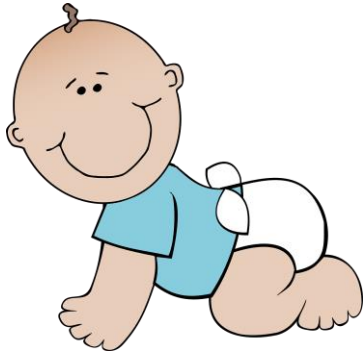


# Predicting the development of autoimmunity in patients with 22q11.2 deletion syndrome using transcriptome analysis and multiplex ELISA assays

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# DiGeorge Syndrome & 22q11.2DS

- DiGeorge Syndrome is a primary immunodeficiency disease characterized by congenital heart disease, hypoparathyroidism, and varying levels of T-cell deficiency
- 35-90% of DiGeorge Syndrome cases are caused by 22q11.2 hemizygous deletions



Distinct facial features  
Congenital heart defect  
Cleft palate  
Hypoparathyroidism  
Kidney abnormalities  
Recurrent infections



Autoimmunity  
Hypothyroid  
Atopy  
Scoliosis  
Developmental delays  
Learning disability  
Psychiatric disorders

Basset et al. J Pediatr. 2011; 159(2):332-9.  
Giardino et al. Blood. 2019; 133(24):2586-2596.  
Jawad et al. J Pediatr. 2001; 139:715-23.  
Sullivan K. Immunological Reviews. 2019; 287:186-201.

# Autoimmunity in 22q11.2DS

- Autoimmunity occurs in 8-9%
- Onset is typically 8 years after 22q11.2DS diagnosis
- ITP is the most common type
- Clinical manifestations vary from patient to patient
- Autoimmune cytopenia significantly correlated with flow cytometry markers indicating a defect in thymic output in a study by Montin et al
- Jawad et al. only found a lower IgG level to be correlated with autoimmunity development
- A precise genotype/phenotype relationship has not been defined



# Transcriptome studies in autoimmune disease

Type of autoimmunity	Upregulated genes	Pathways correlated
<b>Immune thrombocytopenia (ITP)</b>	HLA-DRB5, IGHV3-66, IFI27, FAM212A, PLD5, IFN- $\alpha$ , IFN- $\gamma$ , IL-1 $\beta$ , IL-4	<ul style="list-style-type: none"><li>• Diabetogenesis</li><li>• Intestinal immune network for IgA production</li><li>• Oxidative phosphorylation</li></ul>
<b>Juvenile idiopathic arthritis (JIA)</b>	STAT4, BCL6, STAT3, MHCII, CD74, CD177	<ul style="list-style-type: none"><li>• B cell activation</li></ul>
<b>Inflammatory bowel disease (IBD)</b>	REG1A, REG1B, TLRs, NLRs, DEFA6, IDO1, EXOSC1, CXCLs, MMPs	<ul style="list-style-type: none"><li>• Diabetogenesis</li><li>• Bacterial signals</li><li>• Innate immunity</li><li>• Inflammation</li><li>• Matrix degradation</li></ul>

# Hypotheses

- There are signatures of differential gene expression and corresponding biomarkers in patients with 22q11.2DS and autoimmunity
- Such signatures may overlap with what has been found in patients with autoimmunity without 22q11.2DS
- Whether signatures correlate with flow cytometry or immunoglobulin levels will also be of interest

# Work-flow

## Chart review

- 22q11.2DS patients seen at Duke
- Characterize clinical and laboratory features

## RNA sequencing

- Currently collecting peripheral blood samples for sequencing
- Groups: Healthy, ITP only, 22q11.2DS +/- Autoimmune

## Sequencing analysis

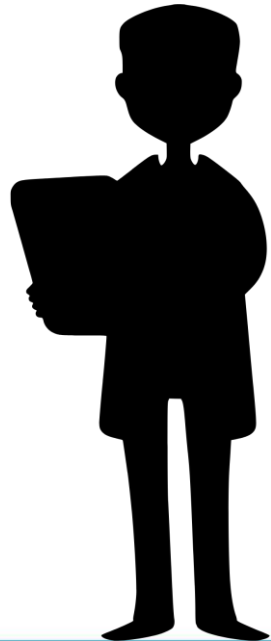
- Gene expression quantification & Differential gene expression
- Pathway analysis

## Validation

- Multiplex ELISA to validate findings of relevant pathways of differentially expressed genes



# Our cohort



Partial DiGeorge Patients (N=51)		
	N	%
Sex		
Males	25	49
Females	26	51
Race		
Caucasian	38	75
African American	6	12
Hispanic	2	4
American Indian	2	4
Mixed/unknown race	3	6
Age		
Mean age at diagnosis of 22q11.2DS in years (range)	3.2 (0-45)	
Mean current age in years (range)	13 (0.4-36)	
Common clinical features		
Characteristic (abnormal) facies	49	96
Developmental delay	46	90
Congenital heart disease	40	78
Psychiatric diagnosis	20	39
Hypoparathyroidism	17	33
Cleft lip/palate	16	31
Recurrent AOM + Tube placement	11	22
Autoimmune diagnosis	13	25
ITP	7	14
Psoriasis	3	6
JIA	2	4
Neutropenia	1	2
Vitiligo	1	2
Raynoud's	1	2

# RNA Sequencing



Collect patient  
peripheral blood  
in tube that  
stabilizes RNA



Isolate RNA  
↓  
Fragment RNA  
↓  
Create cDNA  
↓  
Add adapters

Prepare a library  
for sequencing



Sequence library



Duke Children's



# Sequencing Analysis



Quality control measures (FastQC)



Mapping reads to reference genome (STAR)



Gene expression quantification (HTSeq)



Sample clustering analysis



Differential gene expression analysis



Pathway analysis of differentially expressed genes

# ELISA multiplex

- Bead-based assay using the same basic principles of sandwich immunoassays
- Allows for analysis of multiple samples with multiple markers simultaneously

# Significance

- Identification of genes and pathways involved in autoimmunity in 22q11.2DS patients
- Begin to understand the mechanism of autoimmune development in 22q11.2DS
- Identification of a biomarker(s) to predict autoimmunity development in 22q11.2DS

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  - T32 training grant (AI007062-38)

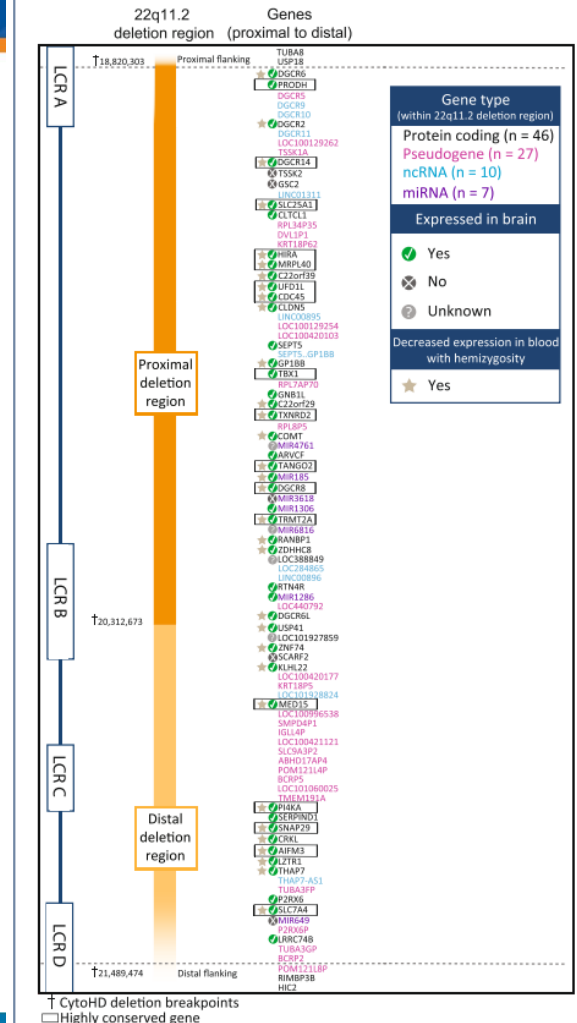
# Extras

# 22q11.2 Deletion Structure

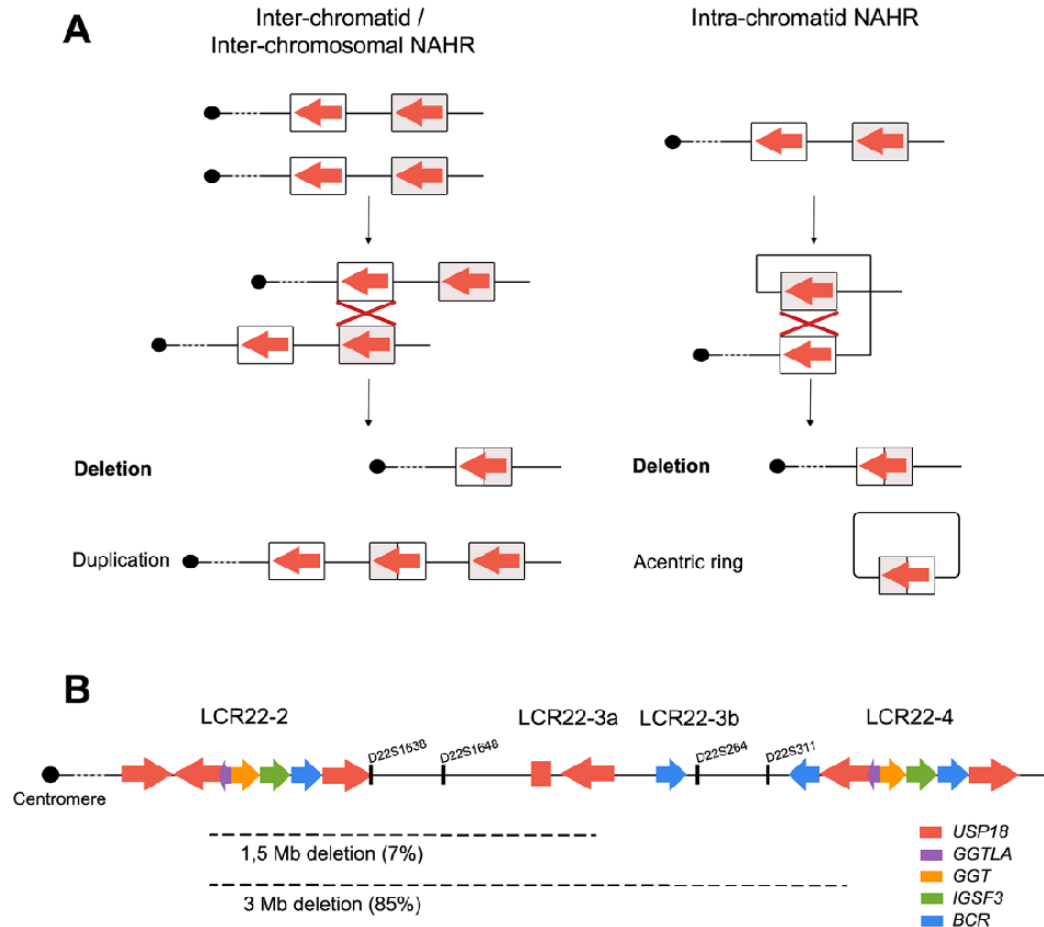
- 85% have hemizygous deletion of 3Mb spanning LCR A-D
  - 45 functional genes
  - Can be detected with FISH
- The remainder have smaller nested deletions
  - Can't be detected with FISH
    - Need microarray testing
  - Similar clinical phenotype

Basset et al. J Pediatr. 2011; 159(2):332-9.

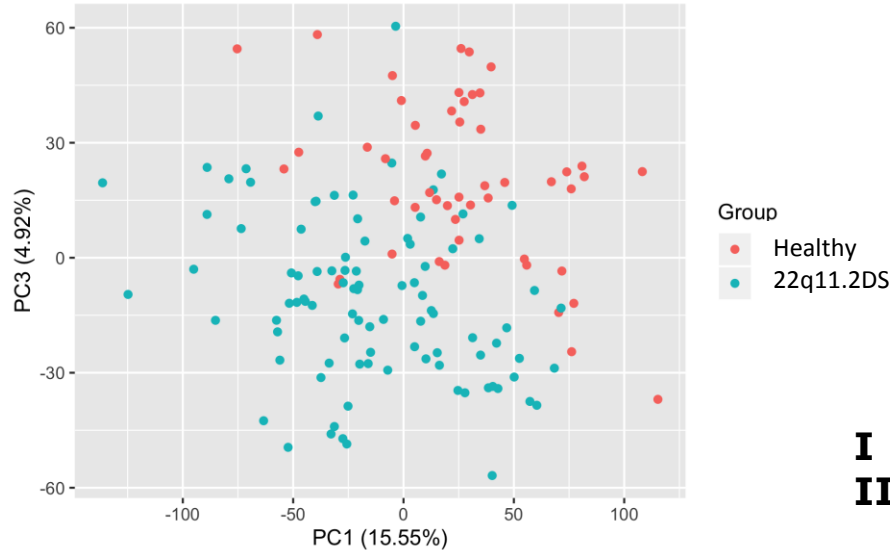
Guna et al. Journal of Neurodevelopmental Disorders (2015) 7:18.



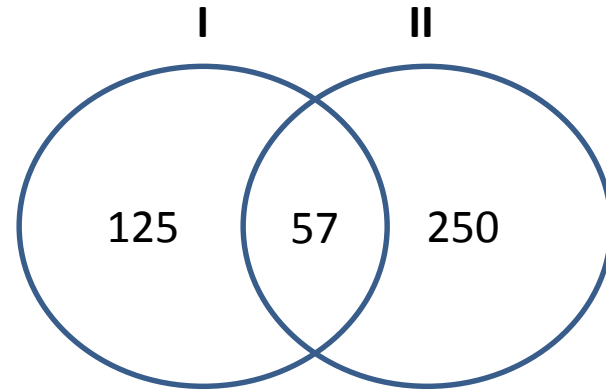
# Genomic rearrangements in the 22q11.2 critical region



# Differential Gene Expression Analysis



PCA plots can help visualize differentially expressed genes in different populations



**I** = healthy vs 22q11.2 without autoimmunity

**II** = healthy vs 22q11.2 with autoimmunity

Venn-Diagrams can help quantify, compare, and contrast differentially expressed genes in different populations



Network analysis of  
signaling events can  
correlate RNA  
expression with  
transcription factors  
and cytokines

