Severe combined immunodeficiencies

DESPINA MOSHOUS

Severe combined immunodeficiencies clinical aspects

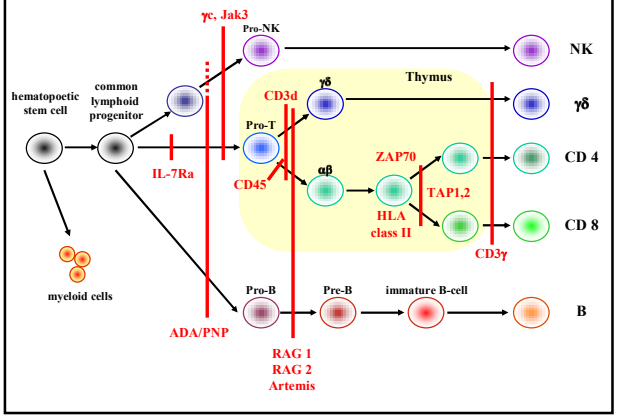
- first clinical signs early, in general < 6 months
- without treatment, lethal in the first year of life
- bacterial, fungal, viral infections
- opportunistic pathogens
- recurrent & therapy resistant infections
- profound / disseminated Candida infections
- interstial pneumopathy (pneumocystis jirovecii +++)
- persisting or recurrent diarrhoea
- denutrition ++
- persisting viral infection (respiratory virus, CMV)

Severe combined immunodeficiencies clinical aspects - 2 -

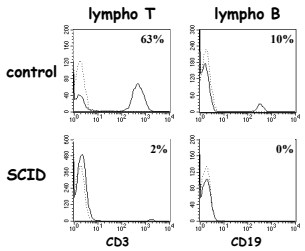
- absence of lymphoid tissue
- absence of thymus

- generalised erythrodermia
- disseminated BCGitis

- anamnesis: positive family history ?
- consanguinity ?
- lymphopenia +++
- hypogammaglobulinemia

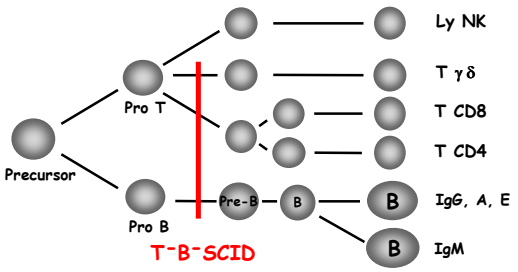


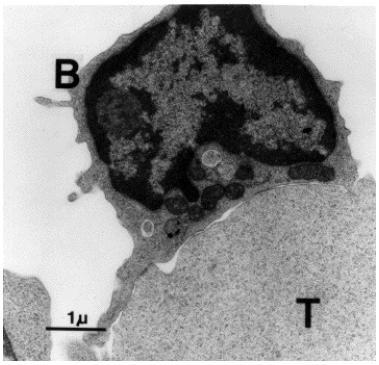
Severe combined immunodeficiency T⁻B⁻NK⁺

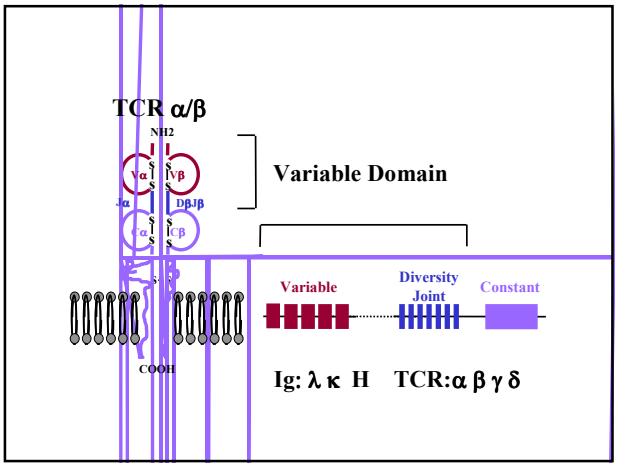


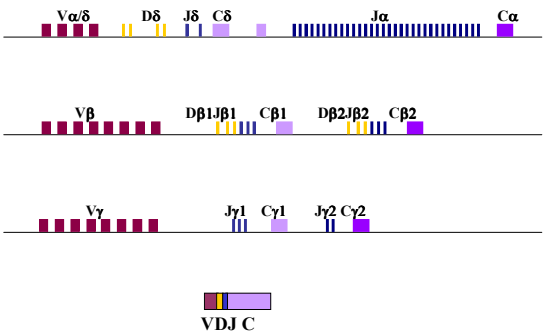
- absence of T and B lymphocytes
- presence of NK cells
- lethal in the first year of life
- 20% of human SCID
- autosomic recessive transmission
- curative treatment BMT

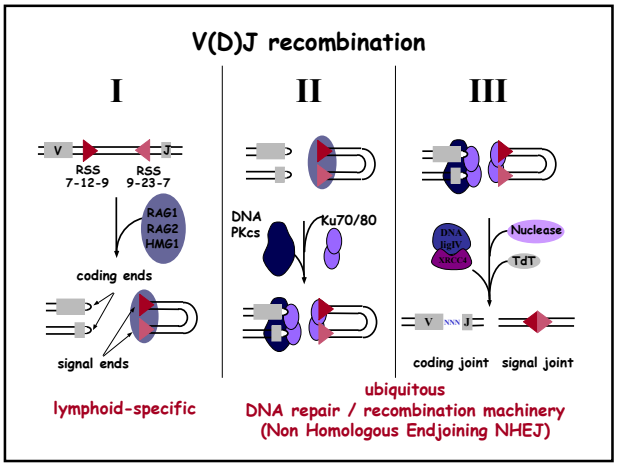
Lymphoid differentiation

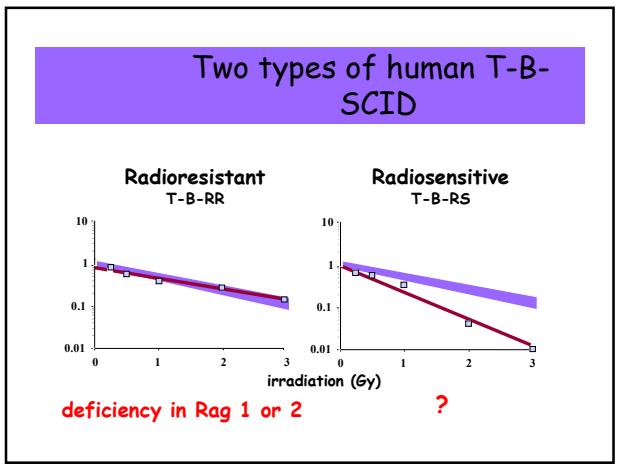


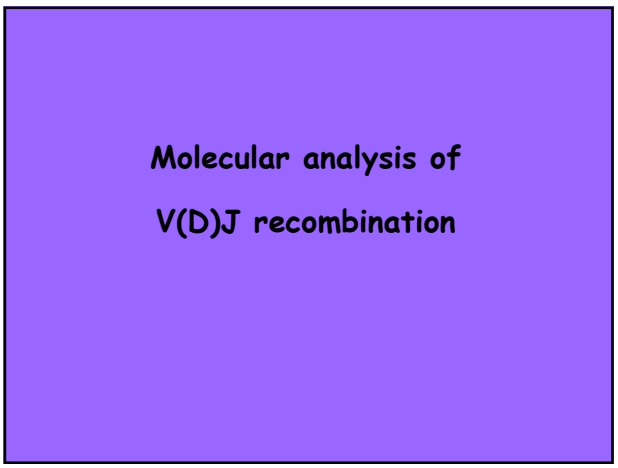

B
T
1μ


TCR α/β
NH2
Variable Domain
COOH
Variable
Diversity
Joint
Constant
Ig: λ κ H TCR:α β γ δ

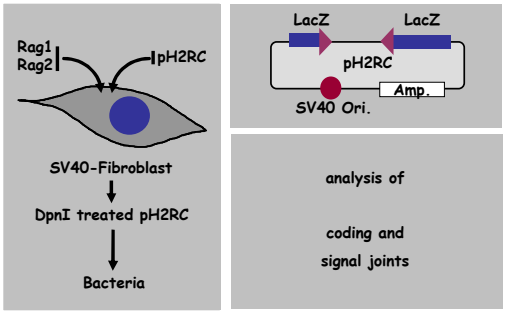

Vα/δ
Dδ
Jδ
Cδ
Jα
Cα
Vβ
Dβ1Jβ1
Cβ1
Dβ2Jβ2
Cβ2
Vγ
Jγ1
Cγ1
Jγ2 Cγ2
VDJ C


V(D)J recombination
I
II
III
RSS
7-12-9
RSS
9-23-7
RAG1
RAG2
HMG1
coding ends
signal ends
DNA
PKcs
Ku70/80
Nuclease
TdT
coding joint
signal joint
lymphoid-specific
ubiquitous
DNA repair / recombination machinery
(Non Homologous Endjoining NHEJ)


Two types of human T-B-SCID
Radioresistant
T-B-RR
Radiosensitive
T-B-RS
irradiation (Gy)
deficiency in Rag 1 or 2
?


Molecular analysis of
V(D)J recombination

V(D)J recombination assay



analysis of

coding and

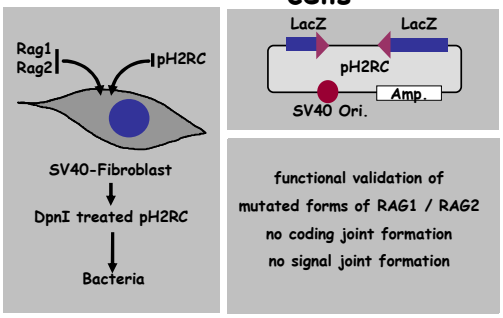
signal joints

Analysis of the first steps of V(D)J recombination

« radioresistant » T⁻B⁻SCID

(RR-SCID)

Defect of the V(D)J recombination in RR-SCID cells



functional validation of

mutated forms of RAG1 / RAG2

no coding joint formation

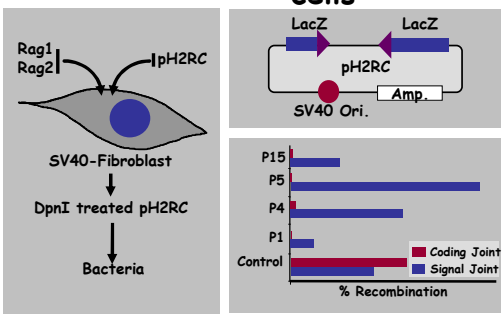
no signal joint formation

Analysis of the later steps of V(D)J recombination

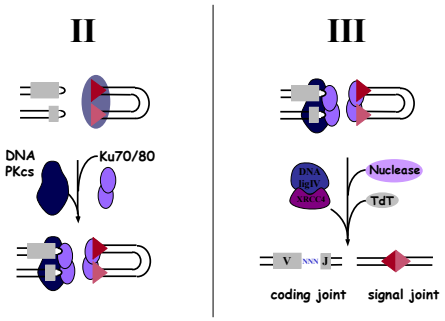
« radiosensitive » T⁻B⁻SCID

(RS-SCID)

Defect of the V(D)J recombination in RS-SCID cells



Defect in T and B cell development	+
Defect in V(D)J Recombination (CJ>SJ)	~~CJ~~ SJ
Radiosensitivity to γ rays	+
Defect in DNA-dsb Repair	(?)
Mutation in the DNA-PKcs gene	-

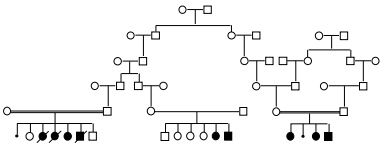


ubiquitous DNA repair/ recombination machinery
NHEJ

Analysis of the gene candidates for the radiosensitive T-B- SCID

- genetics
- sequence
- function

RAG1 RAG2 Ku70 Ku80 DNA-PKcs XRCC4 LigaseIV LigaseI LigaseII/III BLM TopoI TopoII TopoIII DNA-Polα DNA-Polβ DNA-Polδ DNA-Polε Rad21 Rad52 Rad54 c-Abl Rad50 MRE11 NBS1.....

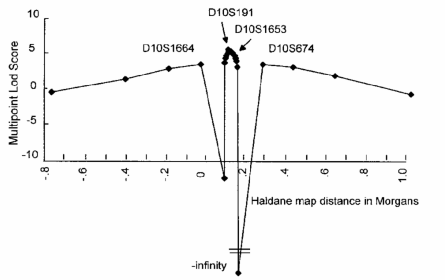


⇐ "tour du génome"
genetic approach to find the locus

The Gene for Severe Combined Immunodeficiency Disease in Athabascan-Speaking Native Americans Is Located on Chromosome 10p

Lanying Li,[1] Dennis Drayna,[2] Diana Hu,[3] Anthony Hayward,[4] Shiela Gahagan,[3,*] Henry Pabst,[5] and Morton J. Cowan[1]

Am. J. Hum. Genet. 62:136–144, 1998

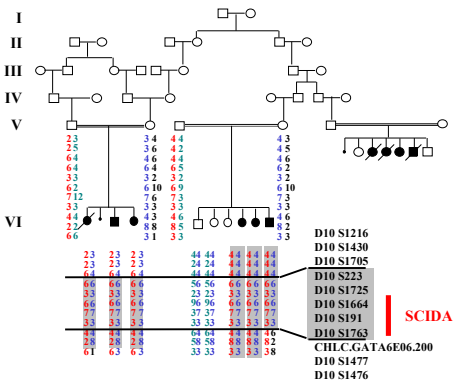


Athabascan-SCID (A-SCID)

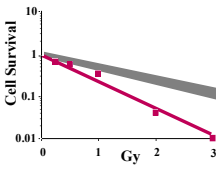
- Navajo, Apache Native American Indians
- ~1/2,000 births (Navajo)
- autosomal recessive transmission
- severe lymphopenia (T and B)
- infections
- treatment BMT

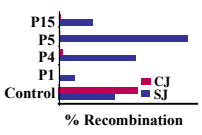
- Rag1 et Rag2 excluded
- Ku70, Ku80, DNA-PKcs excluded
- XRCC4 excluded

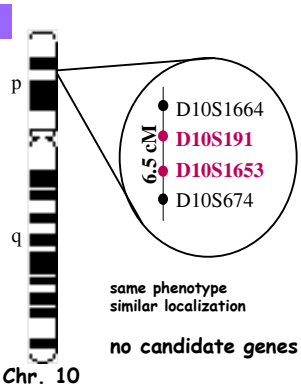
- localization Chromosome 10p



RS-SCID/A-SCID



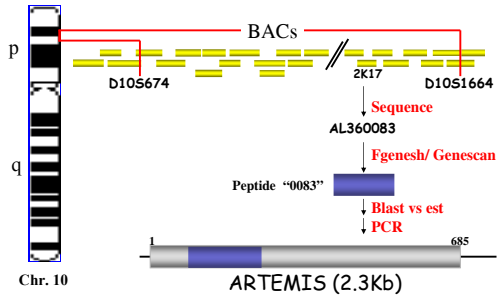




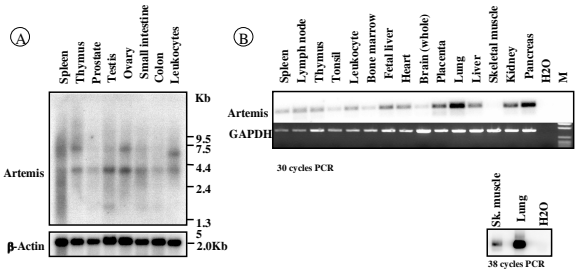
same phenotype
similar localization

no candidate genes

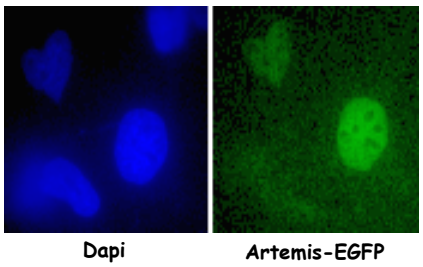
Cloning of Artemis



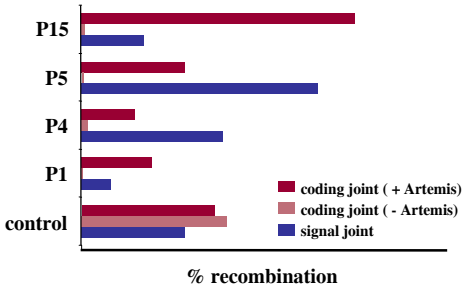
Artemis is ubiquitously expressed



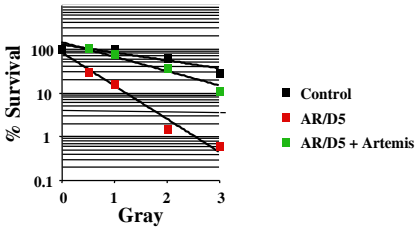
Artemis nuclear localization



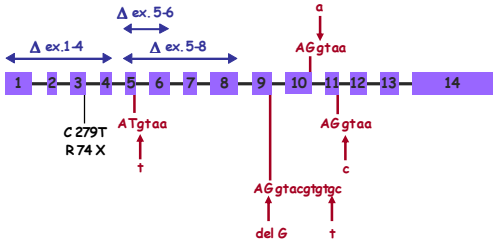
Artemis complements the RS-SCID V(D)J recombination defect



Artemis complements the radiosensitivity of RS-SCID fibroblasts



Artemis is mutated in human RS-SCID



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- Barbara Corneo
- Régina de Chasseval
- Nathalie Nicolas

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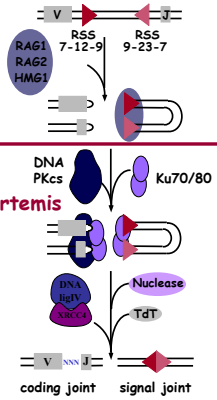
- Françoise Le Deist
- Marina Cavazzana-Calvo
- Nada Jabado

- Isabelle Callebaut (CNRS, Paris)
- Morton J. Cowan (UCSF)
  Lanying Li
- Steve Jackson (Welcome/CRC)
  Nicholas J. Finnie
- Dora Papadopoulo (Curie, Paris)
- Noel Philippe (Debrousse, Lyon)
  Yves Bertrand
- Ozden Sanal,Ilhan Tezcan,
  (Hacettepe U., Turkey)
- Sanger Center (UK)

atypical forms of

« radiosensitive » T^-B^- SCID



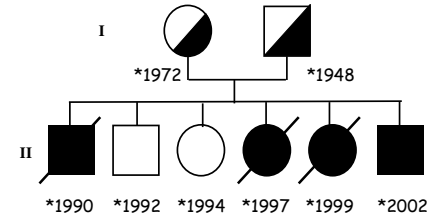
lymphoid specific

RR T-B-SCID
defect in RAG1 or RAG2

ubiquitous DNA repair/
recombination machinery

RS T-B-SCID
defect in Artemis

atypical RS-SCID



died at the age
of 11 months

intrauterine growth delay

at birth	P1	P2	P3
head circumference	32cm (<3.P)	33,5cm (3.-10.P)	33cm (3.-10.P)
height	48 cm (3.-10.P)	49 cm (3.-10.P)	47cm (3.P)
weight	2860g (3.-10.P)	3260g (10.-25.P)	2830g (10.-25.P)

parents' head circumference: mother: 50-90.P
father: 90.P

atypical RS-SCID (μSCID)

P1 - died at the age of 11 months (interstitial pneumonia)

P2 - admission at 16 m: repeated infections,
- failure to thrive, severe growth delay
- hypogammaglobulinemia (IgG and IgA)
- 200 lympho, <1%CD19, 10%NK, 80%CD3+, 77%TCRαβ, 24%TCRγδ, 40-50%HLADr+

P3 - admission in the age of 1,5 months
- 600 lympho,
  2%CD19, 27%NK, 57%CD3+, 80%TCRαβ

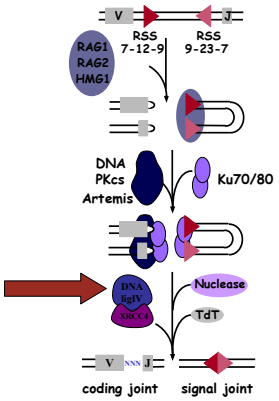
V(D)J recombination

in vivo

- normal proliferation upon PHA-stimulation
- diversified Vbeta repertoire (P2 and P3)
- normal TCR junctions (presence of αβ and γδ T)
- but progressive T and B lymphopenia

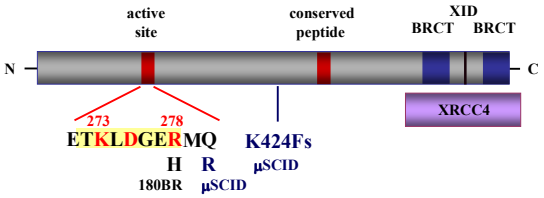
in vitro

- normal frequency of cj and sj-formation
- imprecise signal joint formation



ubiquitous DNA repair/ recombination machinery

DNA Ligase IV mutations in siblings with atypical RS SCID (µSCID)



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- Barbara Corneo
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Alain Fischer

- Françoise Le Deist
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clinical manifestations

V(D)J recombination defects

Nature of the mutation

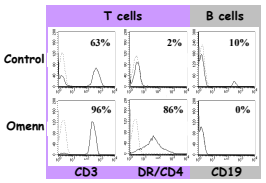
V(D)J activity

completely abolished → „typical" T-B-SCID

residual activity → ?

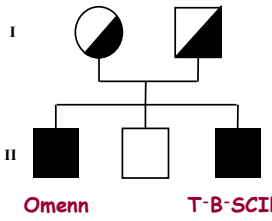
Omenn Syndrome

severe combined immunodeficiency with diffuse and exsudative erythrodermia, lymphoadenopathy, hepatosplenomegaly, protracted diarrhea, failure to thrive



- low IgM, IgA, IgG but hyper-IgE
- absence of peripheral B cells,
- hypereosinophilia
- hyperlymphocytosis T with activated T cells infiltrating tissues (skin, gut)
- restricted heterogeneity of T cell repertoire, oligoclonality

T⁻B⁻ SCID and Omenn Syndrome in the same family

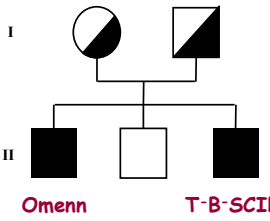


Omenn T⁻B⁻SCID

T cells	6,300	1,900	0
B cells	0	180	0
/ mm³			

(de Saint Basile et al., J. Clin.Invest., 1991)

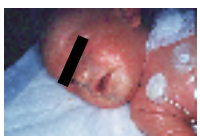
T⁻B⁻ SCID and Omenn Syndrome in the same family

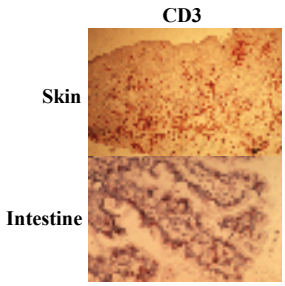


Omenn T⁻B⁻SCID

mutation in RAG2 (R39G/R229Q)

What makes a T-B-SCID develop a Omenn syndrome ?





V(D)J recombination

residual activity generates some lymphocyte clones

no stimulation → no/few circulating lymphocytes T⁻/low B⁻ SCID

stimulation by a second factor → clonal expansion of lympho. Omenn Syndrome

Omenn syndrome = leaky SCID

- „historically" defect in Rag1 and Rag2,
- but also mutations in
  - ARTEMIS
  - DNA ligase 4
  - RNA component of mitochondrial RNA processing endoribonuclease
  - adenosine deaminase
  - IL-2 receptor gamma
  - IL-7 receptor alpha
  - CHD7....

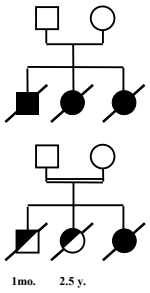
Omenn syndrome = leaky SCID

Genotype	Immunotype	Phenotype
RAG1/RAG2	T+ B- NK+	SCID
Artemis	T+ B- NK+	SCID
ADA	T+ B- NK+	SCID, multisystem involvement
DNA ligase 4	T+ B- NK+	SCID, microcephaly
RMRP	T+ B+ NK+	CHH, SCID
IL-2 receptor γ	T+ B+ NK-	SCID
IL-7 receptor α	T+ B+ NK+	SCID
22q11	T+ B+ NK+	DiGeorge syndrome
CHD7	T- B+ NK+	CHARGE syndrome

atypical

« radiosensitive » T^-B^- SCID

a particular form of RS-SCID

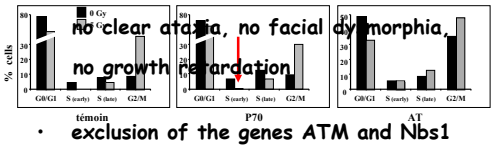


- repeated pulmonary infections
- severe T and B lymphocytopenia
- hypogammaglobulinemia (IgG & IgA)
- polyclonal T and B cell populations
- radiosensitivity
- chromosomal anomalies

- phenotype resembling the immune deficiency in Ataxia teleangiectasia (AT) and NBS syndrome

a particular form of RS-SCID

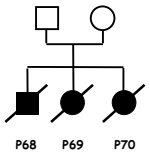
- cell cycle upon irradiation: normal



- exclusion of the genes ATM and Nbs1 by genetic means

no clear ataxia, no facial dysmorphia, no growth retardation

clinical presentation

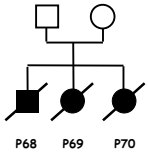


P68:

- candidiasis and protracted diarrhea soon after birth
- lymphadenopathies at age of 9 mo.
- nodular lung infiltrates
- hypogammaglobulinemia
- autoimmune anemia, thrombocytemia
- severe lymphocytopenia

- lymphoproliferation, treated by anti-B cell specific AB,
- patient died 5 days after diagnosis
- massive infiltrations of lung, liver, skeletal muscle (autopsy)

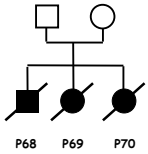
clinical presentation



P69:

- candidiasis, recurrent respiratory and pulmonary infections soon after birth
- at age of 10 months detection of:
  - severe lymphocytopenia
  - hypogammaglobulinemia
- intravenous Ig treatment
- BMT (10/10 MUD) at age of 5 years
- B cell lymphoma of recipient origin localized to the liver day+38 post BMT
- death 12 days later despite anti-B ab

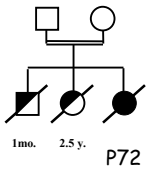
clinical presentation



P70:

- recurrent otitis and broncho-pneumonia from 1 year of age
- bronchiectasis
- at age of 11 years cerebral abcess (Toxoplasma gondii)
- died at age of 13 years because of sepsis and respiratory failure

clinical presentation – family2



P72:

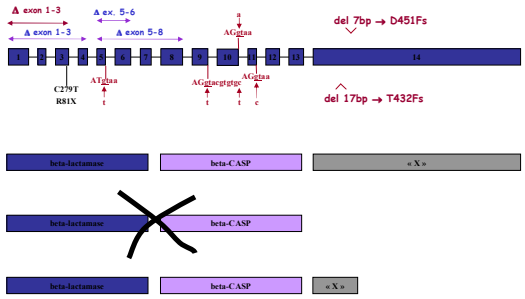
- recurrent pulmonary infections
- bronchiectasis at 4 years of age
- failure to thrive
- at 7 years: cholangitis, liver disease and protracted diarrhea caused by Cryptosporidium infection
- fatal liver cirrhosis leading to death at age of 16 years

immunological data

Patients	P68	P69	P70	P72	Normal values
Lymphocyte counts/mm^3	66	500-1,100	155-610	378-748	2,500-3,500
CD3	8	280-580	77-321	200-350	1,500-2,500
CD4	ND	145-341	21-133	134-185	900-2,000
CD8	ND	90-290	57-212	85-115	400-1,000
CD19	8	8-110	0-5	0-51	200-600
CD16/CD56	18	227	60	ND	100-500
PHA cpmx10^{-3}	35.8 ± 22	26.6 ± 18	16.8 ± 14.6	35.8 ± 22	>40
Tetanus toxoid cpmx10^{-3}	59.4 ± 32	10.0 ± 8.8	3.5 ± 3.9	59.4 ± 32	>15
IgG (g/l)	14.4 (9 m)	1.33 (10 m)	2.91 (5 yrs)	2.46 (8 yrs)	4.2-12.6
IgA (g/l)	0.18	<0.07	<0.06	<0.07	0.4-1.6
IgM (g/l)	6.11	0.56	0.9-3.4	0.43	0.4-1.2

Artemis - structure

T$^-$B$^-$SCID "atypical" T$^-$B$^-$SCID



partial deficiency of Artemis

and...

… EBV associated lymphoma

CD20

Ki67 (proliferative marker)

(lymph node biopsy)

Immunohistological features of lymphomas in P68 and P69

	Sites of involvement (a)	Immunohistochemistry				EBV markers	
		CD20	CD3	CD30	Ki67	LMP1	EBER probe
P68	Cervical lymph node	Large cells	Rare small cells	Rare large cells	Large cells	Some large cells	Large cells
	Liver	+++	+	+	++	+	++
	Lung						
	Striated muscle						
P69	Liver	Large cells	Rare small cells	Rare large cells	Large cells	Some large cells	Large cells
		++	+	+	++	+	++

(a) Lymphoproliferation was characterized as large B cells associated with small lymphocytes.

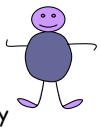
in both patients: clonal IgH proliferation
without evidence of c-myc rearrangement

Cytogenetic analysis
in PHA activated T cells

- **P68 Trisomy Chr. 9 (in lymphoma cells)**
- **P69 Translocation Chr. 7:14**
- **P70 nd**
- **P72 Inversion Chr. 7**

Ig and TCR genes

hypomorphic mutations in Artemis



- partially preserved *in vivo* V(D)J activity
- polyclonal T and B cell populations, albeit reduced in number
- chromosomal instability
- aggressive EBV-associated B cell-lymphoma
  - clonality of B-cell proliferation
  - clonal chromosomal alteration in P68
  - general genomic instability

Artemis

a new « caretaker »

with tumor supressor role

immunodeficiency

lymphoma

defects in NHEJ-factors

Jean-Pierre de Villartay

- Barbara Corneo
- Régina de Chasseval

INSERM U429

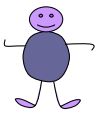
- Nicole Brousse
- Isabelle Callebaut
- Danielle Canioni
- Elizabeth Macintyre
- Christophe Pannetier
- Serge Romana

Alain Fischer

- Françoise Le Deist / C. Picard
- Marina Cavazzana-Calvo
- Jean-Laurent Casanova

and also...

atypical patients with hypomorphic mutations in Artemis



- several patients in the Paris cohort
- (S)CID phénotype with severe lymphopenia
- with defect in T and B cell function
- recurrent infections, ENT, bronchopulm
- chronic diarrhea
- note one patient with pulm. Asp niger
- EBV is a concern...
- MUD in one almost 6 y
- UCB 9,5/10 in a 27 mo

Chronic Inflammatory Bowel Disease as

Key Manifestation of Atypical ARTEMIS Deficiency.

Rohr J, Pannicke U, Döring M, Schmitt-Graeff A, Wiech E, Busch A, Speckmann C, Müller I, Lang P, Handgretinger R, Fisch P, Schwarz K, Ehl S.

J Clin Immunol. 2009 Dec 5. [Epub ahead of print]

Chronic Inflammatory Bowel Disease as

Key Manifestation of Atypical ARTEMIS Deficiency.

- **recurrent diarrhea from the age of 9 months**
- **dx juvenile Crohn's disease (biopsies chronically active inflammation with superficial fissuring ulceration)**
- **immune suppressive therapy (steroids, azathioprin, sulfasalazin, tacrolimus...)**
- **pneumonia, labial abscess**
- **chronic lymphopenia: PID considered at age of 6 years...**

Chronic Inflammatory Bowel Disease as
Key Manifestation of Atypical ARTEMIS Deficiency.

- 761 ly/µl — 63% γδ TCR+/CD3+
- 146 CD3+ — polyclonal repertoire
- 56 CD4+ — only 2,5% naive cells
- 15 CD8+ — proliferation N mitogens
- 40 B
- 530 NK
- normal Ig G A M, positive serological response

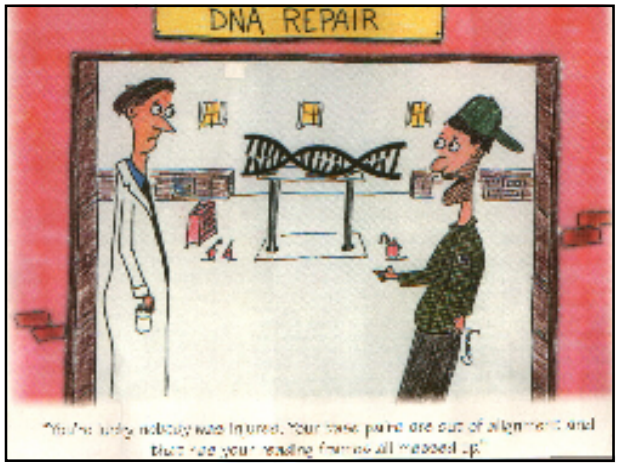
Chronic Inflammatory Bowel Disease as
Key Manifestation of Atypical ARTEMIS Deficiency.

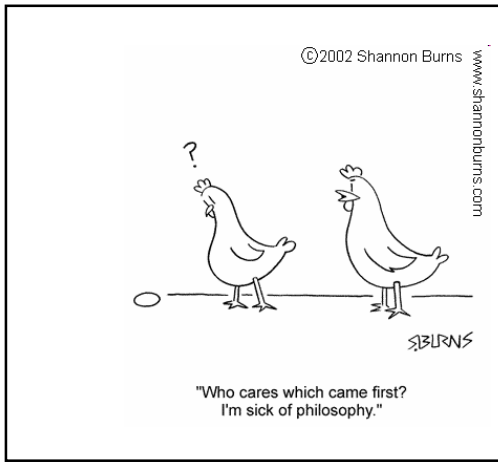
- novel homozygous point mutation Ex 6+1 g>a
- c461+1 g>a, splice donorsite gt -> at
- several alternative transcripts, no normal transcripts
- residual V(D)J activity of some of these transcripts
- HYPOMORPHIC MUTATION

- haplo HSCT (father) follow up 3 years: alive and well

atypical manifestation ?

think about a "leaky (S)CID" …





"Who cares which came first?
I'm sick of philosophy."

SCID T-B-NK+

- **Rag1** ≈ 40%
- **Rag2**
- **Artemis** ≈ 20%

≈ 40%