Inflammation:
One Gene at a Time

Dan Kastner, MD, PhD
Intramural Research Program
NHGRI/NIH/DHHS
February 14, 2017
Mr. Hagopian is a 34 yo 3w referred to us from Dr. Paul
Roche for evaluation of a lifelong history of pauciarticular
arthritis.

The patient states that he has had joint problems
since the time he was crawling. He states that he has
had swelling of the ankles and knees since that time;
and swelling of the ankles and knees since that time;
the arthritis is migratory, usually occurring at one joint
at a time, and accompanied by edema and heat
over the affected joint. The joint problems are sometimes
accompanied by a hypersegmented node over the ankles,
and sometimes by a palpable, reddish eruption on
the palms of the feet. When the joint was in 2nd grade

Impression/Plan:

Have advised pt to contact me
as soon as an attack of acute arthritis
begins. Suspect familial Mediterranean fever.

Drs. Ballentine
In the beginning . . .

YW Kan

David Botstein

Francis Collins
Positional Cloning of \textit{MEFV}, the Gene Mutated in Familial Mediterranean Fever (FMF)
The PYRIN Domain: A Cognate Interaction Motif


Pyrin

IL-1β Converting Enzyme (ICE)

Pro-IL-1β

IL-1β

ASC

Caspase-1

PYRIN

CARD

PYRIN

CARD
IL-1 Inhibition in FMF Amyloidosis

Chae et al PNAS 103:9982, 2006
FMF Knockin Mice: IL-1-Dependent Inflammation

Big Questions Remaining

• What is the function of non-mutated pyrin in the immune system?

• Why are carrier frequencies for *MEFV* mutations so high in certain populations?

• How can we account for the exquisite specificity of colchicine for FMF among the recurrent fever syndromes?
Pyrin inflammasome activation and RhoA signaling in the autoinflammatory diseases FMF and HIDS

Yong Hwan Park, Geryl Wood, Daniel L Kastner & Jae Jin Chae

LPS + C3 toxin

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>Casp1−/−</th>
<th>Asc−/−</th>
<th>Nlrp3−/−</th>
<th>Nlrp4−/−</th>
<th>Aim2−/−</th>
<th>Mefv−/−</th>
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Park et al., *Nat Immunol* 17:914, 2016
PKN1 Phosphorylates Pyrin at S208 and S242

Park et al., Nat Immunol 17:914, 2016
The Binding of 14-3-3 to Pyrin is Substantially Decreased by FMF-Associated Mutations

Park et al., Nat Immunol 17:914, 2016
Colchicine Suppresses the Pyrin Inflammasome

Park et al., Nat Immunol 17:914, 2016
Park et al., Nat Immunol 17:914, 2016
Pyrin S242R in a Family with a Severe Dominantly Inherited Autoinflammatory Disease: PAAND

Pyrin Inflammasome Activation in HIDS

Park et al., Nat Immunol 17:914, 2016
The *Yersinia* Outer Protein YopM Inactivates the Pyrin Inflammasome Through PKN1/PKN2
FMF-KI mice are less susceptible to *Yersinia*

Chae, Bliska, et al., unpublished observations
PAPA Syndrome (Pyogenic Arthritis with Pyoderma Gangrenosum and Acne)

PSTPIP1

Pyin

Shoham et al., *PNAS* **100**:13501, 2003
SoJIA?

Neonatal-Onset Multisystem Inflammatory Disease (NOMID)
Treatment of 18 NOMID Patients with the IL-1 Receptor Antagonist Anakinra

Somatic Mosaicism at *NLRP3*

- 30 – 50% of NOMID/CINCA
- Schnitzler syndrome
  - Middle-age onset
  - Fever, neutrophilic urticaria, bone pain, myalgia, monoclonal gammopathy
- Adult-onset FCAS

Age 9 months, before therapy

3 days post treatment initiation

7 days post treatment initiation

Christina

- 27 y/o Irish woman
- 14 yr hx 3-5 wk febrile episodes
- Periorbital edema, migratory rash, abdominal pain
- Seen 1 wk postpartum
- WBC 29K, ESR 126, CRP 16.3
- Therapeutic response to steroids but not colchicine
TNFRSF1A Mutations Can Cause Dominantly Inherited Periodic Fever

Rheumatology 5th edn, 1645, 2011
The TNF Receptor-Associated Periodic Syndrome (TRAPS)


Rheumatology 5th edn. 1637-57, 2011
Fever with Early-Onset Stroke

A Family 1

B Family 2

Zhou et al. NEJM 370:911, 2014
Early-Onset Stroke and Vasculopathy Associated with Mutations in ADA2
CECR1b Morpholino Injection in Zebrafish: 48 h

Zhou et al. NEJM 370:911, 2014
Anti-TNF Therapy for DADA2

Before anti-TNF: 44 strokes, 1064 patient-months

Since anti-TNF: 0 strokes, 323 patient-months

Barron, Ombrello, et al., unpublished
## Sanger sequencing for CECR1 in PAN cohort

<table>
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<tr>
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<th>Mutations</th>
<th>Additional SNPs</th>
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</table>
| PT (1) | **G47A het**: Rs200930463, MAF: 0.00001649  
**P106S het**: No RS, MAF 0.000008345 | **H335R hom**: Rs2231495, MAF 0.33  
**Y453Y het**: Rs7289170, MAF 0.22 |
| PT (2) | **G383S hom**: no rs (Infevers)        | **N53N hom**: Rs362129, MAF 0.32  
**H335R hom**: Rs2231495, MAF 0.33 |
| PT (3) | **E328K het**: unknown  
**F355L het**: rs116020027, MAF < 0.01 | **L46L het**: Rs7289141, MAF 0.17  
**Y453Y het**: Rs7289170, MAF 0.22 |
| PT (4) | **V349I het**: Rs74317375, MAF < 0.01  | **N53N hom**: Rs362129, MAF 0.32  
**Y453Y het**: Rs7289170, MAF 0.22 |
| PT (5) | **T65M het**: Rs61747288, MAF 0.0004614 | **N53N hom**: Rs362129, MAF 0.32  
**Y453Y het**: Rs7289170, MAF 0.22 |
| PT (6) | **Y220Y het**: rs2231487, MAF < 0.01  | **L46L het**: Rs7289141, MAF 0.17  
**H335R het**: Rs2231495, MAF 0.33 |

Stoffels et al., unpublished
Vibratory Urticaria Associated with a Missense Variant in ADGRE2

Boyden et al., NEJM 374:656, 2016

Loss-of-function mutations in TNFAIP3 leading to A20 haploinsufficiency cause an early-onset autoinflammatory disease


Biallelic hypomorphic mutations in a linear deubiquitinase define otulipenia, an early-onset autoinflammatory disease

Zhou et al., PNAS 113:10127, 2016
Myeloid-Restricted NLRP3 Somatic Mosaicism in Adult-Onset Muckle-Wells Syndrome

A Recurrent Fever Syndrome Caused by Hypomorph Mutations in TRNT1

Digeneic Inheritance in CANDLE

PLCG2 S707Y Defining a New Disorder, APLAID

Zhou et al., Arthritis Rheumatol 67:2482, 2015


Digenic Inheritance in CANDLE

Myeloid-Restricted NLRP3 Somatic Mosaicism in Adult-Onset Muckle-Wells Syndrome

A Recurrent Fever Syndrome Caused by Hypomorph Mutations in TRNT1

Zhou et al., Arthritis Rheumatol 67:2482, 2015
Cleavage-Deficient \textit{RIPK1} Mutations

\textit{De novo} heterozygous p.D324N

Dominant heterozygous p.D324H

Liver Spleen

\textbf{Boyden et al., unpublished}
Genetic Architecture of Common Disease

Behçet’s Disease:
A Genetically Complex Disorder of Inflammation

\[ \lambda_s > 10 \]
Genetic Architecture of Behçet’s Disease

- HLA-B*51
- IL10
- IL23R
HLA-B*51, ERAP1 and Behçet’s Disease Susceptibility

Ombrello et al. PNAS 111:8867-8872, 2014

Genetic Architecture of Behçet’s Disease

- MEFV
- IL23R
- TLR4
- NOD2

- HLA-B*51

- ERAP1

- IL10
- IL23R

- CCR1
- STAT4
- KLRC4

- IL1A-IL1B
- IRF8
- CEBP-PTPN1
- ADO-EGR2
- RIPK2
- LACC1
- FUT2
**Discovery of a Monogenic Form of Behçet’s Disease**

**Exome Sequencing**

- Family 1: European Canadian
  - M1: p.Leu227*
  - P1: WT/WT
  - P2: M1/WT
  - P3: M1/WT

- Family 2: European American
  - M2: p.Phe224Serfs*4
  - P4: WT/WT
  - P5: M2/WT
  - P6: WT/WT

**Sanger Sequencing**

- Family 1
  - Total variants: 30,3499
  - Novel variants: 16,116
  - Dominant inheritance: 80
  - Common gene: 1

- Family 2
  - Total variants: 425,123
  - Novel variants: 17,755
  - Dominant inheritance: 123

- TNFAIP3 (A20)

**Targeted Sequencing**

- Family 3: Turkish
  - M3: p.Arg271
  - P8: WT/WT

- 384 Turkish
  - 384 Japanese Behçet's patients screened

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* Exome sequenced samples

Over 150 patients screened

TNFAIP3, Encoding the A20 Protein
Enhanced Signaling in the NF-κB Pathway

**In Vitro**: A20 Mutations are Associated with Defective K63 Deubiquitinase Activity

293T cells co-transfected with constructs for NEMO (IKKγ), K63 Ub, and either WT-A20 or mutant-A20.

Treatment

Genetic Architecture of Behçet’s Disease

- HLA-B*51
- IL23R
- TLR4
- NOD2
- TNFAIP3 (A20)
- MEFV
- IL10
- IL23R
- CCR1
- STAT4
- KLRC4
- IL1A-IL1B
- IRF8
- CEPB-PTPN1
- ADO-EGR2
- RIPK2
- LACC1
- FUT2
Summary

- Genomic approaches have revolutionized our understanding of the autoinflammatory diseases and their treatment.
- Familial Mediterranean fever is caused by mutations in *MEFV*, encoding the pyrin protein, an activator of IL-1β.
- Bacterial toxins that modify RhoA, as well as loss-of-function mutations in the geranylgeranyl synthetic pathway, lead to pyrin inflammasome activation.
- Next-generation sequencing has revolutionized our ability to “solve” unexplained inflammatory phenotypes.
- Recessive loss-of-function mutations in ADA2 lead to a syndrome of recurrent fever, livedo racemosa, lacunar strokes, and PAN.
- Common low-penetrance variants and rare highly penetrant mutations contribute to Behçet’s disease susceptibility.
- Truncating mutations in A20 define a dominantly-inherited disorder in the Behçet’s spectrum (HA20) with heightened NF-κB signaling and NLRP3 inflammasome activation.
- Our patients continue to present us with new challenges.
NIH Colleagues in the Clinic

- Raphaella Goldbach-Mansky
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- Karyl Barron
  NIAID
- Amanda Ombrello
  NHGRI
- Debbie Stone
  NHGRI
- Paola Pinto
  NHGRI
- Patrycja Hoffmann
  NHGRI
- Bev Barham
  NHGRI
- Anne Jones
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- Tina Romeo
  NHGRI
- Daniella Schwartz
  NIAMS
- Bob Lembo
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  Drexel
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