Children's Hospital and Health System™

Children's Specialty Group™

MEDICAL COLLEGE OF WISCONSIN

WGS in the clinic

David Dimmock

Genetics Center, Children's Hospital of Wisconsin;
Pediatrics, & Human and Molecular Genetics Center,
Medical College of Wisconsin, Milwaukee, WI

Children's Hospital and Health System™

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Human and Molecular Genetics Center

The following relationship(s) exist related to this presentation:

CHW/MCW offers fee for service genetic counseling and whole genome sequencing.

MCW/Presenter hold specific patents related to the tertiary analysis of WGS data

Unapproved Use

David Dimmock has documented that his/her presentation involves comments or discussion of unapproved use.

Whole Genome Sequencing (WGS) and Whole Exome Sequencing devices are not FDA approved in-vitro diagnostic tests.

At this time, the FDA has determined that such approval is not necessary.

Why DNA testing

Utility if Clinically Apparent

- Confidence in the diagnosis to make difficult therapeutic decisions e.g. Trisomy 13
- Ability to avoid screening and testing which may be unsafe/invasive e.g. ARC syndrome Hereditary Fructose Intolerance

Treatment dependent on Molecular results

- Ability to use pharmacologic “read-through” is dependent on mutation type in Cystic Fibrosis

Therapy plan dependent on Molecular results

- SMAD4 mutations as a cause of Juvenile Polyposis Syndrome:
- Cases also require screening for Hereditary hemorrhagic telangiectasia (HHT)

DNA diagnosis allows presymtopmatic Rx

- X-Linked Adrenoleukodystrophy (X-ALD)

Key Benefit to Family

- Reassurance not teratogen
- Identification of others in family that have disease before symptomatic e.g. Hereditary hemorrhagic telangiectasia (HHT), Von Hippel-Lindau (VHL), RB1 mutations

Key problem: Testing is too expensive

- We cannot (yet) test every child for X-ALD before they develop symptoms
- Because testing is too expensive we cannot screen everyone with recurrent nosebleeds for risk of HHT before a family member has an aneurism or catastrophic bleed

- If genetic testing was as cheap and available as TFT's we would not have utility discussions (after all who diagnoses hypothyroidism using reflexes anymore?)
- If we could get as much information as an MRI we would order more comprehensive genetic testing

Types of Sequencing

single gene sequencing	• specific mutations suspected • specific disease suspected
gene panels	• group of disorders suspected • specific pathway suspected
exome sequencing	• coding region of the genome • Includes exons and boundaries
whole genome sequencing	• massive amounts of data • includes introns and exons

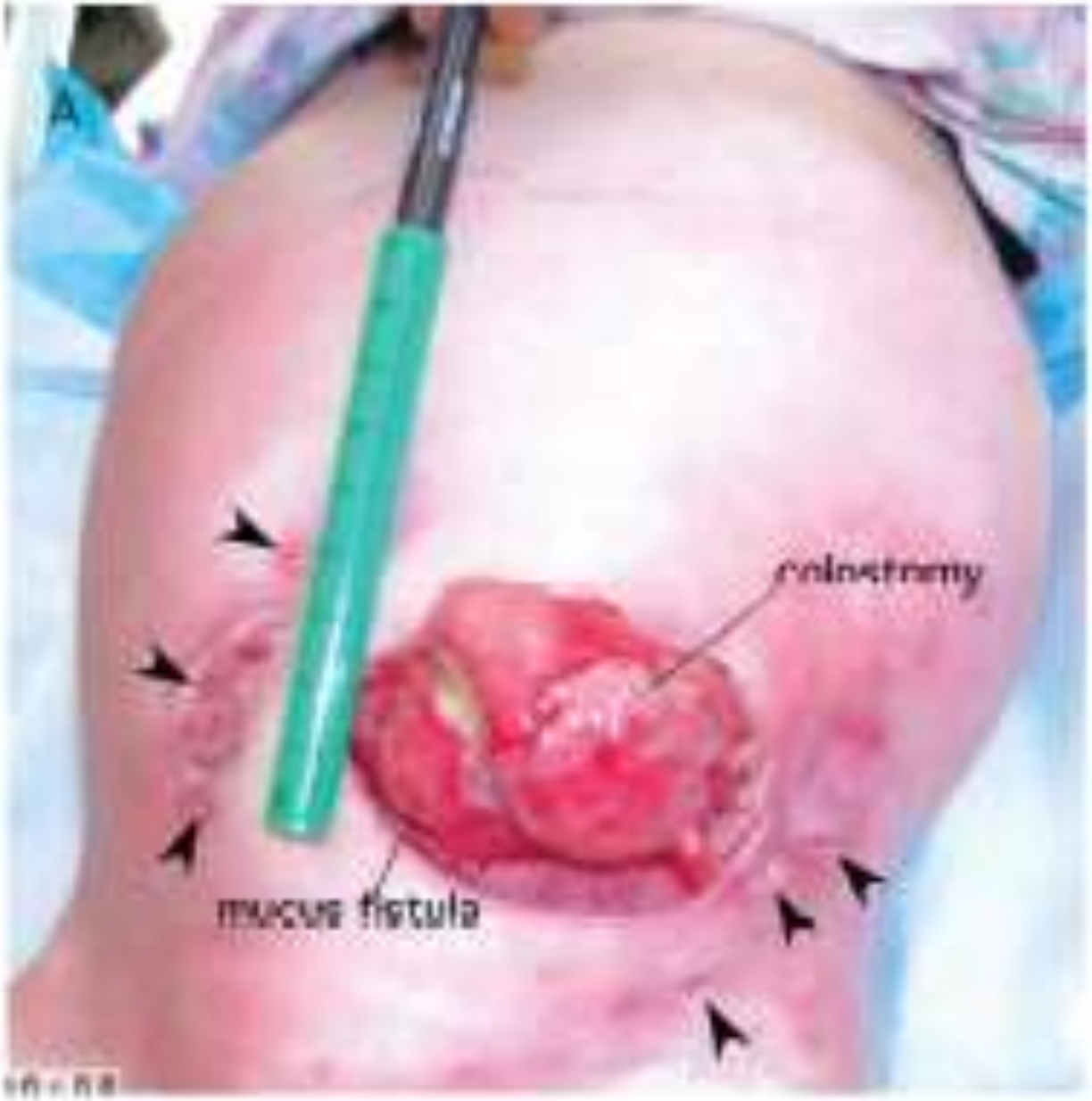
WHERE WE HAVE COME FROM

Index Case – Summer 2009

- 15 month old male presented with poor weight gain and perianal abscess
- Disease progressed within months to refractory inflammatory bowel disease consistent with Crohn's disease in spite of immunomodulatory therapy

Clinical Course

- In spite of aggressive medical and immunomodulatory therapy disease progressed with :
  - mucosal inflammation,
  - strictures,
  - enterocutaneous fistulae,
  - poor cutaneous wound healing,
- ultimately requiring a total colectomy.


A
mucus fistula

Immunological work-up

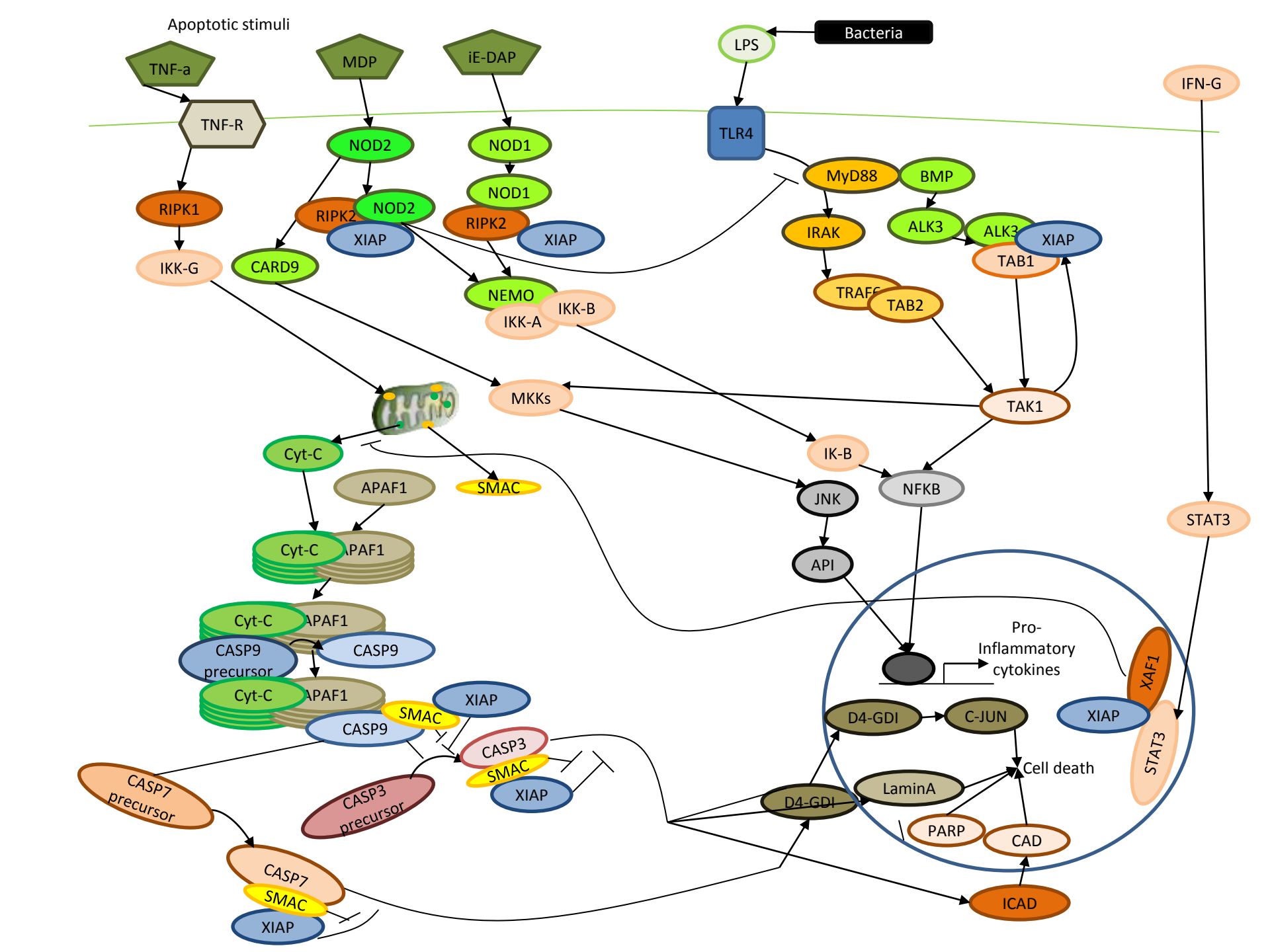
- anti-neutrophil antibody,
- abnormal chemotaxis of neutrophils;
- decreased NK cytotoxicity but no evidence of HLH;
- memory skewing of CD4 cells
- inverted CD4 to CD8 ratio
- Normal genetic testing for autoimmune lymphoproliferative syndrome

Diagnosis?

- Several forms of immune dysfunction have been associated with inflammatory bowel disease.
- May respond to immune reconstitution or require alternate treatment plan dependent on the underlying cause
- Clinical question: Could we use Exome sequencing establish if we should do a bone marrow transplant?

Variant analysis summary

High Confidence variants	16,124
Genic variants	16,012
Protein coding	15,272
Non-synonymous	7,157
Two variants in gene predicted to be damaging	67
-Altering highly conserved amino acids	19
-Not known to contain many missense mutations	5
Novel (dbSNP129) Non-synonymous	878
Homozygous or hemizygous	70
-Predicted to be damaging	17
-Novel (against dbSNP 130)	8
-Altering highly conserved amino acid	4
-Not found in reference genome sequences	2
-Not known to contain many missense mutations	1


Apoptotic stimuli
TNF-a
TNF-R
RIPK1
IKK-G
MDP
NOD2
RIPK2
XIAP
CARD9
iE-DAP
NOD1
NOD1
RIPK2
XIAP
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IKK-B
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TLR4
MyD88
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TRAF6
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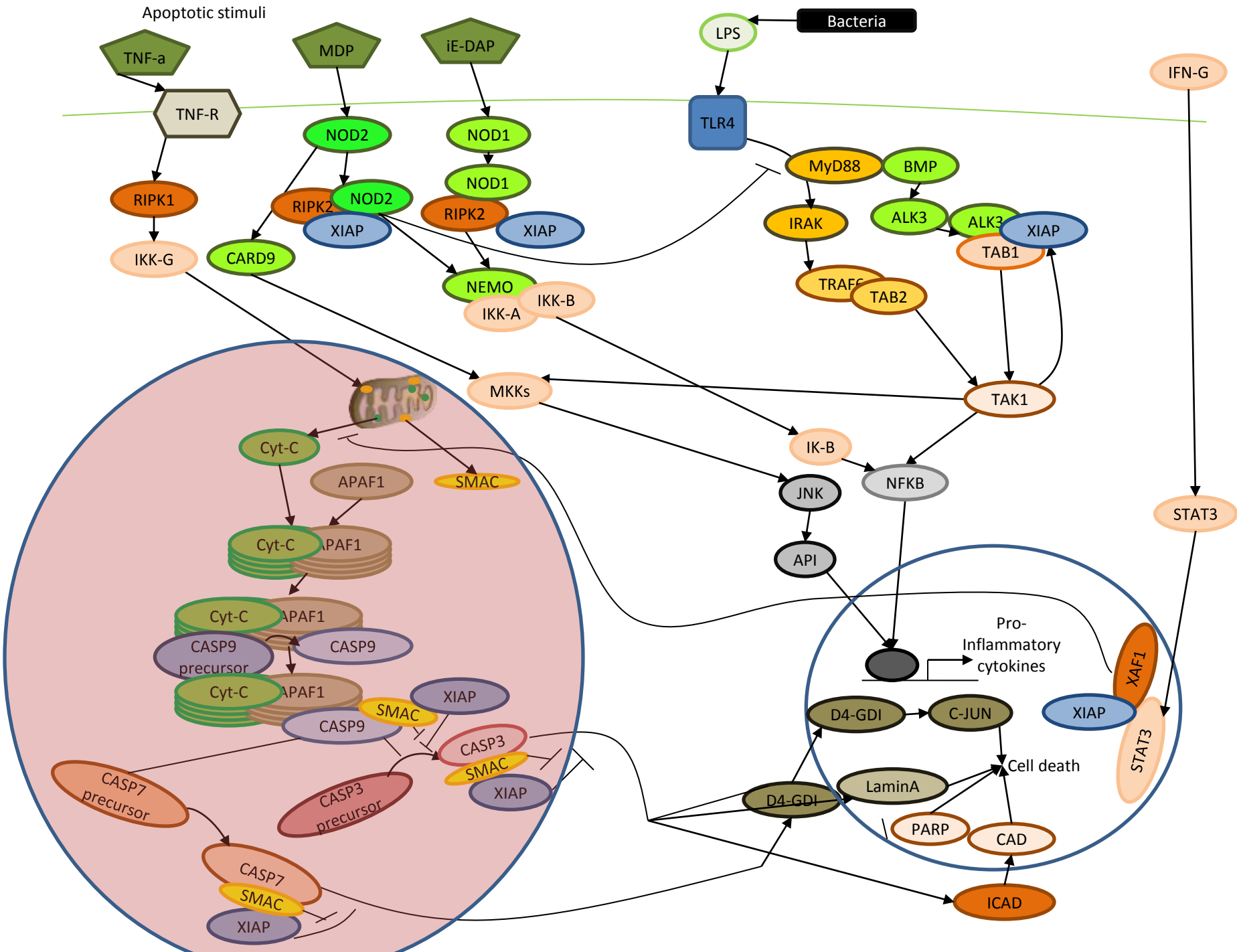
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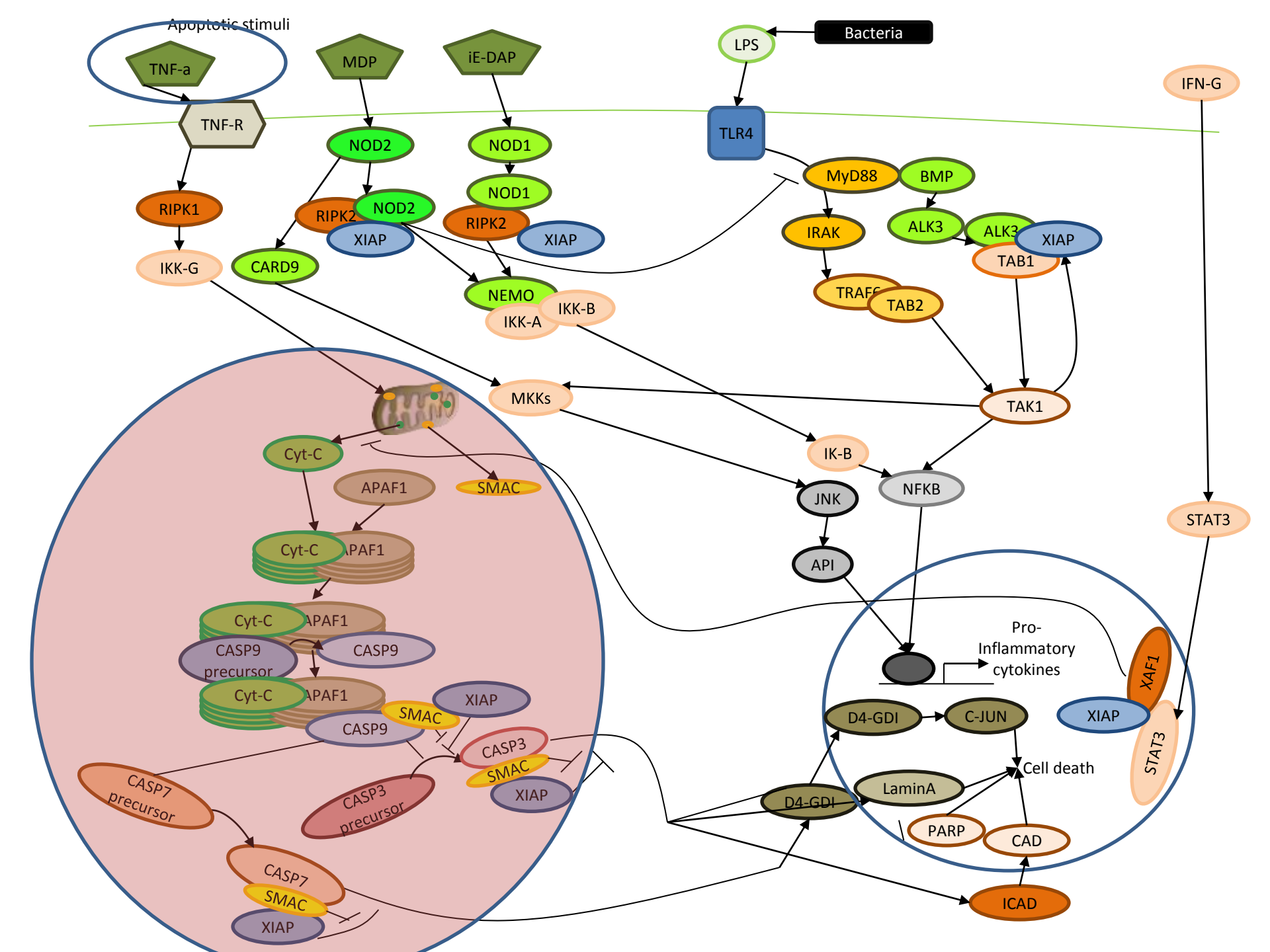
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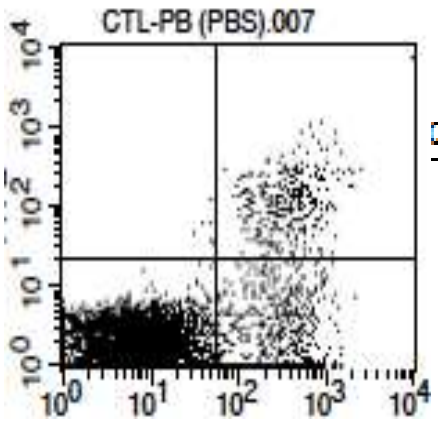
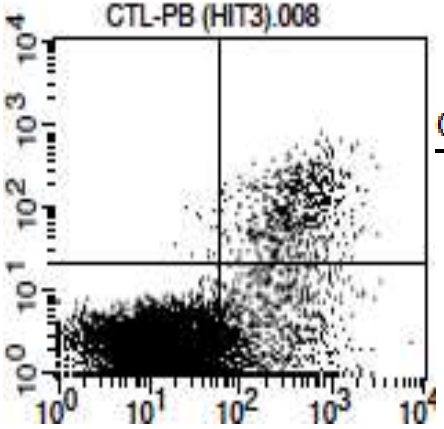
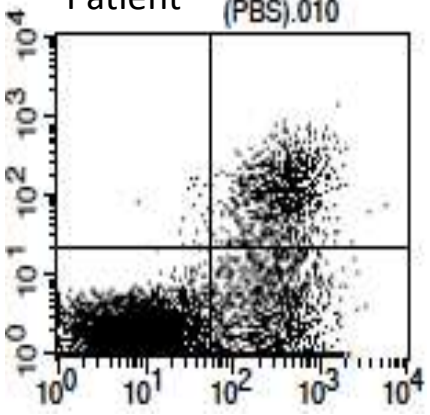
Increased apoptosis



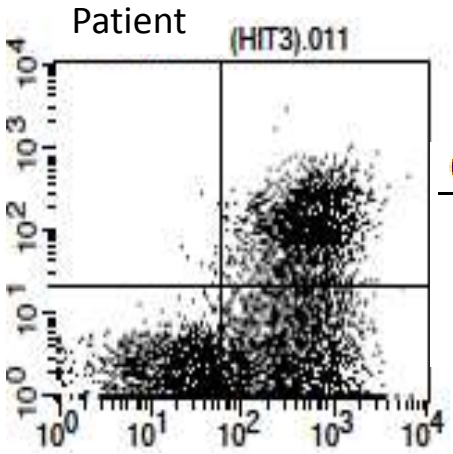
Quad	Events	% Gated	% Total
UL	7	0.13	0.13
UR	312	5.76	5.76
LL	4534	83.73	83.73
LR	562	10.38	10.38



Quad	Events	% Gated	% Total
UL	14	0.16	0.16
UR	577	6.50	6.50
LL	7214	81.24	81.24
LR	1075	12.11	12.11



Quad	Events	% Gated	% Total
UL	21	0.34	0.34
UR	834	13.43	13.43
LL	4055	65.30	65.30
LR	1300	20.93	20.93

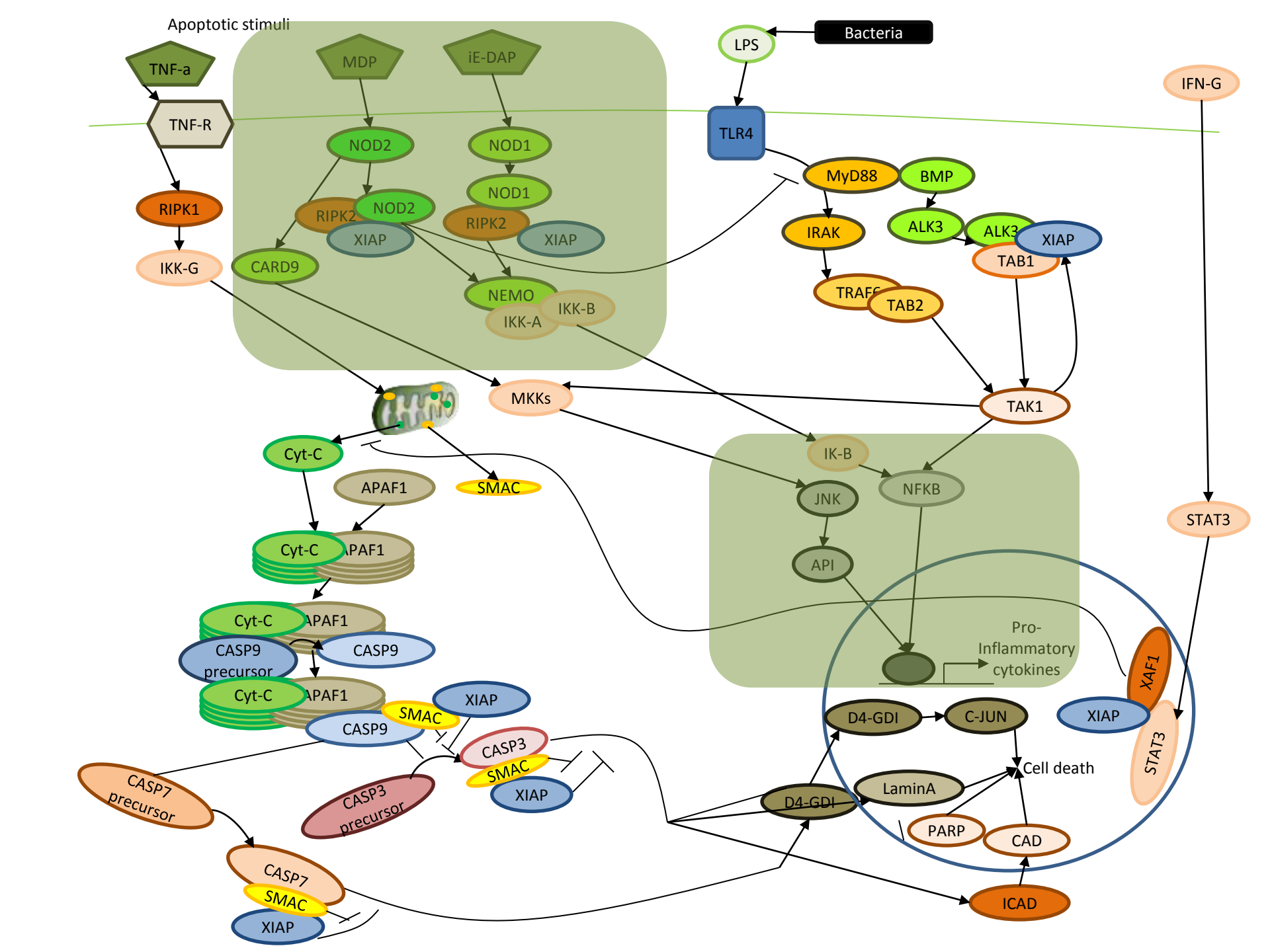


Quad	Events	% Gated	% Total
UL	15	0.24	0.24
UR	1873	30.31	30.31
LL	1718	27.80	27.80
LR	2574	41.65	41.65

Propidium Iodide (late cell death)

Annexin (cell surface indicator of apoptosis)

- The child's PBMCs were activated with PHA and IL-2, fed with fresh IL-2 at day3, then on day 7 cells were restimulated with CD3 antibodies. This resulted in significant death compared with controls.
- The child's cells are more sensitive to activation-induced cell death.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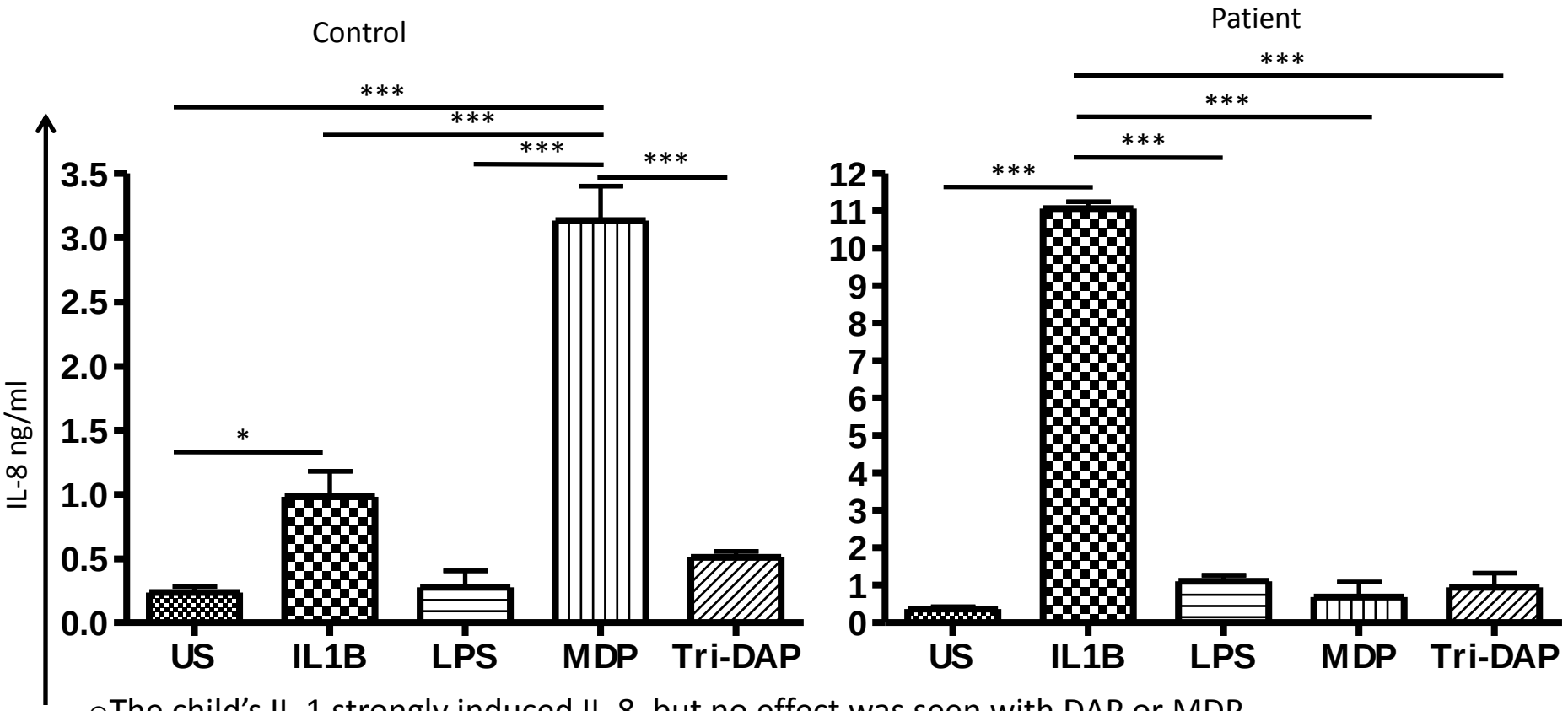
Defective NOD signaling measured by IL-8 production

The child's PBMCs were stimulated with the NOD ligands MDP and DAP. As a control his cells were also stimulated with IL-1. Normal RBMCs were also stimulated with the NOD ligands and separately with IL-1. IL-8 production in response to these stimuli was measured after 16hours.



- The child's IL-1 strongly induced IL-8, but no effect was seen with DAP or MDP.
- In the normal PBMC control, induction of IL-8 was seen with DAP and MDP.
- The child's NOD2 signaling is defective.

 To read the Journal Sentinel's series on Nicholas Volker and the groundbreaking work in DNA sequencing, go to jsonline.com/dna <http://jsonline.com/dna>

Worthey, E.A., et al., *Making a definitive diagnosis: Successful clinical application of whole exome sequencing in a child with intractable inflammatory bowel disease.* Genet Med, 2010.

MCW/CHW WGS Pilot Program

- 57 reviewed, 22 sequenced
- Now routine clinical service

Logistical challenges

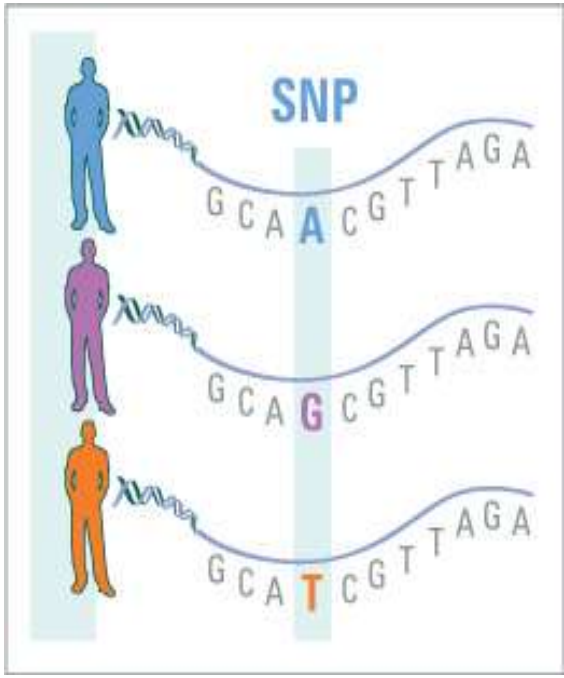
- Counseling
- Consent
- Sequencing
  - Cost
  - Turn Around time
- Analysis
  - Gene list
  - How/What to call a mutation
  - Off target genes results?
- Data return
  - By who, how and when

"better, faster, cheaper"

"**better**, faster, cheaper"

What does it all mean?

- More than 4 million single base pair variants (SNPs) in any individual
  - Which cause the disease we are looking at
  - Will find out other things:
    - Each person a carrier for approximately 5 known severe diseases e.g. Carrier for Cystic fibrosis
    - Multiple variants associate with increased disease risk e.g. Diabetes, Asthma



Establishing Causality

Need to determine :

- this variant
- in this gene
- causes this disease
- in this case

- female with a history of failure to thrive, recurrent infections, poor growth and diarrhea.

- No mutations in *TTC37*, the only gene known to cause THE syndrome
- WGS found interesting variants in 3 genes
- 2 other genes excluded by functional testing

Disruptive variants in Class X gene

Gene	Effects
SKIV2L	Novel stop
SKIV2L	Disrupts canonical splice

SKIV2L Mutations Cause Syndromic Diarrhea, or Trichohepatoenteric Syndrome

Alexandre Fabre,[1,2] Bernard Charroux,[3] Christine Martinez-Vinson,[4] Bertrand Roquelaure,[2] Egritas Odul,[5] Ersin Sayar,[6] Hilary Smith,[7] Virginie Colomb,[8] Nicolas Andre,[9] Jean-Pierre Hugot,[4] Olivier Goulet,[8] Caroline Lacoste,[10] Jacques Sarles,[2] Julien Royet,[3] Nicolas Levy,[1,10] and Catherine Badens[1,10,*]

Syndromic diarrhea (or trichohepatoenteric syndrome) is a rare congenital bowel disorder characterized by intractable diarrhea and woolly hair, and it has recently been associated with mutations in *TTC37*. Although databases report TTC37 as being the human ortholog of Ski3p, one of the yeast Ski-complex cofactors, this lead was not investigated in initial studies. The Ski complex is a multiprotein complex required for exosome-mediated RNA surveillance, including the regulation of normal mRNA and the decay of nonfunctional mRNA. Considering the fact that TTC37 is homologous to Ski3p, we explored a gene encoding another Ski-complex cofactor, *SKIV2L*, in six individuals presenting with typical syndromic diarrhea without variation in *TTC37*. We identified mutations in all six individuals. Our results show that mutations in genes encoding cofactors of the human Ski complex cause syndromic diarrhea, establishing a link between defects of the human exosome complex and a Mendelian disease.

Why Faster?

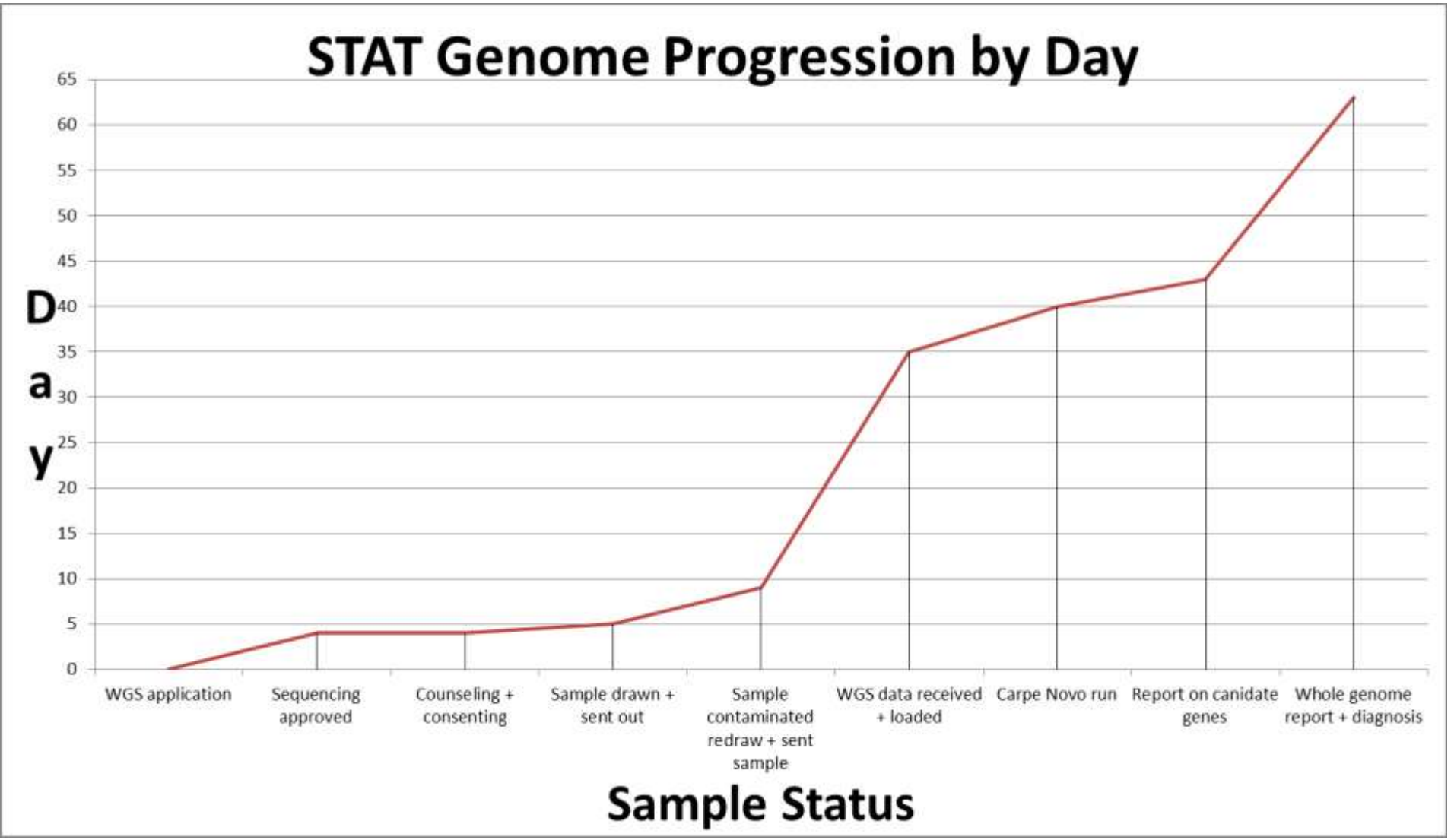
- 2 cases in which management changes could have been made didn’t get results fast enough
- Many cases denied WGS as it would not provide changes in management fast enough
- In approved cases long wait (3 months) is very hard for parents and caregivers

RESEARCH ARTICLE

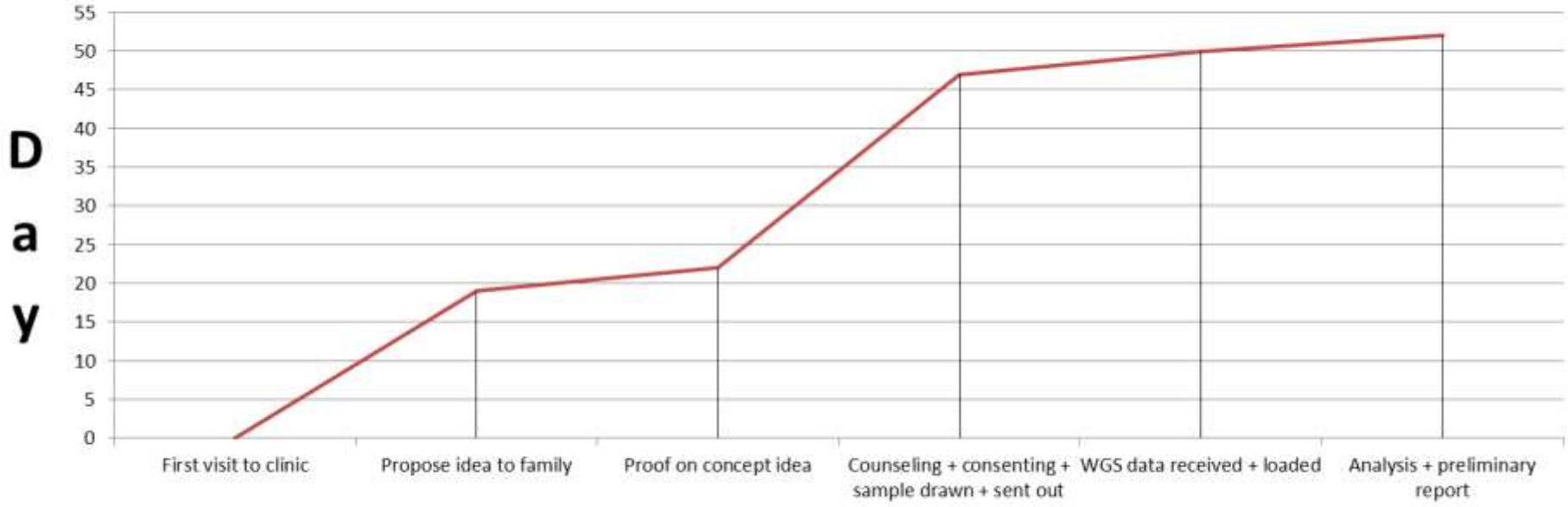
DIAGNOSTICS

Rapid Whole-Genome Sequencing for Genetic Disease Diagnosis in Neonatal Intensive Care Units

Carol Jean Saunders,[1,2,3,4,5]* Neil Andrew Miller,[1,2,4]* Sarah Elizabeth Soden,[1,2,4]* Darrell Lee Dinwiddie,[1,2,3,4,5]* Aaron Noll,[1] Noor Abu Alnadi,[4] Nevene Andraws,[3] Melanie LeAnn Patterson,[1,3] Lisa Ann Krivohlavek,[1,3] Joel Fellis,[6] Sean Humphray,[6] Peter Saffrey,[6] Zoya Kingsbury,[6] Jacqueline Claire Weir,[6] Jason Betley,[6] Russell James Grocock,[6] Elliott Harrison Margulies,[6] Emily Gwendolyn Farrow,[1] Michael Artman,[2,4] Nicole Pauline Safina,[1,4] Joshua Erin Petrikin,[2,3] Kevin Peter Hall,[6] Stephen Francis Kingsmore[1,2,3,4,5]†


STAT Genome Progression by Day
Day
65
60
55
50
45
40
35
30
25
20
15
10
5
0
WGS application
Sequencing approved
Counseling + consenting
Sample drawn + sent out
Sample contaminated redraw + sent sample
WGS data received + loaded
Carpe Novo run
Report on canidate genes
Whole genome report + diagnosis
Sample Status

STAT Genome Progression by Day



"better, faster, **cheaper**"

Cheaper

- Can do more cases, WGS as first tier test
- Improves probability of insurance reimbursement

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