CAMPUS

Deep Blue Team
Researcher Gateway

One of the largest datasets ever produced for any medical condition

Turning data into discoveries

The Power of Multidimensional Datasets

Going beyond the blueprint

Barnes  Kahn  Costello  Kechris  Dowell  Stolovitzky
The project involves cross-sectional and longitudinal aspects, a large multi-dimensional biobank and eventual participation of external sites.

Original timeline proposed in December 2015

LCLs: lymphoblast cell lines, iPSCs: induced pluripotent stem cells
336 participants consented to date!

HTP00001

October 10, 2016

HTP00300

September 7, 2017
Any cool results yet?
Trisomy 21 consistently activates the interferon response

Everywhere we look, it is clear that trisomy 21 causes increased Interferon signaling
What is interferon signaling?

- Interferon signaling is an important part of the innate immune system.
- Interferon activates many different types of immune cells.
- Interferon signaling shuts down RNA and protein synthesis.
What is interferon signaling?

• The bodies of individuals with Trisomy 21 are constantly fighting and infection that isn’t there

• Long term activation of interferon signaling can contribute to autoimmune disorders

• Individuals with Trisomy 21 may mount stronger immune responses to infection than typicals, with potentially adverse effects
Trisomy 21 activates signaling pathways that affect gene expression throughout the genome.

These signaling pathways are dominated by the ‘Interferon response’
4 of the 6 IFN receptors are encoded on chr21!!

Outside the cell

Cell membrane

Inside the cell

Human chromosome 21

APP

IFN receptors

DYRK1A

DSCAM

200 kb
IFNRs are overexpressed in every cell type we tested.

- **Type-I**
  - IFNB1/A1/A2

- **Type-II**
  - IFNG

- **Type-III**
  - IFNLI

**Chr21 gene**

**Skin fibroblasts (n=12)**

<table>
<thead>
<tr>
<th>Gene</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td>IFNAR1</td>
<td>5.03-04</td>
</tr>
<tr>
<td>IFNAR2</td>
<td>4.17e-07</td>
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<tr>
<td>IFNGR2</td>
<td>6.24e-10</td>
</tr>
<tr>
<td>IL10RB</td>
<td>8.23e-05</td>
</tr>
</tbody>
</table>

**mRNA expression (RPKM)**

- **D21:** euploid
- **T21:** trisomy 21

**RNA-seq**

Skin fibroblasts (n=12)
Trisomy 21 cells show massive induction of Interferon Stimulated Genes (ISGs)

IFNRs

IFNRs

JAK1, JAK2, TYK2

Protein kinases

IRFs and STATs

Transcription Factors

Interferon Stimulated Genes

RNA-seq
Skin fibroblasts (n=12)
D21: euploid  T21: trisomy 21

TBX1
p: 4.3e-10

TNFSF10
p: 0.001

CMPK2
p: 4e-04

IDO1
p: 3.22e-07

IFI27
p: 4.8e-11
What would be the consequence of a chronic Interferon response?
Understanding Down syndrome as an immune disorder

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

Kelly D. Sullivan¹,², Donald Evans¹, Ahwan Pandey¹,², Thomas H. Hraha³, Keith P. Smith¹, Neil Markham¹, Angela L. Rachubinski⁴, Kristine Wolter-Warmerdam⁵, Francis Hickey⁵, Joaquin M. Espinosa¹,²,⁶ & Thomas Blumenthal¹,⁶,⁷
Understanding Down syndrome as an immune disorder

On average, people with Down syndrome have significantly elevated levels of inflammatory proteins

On average, people with Down syndrome have significantly elevated levels of inflammatory proteins
Conclusion:

Down syndrome could be understood, in good measure, as an Interferonopathy.

What is an Interferonopathy?
Interferonopathies are a group of genetic disorders characterized by activation 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:

- Neurological dysfunction
- 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
- 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)
Can drugs that block the Interferon response cure some of the co-morbidities associated with Down syndrome?
Blocking the Interferon response

**Ligands**
- Type-I IFNB1/A1/A2

**Receptors**
- IFNAR1
- IFNAR2
- IFNGR1
- IFNGR2
- IFNLR1
- 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

**Monoclonal Antibodies**
- (e.g. Sifalimumab)

**Decoy Receptors**
- Viral IFN-binding Proteins (e.g. B18R)

**JAK inhibitors**
- (e.g. ruxolitinib, baricitinib)

**IDO1 inhibitors**
- and more…

**Genes encoded on chromosome 21**
Inflammatory proteins elevated in Down syndrome can also be inhibited with existing drugs!

These inflammatory proteins can be inhibited with FDA-approved drugs!
Alopecia areata, treated with JAK inhibitors

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
Conclusions

Down syndrome could be classified as an Interferonopathy, along with other genetic conditions leading to gain-of-function alterations in the IFN pathway.

Many of the ill effects of trisomy 21 could be ameliorated, even perhaps eliminated, with inhibitors of the IFN pathway.
Work ahead

1. To define the impact of immune dysregulation on the various traits of Down syndrome.

2. To test the safety and efficacy of immune therapies for Down syndrome.

Both activities will require a combination of approaches, including animal and human research, and the full spectrum of basic science to clinical trials.
Credits

At Crnic:
Tom Blumenthal
Donnie Evans
Neil Markham
Alex Erckenbeck
Keith Smith
Juana Marmolejo
Angela Rachubinski
Keith Smith
Kate Waugh
Ross Granrath
Eric Butcher
Angela Kirkpatrick
Diane Lim

At the Sie Center:
Fran Hickey and the phenomenal staff at the Sie Center, specially Kristy Wolter-Warmerdam

Proteomics: Team at SomaLogic, specially Tom Hraha

Metabolomics: Rani Powers, Jim Costello, Angelo D’Alessandro, Kirk Hansen

CyTOF: Elena Hsieh and the Flow Cytometry Core Team at Biogen, specially Marc Muskavitch

Espinosalab.org:
Special thanks to Kelly Sullivan!
Amanda Hill, Hannah Lewis, Awhan Pandey
Matthew Galbraith, Zdenek Andrysik, Anna Guarnieri
Joseph Cabral and many many more!

Other collaborators: James DeGregori, Roy Parker, Ken Krauter, Michalis Lionakis, Stephanie James.

Hunt Potter and the Rocky Mountain Alzheimer’s Disease Center.
Funding support

Anna and John J. Sie

Michelle Sie Whitten
President and CEO

Dean John Reilly

GLOBAL DOWN SYNDROME FOUNDATION

School of Medicine
UNIVERSITY OF COLORADO ANSCHUTZ MEDICAL CAMPUS
‘People with Down syndrome are a gift. By studying their biology we can help them and the rest of humankind.’
- Tom Blumenthal

‘Nothing is impossible. The impossible just takes a little longer.’
- Winston Churchill