Immune System Dysregulation in Down Syndrome

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People with Down syndrome have a unique disease spectrum

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.

INCREASED incidence compared to typicals

~5x Coronary artery disease. High blood pressure.

Over 10x Solid Malignancies (except testicular cancer).

DECREASED incidence compared to typicals
We know very little about how trisomy 21 causes Down syndrome.
A pan-omics cohort study with deep clinical metadata and a multidimensional biobank

www.trisome.org

Clinical Data

Immune Phenotyping

Chromosome

DNA

Whole Genome Sequencing

SNP Genotyping

Metabolomics

Gene

transcription

RNA

Transcriptomics

Cytokine Profiling

SOMAscan Proteomics
Trisomy 21 consistently activates the interferon response

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A transcriptional signature indicative of hyperactive Interferon signaling is observed in multiple cell types.

Hyperactive T cells have been implicated in the etiology of:

- Hypothyroidism
- Atopic dermatitis
- Alopecia Areata
- Psoriasis
- Hidradenitis suppurativa
- Vitiligo
- Celiac disease
- Type I diabetes
- Down syndrome arthropathy

D21: typical controls
T21: trisomy 21
Interferon, Interferon, Interferon

Type I
IFN-α, β

IFNAR1
IFNAR2

JAK1
TYK2

Type II
IFN-γ

IFNGR1
IFNGR2

JAK1
JAK2

Type III
IFN-λ

IFNL1
IL10RB

JAK1
TYK2

STATs1-6
IRFs1-5, 7-9

Transcription of Interferon Stimulated Genes
Immune Activation, Antiviral Response
Trisomy 21 activates the Interferon response

People with Down syndrome show a hyperactive ‘Interferon response’

The Interferon response is a key aspect of the innate immune system acting throughout the human body

Exacerbated Interferon signaling is known to cause autoimmunity (e.g. during treatment of chronic HCV infections with IFN-α)

Polymorphisms in components of the IFN pathway are commonly associated with autoimmunity

People with Down syndrome are undergoing ‘systemic sterile inflammation’
Trisomy 21 causes changes in the circulating proteome indicative of chronic autoinflammation

Kelly D. Sullivan1,2, Donald Evans3, Ahwan Pandey1,2, Thomas H. Hraha3, Keith P. Smith4, Neil Markham4, Angela L. Rachubinski4, Kristine Wolter-Warmerdam5, Francis Hickey6, Joaquin M. Espinosa1,2,6 & Thomas Blumenthal1,6,7

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

Rani K. Powers1,2,3, Rachel Culp-Hill4, Michael P. Ludwig1,3, Keith P. Smith1, Katherine A. Waugh1, Ross Minter1, Kathryn D. Tuttle5,1, Hannah C. Lewis1, Angela L. Rachubinski1,5, Ross E. Granrath1, Maria Carmona-Iragui6,7, Rebecca B. Wilkerson4, Darcy E. Kahn1, Molishree Joshi8, Alberto Lleo6, Rafael Blesa6, Juan Fortea6,7, Angelo D'Alessandro1,4, James C. Costello2,3, Kelly D. Sullivan1,3,5,8* & Joaquin M. Espinosa1,3,8,9*

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 Enrquez 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,1,5,8 Christopher C. Porter,8 Kelly D. Sullivan,1,2,5 Elena W. Hsieh,1,4,5 and Joaquin M. Espinosa1,2,3,9,*

Trisomy 21 dysregulates T cell lineages toward an autoimmunity-prone state associated with interferon hyperactivity

Paula Araya1,2, Katherine A. Waugh1, Kelly D. Sullivan1,2,3,4, Nicolás G. Núñez3, Emiliano Roselli4, Keith P. Smith5, Ross E. Granrath3, Angela L. Rachubinski1,2, Belinda Enrquez Estrada3,4, Eric T. Butcher3, Ross Minter3, Kathryn D. Tuttle3, Tullia C. Bruno3,4, Mariana Maccioni1,2, and Joaquin M. Espinosa1,2,3,4,*
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. 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
People with Down syndrome show much elevated levels of IFN-inducible cytokines

MesoScale Discovery assay, 129 subjects, 75 with trisomy 21

Each of these cytokines is induced in circulation by Interferon

Each of these cytokines has been implicated in the progression of autoimmune disorders (and Alzheimer’s disease)
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

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November 2019
Topographic analysis highlights global immune dysregulation among individuals with Trisomy 21.

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

- mDCs
- Monocytes
- DN T cells
- CD8+ T cells
- CD4+ T cells
- CD7+ NK cells
- pDCs
- B cells
- Unidentified

Legend:
- Decreased in T21
- No change
- Increased in T21
Adults with Down syndrome display many alterations in immune cell types consistent with a hyperinflammatory state. These changes have been observed in typical people affected by chronic autoinflammatory conditions.

CD8+ T Cells

NK Cells

Fibrocytes

B cells

Time and time again, these alterations could be linked conceptually to IFN hyperactivity.
Widespread overexpression of IFNRs across the immune system of people with Down syndrome

IFNAR1 surface protein expression (CyTOF)

IFNAR1 protein expression

N=36, 18 with trisomy 21

IFNAR2, IFNGR2 and IL10RB are also overexpressed in cells with trisomy 21
People with Down syndrome are hypersensitive to Interferon stimulation

*Ex vivo* IFNα stimulation of fresh blood samples
STAT phosphorylation measured by CyTOF

Immune cells with trisomy 21 are ‘super-responders’ to Interferon
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. Powers¹,²,³, Rachel Culp-Hill⁴, Michael P. Ludwig¹,³, Keith P. Smith¹, Katherine A. Waugh¹, Ross Minter¹, Kathryn D. Tuttle©¹, Hannah C. Lewis¹, Angela L. Rachubinski¹,⁵, Ross E. Granrath©¹, María Carmona-Iragui⁶,⁷, Rebecca B. Wilkerson⁴, Darcy E. Kahn¹, Molishree Joshi⁸, Alberto Lleo⁶, Rafael Blesa⁶, Juan Fortea⁶,⁷, Angelo D’Alessandro¹,⁴, James C. Costello²,³, Kelly D. Sullivan©¹,³,⁵,⁸ & Joaquin M. Espinosa¹,³,⁸,⁹

November 2019
People with Down syndrome display activation of the kynurenine pathway

Plasma metabolomics measuring 91 metabolites
120 participants, 72 with trisomy 21
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
Is there a way to ‘normalize’ the Interferon response in people with Down syndrome?
FDA-approved therapies that decrease the Interferon response: JAK inhibitors

<table>
<thead>
<tr>
<th>Company</th>
<th>Marketed Name</th>
<th>Target</th>
<th>Indication</th>
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<tr>
<td>Lilly</td>
<td>Olumiant® (baricitinib) tablets</td>
<td>JAK1&amp;2</td>
<td>Rheumatoid arthritis (2018)</td>
</tr>
<tr>
<td>abbvie</td>
<td>RINVOQ® (upadacitinib) tablets</td>
<td>JAK1</td>
<td>Rheumatoid arthritis (2019)</td>
</tr>
</tbody>
</table>

Also tested in clinical trials for conditions more common in people with Down syndrome, including:

- Alopecia areata
- Atopic dermatitis
- Depression
- Hidradenitis suppurativa
- Juvenile idiopathic arthritis
- Leukemia
- Psoriasis
- Vitiligo
Off-label use of the JAKi Tofacitinib for alopecia areata in Down syndrome

Before 2 months 7 months

1 month 2 months 3 months

Rachubinski et al, JADCR 2019
Widespread autoimmunity in Down syndrome

>60% of adults with Down syndrome have been diagnosed with at least one autoimmune condition

~50% of people with Down syndrome display hypothyroidism, attributed to autoimmune thyroid disease (AITD)

~25% 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
JAK inhibition in Down syndrome

Joaquín M. Espinosa, PhD
Executive Director
Linda Crnic Institute for Down Syndrome

Cory A. Dunnick, MD
Clinical Trials Director
Department of Dermatology

David Norris, MD
Chair
Department of Dermatology
JAK inhibition in Down syndrome

• Phase II, single arm, open-label
• 16-week treatment with Tofacitinib and 2-week follow-up
• IND exempted
• Adults with Down syndrome ages 18-60
• 10 participants during R61, additional 30 during R33
• Active autoimmune skin conditions:
  o Alopecia areata
  o Psoriasis
  o Vitiligo
  o Hidradenitis suppurativa
  o Atopic dermatitis

**Aim 1:** Define the safety profile in Down syndrome.
**Aim 2:** Determine the impact on immune dysregulation.
**Aim 3:** Define the impact on immune skin conditions.
**Aim 4:** Characterize impact on cognition and quality of life.
Timeline

Quarterly milestones and status updates

- Study binder materials, regulatory documents, training
- Identify potential participants, recruitment, pre-screening
- Screening and enrollment
- Completion of all study visits for 10 participants
- Data generation and analysis
- Final report for NIH, application for R33 phase

Timeline:

- Y1Q1: Submit study documents for review to KAI
- Y1Q2: Complete training IRB approval Identify participants
- Y1Q3: Begin prescreening Enroll first participant
- Y1Q4: Enroll nine additional participants
- Y2Q1: Earliest participants begin completing study
- Y2Q2: Complete all study visits Begin data generation
- Y2Q3: Complete data generation Submit final report
- Y2Q4: Continue recruitment and pre-screening for R33 phase

Dates:

- Sep 2019: Y1Q1
- Dec 2019: Y1Q2
- Mar 2020: Y1Q3
- Jun 2020: Y1Q4
- Sep 2020: Y2Q1
- Dec 2020: Y2Q2
- Mar 2021: Y2Q3
- Jun 2021: Y2Q4
- Sep 2021: Y2Q4
Credits

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- CDIFund
- School of Medicine
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