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Effects of Processing Delays on Global Gene Expression Patterns in PBMCs

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Overview

1. **Project – Our interest**
2. Sample handling and processing
3. Study results

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Gene Expression in Pediatric Arthritis (NIAMS: P01AR048929)

Overall Objective: Use gene expression analysis to investigate peripheral blood samples from patients with JIA

1. Determine differences **between** controls and current JIA classes
2. Assess heterogeneity **within** the current JIA classes

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8 sub-classes of JIA

1. **Persistent Oligoarticular (< 5 joints ever)**
2. Extended Oligoarticular (evolves to ≥ 5 joints)
3. **RF- Polyarticular (≥ 5 joints)**
4. RF+ Polyarticular (≥ 5 joints)
5. Enthesitis related arthritis (ERA): Arthritis with enthesitis, HLA-B27+
6. Systemic: Arthritis with systemic features
7. Psoriatic arthritis: Arthritis with psoriasis
8. Undifferentiated: Do not fit any category or fit more than 1 category

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Why study peripheral blood?

- Has been previously used to investigate gene expression
- “Surveys” entire body
- Easily obtainable
  - Collected from patients in clinic
  - Often collected during routine blood draws
  - Minimal risk
- Alternatives
  - Synovial fluids (few)
  - Synovial tissues (very few)
- Appropriate for diagnostic testing

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Multi-Center Study

- Power analysis: ~280 subjects

- Single institution
  - Not enough patients
- 5 clinical centers

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Collaborating Centers

- Cincinnati Children's Hospital (CCHMC)
- Children's Hospital of Philadelphia (CHOP)
  - David Sherry, MD
- Children's Hospital of Wisconsin
  - Judyann Olson, MD
- Schneider Children's Hospital (New York)
  - Beth Gottlieb, MD MS
  - [Normal Ilowite, MD (Montefiore)]
- Toledo Children's Hospital
  - Thomas Griffin, MD, PhD

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Our study: issues with multi-center

- Power analysis: ~280 patients

- Single institution
  - Not enough patients
- 5 clinical centers
  - Lab locations

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Our study: issues with multi-center

- Power analysis: ~280 patients

- Single institution
  - Not enough patients
- 5 clinical centers
  - Lab locations
  - Specific research lab Vs GCRC
  - Research group Vs Single person

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Overview

1. Project
   A. Determine differences between and within JIA subclasses
   B. Gene expression from peripheral blood
   C. Multi-center study
2. **Sample handling / processing**
3. Study results

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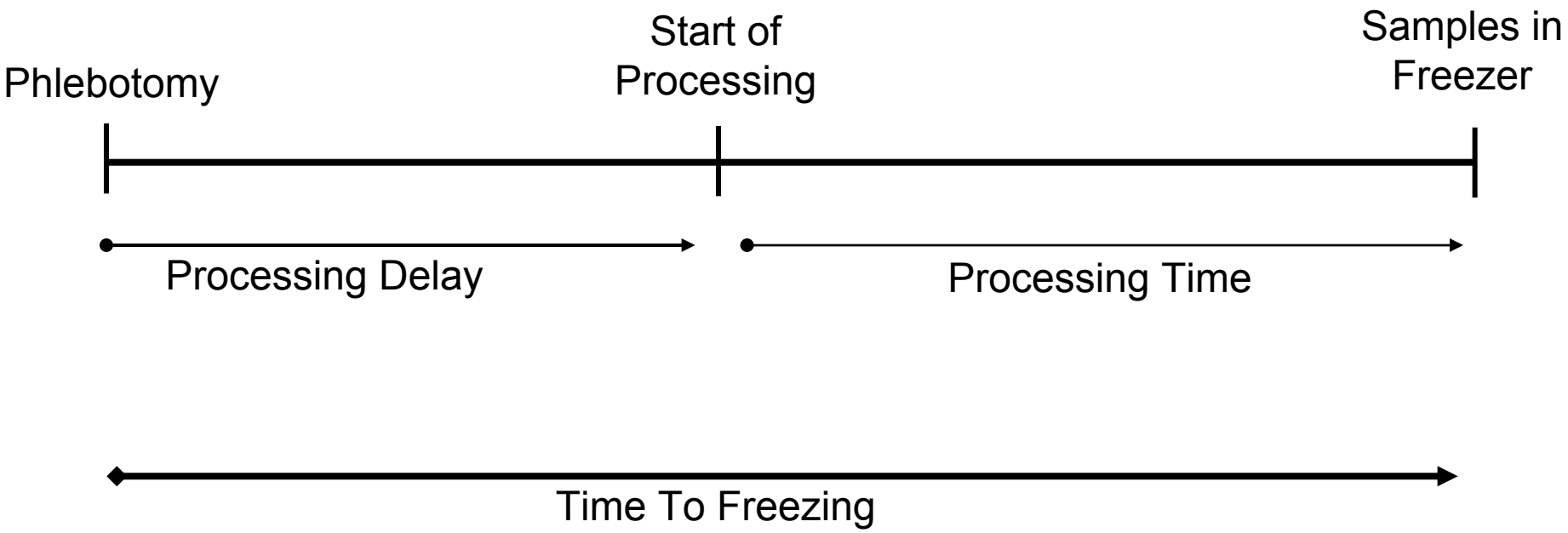
Definitions



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Ficoll isolation of PBMC

- Positive
  - Standard protocol with long history
  - Cells for downstream applications (flow, FACS, transformation, etc)
- Negatives
  - Ficoll takes time
    - Processing delay: Sample transport (Variable)
    - Processing time (into Trizol): ~1-1.5 hours
    - Cells activated and expression profiles can change
    - RNA not stabilized until samples put in Trizol

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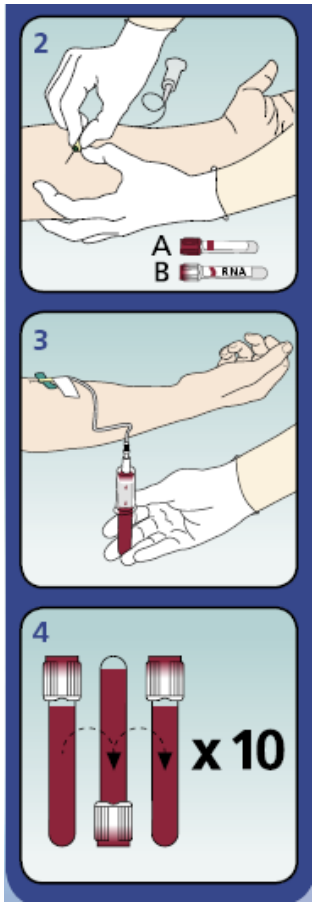
PAXgene

- PAXgene Blood RNA
  - Positive
    - Simple
    - "Instant" stabilization
      - Processing delay 1 minute
      - Processing time 1 minute
    - Stable over time
  - Negative
    - Must be d[illegible]last
    - Does not [illegible]t cells for flow cytometr[illegible]FA[illegible]S
    - Globin[illegible]ect

- Tempus did not exist (launched August 31, 2004)



http://www.preanalytix.com/pdf/PAXgene_Blood_RNA_Brochure_2.pdf

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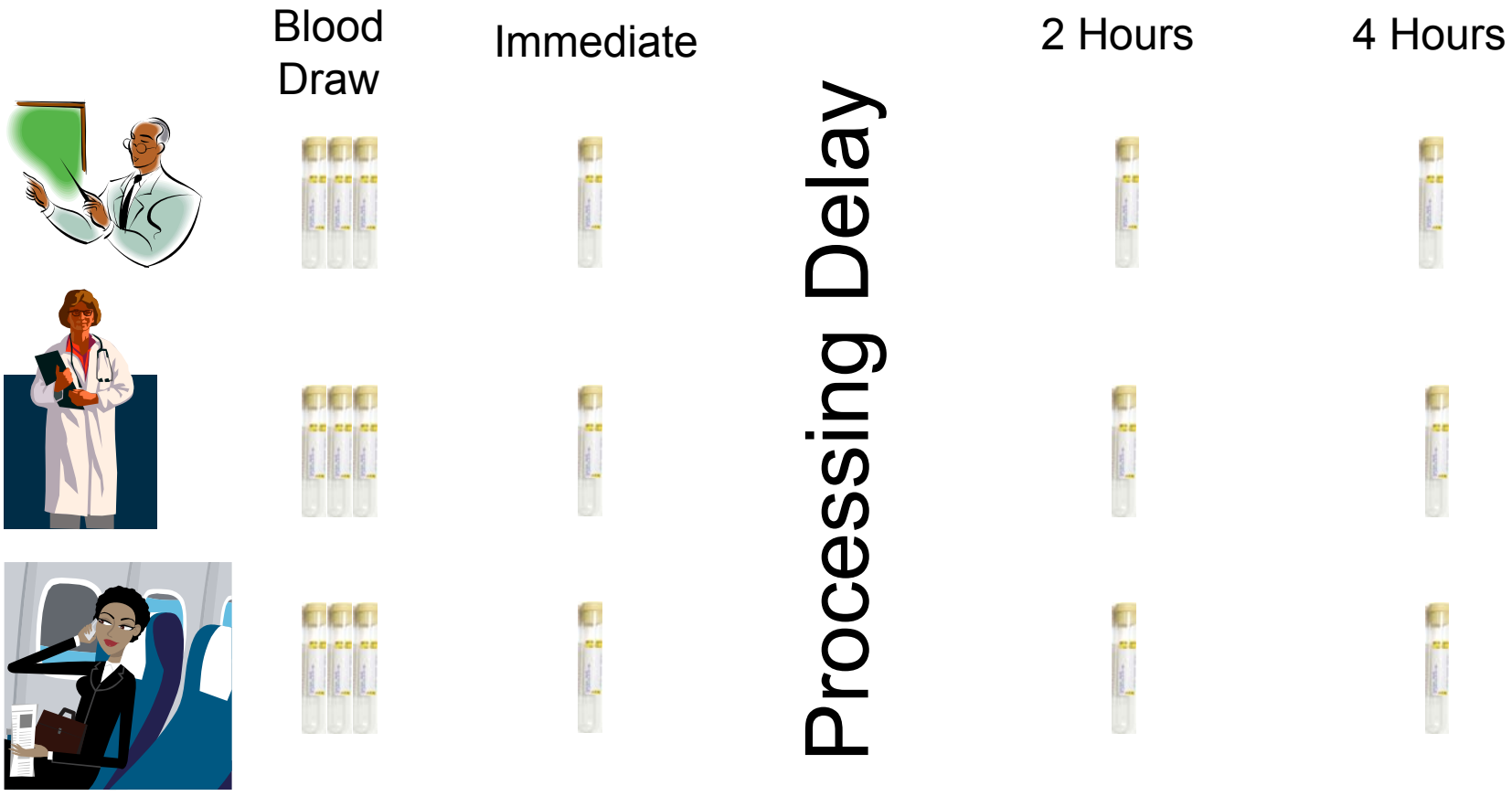
How much of an issue is variation in processing time?



*Similar study done by EC Baechler, et al looking at effect of overnight shipping. Genes and Immunity (2004) 5, 347-353.

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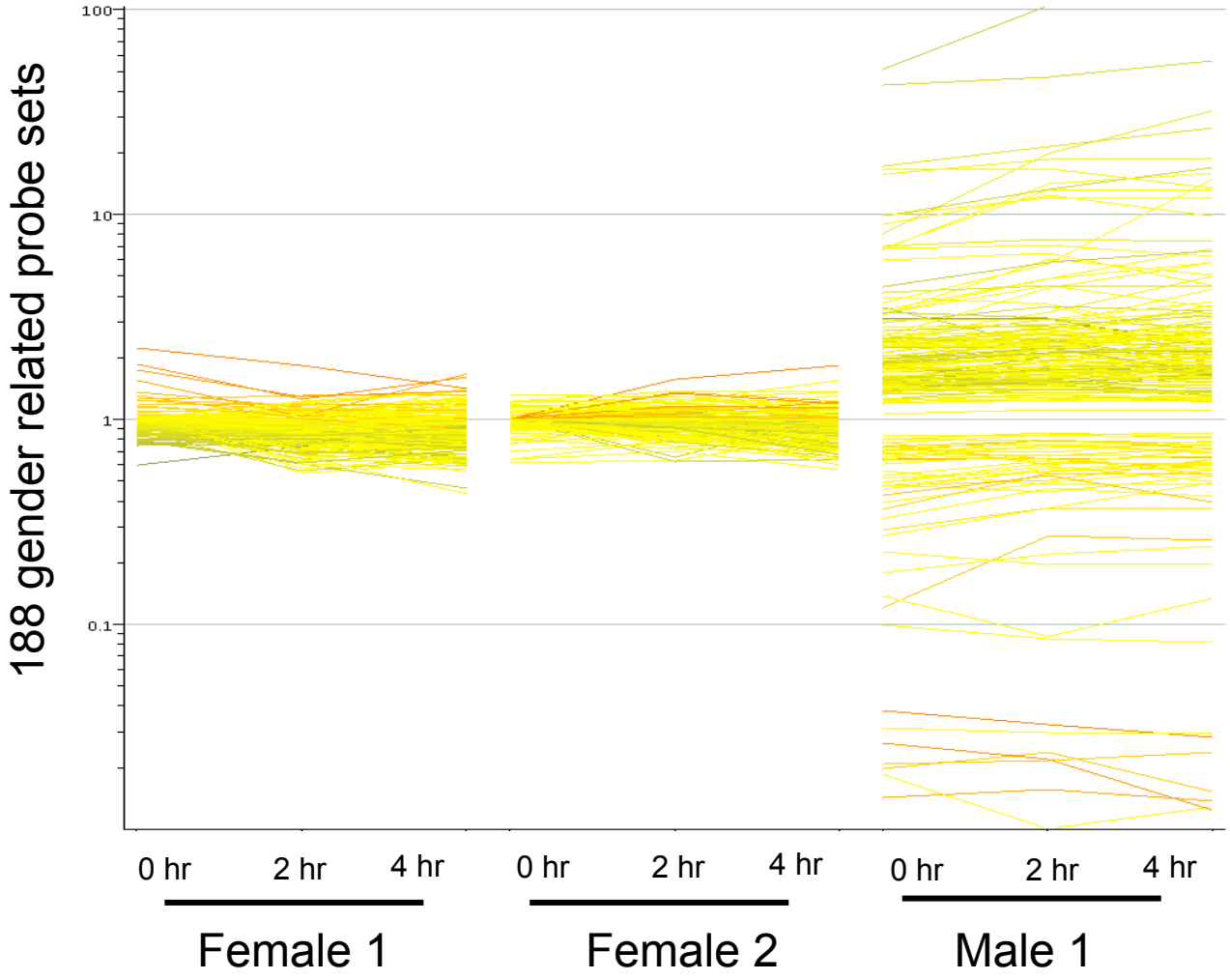
Gender differences can still be detected



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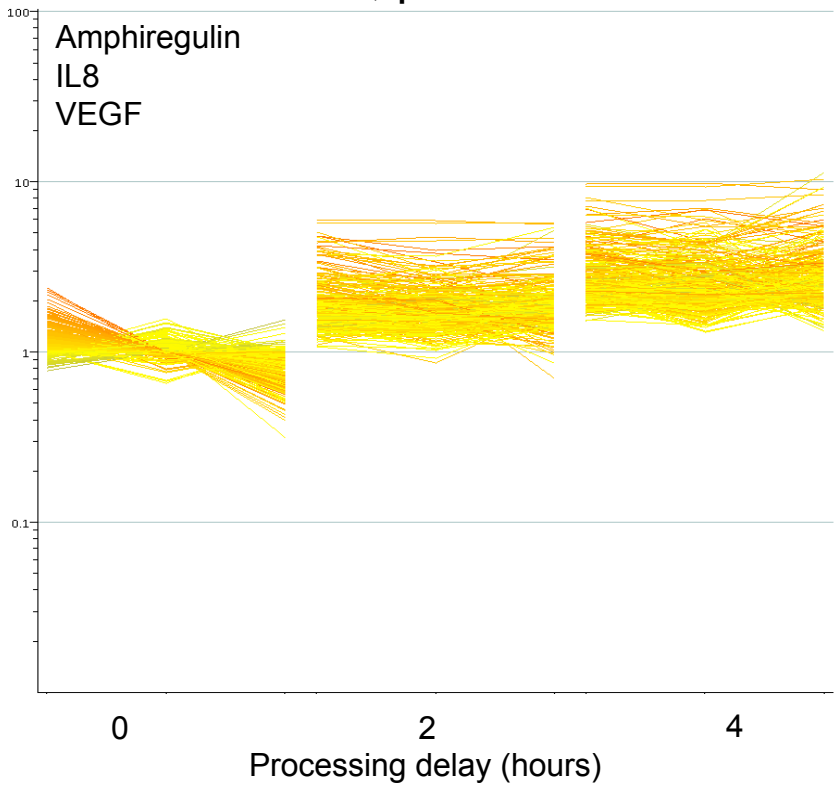
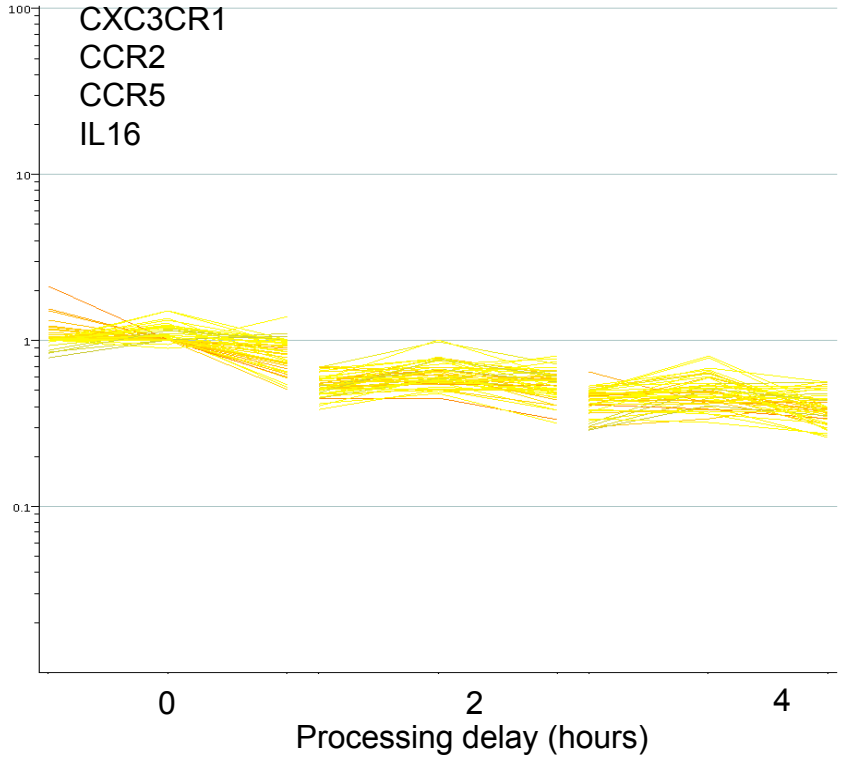
Processing Delays Cause Changes in Global Gene Expression Patterns from PBMC

311 probe sets increased
2-fold; p < 0.05



45 probe sets decreased
2-fold; p < 0.05



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Overview

1. Project – Our interest
2. Sample handling and processing
   A. Use Ficoll isolated PBMC
   B. Large biological effects can still be identified
   C. Processing time affects gene expression

3. Study results

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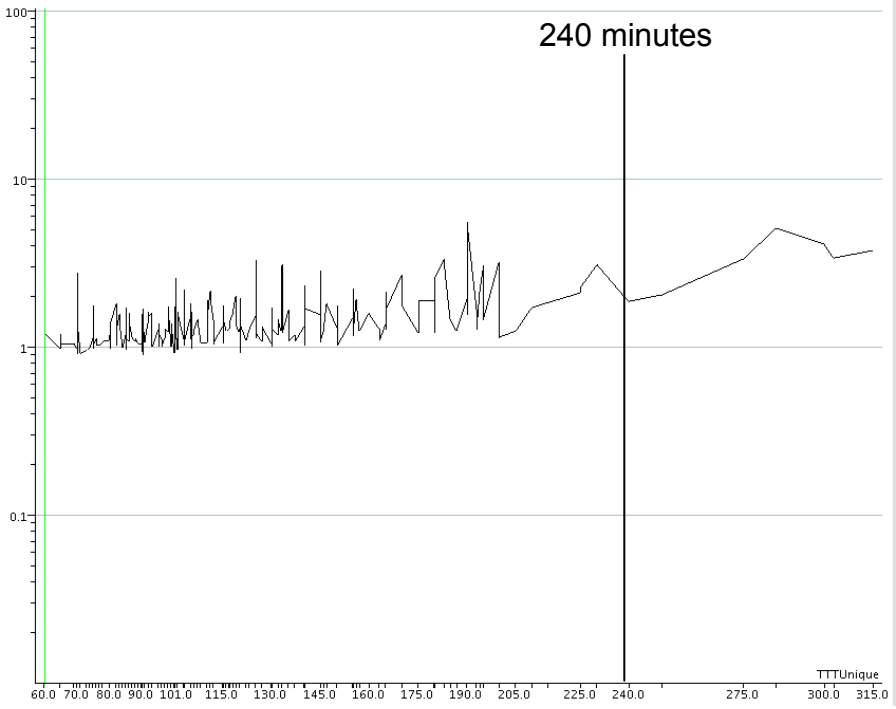
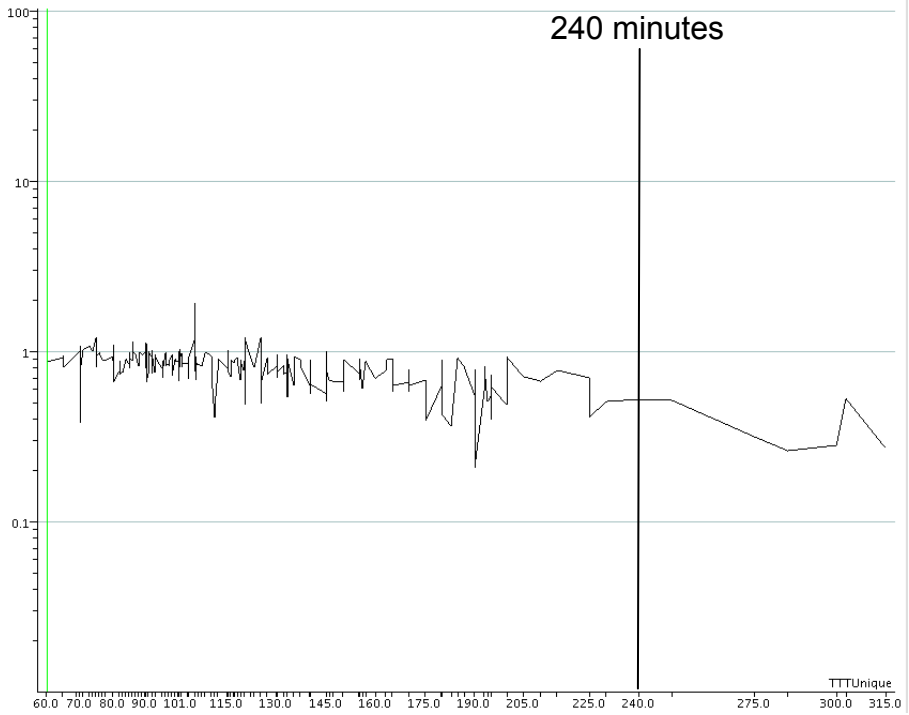
Trend in TTF genes is maintained in group of 267 arrays



Time to freezing



Time to freezing

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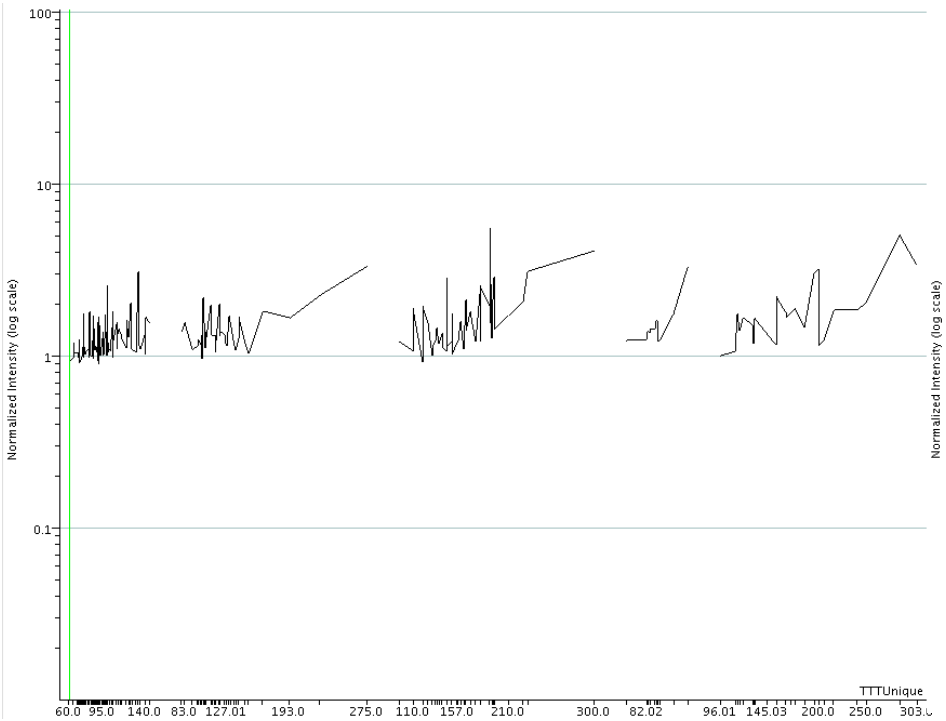
Trend is not hospital specific



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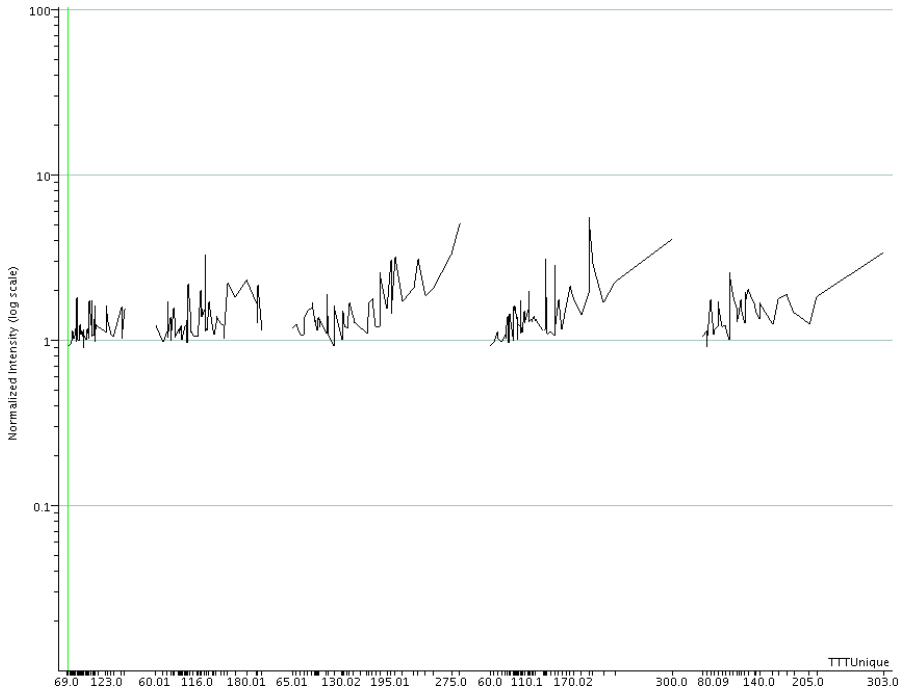
Trend is not JIA subtype specific



Control ERA Persistent Oligoarticular RF-Polyarticular Systemic

Control ERA Persistent Oligoarticular RF-Polyarticular Systemic

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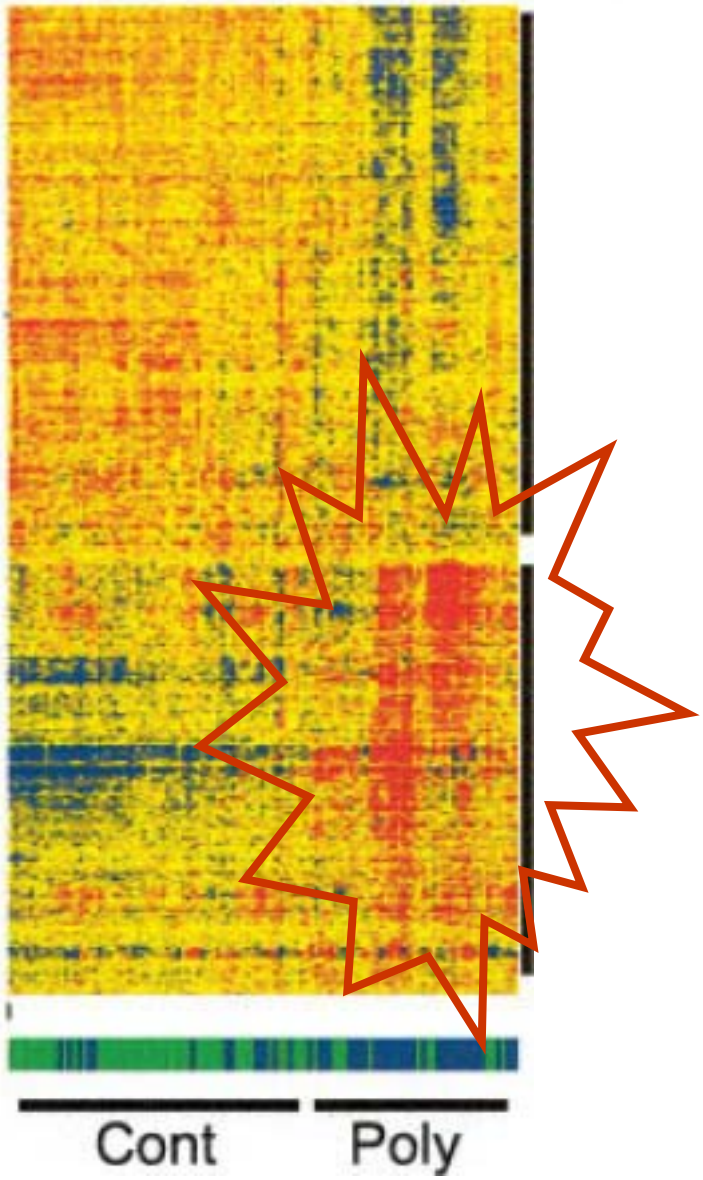
ARTHRITIS & RHEUMATISM
Vol. 60, No. 7, July 2009, pp 2102–2112

Subtype-Specific Peripheral Blood Gene Expression Profiles in Recent-Onset Juvenile Idiopathic Arthritis

Michael G. Barnes,[1] Alexei A. Grom,[1] Susan D. Thompson,[1] Thomas A. Griffin,[1] Paul Pavlidis,[2] Lukasz Itert,[1] Ndate Fall,[1] Dawn Paxson Sowders,[1] Claas H. Hinze,[1] Bruce J. Aronow,[1] Lorie K. Luyrink,[1] Shweta Srivastava,[1] Norman T. Ilowite,[3] Beth S. Gottlieb,[4] Judyann C. Olson,[5] David D. Sherry,[6] David N. Glass,[1] and Robert A. Colbert[1]



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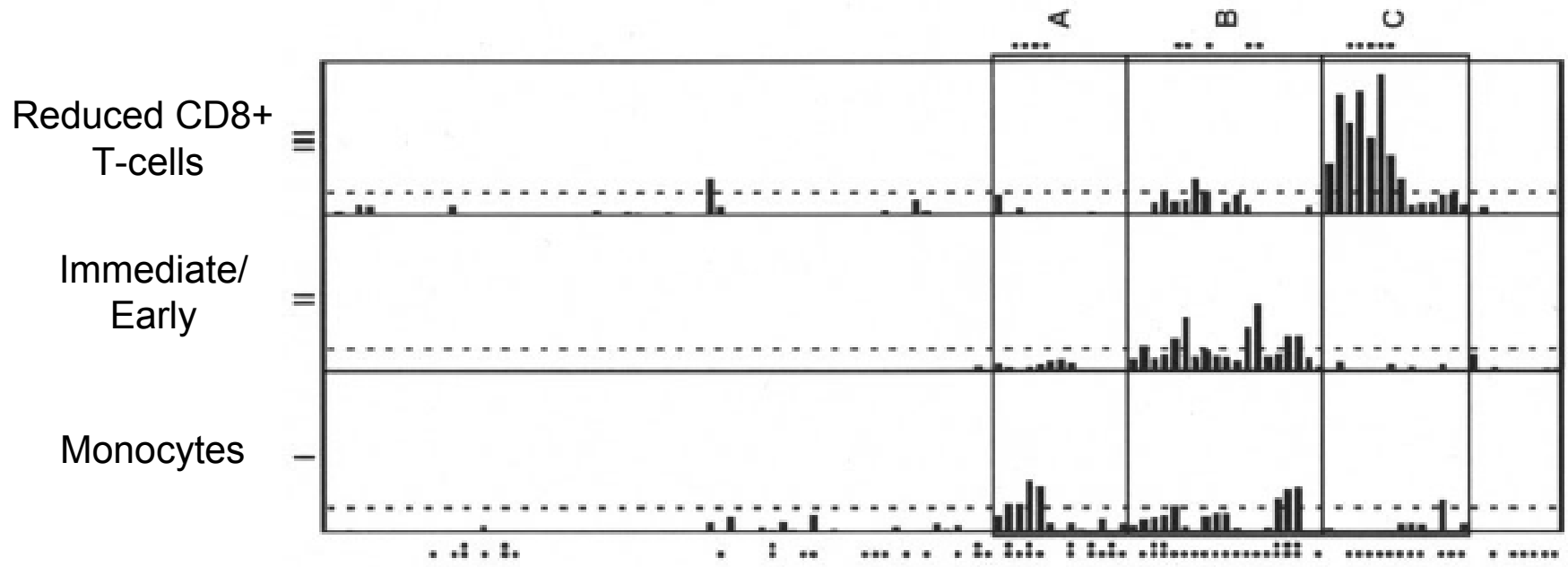
Gene Expression Signatures in Polyarticular Juvenile Idiopathic Arthritis Demonstrate Disease Heterogeneity and Offer a Molecular Classification of Disease Subsets

ARTHRITIS & RHEUMATISM
Vol. 60, No. 7, July 2009, pp 2113–2123

Thomas A. Griffin,[1] Michael G. Barnes,[1] Norman T. Ilowite,[2] Judyann C. Olson,[3] David D. Sherry,[4] Beth S. Gottlieb,[5] Bruce J. Aronow,[1] Paul Pavlidis,[6] Claas H. Hinze,[1] Sherry Thornton,[1] Susan D. Thompson,[1] Alexei A. Grom.[1] Robert A. Colbert.[1] and David N. Glass[1]



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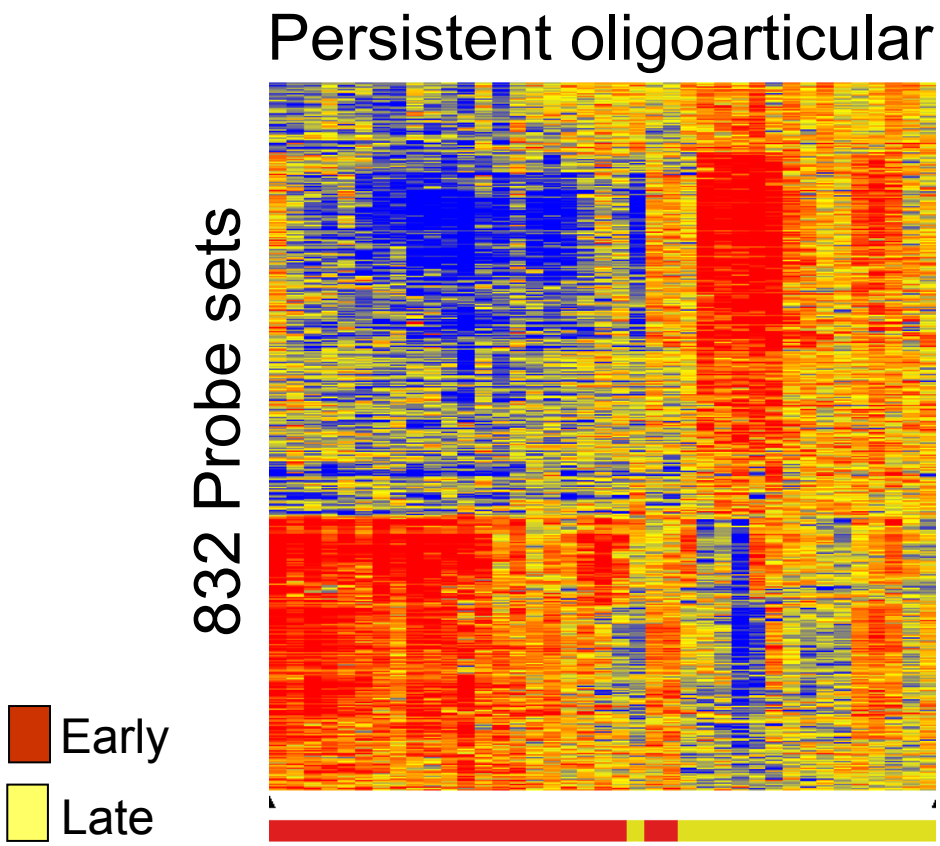
Can we identify gene expression signatures indicative of age-at-onset?



Not explained by:

- Sex
- Hospital
- Current joint count
- X-ray results
- CRP
- ANA

- No overlap with TTF genes
- No overlap with Signature II

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Overview

1. Project – Our interest
2. Sample handling and processing
3. Study results
   A. Important to track sample processing time points and include during data interpretation
   B. A large biological effect can still be identified in spite of processing variations
   C. Limited number of extended TTF samples have limited effect on results

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How can we handle the effect of time to freezing?

- ~~Ignore the issue~~
- Only use “best” samples (< 4 hours TTF)
- Filter TTF genes[1]
- Other collection tubes:
  - PAXgene/Tempus
    - Globin reduction protocols
    - Globin effect “solved” with NuGEN labeling
  - CPT
- Standardize processing delay (realistic...)
- Statistical modeling

[1]Baecheler, EC. PNAS (2003) 100: 2610-2615.

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