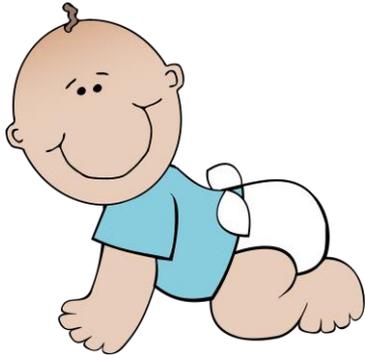


Predicting the development of autoimmunity in patients with 22q11.2 deletion syndrome using transcriptome analysis and multiplex ELISA assays

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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

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
Immune thrombocytopenia (ITP)	HLA-DRB5, IGHV3-66, IFI27, FAM212A, PLD5, IFN- α , IFN- γ , IL-1 β , IL-4	<ul style="list-style-type: none">• Diabetogenesis• Intestinal immune network for IgA production• Oxidative phosphorylation
Juvenile idiopathic arthritis (JIA)	STAT4, BCL6, STAT3, MHCII, CD74, CD177	<ul style="list-style-type: none">• B cell activation
Inflammatory bowel disease (IBD)	REG1A, REG1B, TLRs, NLRs, DEFA6, IDO1, EXOSC1, CXCLs, MMPs	<ul style="list-style-type: none">• Diabetogenesis• Bacterial signals• Innate immunity• Inflammation• Matrix degradation

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

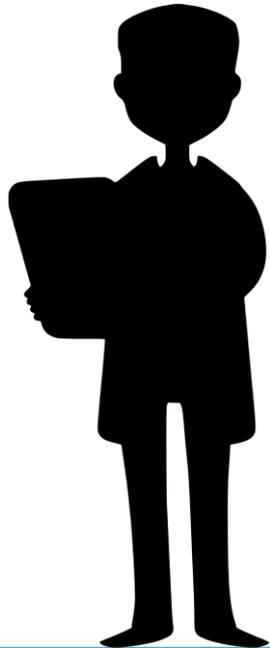
Sequencing analysis

- 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
Raynaud'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



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

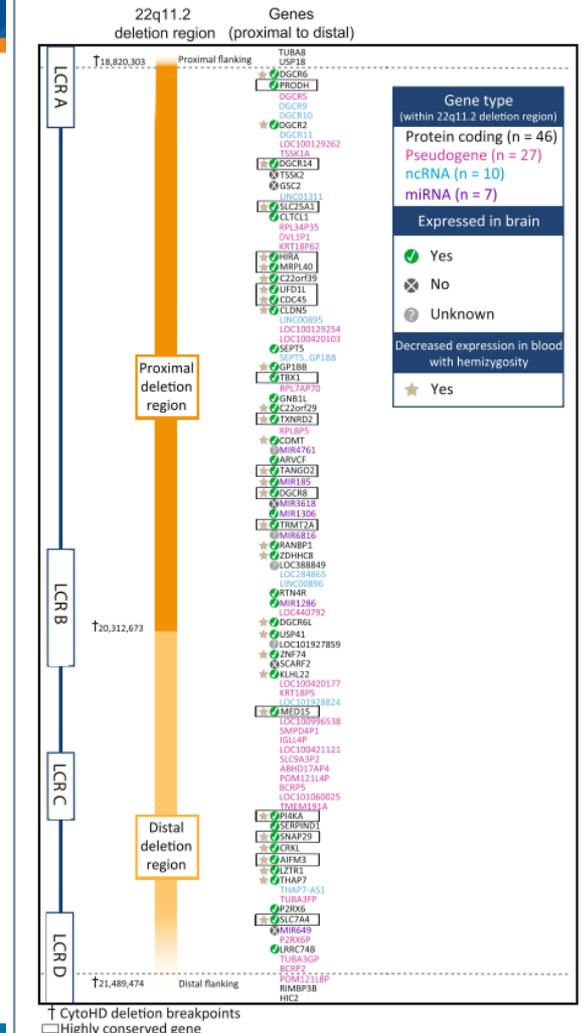
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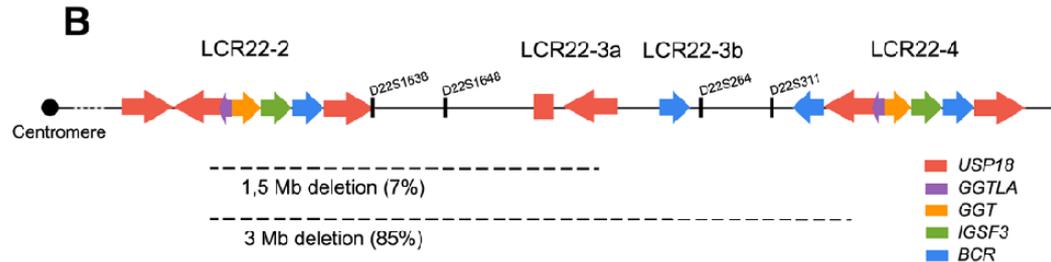
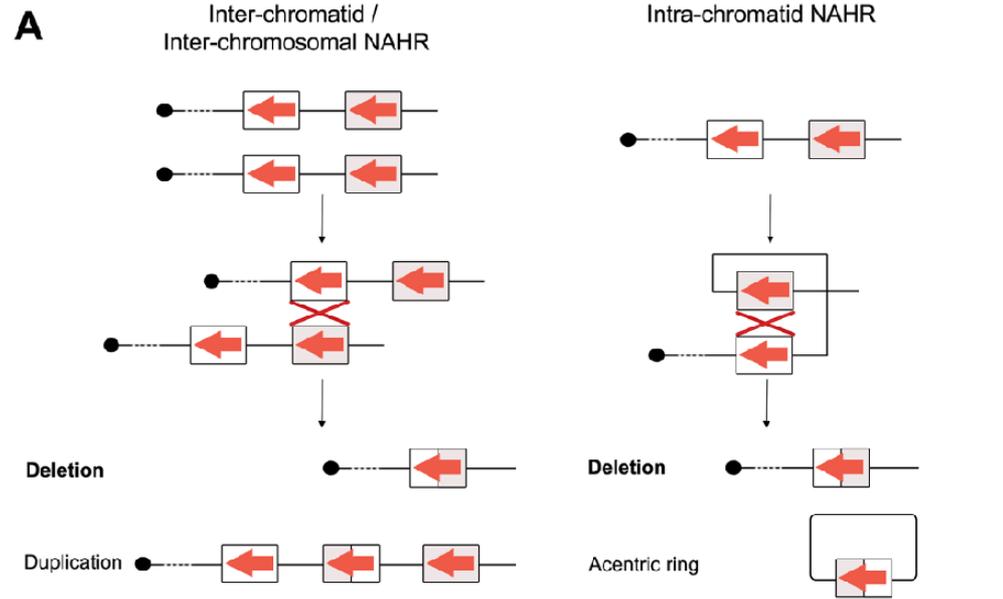
Extras

22q11.2 Deletion Structure

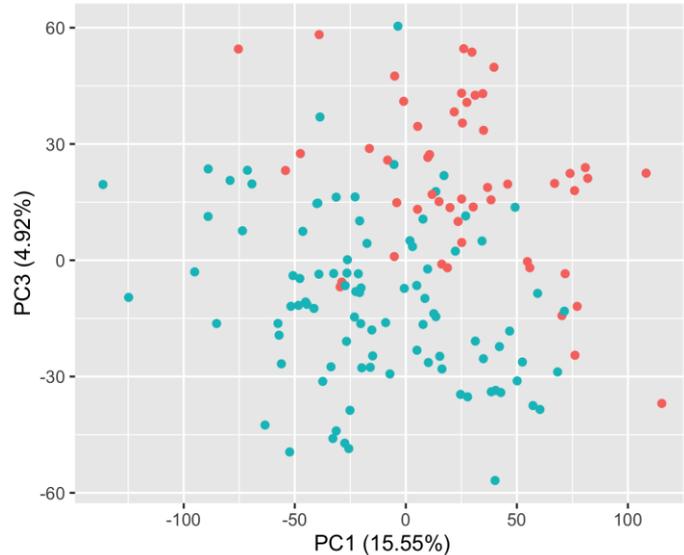
- 85% have hemizygous deletion of 3Mb spanning LCR A-D
 - 45 functional genes
 - Can be detected with FISH
- The remainder have smaller nested deletions
 - Can't be detected with FISH
 - Need microarray testing
 - Similar clinical phenotype



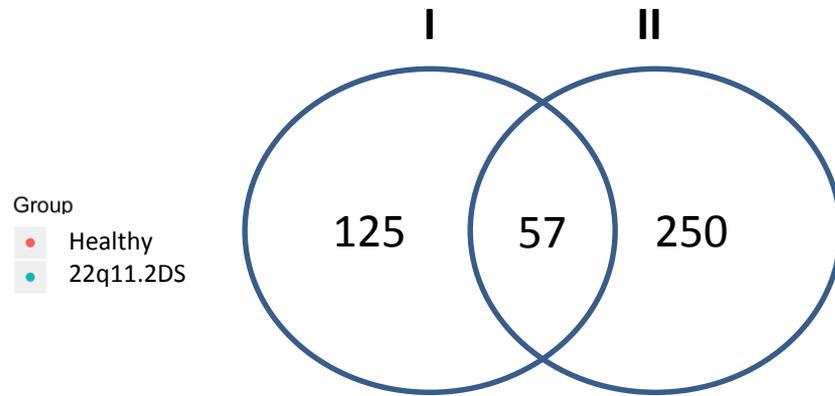
Genomic rearrangements in the 22q11.2 critical region



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

