

3) Search for a causal relationship

The evidence presented above demonstrates a correlation between the decrease of infections or factors predisposing to infections and immune disorders. A causal relationship remains to be established. Three lines of evidence argue in this direction.

a) A number of anti-infectious interventions have led to increased allergies. Thus, anti-helminthic treatment and the anti-pneumonia vaccine (just mentioned) were reported to increase incidence. There are also some data related to antibiotics, but they are more controversial, perhaps because of the difficulty in separating the effect of antibiotics and that of the infections at the origin of the antibiotics treatment. Note however that kanamycin treatment in mice increases the IgE level ⁽⁸⁾.

In the same vein, the prevention or even healing of atopic dermatitis by administration of non-pathogenic lactobacilli is very telling ⁽⁹⁾.

The best piece of evidence, though, has been derived from the prevention of a number of animal models of allergic or autoimmune diseases by infections in animal models. Even more striking is the observation, reminiscent of the Pakistani immigrants to the UK, that non-obese diabetic (NOD) mice which show a low incidence of IDDM in conventional facilities immediately show a jump to 100% disease incidence when bred in germ-free conditions ⁽¹⁾.

4) Putative mechanisms

A number of mechanisms probably explain the protective role of infections discussed above.

Antigenic competition is the most obvious possibility. It has been known for several decades that a given ongoing immune response may compete with other immune responses. The underlying mechanisms are not clear but probably involve MHC peptide competition and cytokine consumption, notably of IL-7, a cytokine playing a major role in homeostatic-driven lymphocyte activation and proliferation.

Bystander suppression is another possibility. Immune responses generate the activation of various subsets of regulatory T cells, the effects of which are not limited to the immune response against the initial antigen.

Last but not least, infectious agents might influence immune responses after stimulation of various receptors independently from the evoked immune response. Toll-like receptors are major candidates in this respect, all the more so because several TLR agonists have proven capable of preventing IDDM in NOD mice. Virus receptors could also be instrumental as previously shown for the measles virus and more recently for the hepatitis A virus (HAV). Atopy is less frequent in HAV seropositive subjects.

Genetic predisposition to asthma involves a gene on chromosome 5 which has recently been shown to code for a protein, named TIM-1, expressed by Th2 cells ⁽¹⁰⁾. TIM-1 polymorphism does not differ between atopic and non-atopic subjects, considered as a whole, but does it when one considers HAV seropositive subjects separately ⁽¹¹⁾?

5) Practical implications and applications

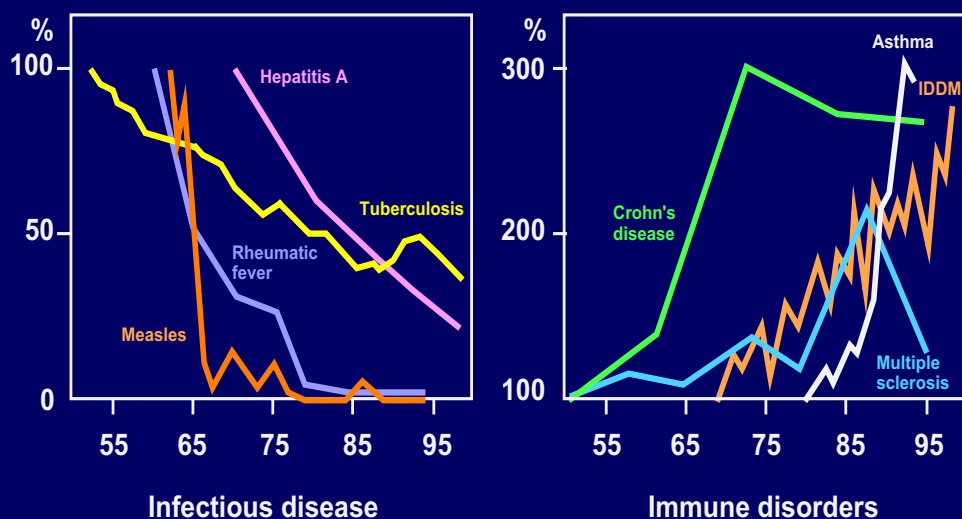
The data presented above clearly show that some of the infections primarily observed in Western countries had a favourable role which is missing today. What could the practical consequences be of such an observation? It is obviously out of question to reconsider hygiene measures which allowed the remarkable decrease of severe childhood infections or efficient modern vaccination programs. One may note, however, that extreme measures are probably not justified, as is sometimes observed in a somewhat obsessive fashion. One should be more critical about unnecessary measures, notably unjustified antibiotic treatment which, in addition to creating antibiotic resistance, may contribute to increased frequency of immune disorders.

Lastly, one should conduct active research for immunostimulatory therapies which could substitute for infections. One may think of bacterial extracts. There have been some interesting preliminary data with mycobacterium vaccae extracts in asthma, atopic dermatitis and psoriasis⁽¹²⁻¹⁴⁾. One may also explore the effect of probiotics as suggested by two very promising randomised trials^(15, 16). More generally, one should pay more attention to the maintenance of a physiological gut flora.

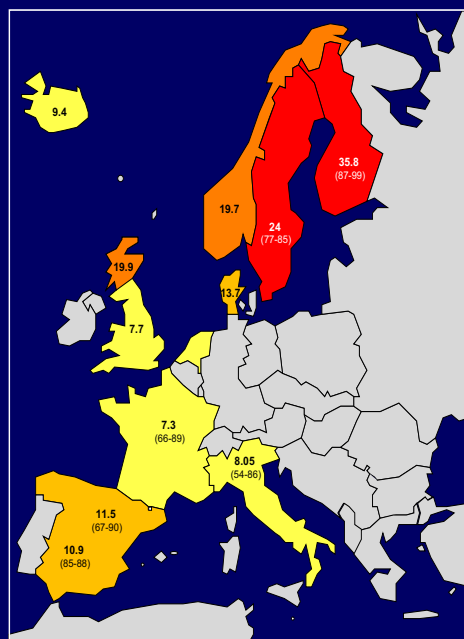
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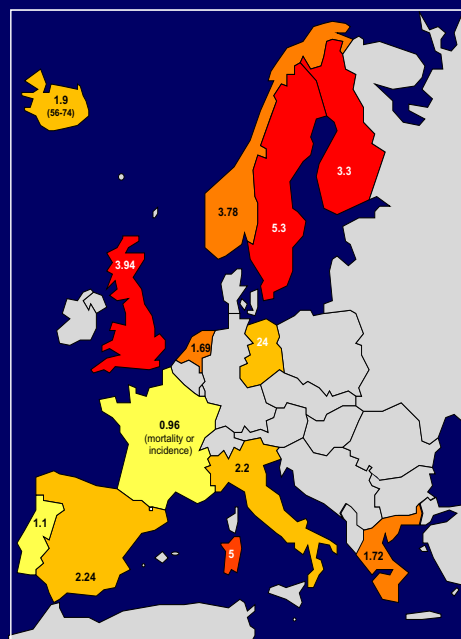
Incidence of prototype infectious disease and immune disorders over 4 decades



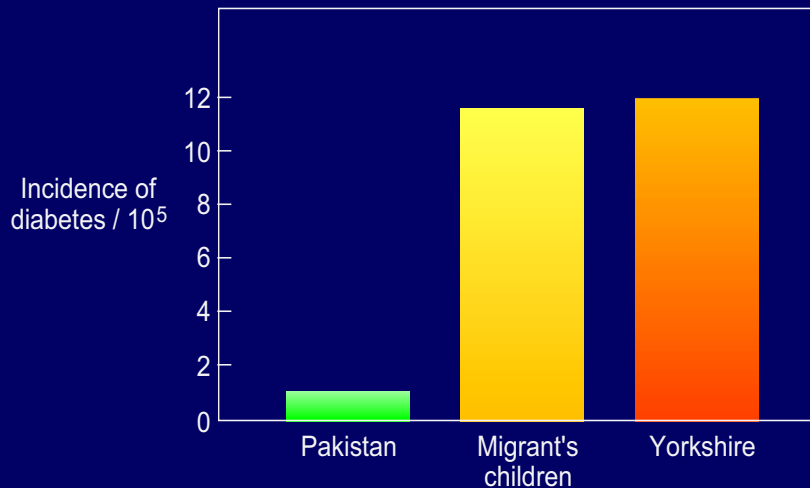
Incidence of IDDM
(per 100,000)



Incidence of multiple sclerosis
(per 100,000)



IDDM incidence in children of migrants from Pakistan to Yorkshire



Staines A. (1997) and Bodansky H.J. (1992)

PREVALENCE OF ASTHMA AND ATOPY IN TWO AREAS OF WEST AND EAST GERMANY

	West	East
• ATOPIC SENSITIZATION	36.7%	18.2%
	OR 2.6	
	<u>p<0.0001</u>	
• CURRENT ASTHMA AND HAY FEVER	5.9%	3.9%
	8.6%	2.7%
	OR 1.5 p<0.0001	
	OR 3.4 p<0.0001	

N=5.030 West

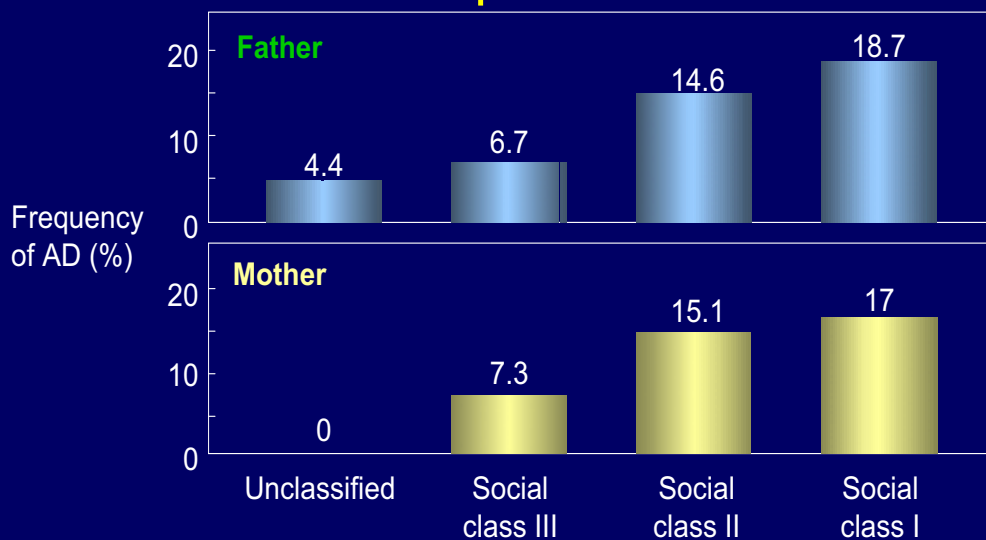
N=2.623 East

E. Von Mutius, Am J Respir Crit Care Med. 1994

IBD & Industrialization and urbanization



Correlation between incidence of AD and parents' social level



Werner et al., 2002

CORRELATION BETWEEN HYGIENE INDICES AND IBD (ODDS RATIO)

<u>RISK FACTORS</u>	CROHN'S DISEASE	ULCERATIVE COLITIS
WATER TAP	1.8	0.9
HOT WATER TAP	5.0	1.3
SEPARATE BATHROOM	3.3	1.3
FLUSH TOILET	1.1	1.4
MAINS DRAINAGE	2.6	1.2

(A.E. Gent et al., 1994)

DAY CARE ATTENDANCE IN EARLY LIFE AND RISK OF ASTHMA AT 6 YEARS

	O.R.
Atopic dermatitis	0.3
Asthma at 6 years	0.3
Recurrent wheezing	0.3

Celedon JC, Am. J. Respir. Crit. Care
Med. 2003

FACTORS CONTRIBUTING TO THE APPEARANCE OF INFECTIONS

Sources of pathogenic agents

- drinking water
- food (cold storage)
- climate
- housing conditions

Anti-infectious defense

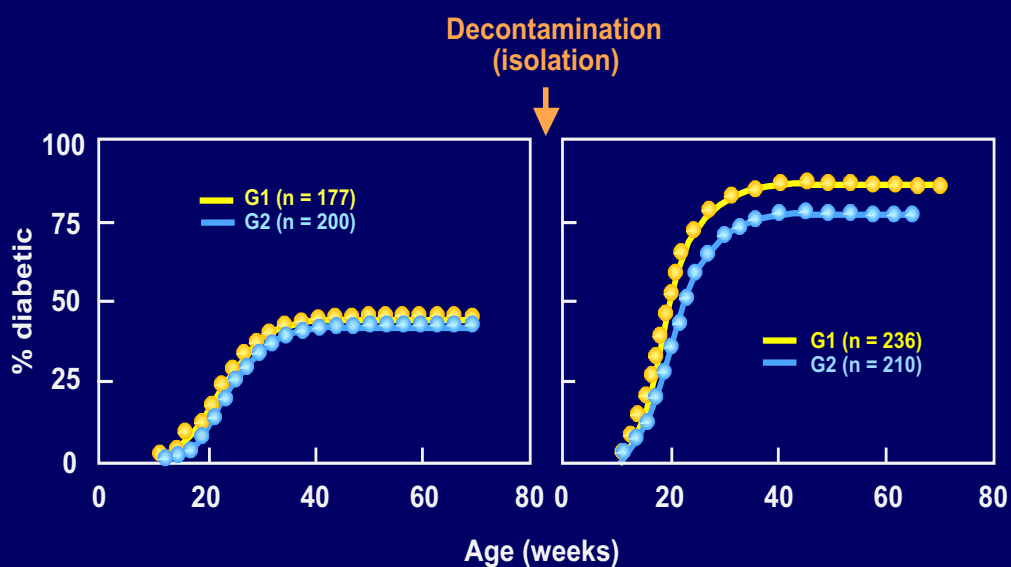
- genetic factors
- nutrition
- antibiotics
- vaccination

CAUSAL RELATIONSHIP

- **ANIMAL MODELS**
- **CLINICAL TRIALS
(THERAPEUTIC PROOF OF
PRINCIPALE)**



Effect of infections on diabetes incidence in female NOD mice

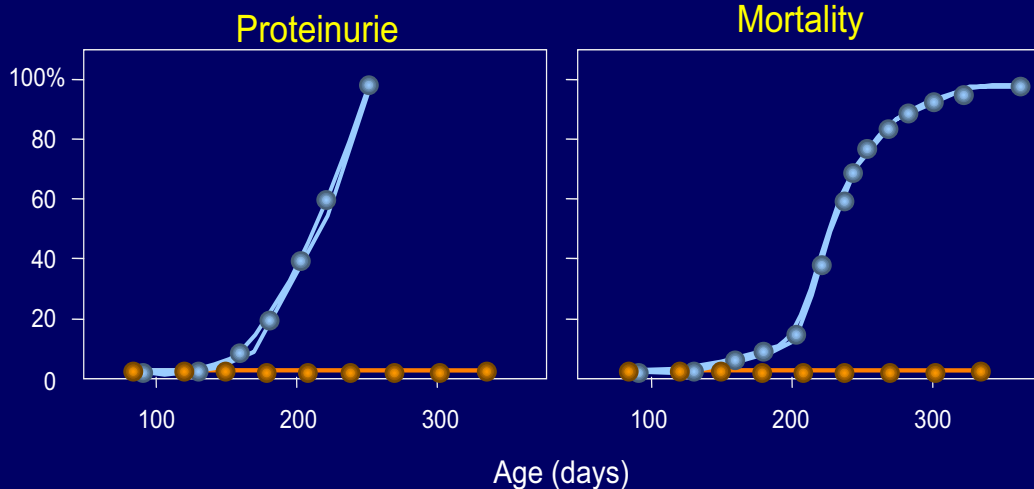


PREVENTION OF IDDM IN NOD MICE BY INFECTIOUS AGENTS

Bacteria	streptococci salmonella mycobacteria (CFA, BCG, ...)
Viruses	LCMV MHV LDHV
Parasites	schistosoma oxyures

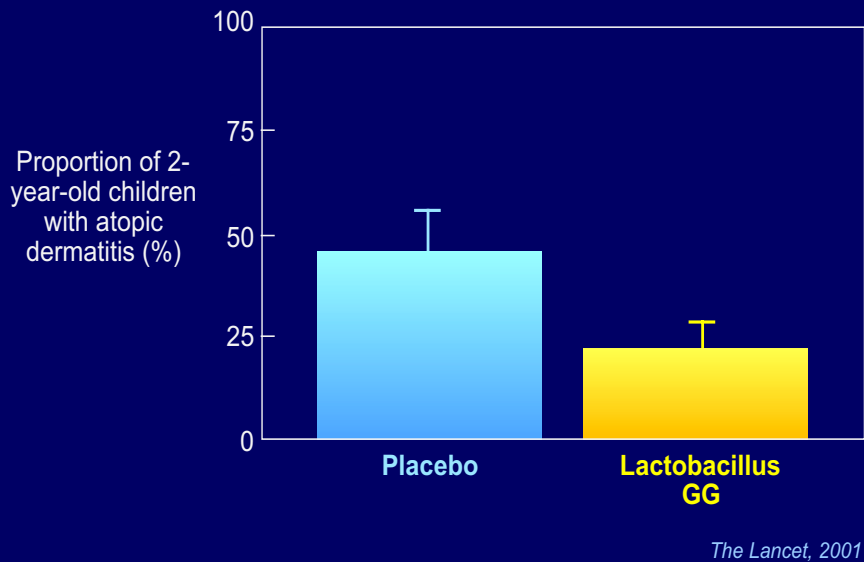
Effect of malaria in B / W mice

● Controls
● Mice infected

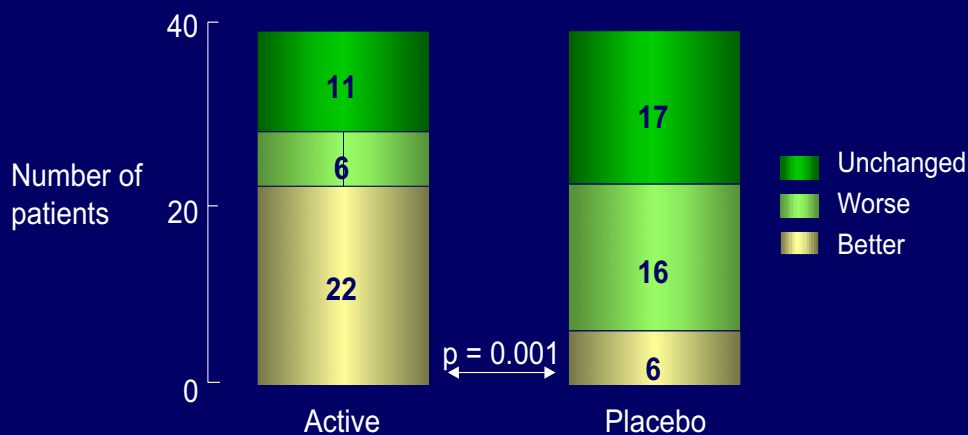


B. M. Greenwood et al, 1970

Treatment effect of Lactobacillus GG on atopic dermatitis



Effect of a 6-week treatment of atopic dermatitis in Danish children (aged 1-13) by probiotics (L rhamnosus and L reuteri)



MECHANISMS

MECHANISMS OF THE PROTECTION FROM AUTOIMMUNE DISEASES AFFORDED BY INFECTIONS

1. Antigenic competition

Effect on antigen presentation

- preemption (phagocytosis)
- antigen processing
- antigen binding to MHC molecules

Competition for homeostatic signals

MECHANISMS OF THE PROTECTION FROM AUTOIMMUNE DISEASES AFFORDED BY INFECTIONS

2. Antigen-induced immunoregulation (by-stander suppression)

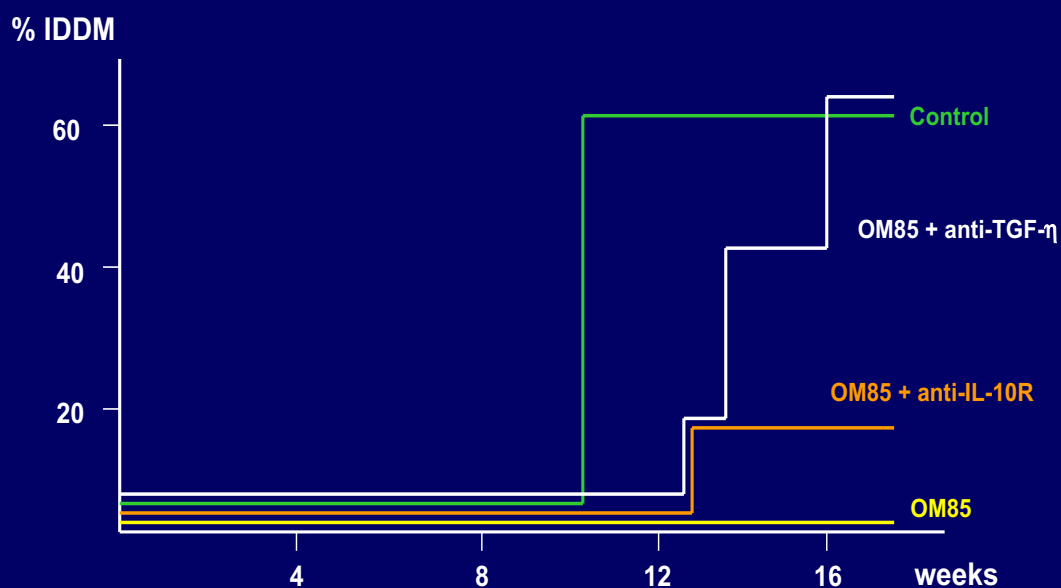
Th1/Th2 cells

Th3 cells

NKT cells

CD25⁺ T cells

cytokine dependency of the OM85-induced diabetes protection



MECHANISMS OF THE PROTECTION FROM AUTOIMMUNE DISEASES AFFORDED BY INFECTIONS

3. Non-antigen-mediated effects

Toll-like receptors (regulatory cytokines)

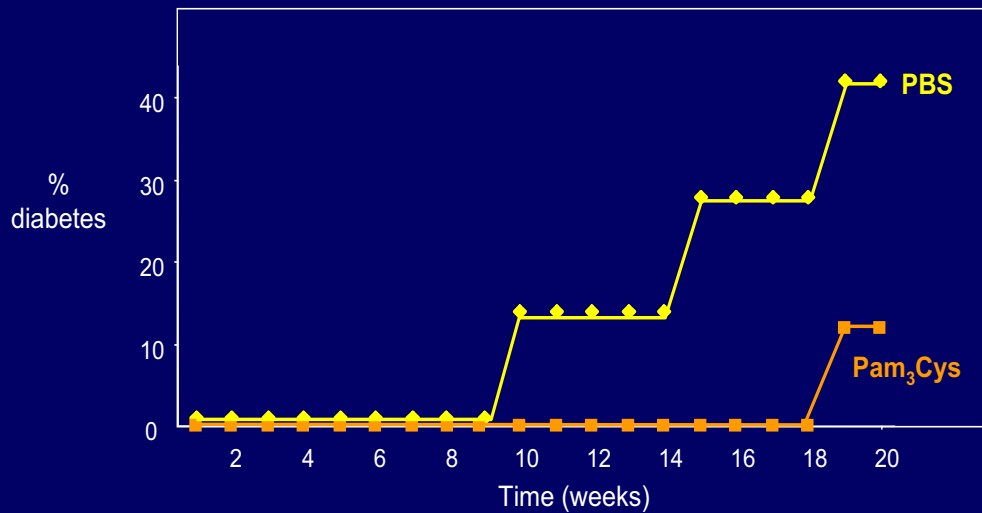
Superantigens (depletion of T cell subsets)

Immunosuppressive proteins

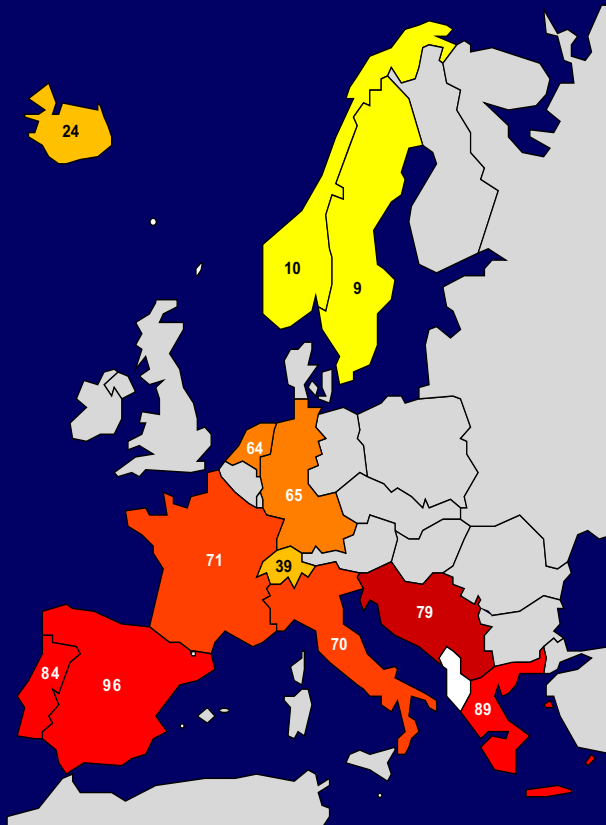
TOLL-LIKE RECEPTORS

TLR Family	Ligands (examples)
TLR1	Tri-acyl lipopeptides (bacteria, mycobacteria)
TLR2	Lipoprotein/lipopeptides (a variety of pathogens) Peptidoglycan and Lipoteichoic acid (Gram-positive bacteria) Glycoinositolphospholipids (Trypanosoma Cruzi) HSP70 (host)
TLR3	Double-stranded RNA (virus)
TLR4	LPS (Gram-negative bacteria) Taxol (plant) Fusion protein (RSV) HSP60 (host)
TLR5	Flagellin (bacteria)
TLR6	Di-acyl lipopeptides (mycoplasma)
TLR7	Imidazoquinoline (synthetic compounds)
TLR8	?
TLR9	CpG DNA (bacteria)
TLR10	?

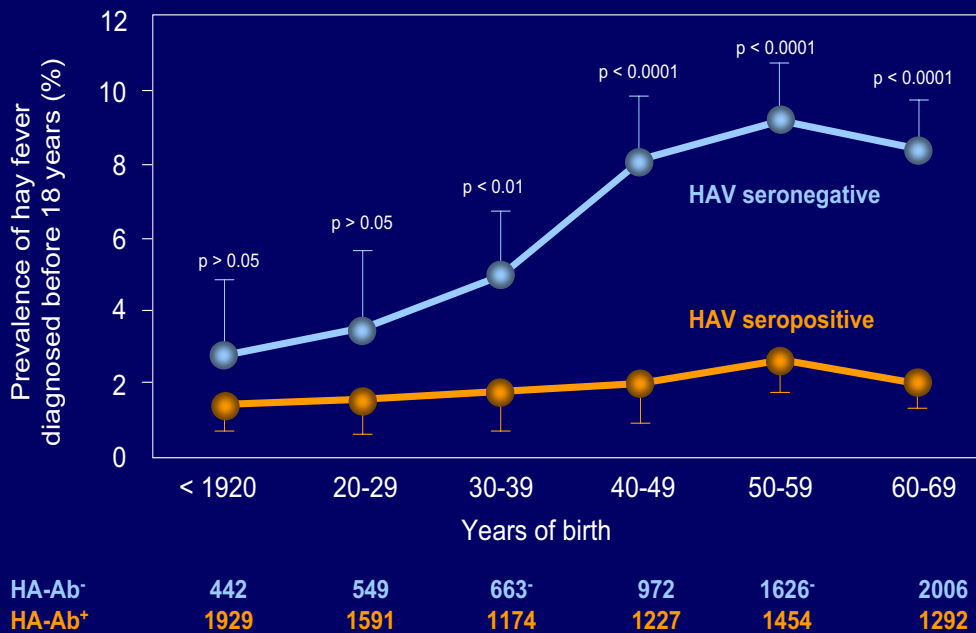
Prevention of diabetes in NOD mice by administration of Pam₃Cys, an agonist of TLR2



Prevalence of anti-hepatite A virus antibodies in Europe (%)



Hay fever at/or before 18 years in the USA general population according to serology for hepatitis A virus (NHANES-III)



S. Bonini et al. JACI, 2002

Asthma, HAV and TIM-1

- Major asthma susceptibility gene at human chromosome 5q23-35
- Production of congenic mice differing at the homologous chromosomal segment
- Identification of a Mendelian trait expressed by TH2 cells controlling airway hyperreactivity
- Positional cloning identified a gene family that encodes TIM proteins
- The human homologue of TIM-1 is Hepatitis A Virus (HAV) receptor

(JJ.McIntire et al., Nature Immunology, 2001)

CONCLUSIONS

- The decline of infectious diseases in developed countries parallels the increase of allergic and autoimmune disorders
- High socio-economic level is a risk factor for immune disorders

CONCLUSIONS (Continued)

- Collective and possibly also individual hygiene is a risk factor
- Underlying mechanisms are multiple including homeostasis, bystander suppression, Toll-like and HAV receptors

Familial Mediterranean Fever and the Expanding Spectrum of Hereditary Autoinflammatory Disorders

Dan Kastner, MD, PhD
Genetics and Genomics Branch
NIAMS, NIH, DHHS

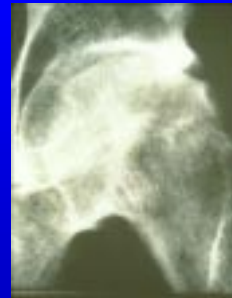
FMF: Clinical Features

1-3 day episodes of fever with:

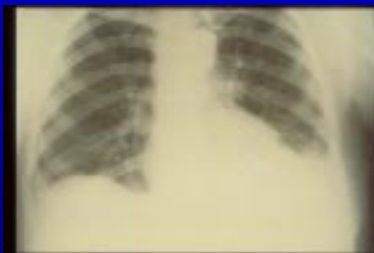
- Abdominal pain
- Chest pain
- Arthritis
- Rash



Peritonitis (air-fluid levels)



Chronic arthritis of the hip



Pleurisy (left pleural effusion)

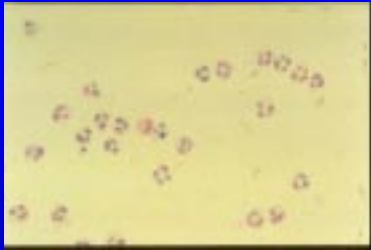


Posterior pericardial effusion

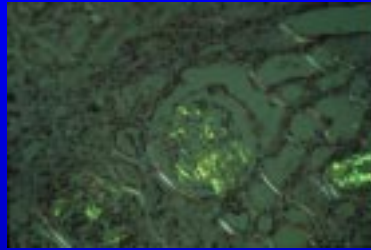


Erysipeloid erythema

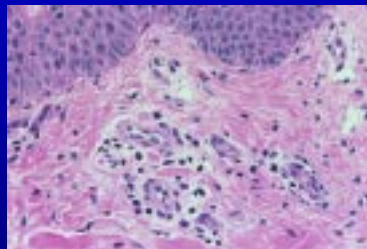
FMF: Histology



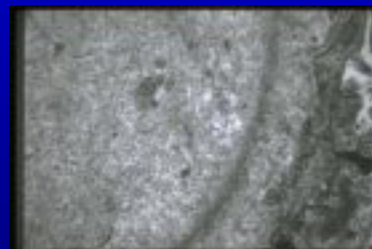
PMNs, synovial fluid



Renal amyloid, Congo red



Erysipeloid erythema

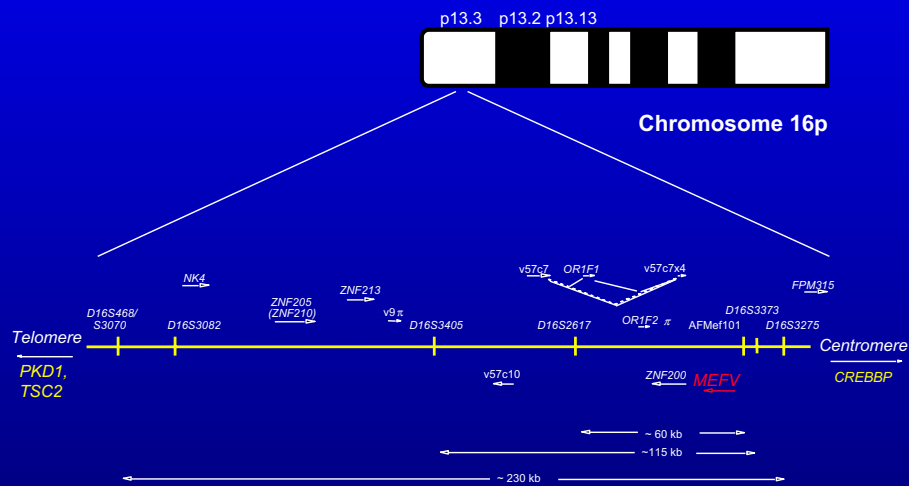


Renal amyloid, EM

FMF Family Studies



Positional Cloning of *MEFV*, the FMF Gene



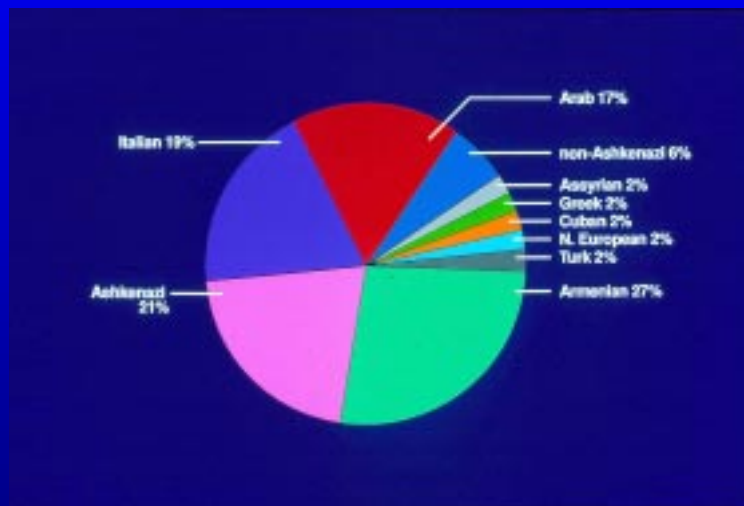
FMF Protein: Pyrin or Marenostin

Spread of Ancient Ancestral Mutations in FMF



Hospital Practice 33:131, 1998

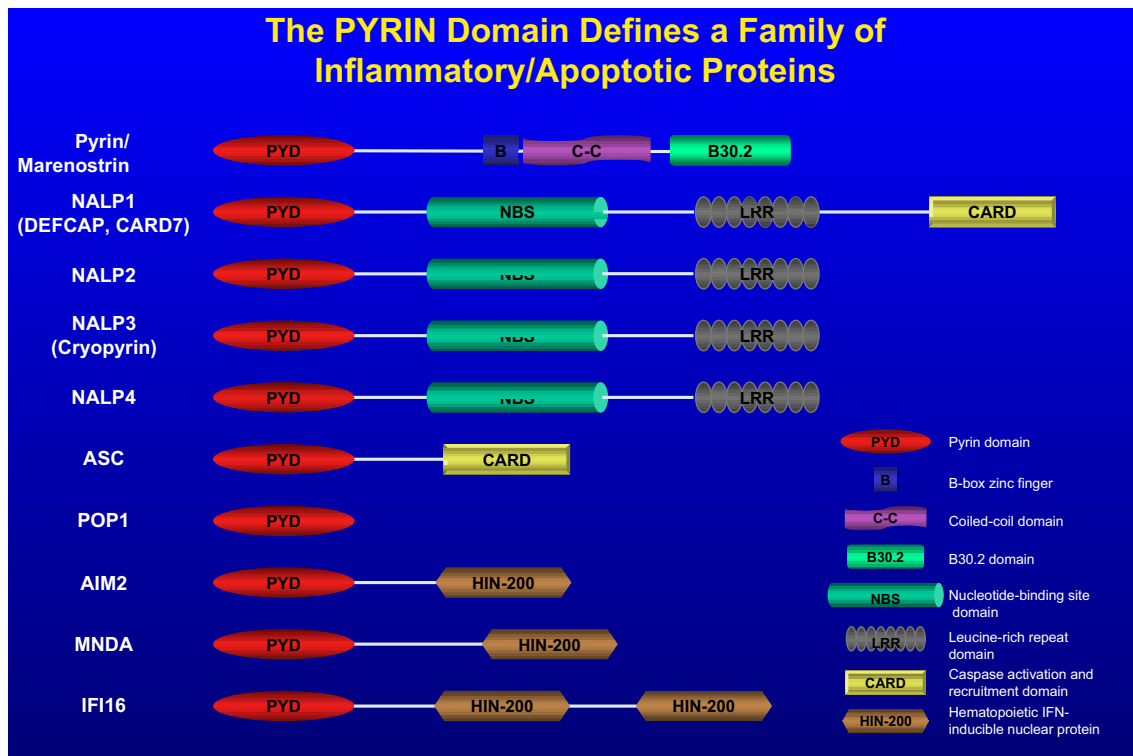
DNA Diagnosis Expands the Clinical Spectrum of FMF



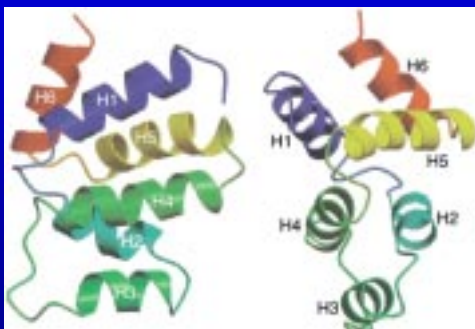
Medicine (Baltimore) 77:268, 1998

Genetic Testing In FMF

- 41 mutations currently known
- “Hot spots” in exon 10 and, to a lesser degree, exon 2
- Two mutations required for genetic diagnosis
- Only about 70% of patients with clinical FMF have two identifiable mutations
- Strong possibility of locus heterogeneity
- Clinical judgment still required to make the diagnosis



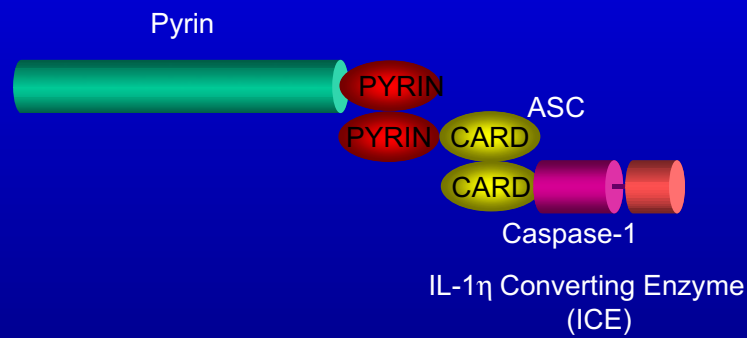
Structure of the Death Domain-Fold Motifs



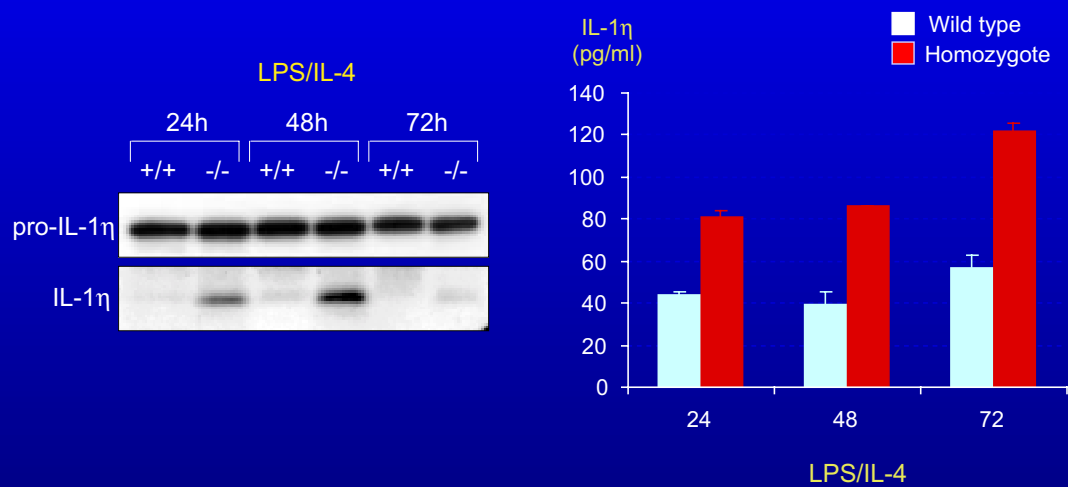
Protein Science 10:1911, 2001

- Adaptor domain architecture important in homotypic protein-protein interactions
- Death domains
- Death effector domains
- Caspase activation and recruitment domains (CARDs)
- Pyrin domains

ASC Can Act as a Link between the PYRIN Domain and Apoptotic and Inflammatory Pathways



Pyrin-Deficient Mice Produce More IL-1 η Than Wild-Type

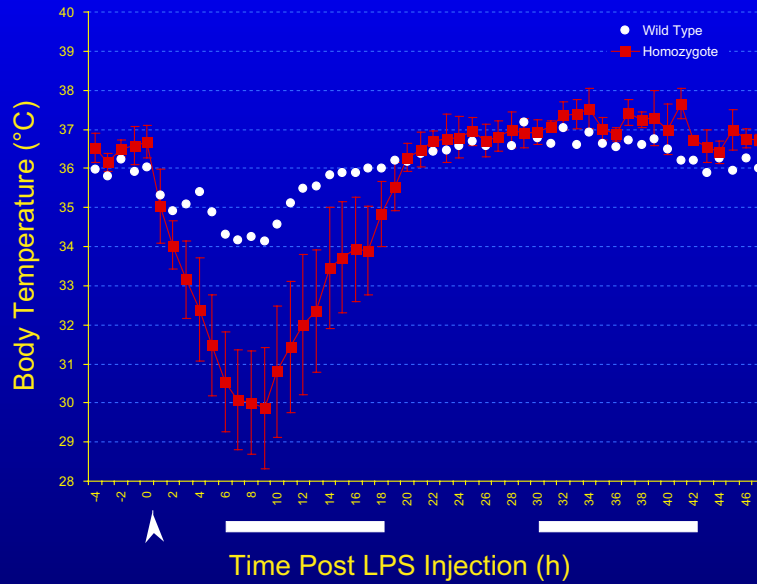


Molecular Cell 11:591, 2003

D2

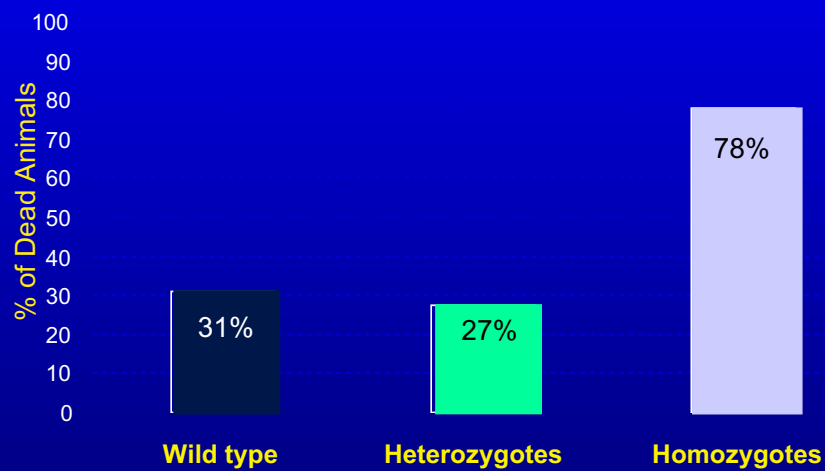
INTRODUCTORY
WORKSHOPS II

Pyrin-Deficient Mice Are Hypersensitive to Endotoxin

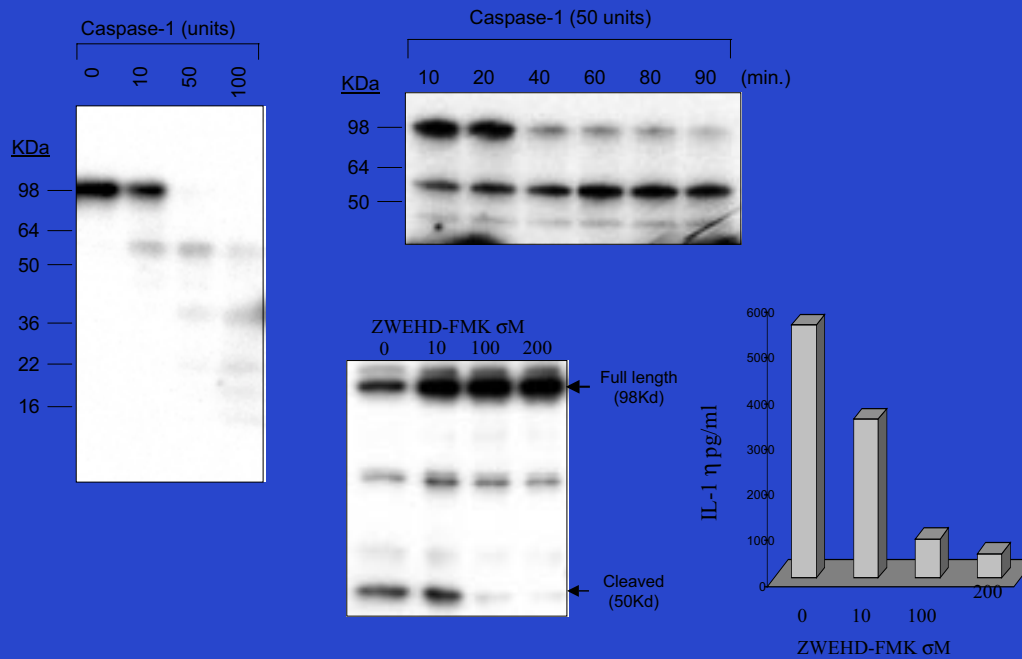


Molecular Cell 11:591, 2003

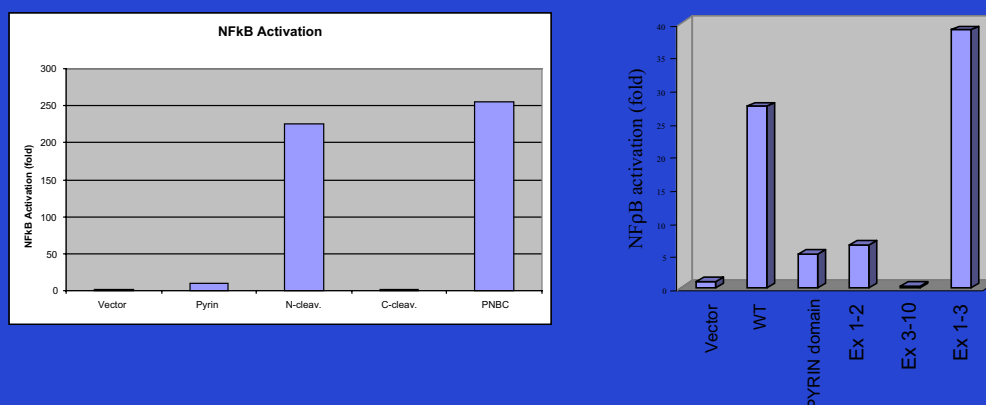
Susceptibility of Pyrin-Deficient Mice to Septic Shock



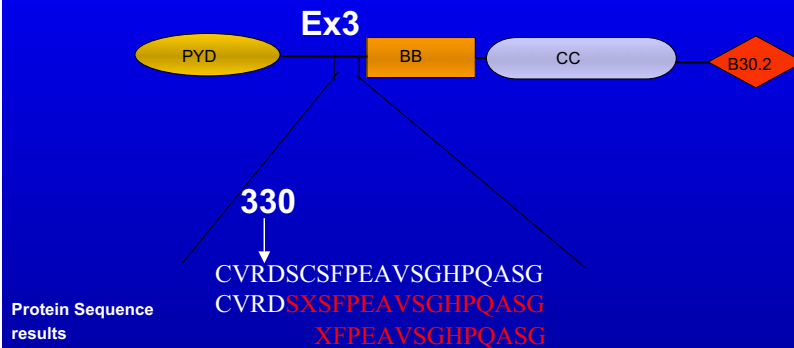
Pyrin is Cleaved by Caspase-1



The N-Terminal Caspase-1 Cleavage Fragment of Pyrin Activates NF- κ B

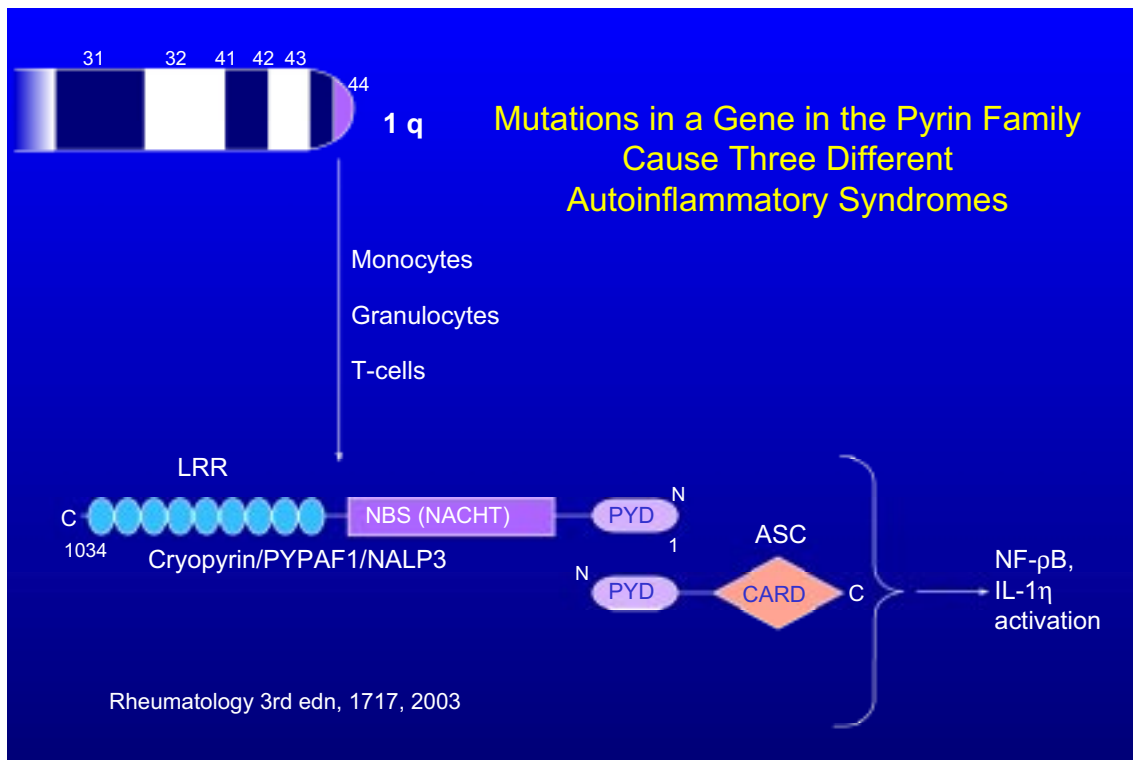


Identification of the Caspase-1 Cleavage Site in Pyrin



Molecular Pathogenesis of FMF

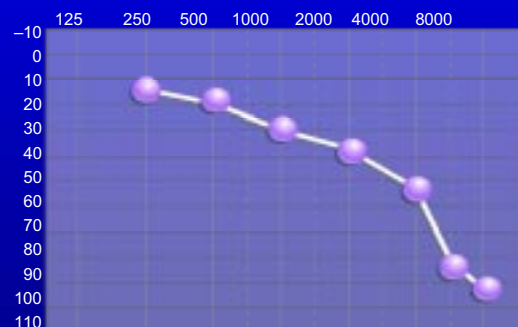
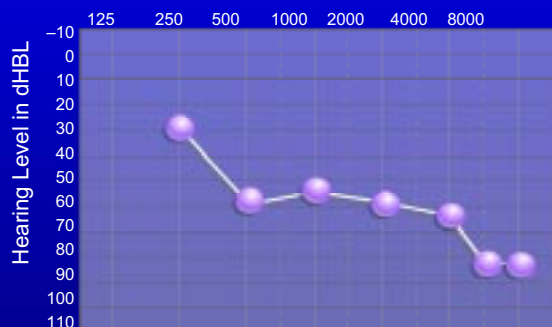
- Caused by mutations in pyrin/marenostrin
- Pyrin/marenostrin regulates:
 - IL-1 η production
 - NF- ρ B activation
 - Monocyte apoptosis
- The PYRIN domain defines a family of inflammatory regulators



Muckle-Wells Syndrome



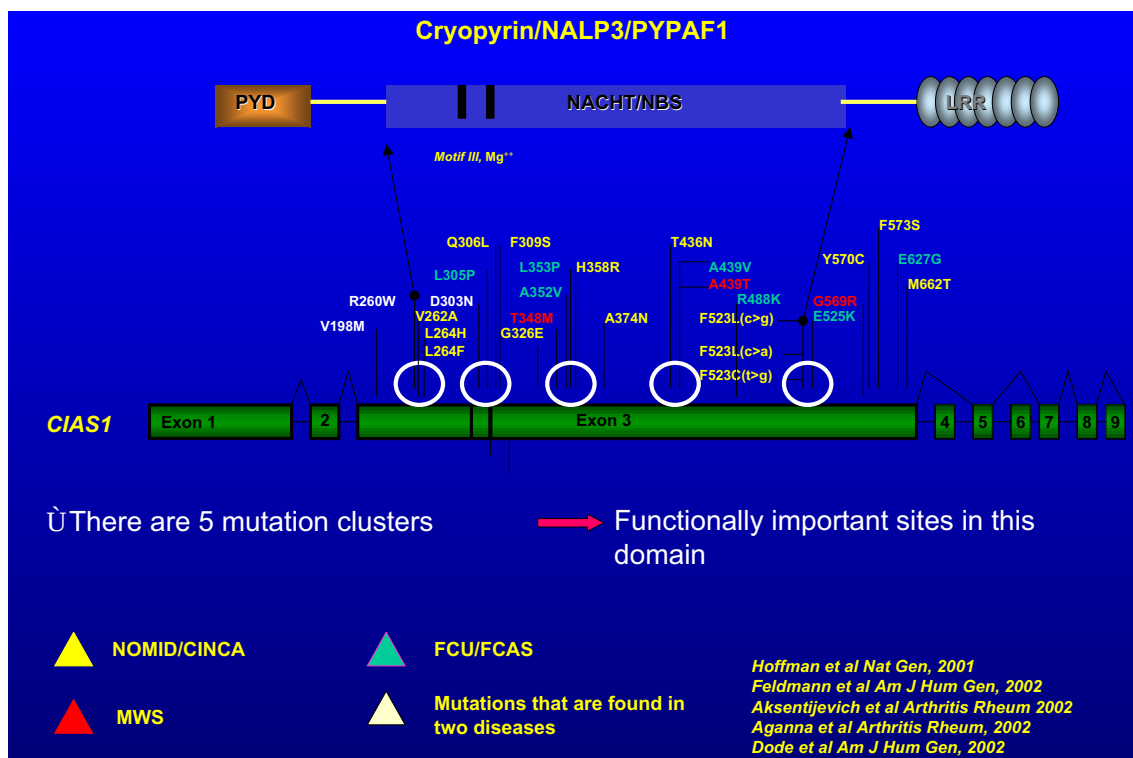
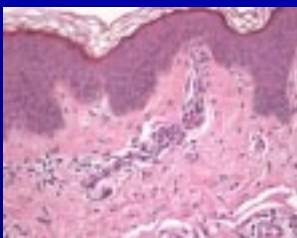
- Febrile attacks
- Urticarial rash
- Sensorineural deafness
- AA amyloidosis



D2

INTRODUCTORY
WORKSHOPS II

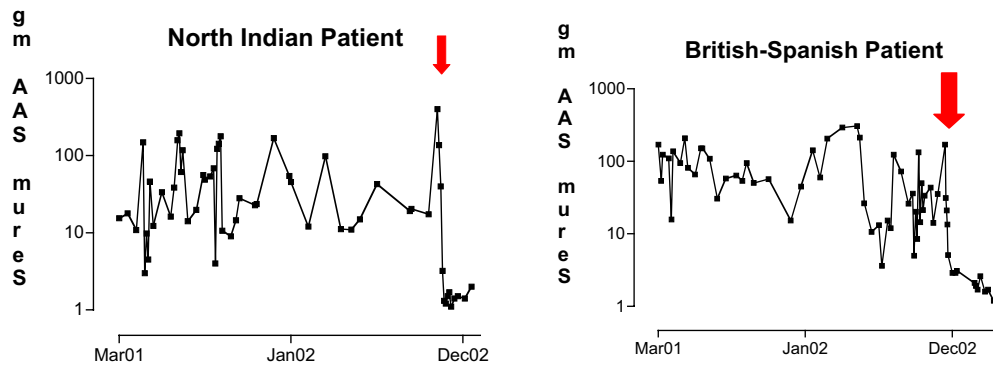
Chronic Infantile Neurologic Cutaneous and Articular (CINCA) Syndrome



Interleukin-1 Receptor Antagonist in Two Patients with R260W *CIAS1* Mutations

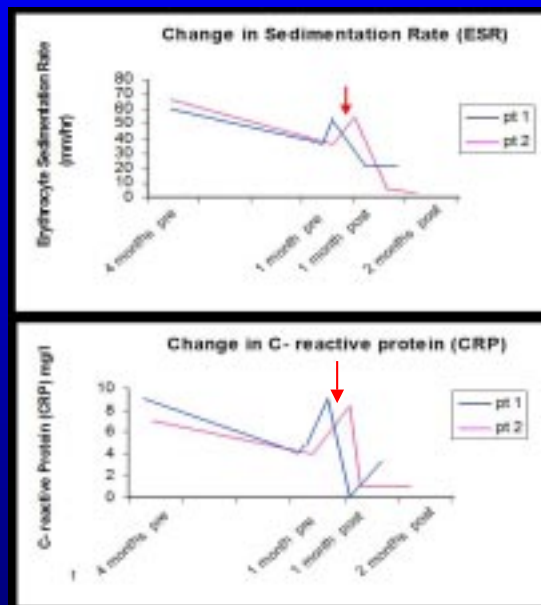
D2

INTRODUCTORY
WORKSHOPS II



Hawkins et al., NEJM 348:2583, 2003.

Response to the IL-1 Receptor Antagonist in Two Patients with NOMID/CINCA

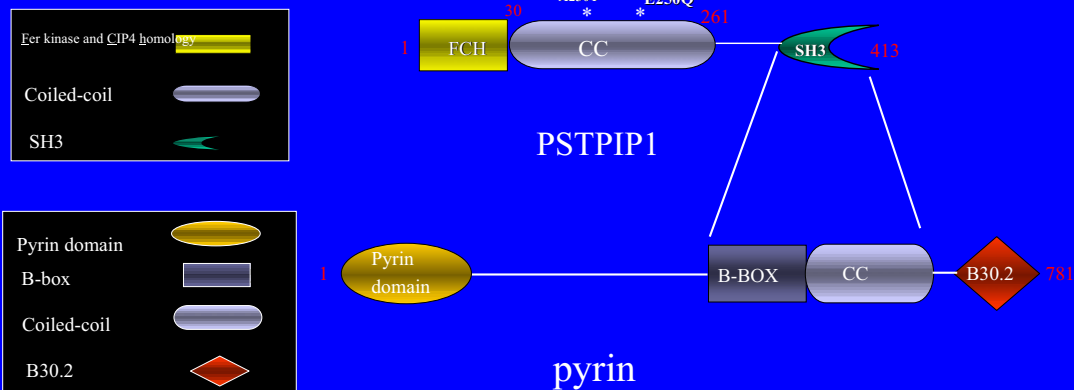


PAPA Syndrome

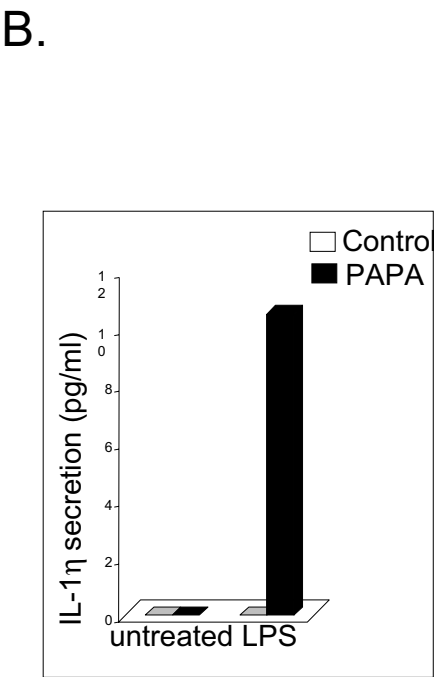
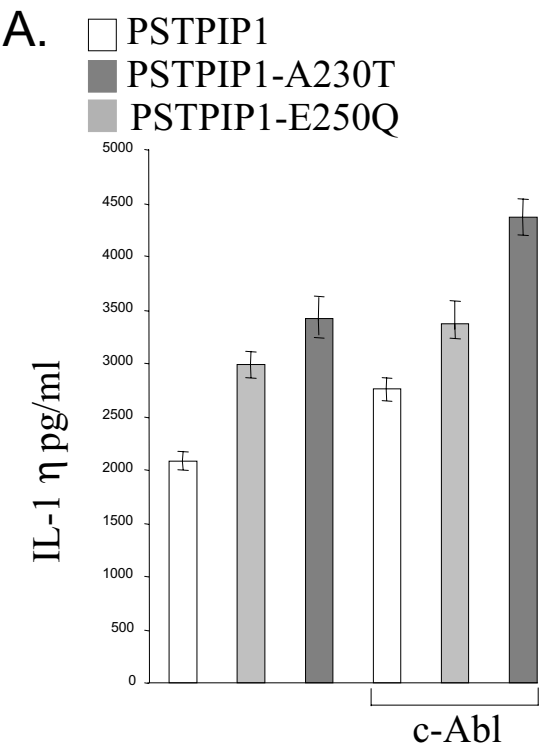
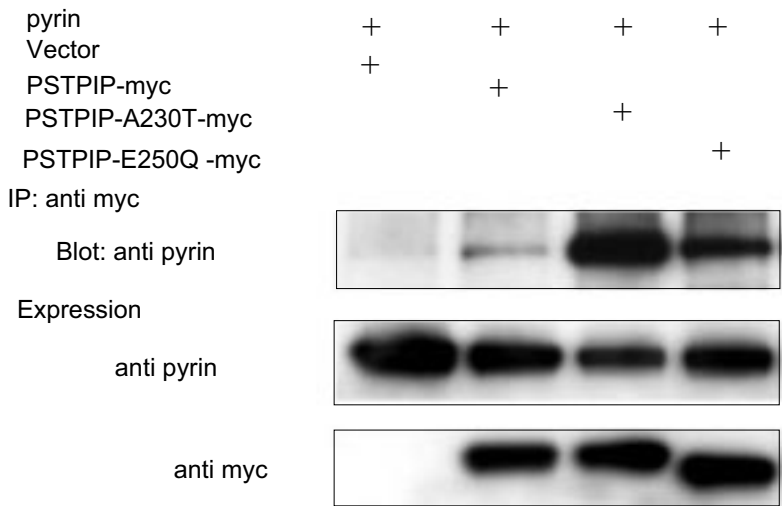
- Pyogenic arthritis
- Pyoderma gangrenosum
- Acne
- Autosomal dominant inheritance
- Caused by mutations in PSTPIP1



Extending the Pyrin Pathway: Proline Serine Threonine Phosphatase Interacting Protein 1 (PSTPIP1/CD2BP1)



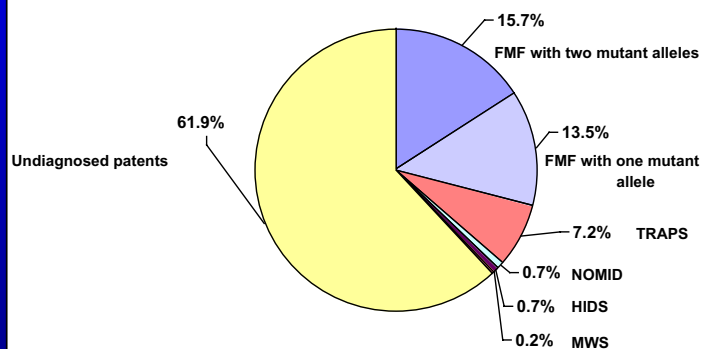
PAPA-Associated PSTPIP1 Mutations Cause Increased Interaction with Pyrin



FMF and Family: Lessons on the Control of Inflammation

- Mutations in pyrin/marenostrin and related proteins have been found in FMF, FCAS (FCU), MWS, CINCA/NOMID, and PAPA syndrome
- The N-terminal PYRIN domain defines a new family of proteins involved in inflammation and apoptosis
- The proteins encoded by these genes may prove to be important targets for therapeutic intervention

NIH Periodic Fever Patients





D2

**INTRODUCTORY
WORKSHOPS II**

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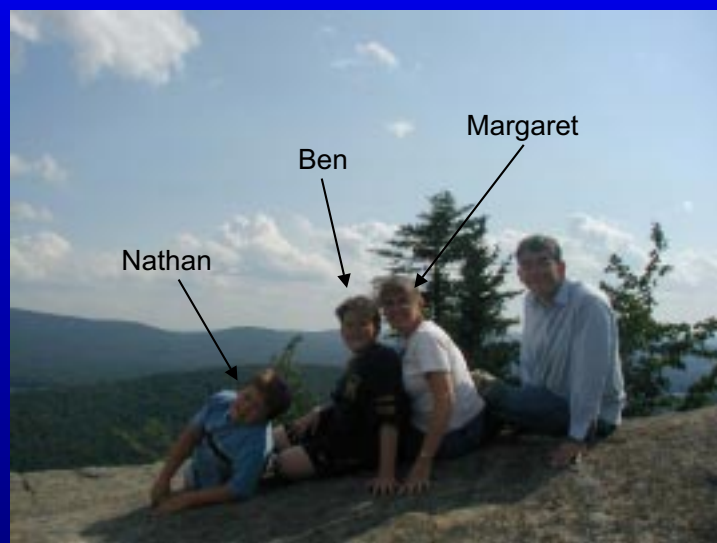
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Dan Lovell

Balu Athreya

Robert Warren

Raphaela Goldbach-Mansky





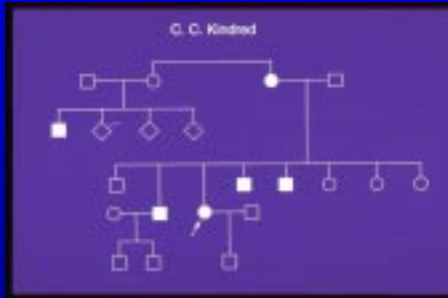
December, 2003: The Hereditary Periodic Fever Genes

Disease	Gene	Chr	Protein	Known Mutations
FMF	<i>MEFV</i>	16 p13.3	pyrin (marennostatin)	41
HIDS	<i>MVK</i>	12 q24	mevalonate kinase (MK)	23
TRAPS	<i>TNFRSF1A</i>	12 p13	55 kDa TNF receptor	33
MWS/ FCAS/ NOMID	<i>CIA S1</i>	1 q44	cryopyrin/ PYPA F1 / NALP 3	23

D2

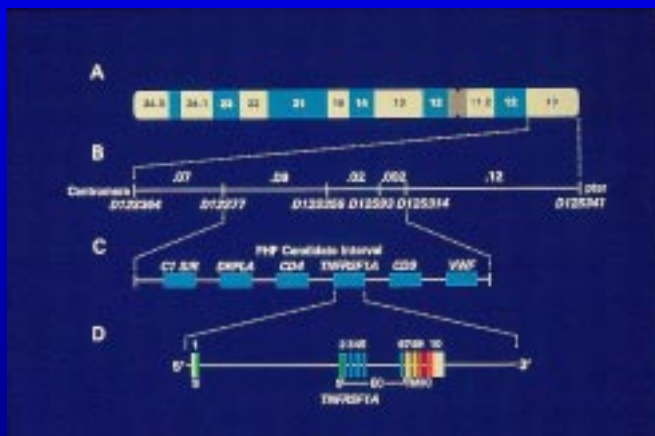
INTRODUCTORY
WORKSHOPS II

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

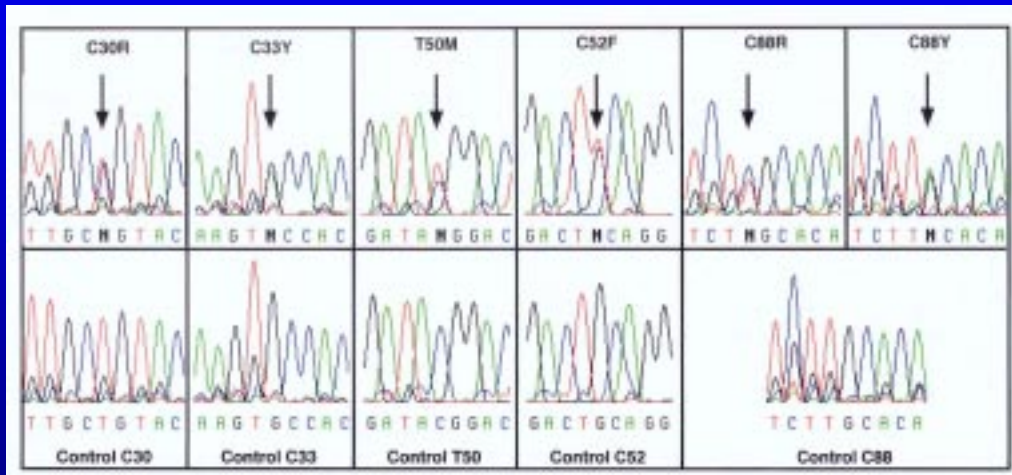
The Gene Causing the Dominant Periodic Fevers is Located on the Short Arm of Chromosome 12



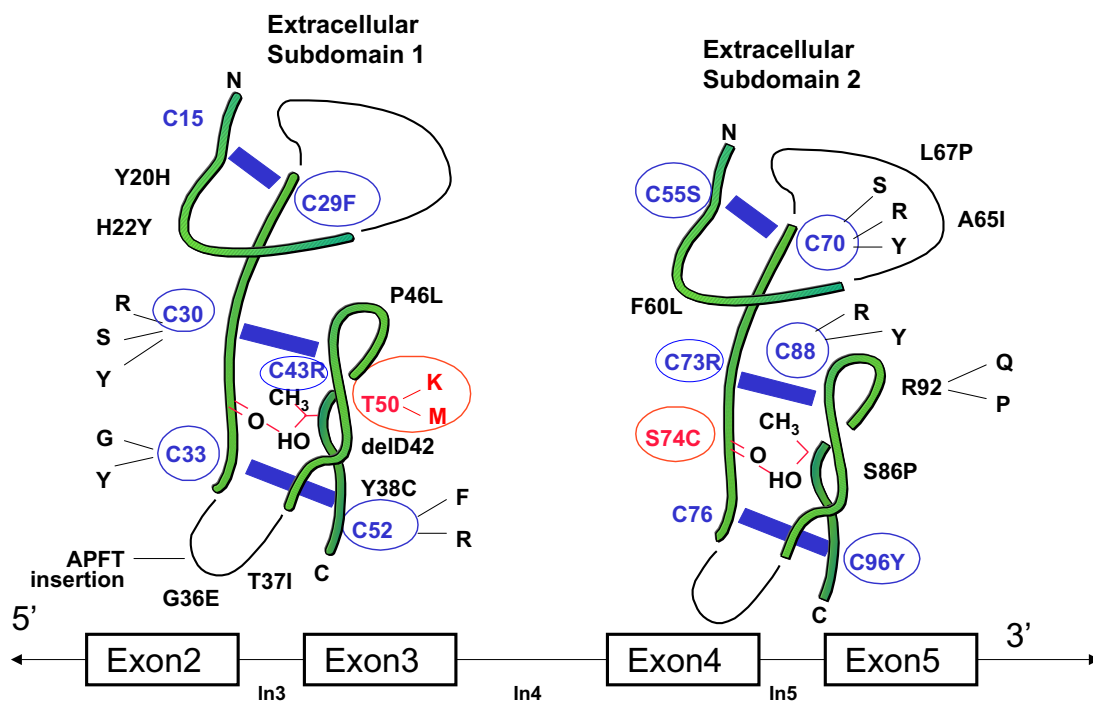
Mike McDermott

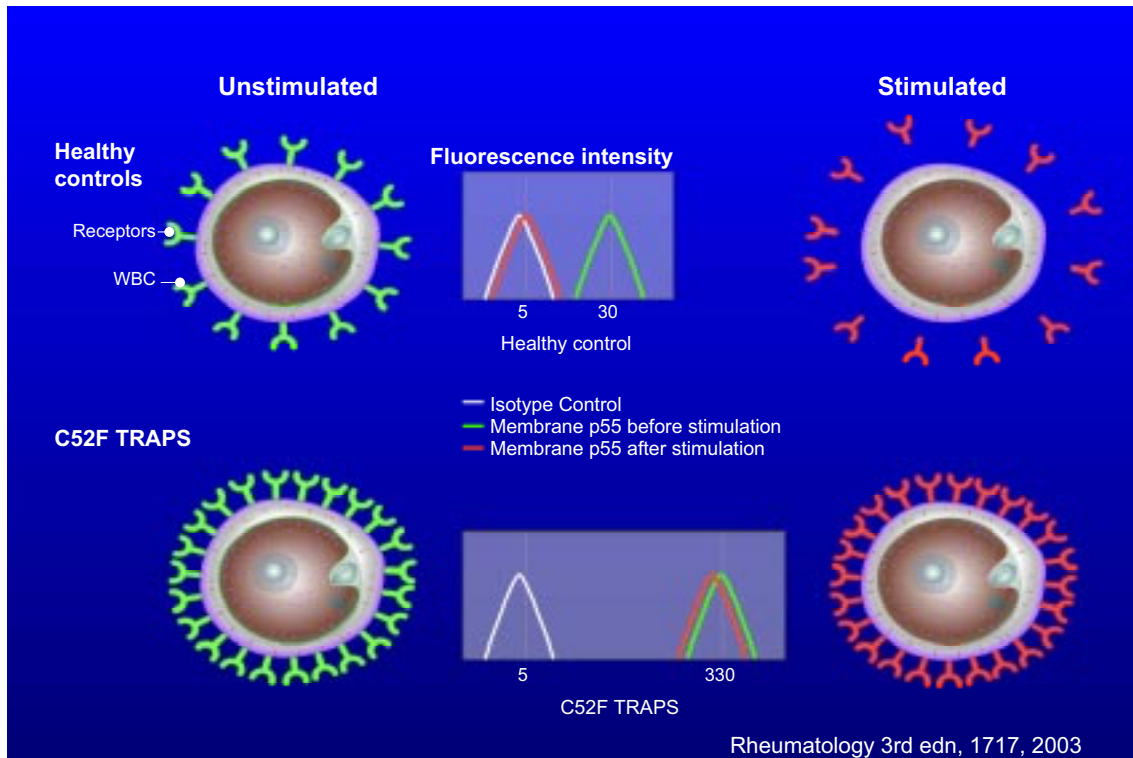
Curr Opin Immunol 12:479, 2000

p55 TNF Receptor Mutations in Patients with Dominantly Inherited Periodic Fevers

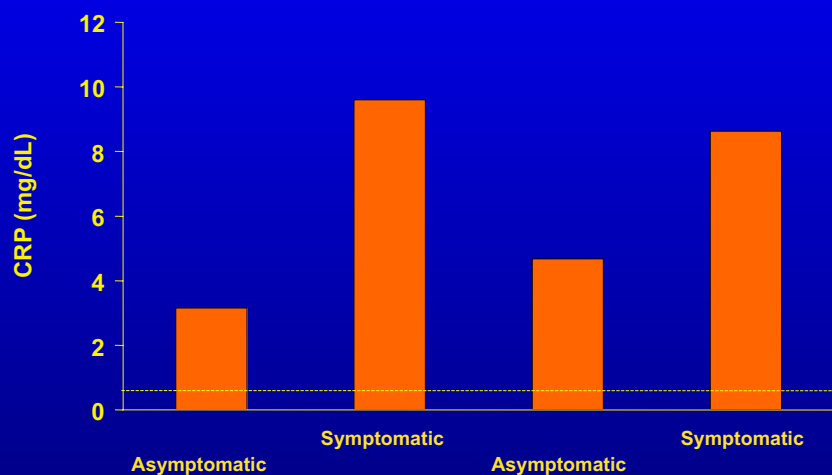


Cell 97:133, 1999



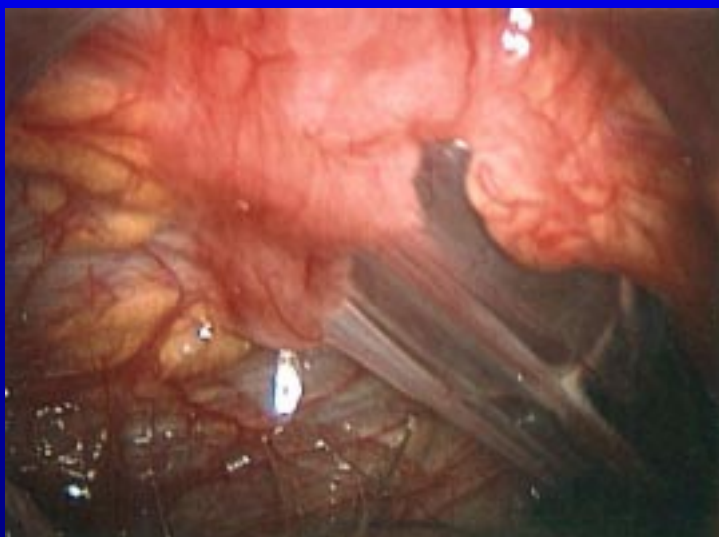


TRAPS Attacks: The Tip of the Iceberg

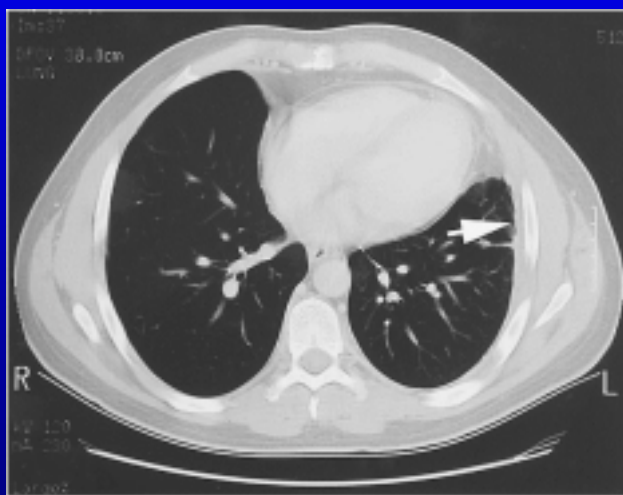


Medicine 81:349, 2002

TRAPS: Peritoneal Adhesions



TRAPS: Chronic Pleural Reaction

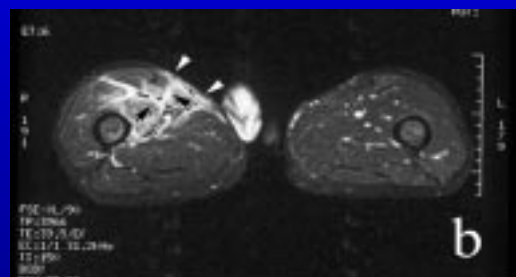
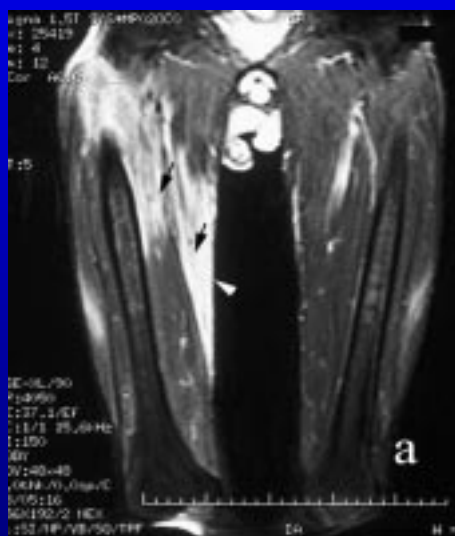


TRAPS: Migratory Rash and Myalgia



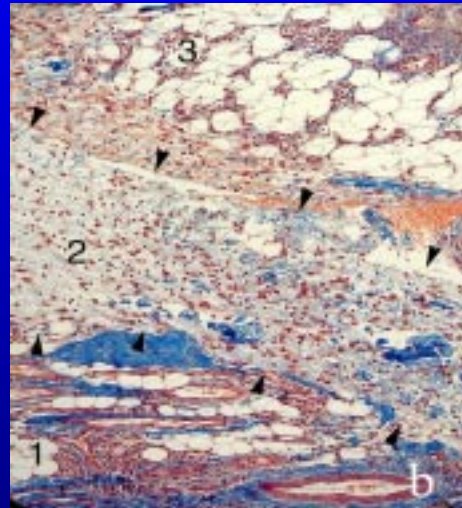
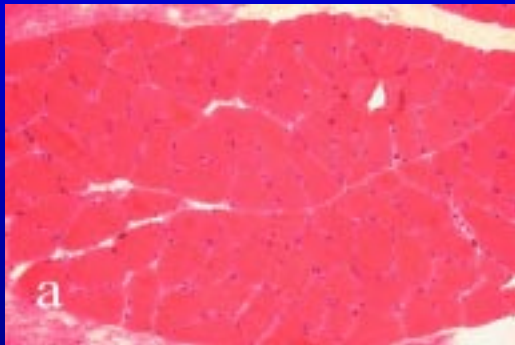
Arthritis Rheum 46:2189, 2002

Myalgia in TRAPS



Medicine 81:349, 2002

Monocytic Fasciitis in TRAPS



Medicine 81:349, 2002

TRAPS: Ocular Findings



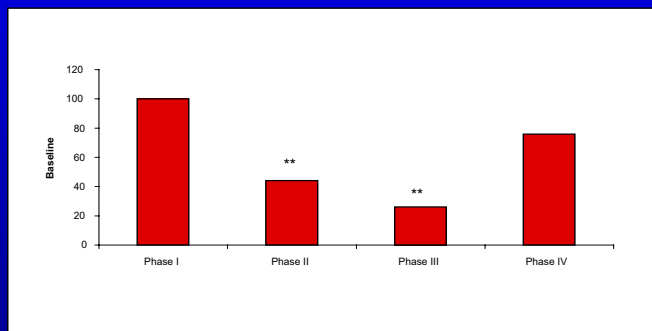
Conjunctivitis



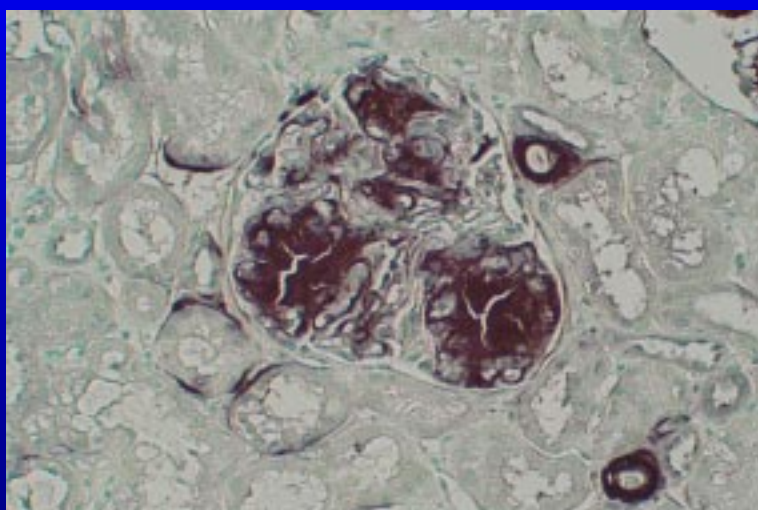
Periorbital edema

Medicine 81:349, 2002

TRAPS-Etanercept Trial



Progression of Amyloidosis on Etanercept Therapy in a Patient with Marked Clinical Improvement without Normalization of SAA Levels



Rheumatology 3rd edn, 1717, 2003

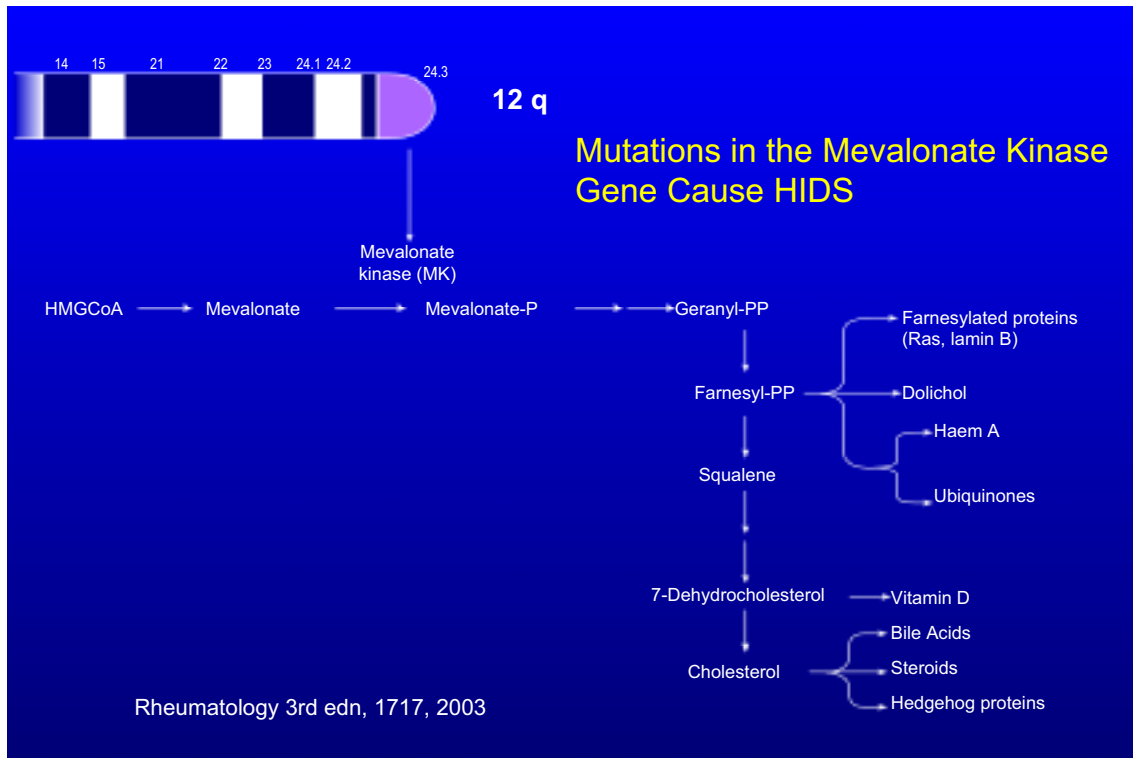
Case 3: Liri

- Irish/Albanian ancestry
- Age 6 wks: 7 day attacks of fever, erythematous maculopapular rash, diarrhea
- Subsequent attacks:
 - Fever
 - Rash
 - Abdominal pain, diarrhea
 - Generalized arthralgia, myalgia
 - Tender, enlarged cervical LN
 - Sometimes oral ulcers
- Last 4 - 10 days, occur q 4 - 6 wks

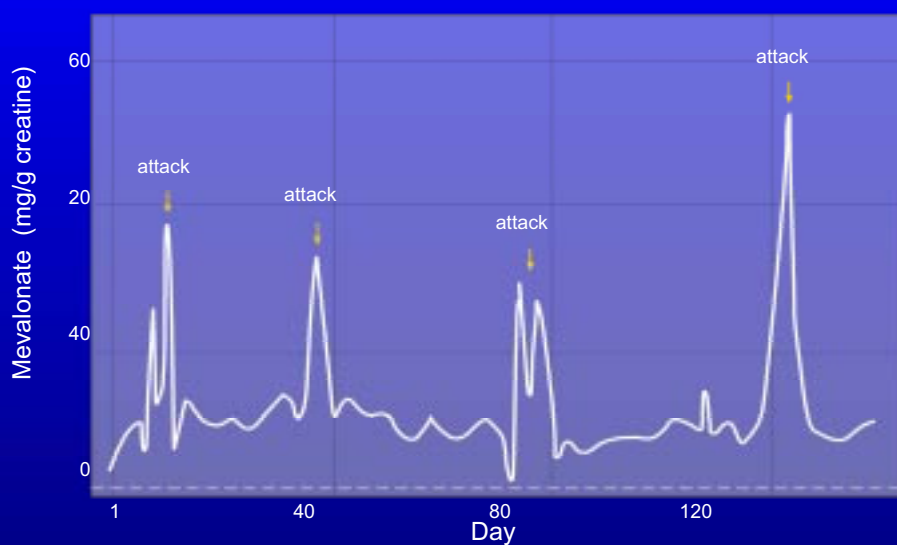
Liri



Arthritis Rheum 48:2645, 2003



Correlation of HIDS Attacks with Urinary Mevalonate Levels



Diagnosing HIDS

- “Classic HIDS”---elevated IgD, increased urinary mevalonate, *MVK* mutations
- “Variant HIDS”---increased IgD with normal urinary mevalonate, no *MVK* mutations
- Rarely---increased urinary mevalonate, *MVK* mutations with normal IgD
- If clinical suspicion is high, order serum IgD and either *MVK* genetic testing (V377I) or urinary mevalonate