

Gulbu Uzel, MD Update on Primary Immunodeficiencies: Recent Discoveries

**Viewing Time**

The program will take up to one hour to complete.

**Target Audience**

This program is designed for primary care physicians.

Other health care professionals working with patients and their families may also find this program of interest.

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**Update on Primary Immunodeficiencies: Recent Discoveries**

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**Update on Primary Immunodeficiencies: Recent Discoveries**

*A lecture about recent discoveries in the study of unusual immune disorders.*

# Gulbu Uzel, MD Update on Primary Immunodeficiencies: Recent Discoveries

## Program Objectives

*Upon completion of this program, participants should be able to:*

- Understand the recent discoveries in primary immunodeficiencies especially Job's syndrome, LAC-1/CGD and SCID
- Identify unusual patients with unusual immune disorders and know how to carry out his/her investigation of patient's immunodeficiency

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## Receiving CME Credit

To receive CME credit you must view the entire program and complete the evaluation form at the end.

## Updates on Primary Immunodeficiencies



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**OUTLINE:**

- UPDATES ON LAD-1
- UPDATES ON CGD
- THE WORLD REIGNED BY JOB
- OTHER FACE OF RAG1/RAG2 DEFECTS

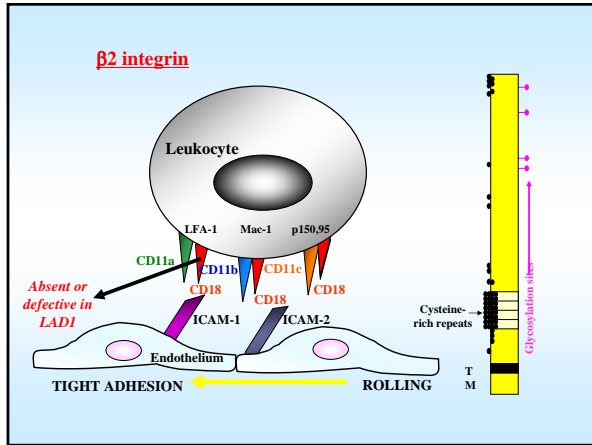
**PEDIGREE**

**Sibling A**

- 5 year old girl
- Omphalitis, perianal abscess in the neonatal period
- History of numerous skin infections, pneumonia and recurrent otitis media
- Frequent hospitalizations in spite of aggressive prophylaxis
- Severe gingivitis, periodontitis

**Sibling B**

- 3 year old boy
- Omphalitis and elevated WBC in the neonatal period
- Staphylococcal sepsis, typhilitis
- Nonhealing skin ulcer following staphylococcal cellulitis
- Gram negative bacterial arthritis, cellulitis
- Multiple admissions



**Clinical features:**

- Extreme leukocytosis (15,000 to 70,000/mm<sup>3</sup>)
- Delayed separation of the umbilical cord, omphalitis
- Severe gingivitis, periodontitis

- Recurrent necrotic skin, soft tissue and organ infections, inflammatory bowel disease
- Poor wound healing with dysplastic cutaneous scars

**Flow cytometric analysis of CD18 for SIB A**

Surface expression (Unstimulated)

Cell Type	Parameter	NL (Normal)	Sibling A
PMNs	CD18 FITC	High expression (pink peak)	Low expression (pink peak)
	CD18 PE	High expression (pink peak)	Low expression (pink peak)
Lymphocytes	CD18 FITC	High expression (green peak)	Low expression (green peak)
	CD18 PE	High expression (green peak)	Low expression (green peak)

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## Patient 1:

- Caucasian male born to non-consanguineous parents
- Delayed cord separation at 2-1/2 mos
- Pseudomonas sepsis at 10 months of age
- Persistent groin ulceration requiring debridement and multiple skin grafts
- Developed colonic and perirectal ulcers
- Matched unrelated bone marrow transplantation at age 21 was complicated by severe graft versus host disease and death.



## Case Presentation

### Patient 2:

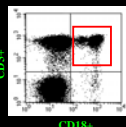
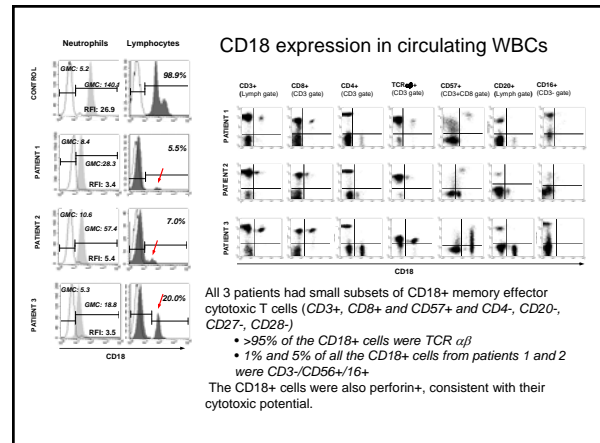
- 29 year old Caucasian female born to non-consanguineous parents
- 10 days of age, developed omphalitis
- Diagnosed with LAD-1 at 9 years of age
- Developed subglottic abscess at age 10 and osteomyelitis of left ankle at age 14
- At age 16 had extensive colitis with perianal fistula formation
- Extensive gingivitis and loss of most permanent teeth



## Case Presentation

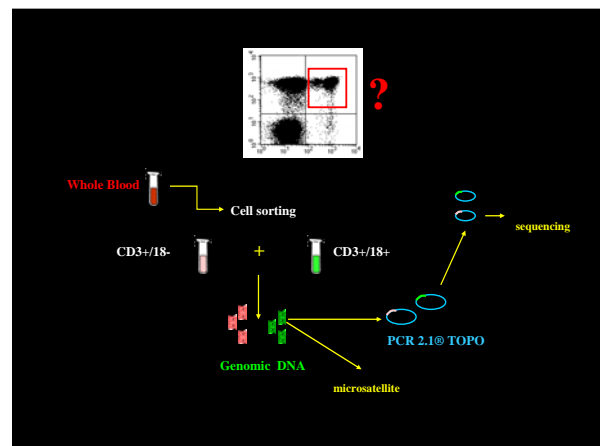
### Patient 3:

- 37 year old Caucasian male born to nonconsanguineous parents
- At age 10, presented with poor wound healing after tracheostomy for complicated croup
- At age 18 exploratory laparotomy for presumed appendicitis, diagnosed with Crohn's Disease.
- Recurrent poor healing ulcers on the legs and thighs
- Severe gingivitis and periodontitis, loss of permanent teeth.
- At age 27 emergency laparotomy for ileocecal stenosis resulted in right hemicolectomy
- At age 35, he was started on infliximab for LAD-1 associated colitis

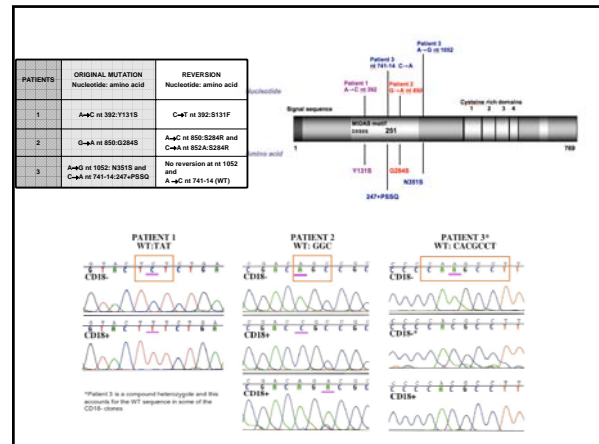
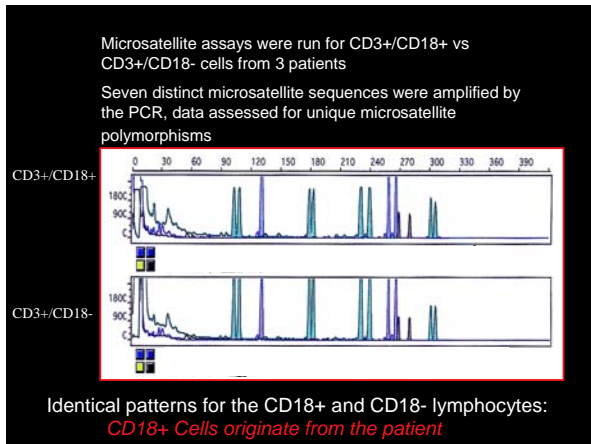


## WHERE DO THE CD 18+ LYMPHOCYTES COME FROM?

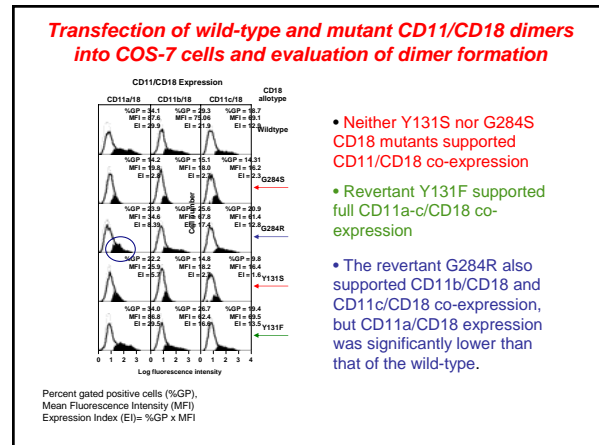
- Are they maternal?
- Could they be transfusion related?
- Is this a reversion/mosaicism?



# Gulbu Uzel, MD Update on Primary Immunodeficiencies: Recent Discoveries



All 3 patients had reversions of their mutant alleles that arose in them primarily and appear to be limited to the hematopoietic compartment



**RESULTS:**

We have identified three adult LAD-1 patients with somatic mosaicism who survived into adulthood without allogeneic bone marrow transplants.

Allelic change was observed only in the genomic DNA from a small subset of circulating CD8+ T cells.

Microsatellite analyses proved that the lymphocytes carrying both the mutant and revertant CD18 alleles were endogenous in each patient.

In two patients with different homozygous missense mutations, reversion was not to the wild-type but to a third amino acid.

In contrast to reports in patients with immunodeficiency, our patients 1 and 2 had novel reversions that partially or fully restored the function but were not WT.

It is the reversion to relatively normal function that is important in the expansion of these cells, and not the reversion to WT sequence.

**What is reversion or somatic revertant mosaicism?**

Rare cases of somatic mosaicism resulting from reversion of inherited mutations have been reported that lead to attenuation of blood-cell disorders.

An act of nature- leading to a sort of somatic gene therapy

The impact of the revertant cells, particularly their representation in the peripheral blood pool, serves to predict the number of revertant cells needed to gauge the lower limits needed for successful gene correction or transplantation.

The revertant mosaic is a mutant that has regained the wild-type phenotype, either partially or completely

Somatic revertant mosaicism have been described in:

- Epidermolysis bullosa,
- Tyrosinaemia type 1,
- Duchenne muscular dystrophy,
- Lesch- Nyhan disease
- Charcot-Marie-Tooth disease type 1A
- Fanconi anemia

Revertant mosaicism has been described in primary T cell defects:

- Adenosine deaminase (ADA)deficiency
- X-linked severe combined immunodeficiency (X-SCID)
- Wiskott-Aldrich Syndrome (WAS)

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Spontaneous reversion of mutations are more valuable if the corrected cells acquire a selective survival or growth advantage. A similar survival or growth advantage may have helped these long-lived memory effector T cells populate and persist in the peripheral blood in LAD-1.

The limitation of reversion mutations to subsets of cytotoxic lymphocytes in LAD-1 suggests that the cytotoxic T cells have some predisposition to mutation through their propensity for DNA rearrangement and to positive selection, possibly through their anti-infective capacity.

Inflammatory bowel disease in all three patients leaves open the possibility that these cells may be disadvantageous to the host.

We are unable to determine the earliest time point these cells were detectable in peripheral blood since we do not have PBMCs dating as early as birth or infancy.

Whether these reversion mutations have any effect on patient survival remains to be determined.

Could these reversions be the cause or consequence of long-term survival in this severe immunodeficiency?

## Chronic Granulomatous Disease (X, AR)

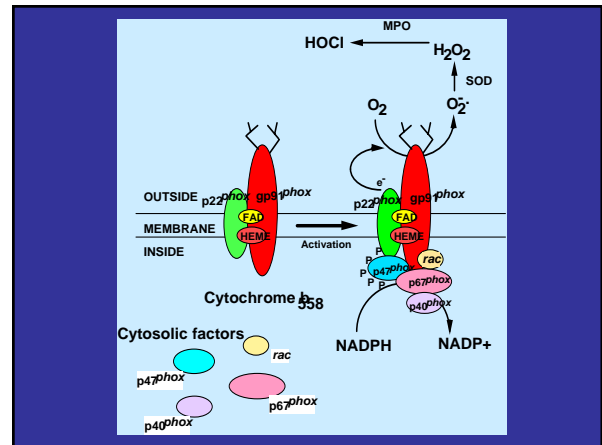
- Recurrent life-threatening infections with catalase-positive bacteria and fungi and tissue granuloma formation
- Infections: pulmonary, cutaneous, lymphatic, hepatic, bone
- Bacteremia rare

### Frequent offenders (5 most common):

- S. Aureus* \*most common pathogen
- S. marsecens*
- B. cepacia*
- Nocardia* spp.
- Aspergillus* spp. \*\*invasive pulmonary aspergillosis is the primary cause of death in North American CGD

### Others:

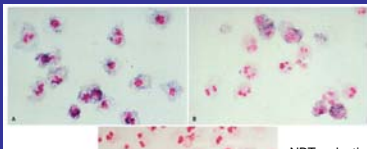
- Chromobacterium violaceum*
- Paecilomyces* spp.
- Granulobacter bethesdensis*



## Diagnosis of Chronic Granulomatous Disease (X, AR)

- Dx- PMN nitroblue tetrazolium reduction (NBT)  
 PMN dihydrorhodamine 123 oxidation (FACS),  
 Chemiluminescence, *Staph* killing

NBT reduction by purified normal neutrophils following stimulation with PMA and calcium ionophore



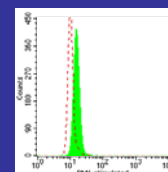
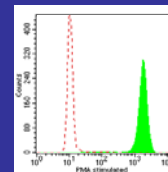
NBT reduction by purified neutrophils from an X-linked CGD carrier



NBT reduction by purified neutrophils from a CGD patient fail to reduce the NBT dye and appear clear

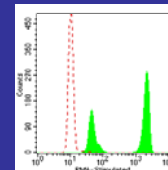
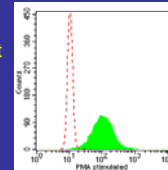
## Diagnosis of CGD: DHR Assay

Normal



*gp91<sup>phox</sup>* deficient CGD (X-linked)

*p47<sup>phox</sup>* deficient CGD (AR)



X-linked CGD carrier

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## Genetics of Chronic Granulomatous Disease (X, AR)

X-linked, chr. Xp21 (70% of cases)

Defect in gp91*phox*

Carrier females are mosaic (Lyonization)

About 1/3 of carriers are sporadic, from sperm

Autosomal recessive (30% of cases)

Defect in p47*phox*: 20-30% (Chr 7)

Defect in p67*phox*: <5% (Chr 1)

Defect in p22*phox*: <5% (Chr16)

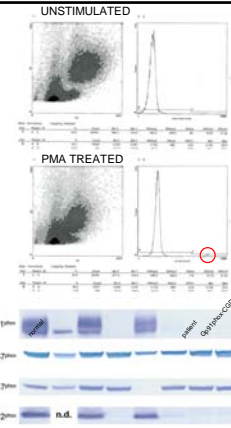
1/2000 carry the gene for the most common AR form

## SOMATIC MOSAICISM IN CGD

A 20-year-old man with X-linked CGD and severe inflammatory bowel disease requiring colonic diversion.

He had two distinct populations of neutrophils by neutrophil oxidative burst testing of peripheral blood. In two independent measurements of neutrophil oxidative burst by dihydrorhodamine (DHR) assay, 98% of peripheral blood neutrophils failed to oxidize dihydrorhodamine (DHR-), while 2% had a normal oxidative capacity (DHR+).

Sorted DHR+ and DHR- cells were studied by microsatellite analyses, which proved patient origin of both the DHR+ and DHR- cells.



- The DHR- cells, buccal mucosal epithelial cells and peripheral blood mononuclear cells had the mutation 676C>T causing a premature stop (R226X) in CYBB.
- DHR+ cells had the wild-type sequence, 676C leading to a normal protein.
- His mother is not a carrier by both DHR and sequencing so patient represents a *de novo* mutation
- Majority of his peripheral blood and all his buccal cells were mutant, and only a small percentage reverted to normal
- We believe that he has reverted the mutation in a small population of his neutrophil precursors.
- Exploration of other cell lines (lymphoid, monocytes, etc) is underway.
- This is the first report of a reversion mutation in X-linked CGD, and the only report of a reversion mutation affecting neutrophils in a primary phagocyte defect.

## 27 year old woman

Referred from her internist for evaluation of recurrent cutaneous boils with *S. aureus* and an IgE of 12,376 IU. "Bronchitis and sinusitis at least once a year" and persistent eczema requiring topical steroids. Never been hospitalized but is having "more trouble" lately.

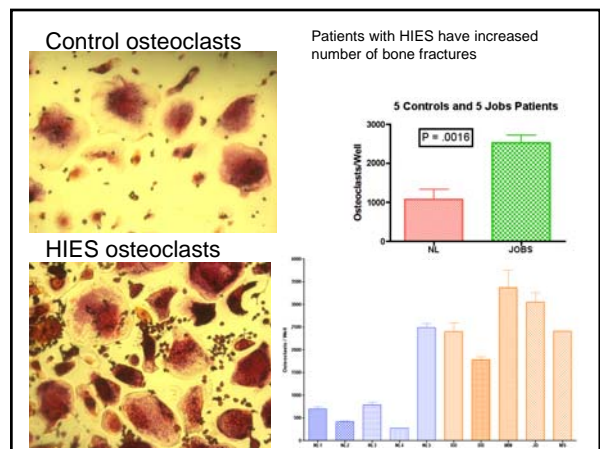
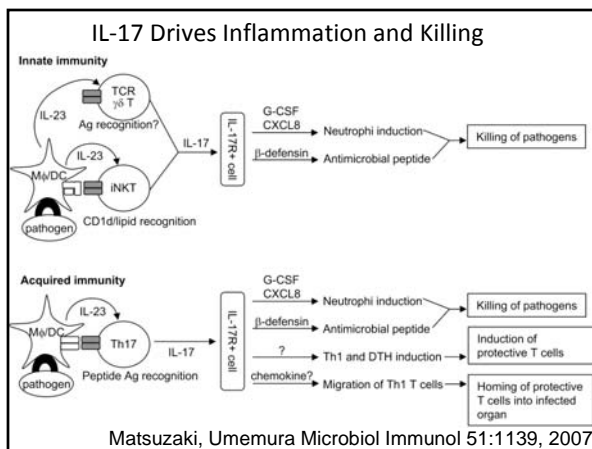
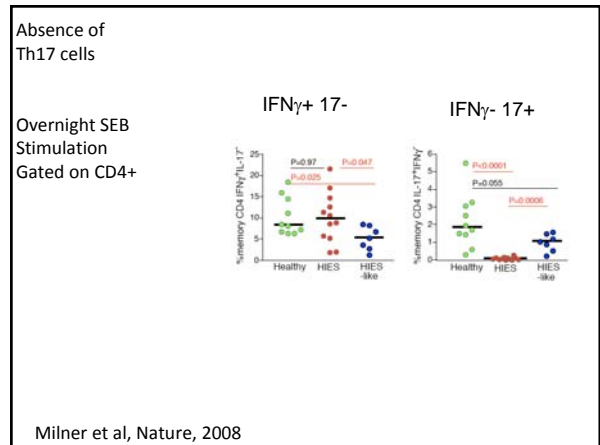
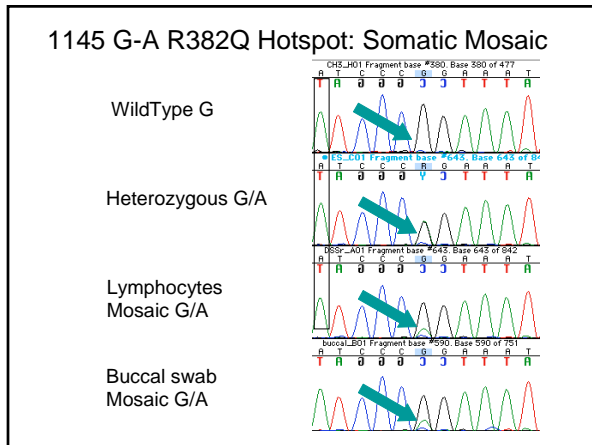
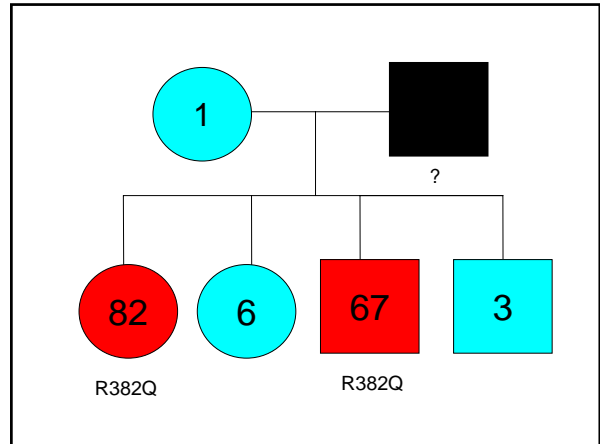
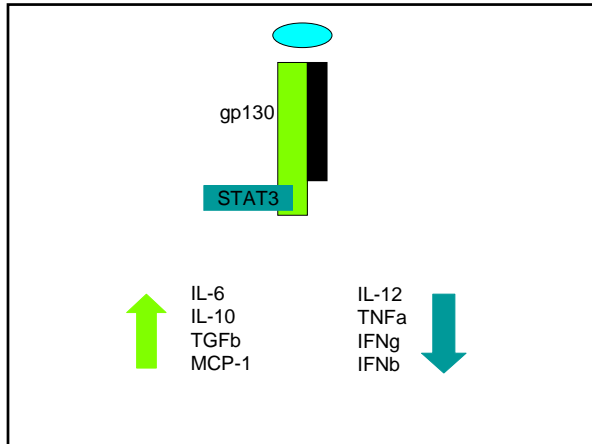
## Hyper IgE Syndrome (HIES)

- Autosomal Dominant disorder, formerly known as Job's Syndrome
- Unidentified in 2007
- Clinical features:
  - \* Eczema
  - \* Eosinophilia
  - \* Elevated IgE
  - \* Staphylococcal skin infections
  - \* Lung cysts.
  - \* Recurrent infections.





# Gulbu Uzel, MD Update on Primary Immunodeficiencies: Recent Discoveries



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## HIES

- Stay tuned: more molecular work being done
- Mosaic patient may be critical to help explain pathophysiology

THE NEW ENGLAND JOURNAL OF MEDICINE

BRIEF REPORT

### An Immunodeficiency Disease with RAG Mutations and Granulomas

Christina Schuetz, M.D., Kristen Huck, M.D., Sonja Gudowius, M.D.,  
 Mosaif Megahed, M.D., Oliver Fegen, Ph.D., Bernd Hubner, Ph.D.,  
 Dominik T. Schneider, M.D., Burkhard Marfas, M.D., Ulrich Pannicka, Ph.D.,  
 Ben Willemze, M.D., Ruth Knäuper, M.D., Ulrich Göbel, M.D.,  
 Amgar Schulz, M.D., Arndt Burkhardt, M.D., Wilhelm Friedrich, M.D.,  
 Klaus Schwarz, M.D., and Tim Nishanvi, M.D.

#### SUMMARY

We describe three unrelated girls who had an immunodeficiency disease with granulomas in the skin, mucosa membranes, and internal organs. All three girls had severe complications after viral infections, including B-cell lymphoma associated with Epstein-Barr virus (EBV). Other findings were hypogammaglobulinemia, a diminished number of T and B cells, and sparse thymic tissue on ultrasonography. Molecular analysis revealed that the patients were compound heterozygotes for mutations in recombinases activating gene 1 or 2 (RAG1 or RAG2). In each case, both parents were heterozygous carriers of a RAG mutation. The mutations were associated with reduced function of RAG *in vitro* (3 to 30% of normal activity). The parents and one sibling in the three families were healthy.

#### Patient 1:

5 year old Caucasian male referred for immune evaluation

#### Medical history significant for:

- Evans syndrome at 10 months (Treated with steroids, IVIG, cyclosporine, vincristine)
- Neutropenia at 20 months
- Guillain-Barre at 2 ½ years (treated with IVIG)
- Persistent hemolytic anemia, thrombocytopenia and neutropenia at 3 years (received 4 doses of Rituximab combined with steroids and cyclosporin)
- Psoriasis
- Vitiligo at 4 years

#### Patient 1:

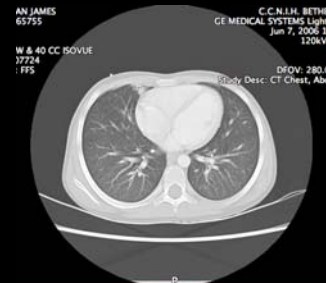
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- Neutropenia at 20 months
- Guillain-Barre at 2 ½ years (treated with IVIG)
- Persistent hemolytic anemia, thrombocytopenia and neutropenia at 3 years (received 4 doses of Rituximab combined with steroids and cyclosporin)
- Psoriasis at 3.5 years
- Vitiligo at 4 years

#### Infectious Disease hsx:

- 2 recent pneumonias, bronchoscopy revealed *H. influenza*
- No opportunistic infections
- Nonspecific colitis, bacterial overgrowth



Chest CT: mild bronchiectasis  
scarring from previous infections

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LABS:  
 Low IgG level at 4 years (IgG: 391, normal IgA, normal IgM, IgE undetectable),  
 Protective diphtheria and tetanus titer, unprotective pneumococcal titers.  
 He was started on IVIG q4weeks

LABS:  
 WBC: 3.5K, 4% Eos

CD3+/CD4+: 115/mm3  
 CD3+/CD8+: 800/mm3  
 CD19+: 120/mm3  
 CD3-/CD16CD56: 100/mm3  
 CD3+HAL-DR+%: 12%

### Classification of SCID

Disease	Relative frequency	Inheritance	Cells affected	Gene Product
Reticular dysgenesis	<1%	AR	T, B, NK, PLTS	?
Alymphocytosis	10%	AR	T, B	RAG1, RAG2
Absence of T lymphocytes	50%	XL	T, NK	$\gamma$ c chain
	10%	AR	T, NK	JAK3
	1%	AR	T	IL-7 R $\alpha$ Chain
	<1%	AR	T+/-NK	?
ADA deficiency	20%	AR	T, B, NK	ADA

Mitogen stimulation: significantly low proliferation

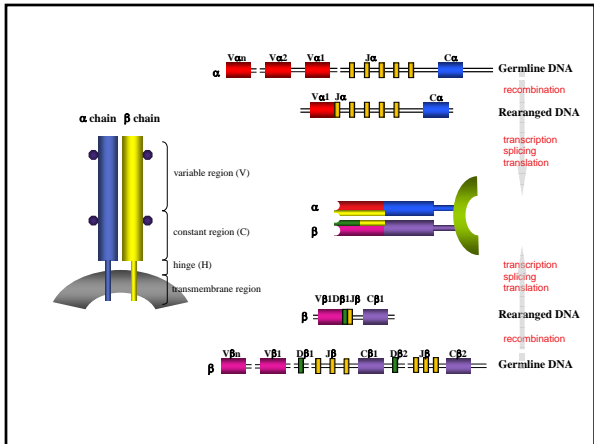
TCR VB: all TCR VB families represented

TRECS: very low copy numbers

?

SEQUENCING OF RAG1 AND RAG 2 :

Compound heterozygote:  
 M435V (paternal allele) and R699W (maternal allele)



Mutations in recombination activating genes 1 and 2 (RAG1 and RAG2): cause a spectrum of severe immunodeficiencies

Classical (T(-)B(-)SCID) and Omenn syndrome (OS)

Increasing number of peculiar cases.

Hypomorphic mutations leading to reduced V(D)J recombination may lead to autoimmunity while lacking the characteristic features of Omenn syndrome and maintaining the ability to form specific antibodies

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Patient has received bone marrow transplant  
Donor was HLA identical unaffected new born sibling

**The Clinical spectrum of hypomorphic RAG mutations.**

Patient 2:  
-Disseminated *M. avium* complex infection with hepatosplenomegaly, persistent sterile granulomatous skin lesions, and diarrhea.  
-Low T and B cells with elevated NK cells.  
-Abnormal lymph node and white spleen architecture and had a fatty thymus without Hassel's corpuscles.  
-She was compound heterozygous for two RAG1 mutations (R396C, R975Q) previously reported in Omenn syndrome

Patient 3  
-History of multiple severe bacterial pneumonias, herpetic gingivitis, and zoster after age 2.  
-Absent naive T cells and normal levels of other lymphocytes. Normal immunoglobulins with normal specific antibody titers.  
-She was compound heterozygous for RAG1 R474C and del256-7 causing K86fs.

These distinct presentations of hypomorphic RAG mutations highlight the diverse role of RAG in immune protection and autoimmunity. Recognizing patients with these RAG mutations is key to earlier diagnosis and treatment, and improving patient survival.

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Martha R Kirby

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and  
Questions**

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