Novel genetic models of common variable immune deficiency (CVID)

CAGPM Presentation
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Outline

• Defining CVID
• Why CVID research matters
• Defining the genetic basis of CVID through next generation sequencing
• Creation of a humanized mouse model that reflects the genetic heterogeneity of CVID
Defining CVID
Primary immunodeficiency diseases (PIDDs)

• A genetic defect leading to an alternation of the immune system and increased risk of infection

• PIDDs estimated to occur in 1-2% of the population.

• CVID is the most common PIDD (nearly 1/3).


What is CVID?

• Heterogeneous classification of disorders where patients have low antibody levels with poor antibody function leading to an increased risk of infection along with a predisposition to autoimmunity and malignancy development
Genetic basis of CVID

- CVID is a genetic disease
- This is evidenced by the following observations:
  - Disease can be familial
  - Vast majority of patients are diagnosed in the first three decades of life, unlike most chronic diseases

Why CVID research matters
CVID - Mortality

A. Age at death distribution:
- Pediatric: 13, Adult: 4

B. Gender distribution:
- Female: F, Male: M

C. Cause of death:
- Infection: 7, Lung Disease: 1, Liver Disease: 1

D. Number of subjects by condition:
- All Cancer Types, Lymphoma/Leukemia, Autoimmune Disease, Infectious Disease, Gastrointestinal Disease, Lung Disease, Allergy, Depression

E. Odds Ratio:
- All Pediatric, Pediatric Females, Pediatric Males

All data visualizations are color-coded to represent the gender and condition distribution.
CVID – Mental health

- Increased health care utilization regardless of age
- Report lower physical and social functioning than healthy controls
- Have lower mental health scores than age and sex matched diabetic and congestive heart failure patients
- Duke data:

Defining the genetic basis of CVID through next generation sequencing
Next generation sequencing

• Whole exome sequencing has been performed for a total of 219 CVID patients
• 168,433 variants have been identified
• The challenge: determination of which variants are likely to be pathogenic
Exac Frequency Profile for MADCAM1

Considering rare variants Exac freq<0.1, we see that CADD score is correlated with rareness score.
Creation of a humanized mouse model that reflects the genetic heterogeneity of CVID

Three steps:
1. Creation of stem cells
2. Conversion of stem cells to hematopoietic stem cells
3. Transplantation of hematopoietic stem cells into immune deficient mice
Creation of stem cells

- PBMC proliferation
- Nucleofection (Plasmid contains Oct3/4, Sox2, Klf4, c-Myc)
- iPSC growth
- Cell staining for pluripotency markers
- Karyotyping
- Sanger Sequencing
- Teratoma
iPSC Growth & Staining

Day 11  Day 12  Day 13

Day 14  Day 15  Day 16

DAPI

SSEA4

Tra-1-60

NANOG

SOX2
Cartilage
Pigmented cells
Adipose (fat)
Glandular tissue
Muscle
Red blood cells
Epithelium
Cartilage
Adipose (fat)
Glandular tissue
Early bone?
Creation of hematopoietic stem cells

iPSC proliferation

Transfection with RUNX1, ERG, HOXA9, HOXA5, & LCOR

Subcutaneous injection of cells into mouse

Evaluate for creation of bone marrow niche within generated teratoma
Upcoming work: Transplantation of hematopoietic stem cells into immune deficient mice

- iHSC proliferation
- Transplantation into liver of 1-2 week old immune deficient mouse
- Examine immune system
- Evaluate response to vaccines and novel therapies
Questions?
Improving Clinical Outcomes through Early Identification of Treatment Resistance in Dermatomyositis

Cory Stingl, MD
Disclosures

• Financial: none
• Medication: there are no FDA-approved treatments for dermatomyositis so any mention of treatment is off-label
Overview

• Brief overview of what a pediatric rheumatologist is

• Project 1 – Dermatomyositis
  • Disease overview
  • Present the clinical problem our research plans to address – treatment response early in disease
  • Discuss our approach for addressing this problem

• Project 2 – Aspirin and ticagrelor
  • Predictive signatures of platelet functional response
What is a Pediatric Rheumatologist?

- Specialists in rare autoimmune diseases
  - Prevalence: 300,000 children
- Prevalence of pediatric rheumatologists
  - 300

Image sources:
Knee: https://jacobsuveitisandjourney.blogspot.com/2012/11/remission-is-offically-over.html
Malar rash: https://www.dermnetnz.org/topics/systemic-lupus-erythematosus-images/
What is Dermatomyositis (DM)?

- Incidence of 2-4/million children
  - About 210 children in US per year
- Incidence of 5-10/million adults
- Combined prevalence
  - ? 50/million in US
- Primarily affects
  - (Proximal) muscles
  - Skin

Sources: Rheumatology image library
Dermatol Online J 2009, vol. 15 (2)
JAMA 2011 vol 305 (2) pp. 183-190
What is Dermatomyositis?

- Weakness
  - Trouble with basic activities
    - Brushing hair
    - Walking
  - Falls
  - Difficulty swallowing
  - Difficulty breathing

Sources: understandingmyositis.org
DM and the immune system

Sources: Skelet Muscle, 2013 vol. 3(1) p.13
Outcomes and Treatment of DM

• Historic
  • Rule of thirds

• Current first-line treatments
  • Steroids
  • Methotrexate
  • +/- Intravenous immunoglobulin

• Newer treatments – biologics
  • Rituximab
  • Others under investigation

• Many patients started on treatment by 1st rheumatology visit

Sources: understandingmyositis.org
Why Dermatomyositis

• 25% of newly diagnosed patients do not respond to first-line treatment*

• Cannot identify who will respond to first-line treatment
  • Leads to over and undertreatment
  • Prolonged exposure to steroids -- added morbidity
  • Morbidity and mortality of prolonged disease activity

• Effective second line therapies exist
  • Use is reserved for refractory cases

• Ideal: target the right treatment to each patient at diagnosis

* Lancet, 2016 vol. 387 (10019) 671-678
Why Dermatomyositis

• No validated clinical or research indicators of early treatment responsiveness
• Ann Reed, MD
  • Expert in DM biomarker research
  • Ran one of the only two clinical trials in dermatomyositis -- rituximab
  • Has longitudinal cohorts of patients with dermatomyositis
Why Dermatomyositis

• J Rheumatology, 2017
  • T-cell gene expression
  • At visit 1
    • + correlation with muscle disease activity: RORC (p = 0.042), IL-17F (p = 0.040), GATA3 (p = 0.044), and STAT4 (p < 0.001)
    • + correlation with global disease activity: RORC (p = 0.018) and STAT4 (p = 0.001)
  • At 6 month follow-up visit
    • - correlation with non-muscle disease activity: STAT6 (p = 0.044), IL17-D (p = 0.010), and BCL6 (p = 0.009)
Why Dermatomyositis

• J Rheumatology, 2017
  • T-cell gene expression changes by medication class
    • DMARDs:
      • Increased levels of IL-1β (p = 0.012), STAT3 (p = 0.005), STAT6 (p = 0.001), and STAT5B (p = 0.037),
      • Decreased levels of IFN-γ (p = 0.027), IL-22 (p = 0.044), and IRF4 (p = 0.023)
    • Glucocorticoids
      • Increases in FOXP3 (p = 0.020), IL-23A (p = 0.013), IRF4 (p = 0.013), and TGF-β1 (p = 0.022),
        and decreases in IL-2 (p = 0.017)
Aims

• Aim 1: Determine gene expression signatures that predict 6-month treatment response in a cohort of newly-diagnosed subjects with DM.

• Aim 2: Determine changes in gene expression from visit one to visit two (4 months) that change with treatment response at 6 months

• Aim 3: Determine clinical markers that predict 6-month treatment response in a cohort of newly-diagnosed subjects with DM.
Approach

• The study design is opportunistic and observational in nature
• 3 cohorts of recently diagnosed patients
  • Combined Duke/Mayo Clinic cohort (Aim 1 & 2)
    • Clinical data and samples
    • N=40 subjects, half received some treatment prior to the 1st rheumatology visit
  • Northwestern (Aim 3)
    • Clinical data
    • N=50
• Clinical data
  • Demographics
  • Exam findings
  • Standard clinical laboratory measures
  • Myositis-specific autoantibodies
Approach

• Outcome measure: 2016 ACR/EULAR criteria for minimum, moderate, and major response in DM at the 6 month visit (range 4-8 months)

• Whole blood RNA sequencing
  • 50 bp, single-end reads, targeting 40 million reads/sample
Data Analysis

• Aim 1: Determine gene expression signatures at visit 1 that predict 6-month treatment response in newly-diagnosed subjects with DM.
  • Dimension reduction: principle components analysis
  • Similarity network fusion
  • Pathway analysis

• Aim 2: Determine changes in gene expression from visit one to visit two (4 months) that change with treatment response at 6 months
  • Dimension reduction: principle components analysis
  • Linear mixed effects model
  • Pathway analysis

• Aim 3: Determine clinical markers that predict 6-month treatment response in a cohort of newly-diagnosed subjects with DM.
  • Dimension reduction: principle components analysis
  • Similarity network fusion
Limitations

• Small sample size
• Some patients received steroids or DMARDs before sample collection
  • But reflects our clinical realities
Future Steps

• Two potential cohorts for validation
  • Childhood Arthritis and Rheumatology Research Alliance (CARRA) JDM registry/biobank
  • British DM study group
Project 2 – Gene Expression Signatures in Aspirin and Ticagrelor-Treated Healthy Volunteers

• Premise
  • Numerous indications for anti-platelet therapy
    • Myocardial infarction
    • Stroke
    • Peripheral arterial disease
  • Approximately 5-15% but as high as 30% of patients are “aspirin-resistant*”
  • Predictors of bleeding risk are unknown

Source: Saulingo https://commons.wikimedia.org/wiki/File:Aspirine_macro_shot.jpg
*Expert Rev Neurother. 2011 February; 11(2): 251–263
Plaque rupture: Int. J. Mol. Sci. 2016, 17(9), 1511
Design

- 58 Healthy volunteers
- Crossover trial
  - Each volunteer gets 35 days of aspirin (or ticagrelor), a 28 day washout, and 35 days of ticagrelor (aspirin)
- Platelet function: assessed with platelet function score (PFS)
  - Light transmission aggregometry using platelet agonists
    - Collagen, arachidonic acid, ADP, epinephrine
  - Platelet function assay (PFA)
- Whole blood, 50 bp, single-end, target depth: 35 million reads/sample
Aims

• Aim 1: Determine pre-treatment gene expression signatures that predict platelet functional responses to aspirin or ticagrelor
• Aim 2: Identify gene expression signatures that change with platelet function score following treatment with aspirin or ticagrelor
• Aim 3: Identify gene expression signatures that change following treatment with aspirin or ticagrelor
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