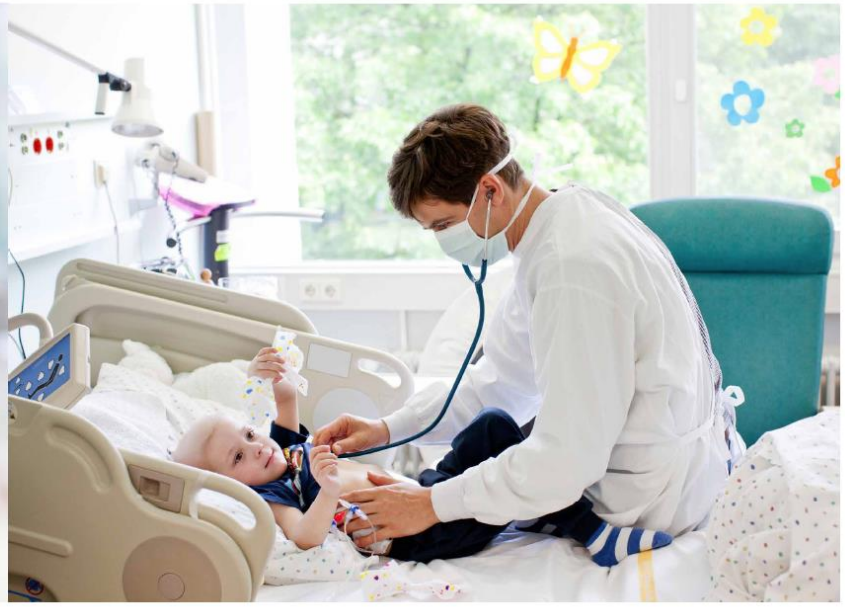
UNIVERSITÄTS KLINIKUM FREIBURG



Bodo Grimbacher

Angeborene Immundefekte, 2. Oktober 2017, Stuttgart

CCI Centre of Chronic Immunodeficiency

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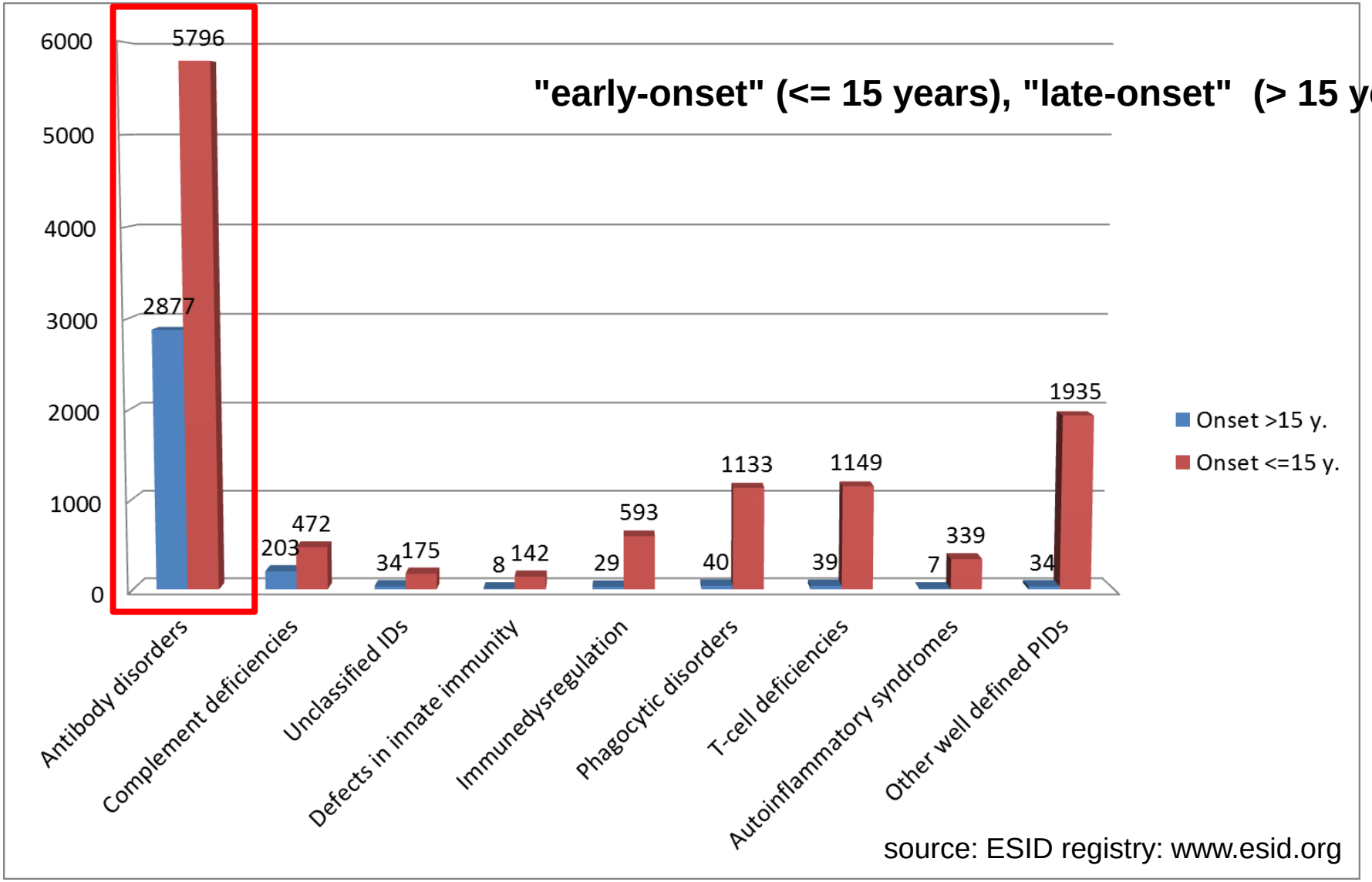
Categories of Primary Immunodeficiencies



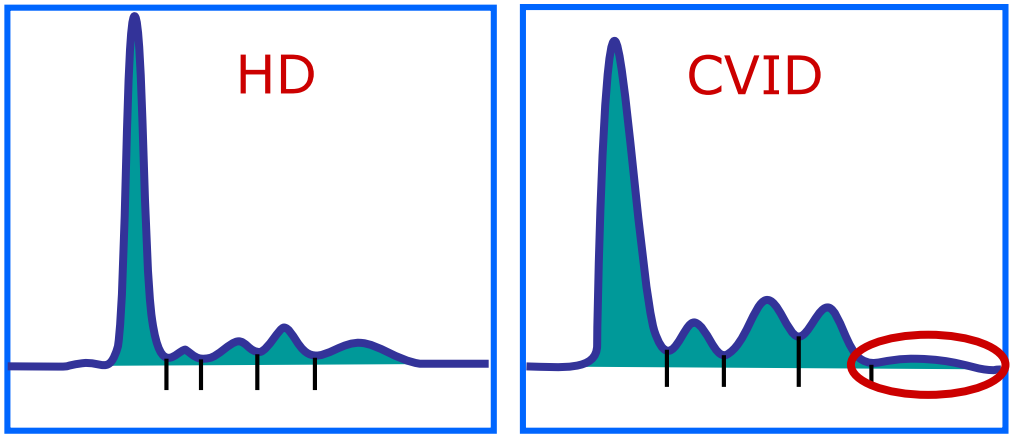
Common Variable Immune Deficiency (CVID)

- **Prevalence:** estimated to be 1:25.000 to 1:100.000
- **Inheritance:** ~10% familial CVID (thereof 75 AD and 25% AR), 25% coincide with familial sIgAD
- **Definition:** Heterogeneous primary antibody deficiency syndrome
  - Serum Ig levels:

    IgA < 0.05 g/l

    IgG < 5g/l

    IgM in ~80% low



- **Clinic:** Susceptibility to recurrent infections of the upper respiratory tract, mainly with encapsulated bacteria

P. Yong et al: „A Rose is a Rose is a Rose, but CVID is not CVID“ Adv in Immunol., 2011

Common Variable Immune Deficiency (CVID)

- Additional clinical manifestations:

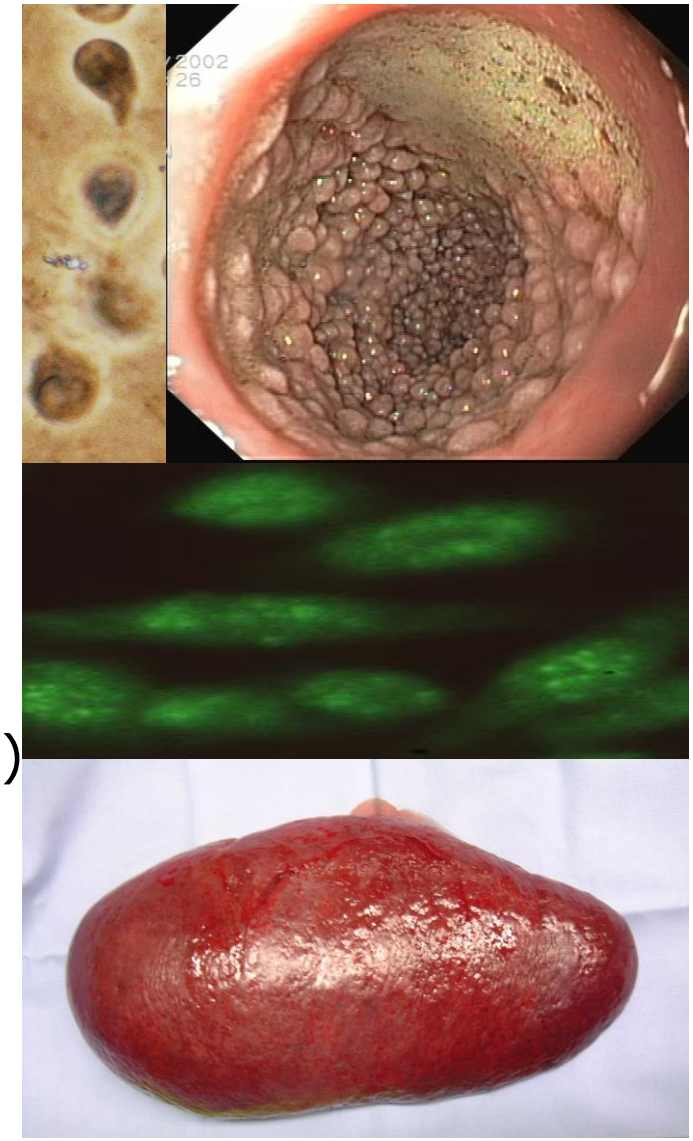
- GIT: diarrhea, gardiasis, nodular lymphatic hyperplasia

- Autoimmune-Phenomena: ITP, AIHA, PSS and others (~20%)

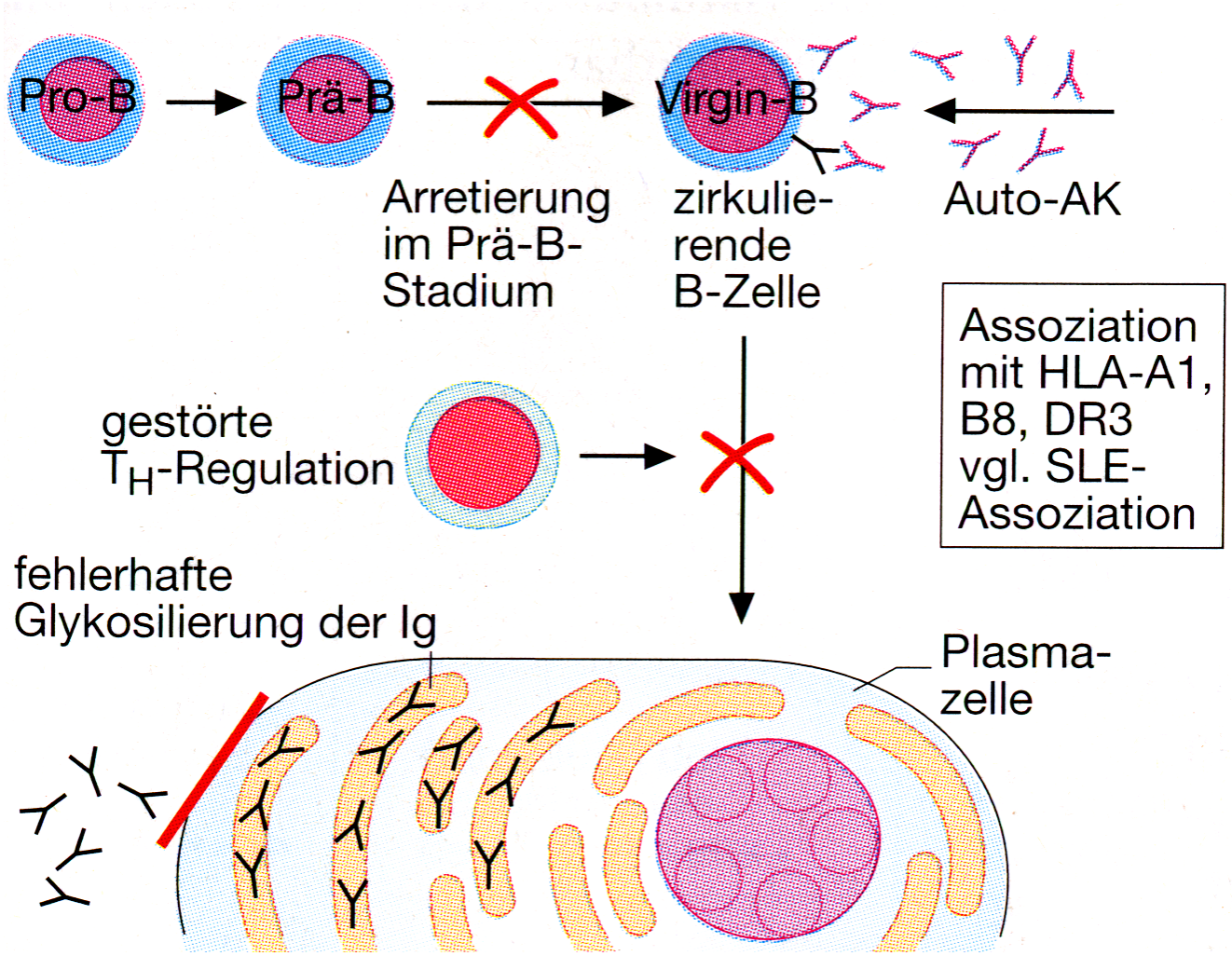
- Splenomegaly (~20%)

- Sarcoid-like granulomas (approx. 10%)

- Malignancies: Lymphomas (<10%), cancer of the stomach

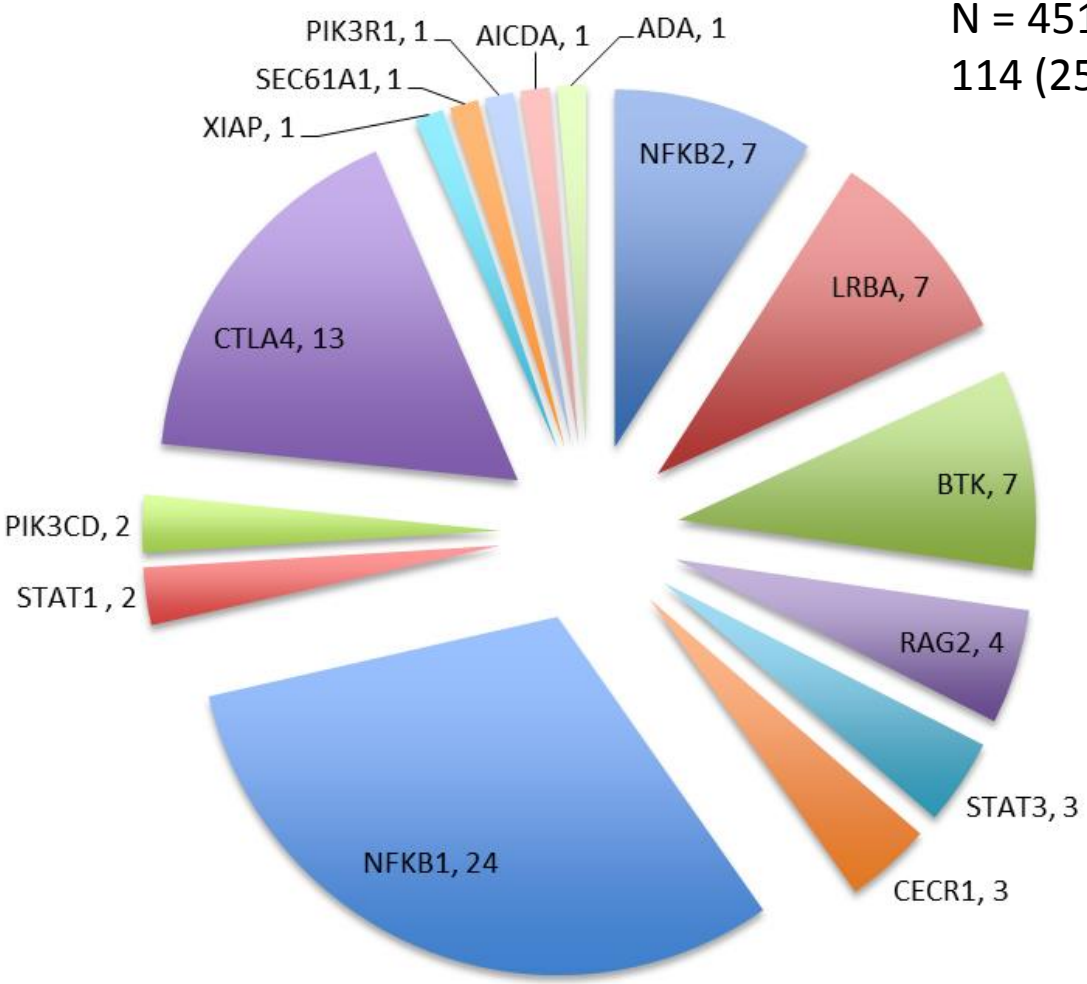


CVID: variable Hypogammaglobulinemia – possible causes



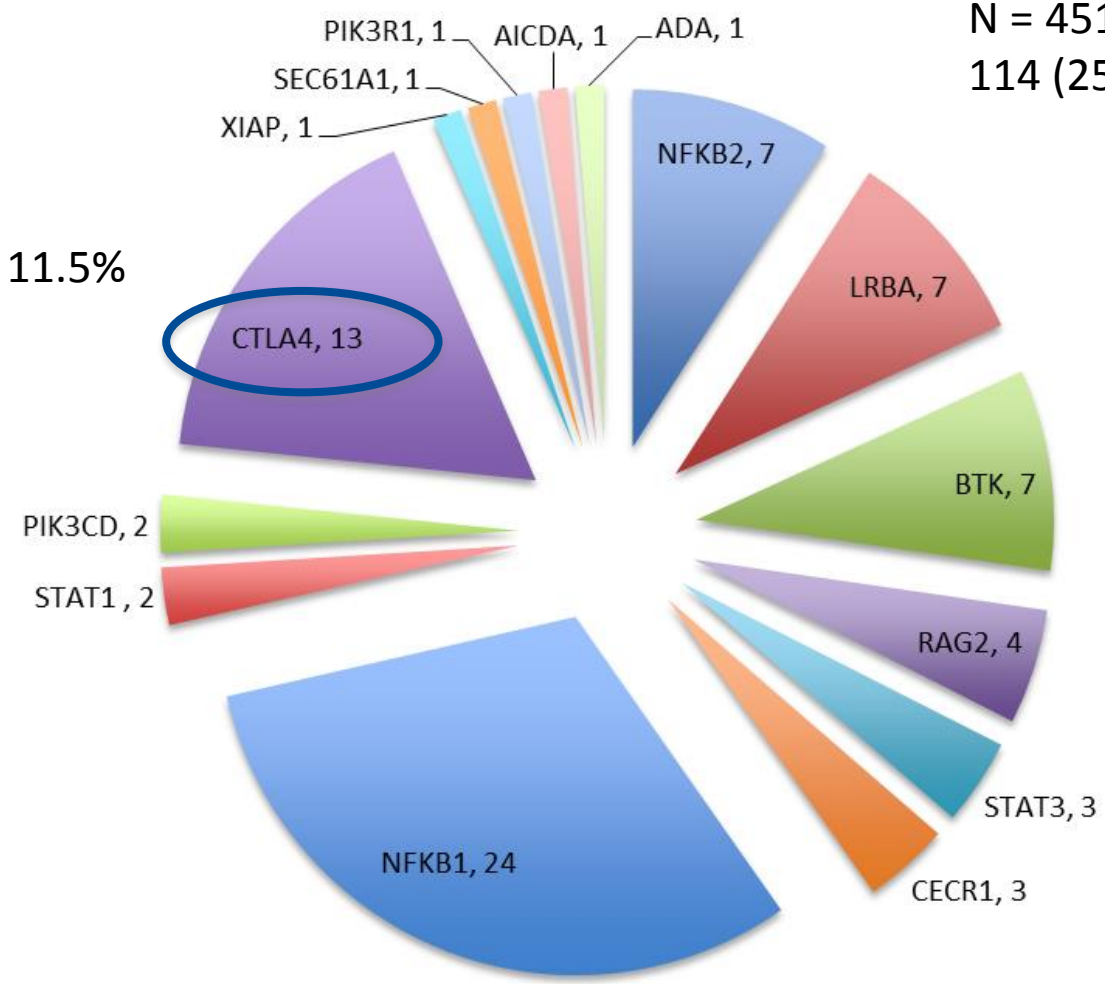
aus: Burmester, Pezzutto:
Taschenatlas der Immunologie

Monogenetic Causes for Hypogamma-/Agammaglobulinemia



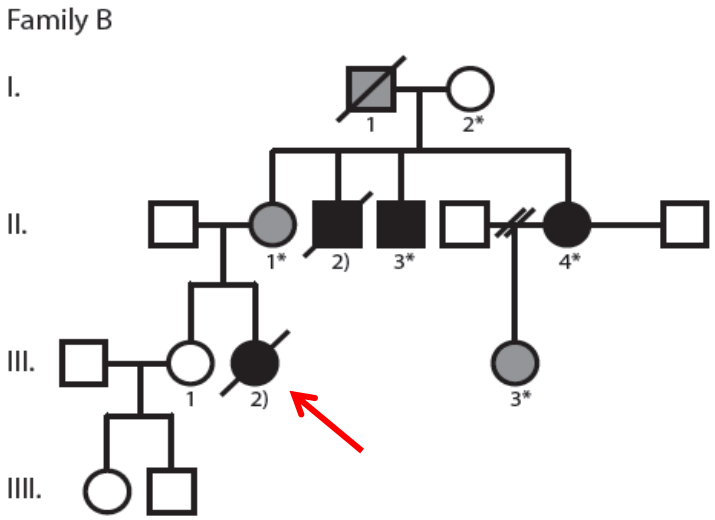
N = 451 adult (!) patients
114 (25%) with clear mutation

Monogenetic Causes for Hypogamma-/Agammaglobulinemia



N = 451 patients
114 (25%) with clear mutation

Heterozygous mutations in CTLA4 can cause immune dysregulation



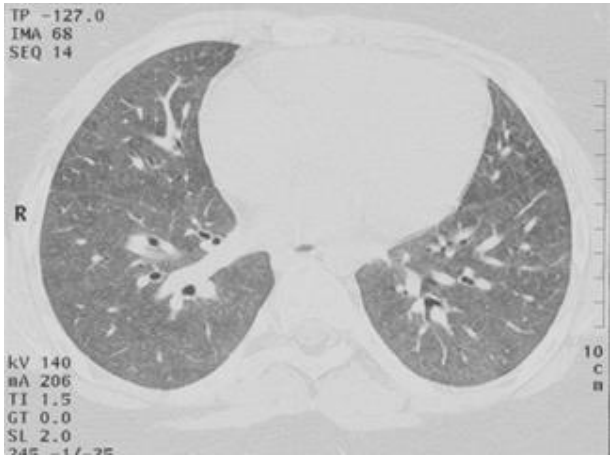
Symptoms at 1st presentation

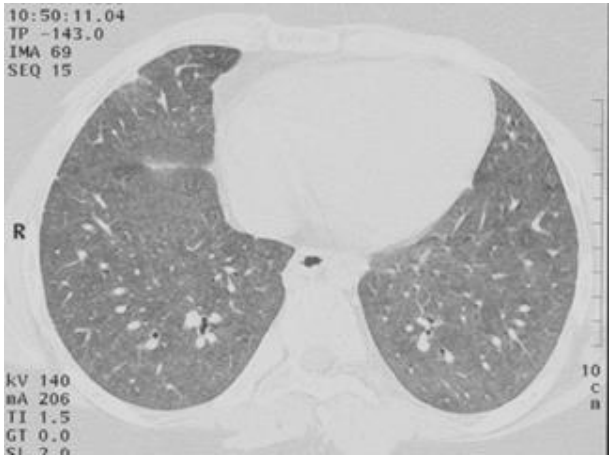
- Age 7y: recurrent sinusitis and otitis
- Age 10y: weight loss (4kg); dyspnea

Clinical findings

- Pathological auscultation and peribronchitis in chest x-ray
- Bronchoscopy: chronic inflammation and atrophy of the bronchial wall
- IgG-antibodies against pigeon droppings, pigeon serum and *aspergillus fumigatus*
- Low IgA

→ **Diagnosis:** Idiopathic pulmonary fibrosis



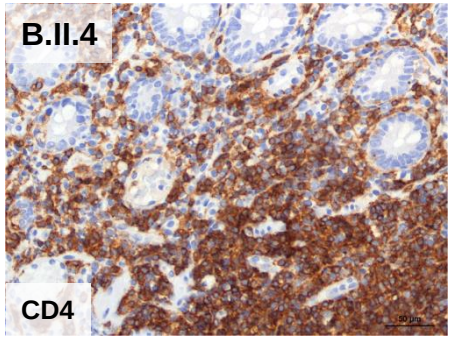


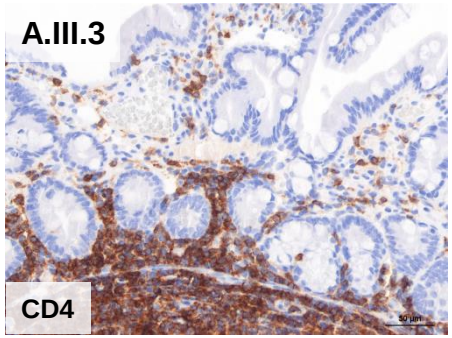
Schubert *et al.*, 2014, Nature Medicine

Clinical manifestations

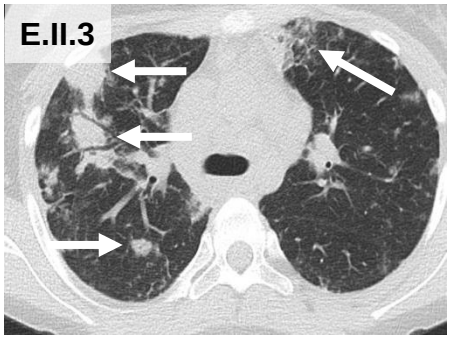
Patients suffer from massive lymphocytic organ infiltrations

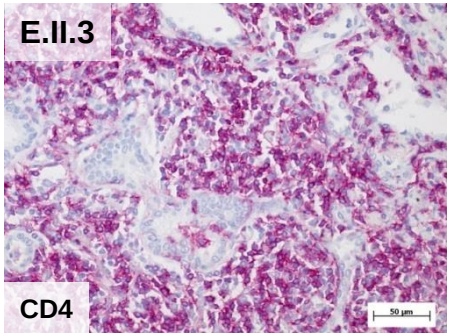
Duodenum



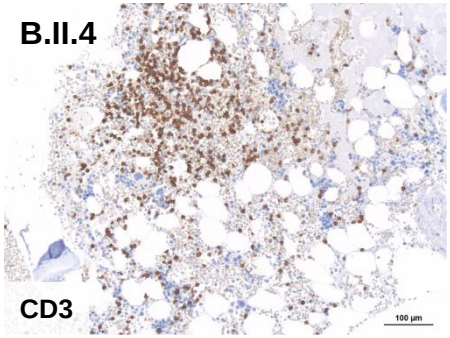


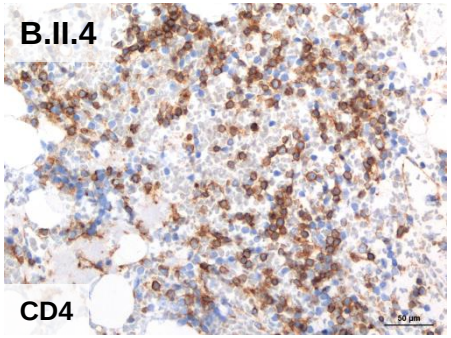
Lung



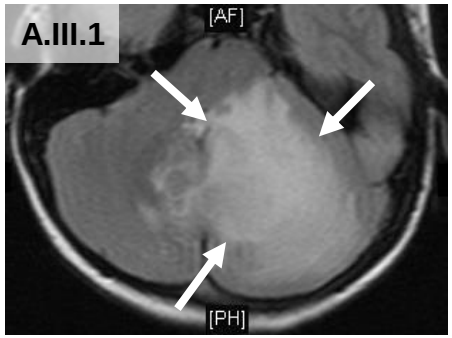


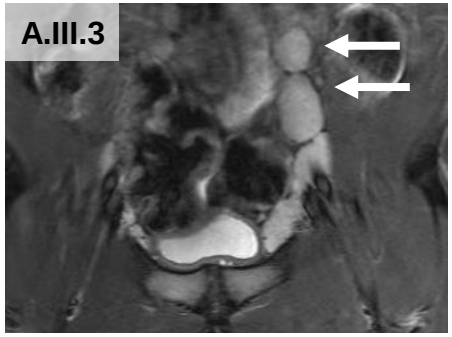
Bone marrow





Cerebellum

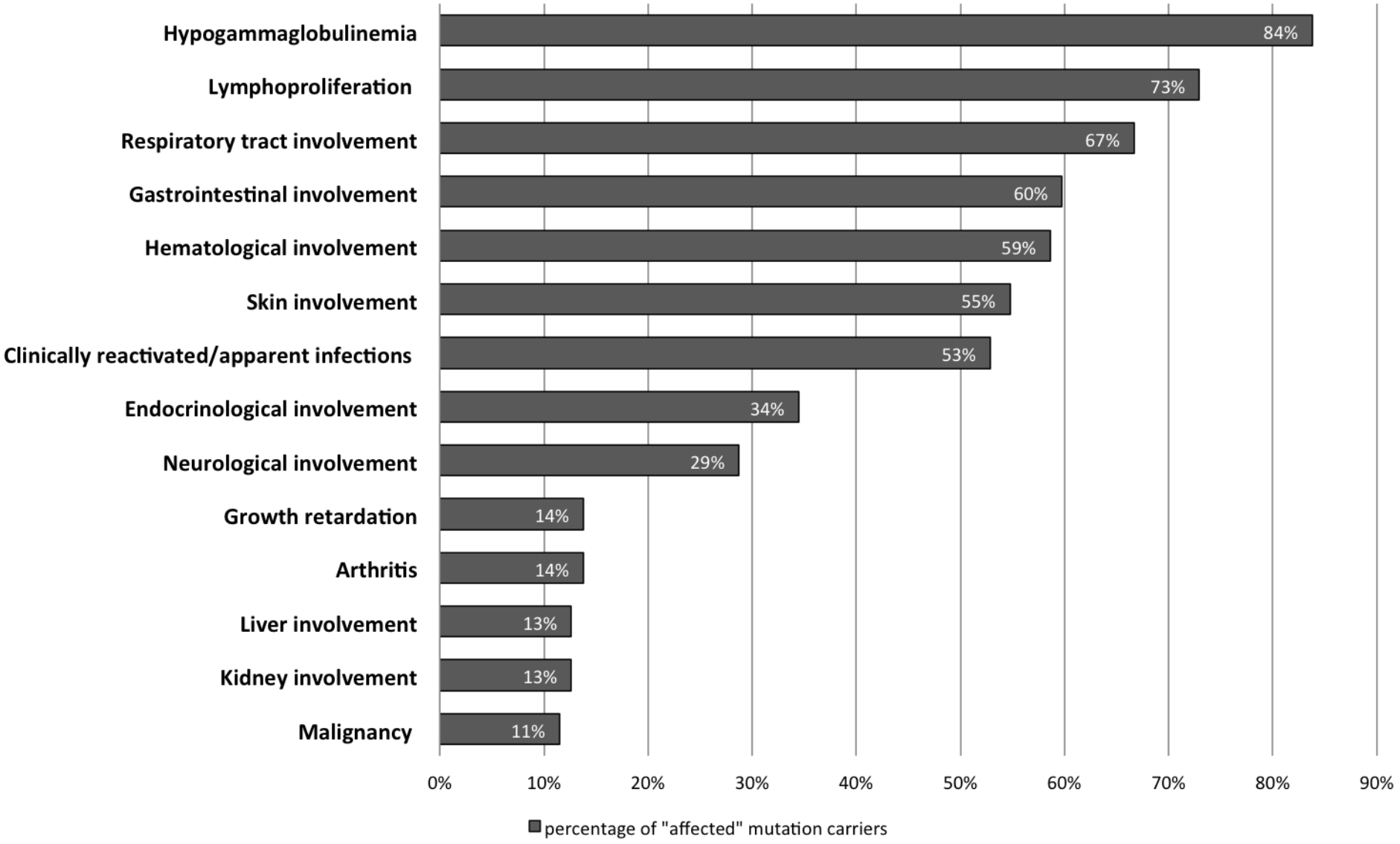


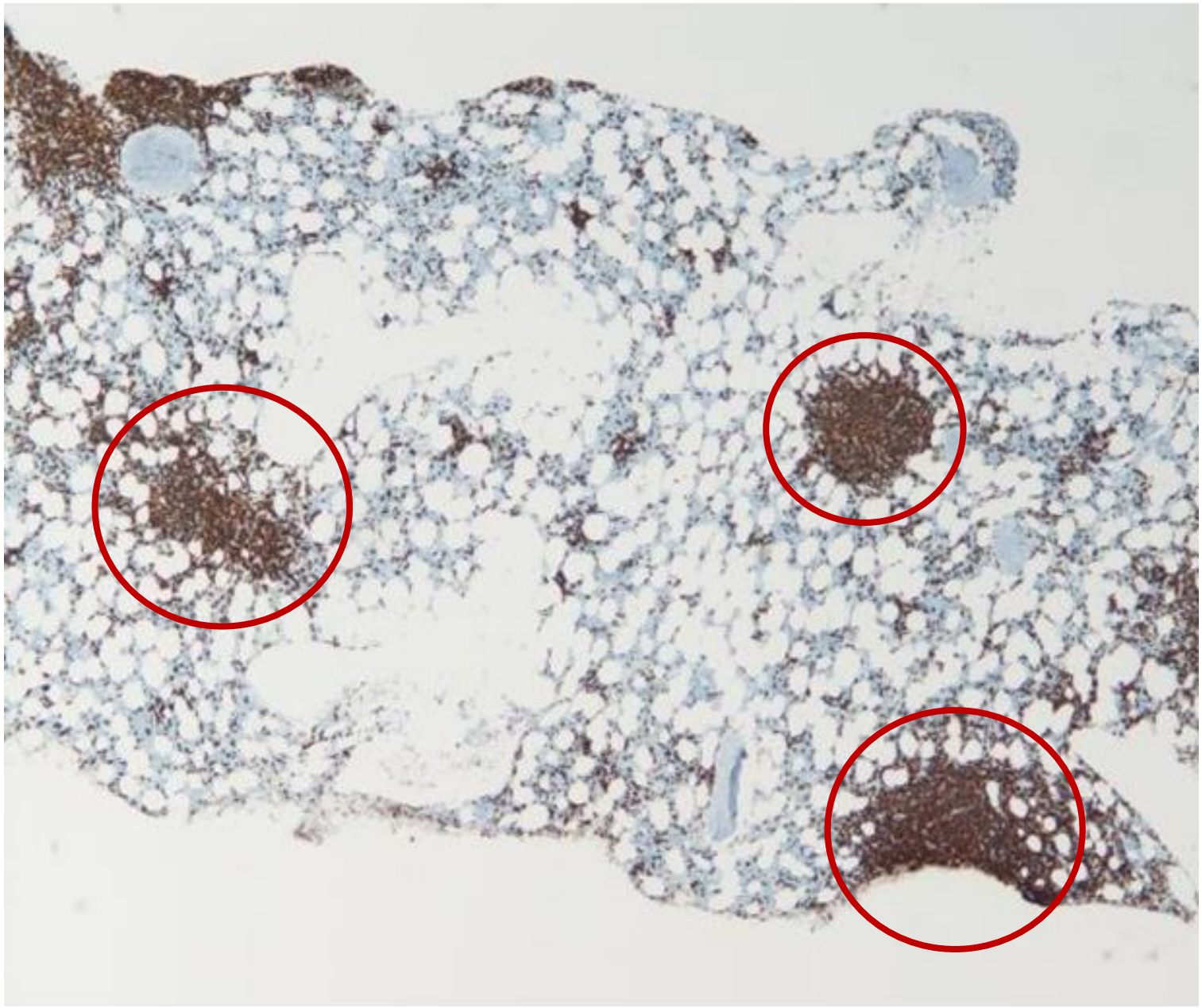


Lymph nodes

Schubert *et al*. 2014 *Nature Medicine* 20;1410–1416

The clinical phenotype is characterized by T-cell-mediated inflammation, lymphoproliferation, and hypogammaglobulinemia

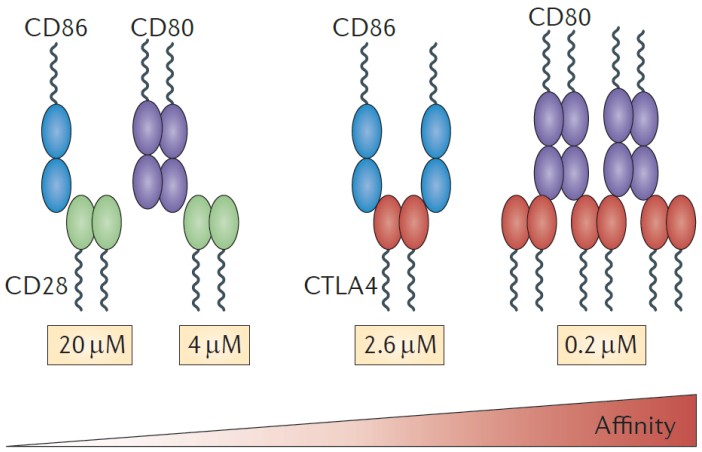


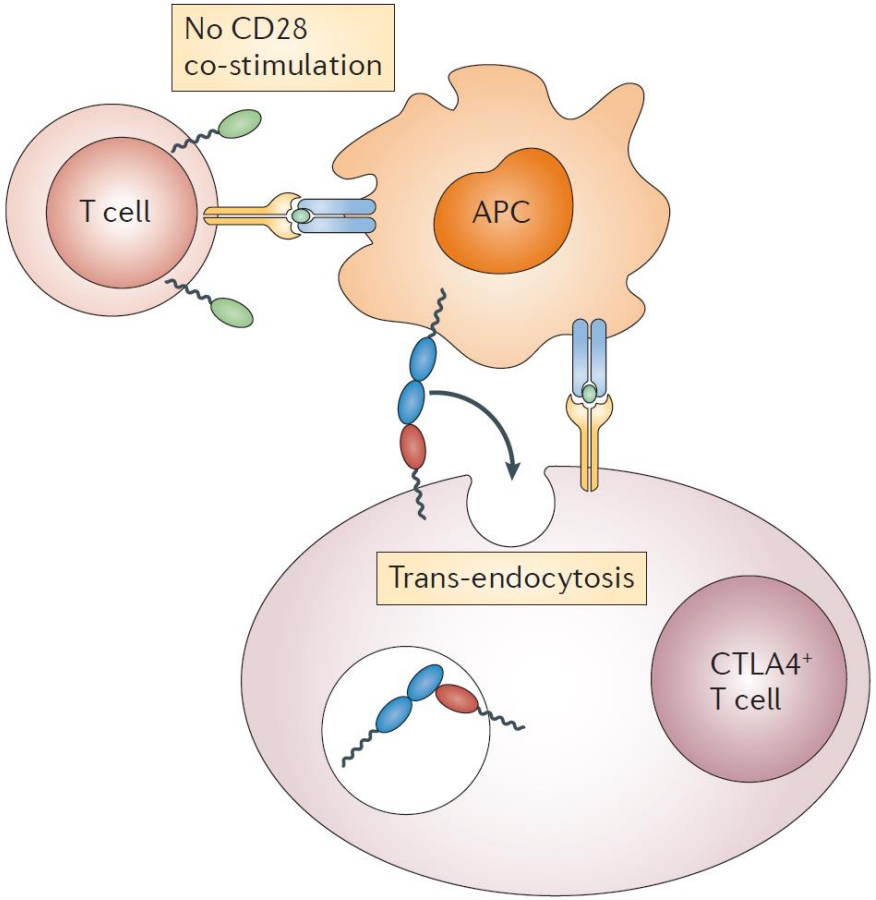


Patient Z.II.2. Nodular T-cell infiltration(bone marrow).

CTLA-4 – an essential inhibitory receptor on Tregs

CTLA-4 captures its ligands CD80 and CD86 from the surface of APCs



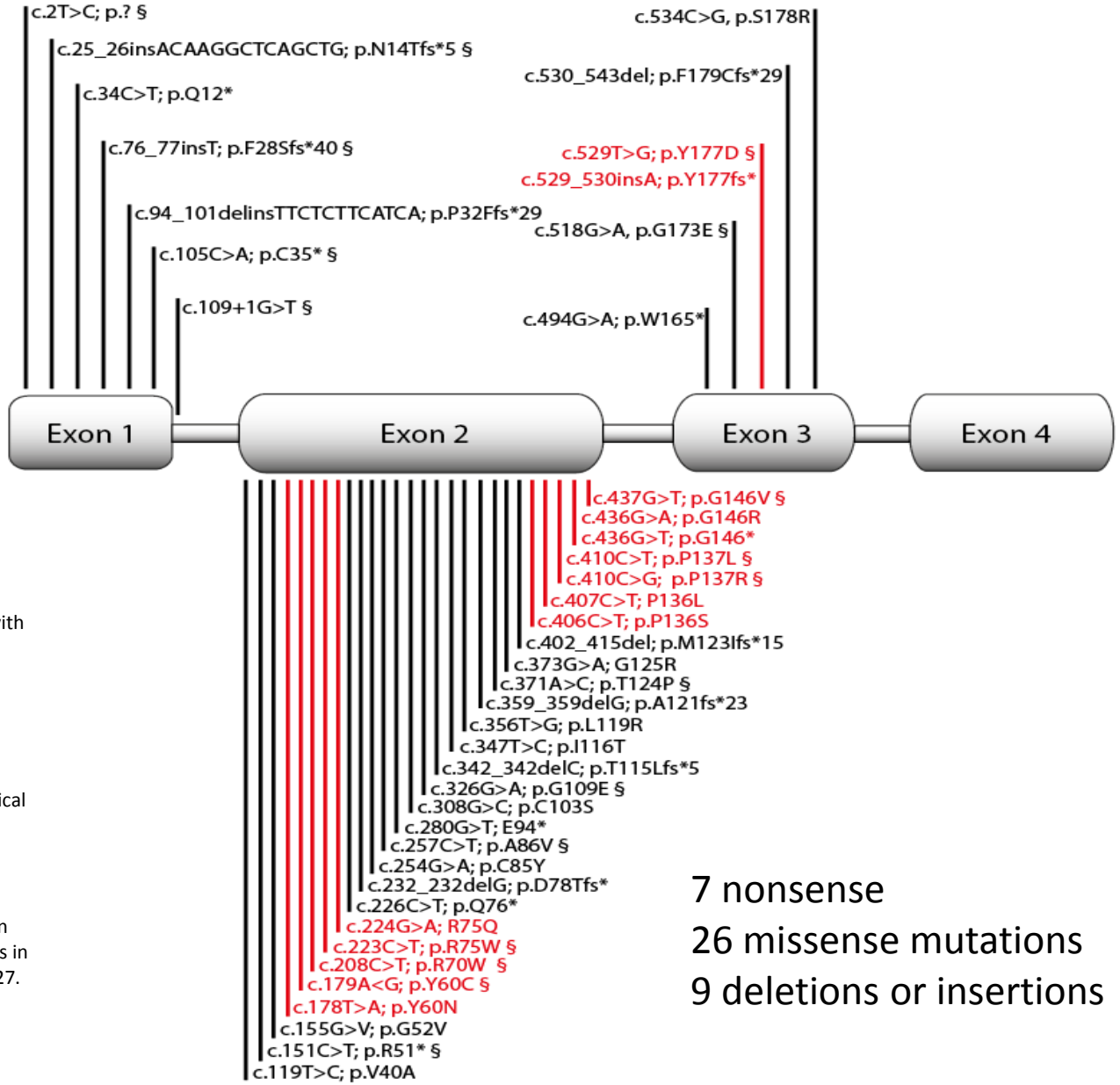


Sansom and Walker, Nat Rev Immunol. 2011 Nov 25;11(12):852-63.

130 mutation carriers with 42 different mutations

Leader peptide and extracellular domain (exon1+2): 36 mutations

Transmembrane domain (exon3): 6 mutations



§ , have previously been described

1.Schubert D, Bode C, Kenefeck R, et al. Autosomal dominant immune dysregulation syndrome in humans with CTLA4 mutations. Nat Med 2014;20:1410-6.

2.Zeissig S, Petersen B-S, Tomczak M, et al. Early-onset Crohn's disease and autoimmunity associated with a variant in CTLA-4. Gut 2015;64:1889-97.

3.Slatter MA, Engelhardt KR, Burroughs LM, et al. Hematopoietic stem cell transplantation for CTLA4 deficiency. Journal of Allergy and Clinical Immunology;138:615-9.e1.

4.Hayakawa S, Okada S, Tsumura M, et al. A Patient with CTLA-4 Haploinsufficiency Presenting Gastric Cancer. Journal of Clinical Immunology 2016;36:28-32.

5.Kuehn HS, Ouyang W, Lo B, et al. Immune dysregulation in human subjects with heterozygous germline mutations in *CTLA4*. *Science (New York, NY)*. 2014;345(6204):1623-1627. doi:10.1126/science.1255904.

7 nonsense
26 missense mutations
9 deletions or insertions

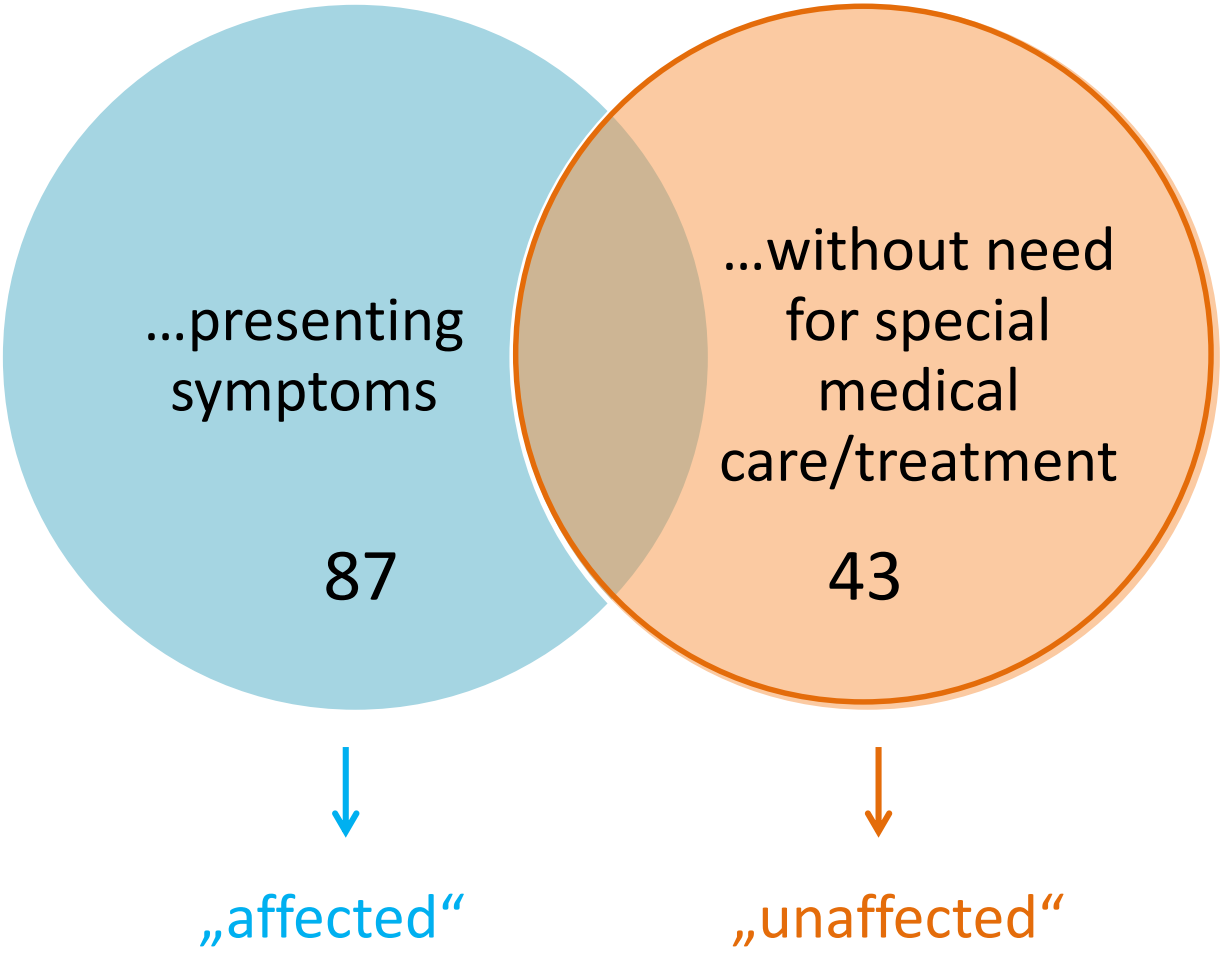
Disease is a continuum,
and health is a subjective measure...

mutation carriers…

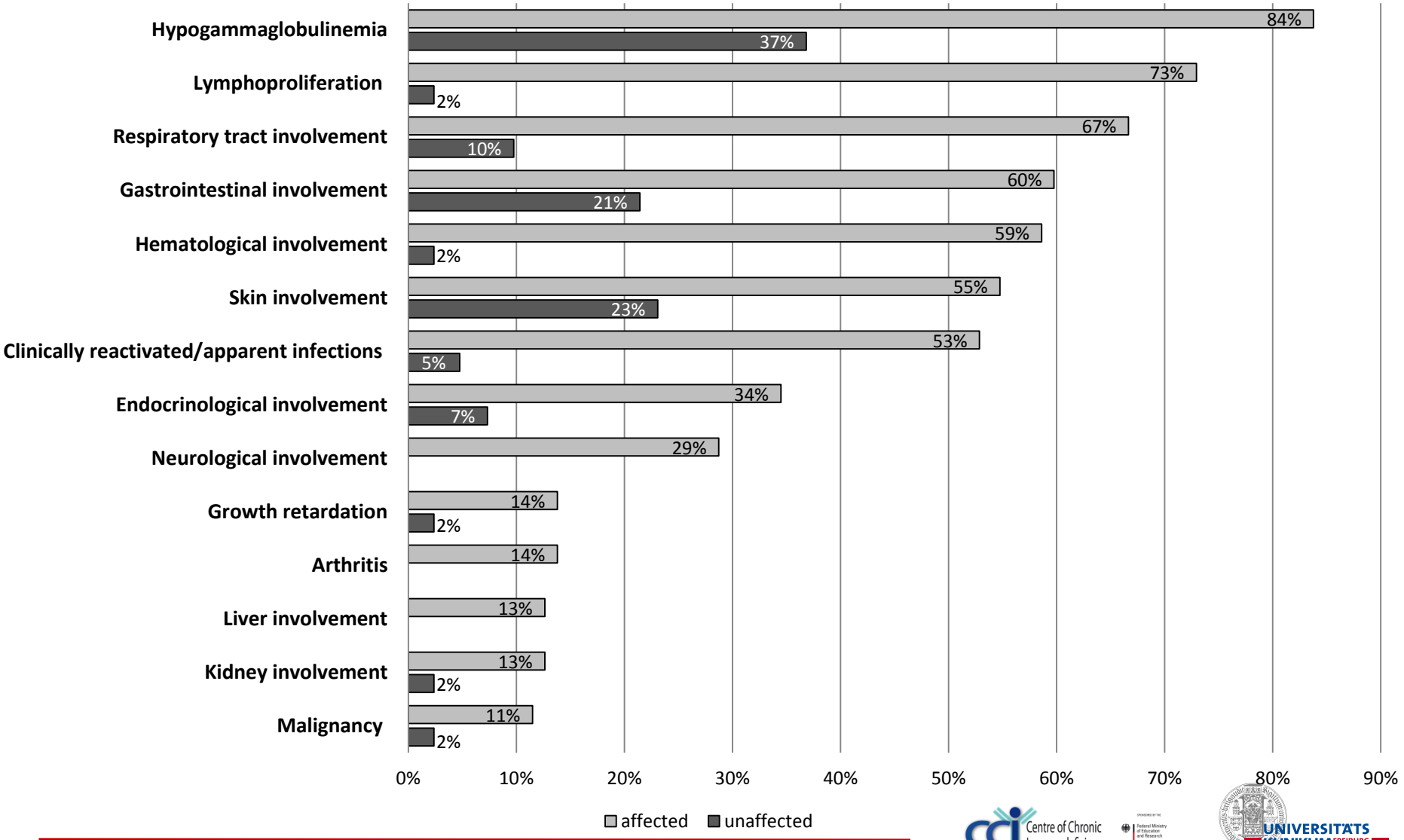
No symptoms ... few symptoms … severe symptoms

Classification into affected and unaffected

130 mutation carriers…



Hypogammaglobulinemia is most common symptom in affected and unaffected mutation carriers whereas lymphoproliferation occurs primarily in the affected individuals



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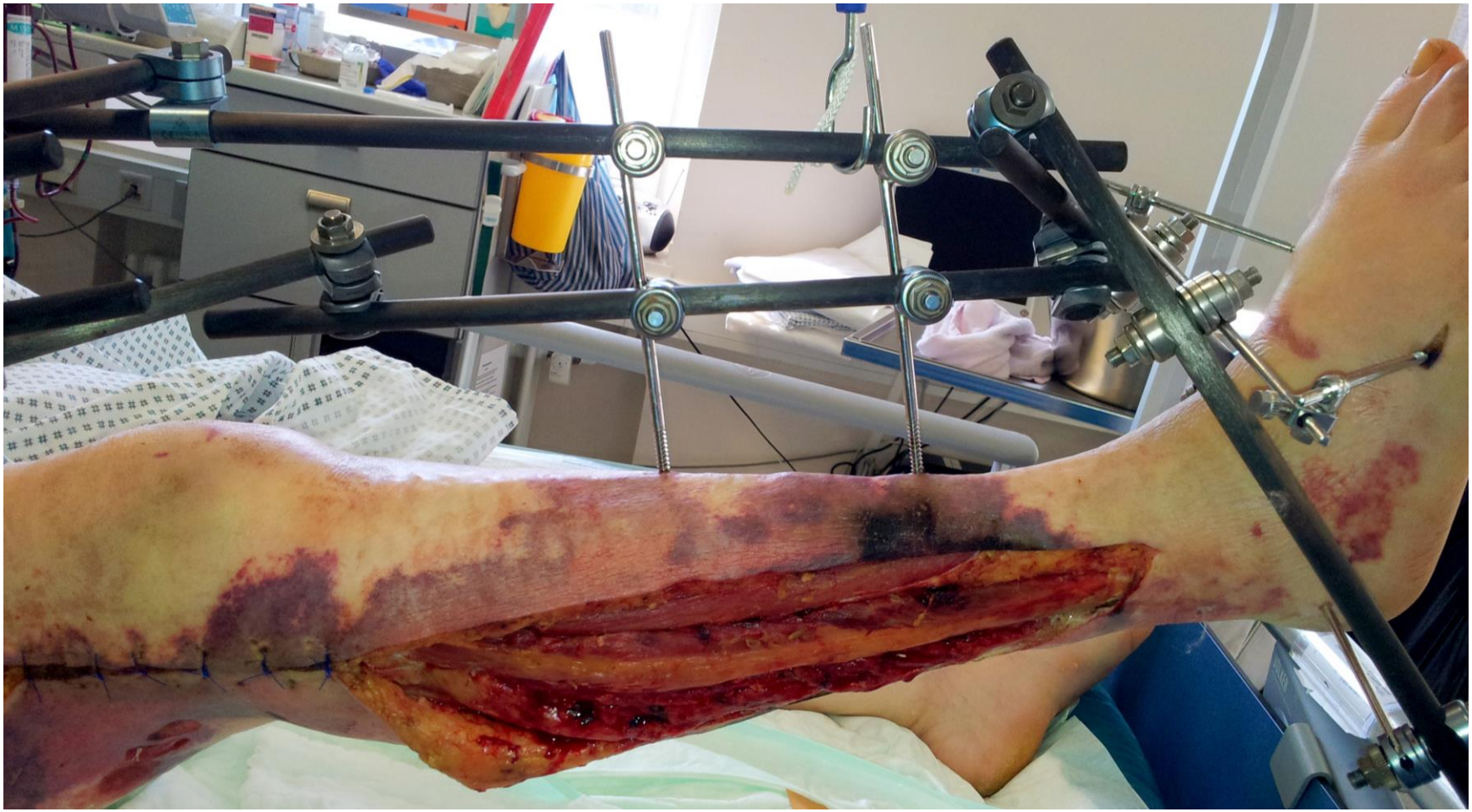
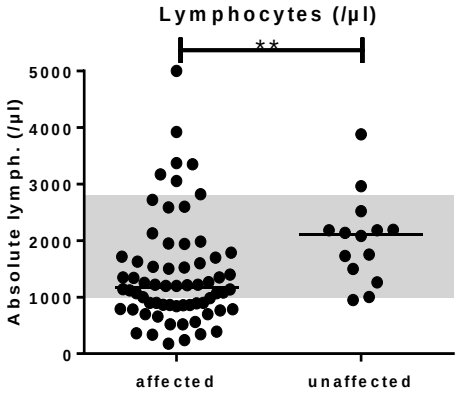
Previously unaffected CTLA4 insufficiency

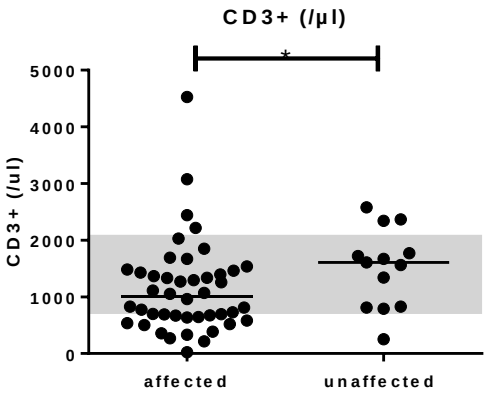
Necrotizing fasciitis, *S. pyogenes* cultured

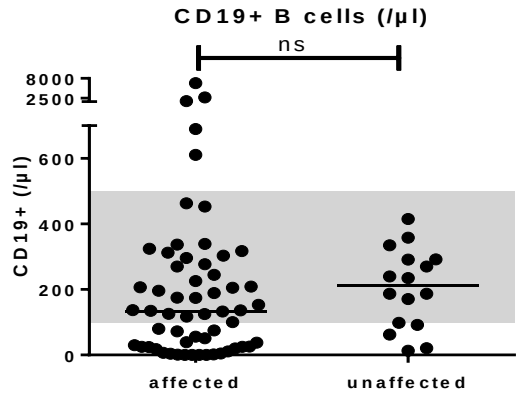


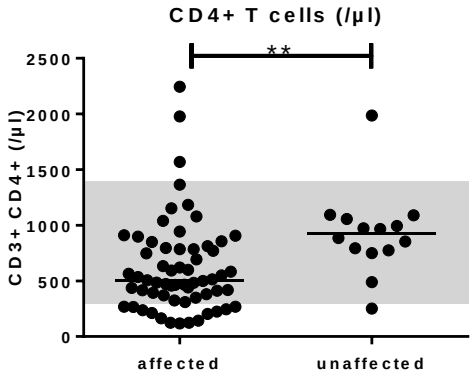
Immunological phenotype

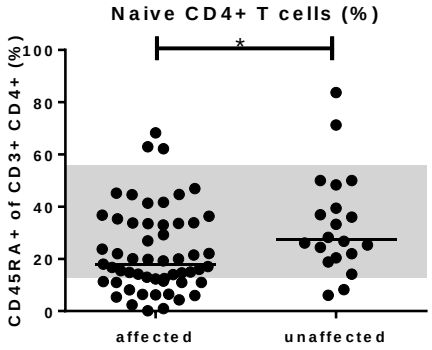
Lymphopenia, decreased CD4+T cells, reduced B cells, increased FoxP3+ Tregs (%)

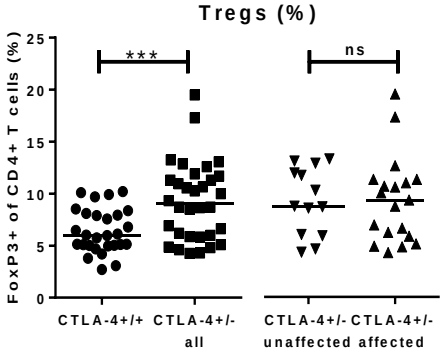


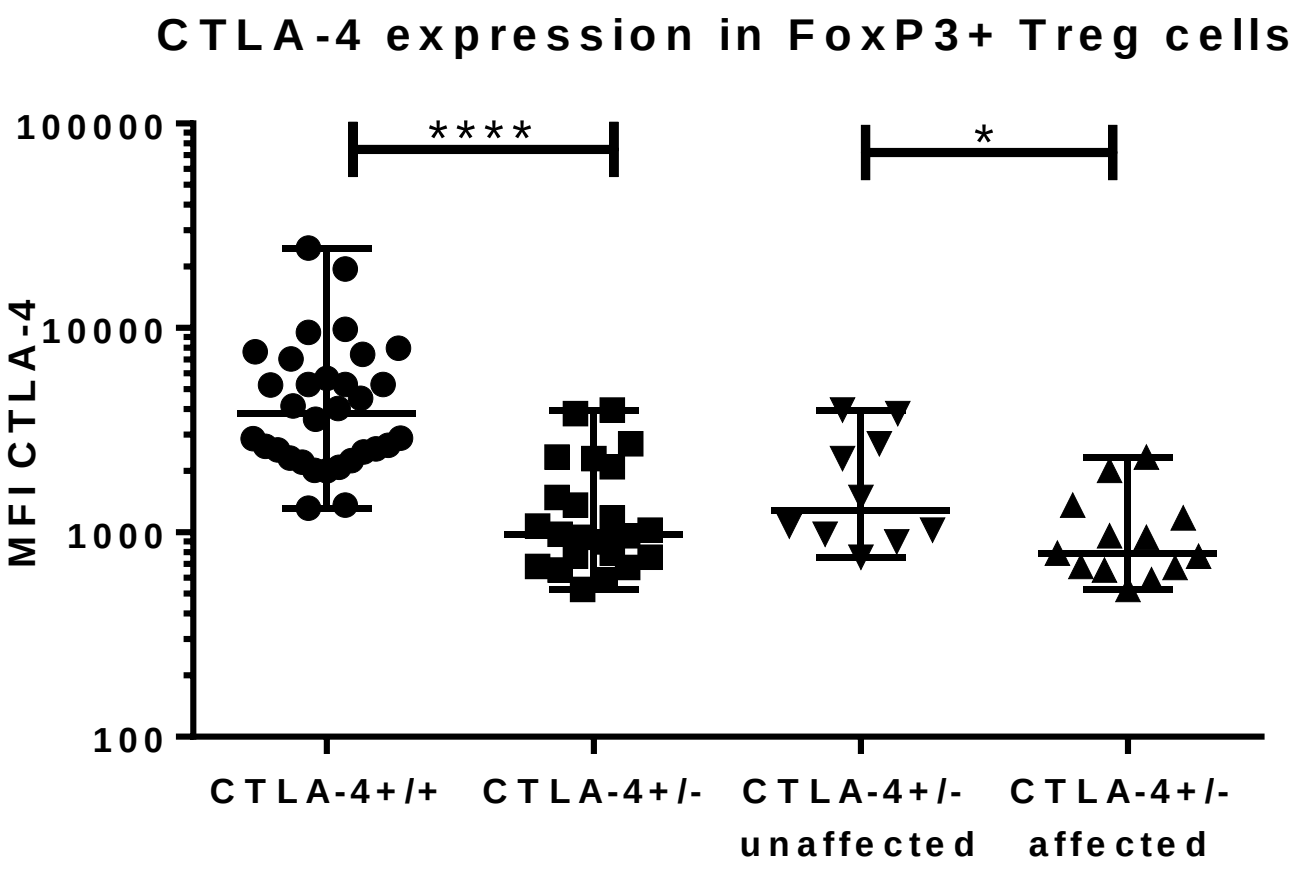











CTLA-4 expression in FoxP3+ Treg cells
MFI CTLA-4
100000
10000
1000
100
****
*
CTLA-4+/+
CTLA-4+/-
CTLA-4+/- unaffected
CTLA-4+/- affected

Question: How to diagnose CTLA4-insufficiency?

Answer: Gene sequencing. CTLA4 has only 4 exons; so far, all mutations are in exons 1-3.

Question: In case I find a missense mutation in CTLA4, does this mean the patient has to have CTLA4-insufficiency?

Answer: No. There may be muissense variants in *CTLA4* which do not impair CTLA4 biology. Example polymorphism at position 17.

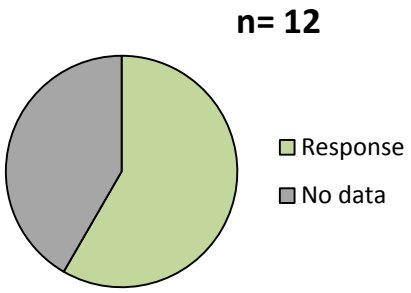
Question: How to treat CTLA4-insufficiency?

Answer: There is no easy answer, as the phenotype is so variable.
Obviously the hypogammaglobulinemia needs immunoglobulin replacement,
CNS involement required high dose corticosteroids,
with bowel involvement one may try first with topical budesonide,
but...

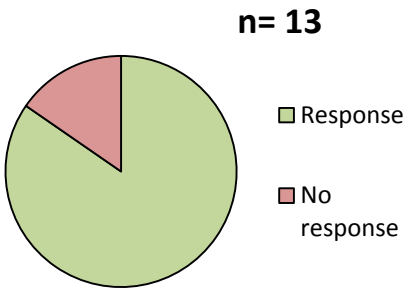
Abatacept, sirolimus, and rituximab are all targeted treatment options in CTLA-4 insufficiency

→ EBV reactivation may be a risk, therefore viral load should be monitored carefully

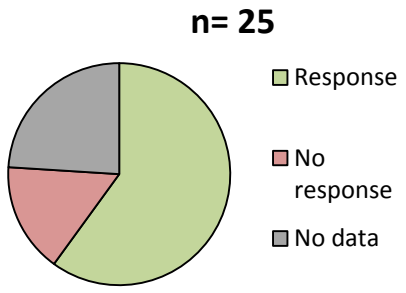
Sirolimus



Abatacept/Belatacept



Rituximab



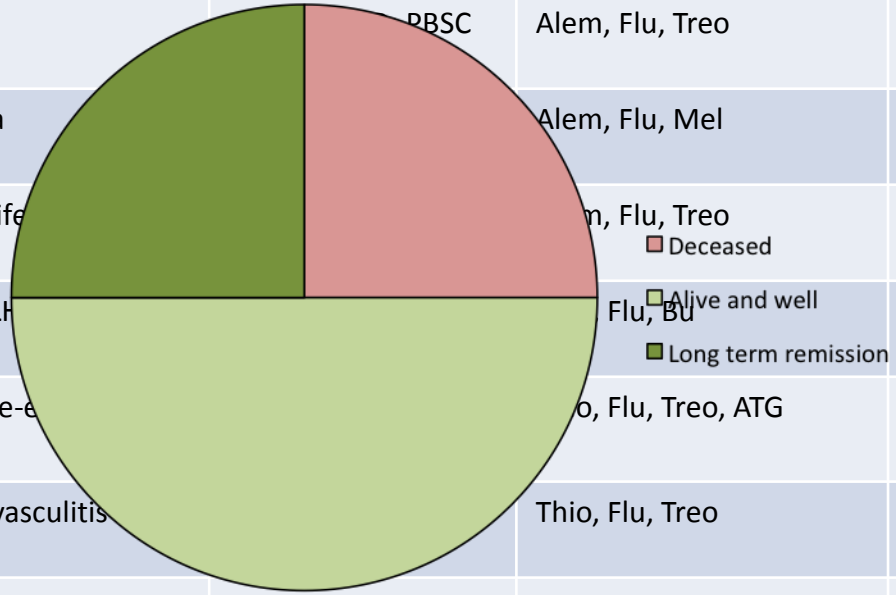
Drug	Total patients	Good response	Positive effects	Transient effect	Side effects
Rituximab	25	15	Cytopenia (3), GI symptoms (1)	0	B cell loss in general
Sirolimus	12	7	GI symptoms (3), splenomegaly (2), cytopenia (2), CMV load (1), lymphadenopathy (1)	1	Raising CMV load (1), maintenance of lymphopenia (1), infections (1), sepsis (1)
Abatacept/ Belatacept	13	11	GI symptoms (5), respiratory symptoms (2), lymphadenopathy (1), dropping of sIL2 receptor (1)	1	EBV reactivation (2) → HLH (1)

Patient (sex)	Age of onset (y)	Primary manifestations (first symptom)	Age of death (y)	Primary cause of death
P1 (m)	12	CVID; lung disease; neurological involvement; enteropathy	34	Wasting enteropathy and lung disease
P2 (f)	17	CVID; respiratory involvement	37	Relaps of disease following lung transplantation
P3 (m)	15	Enteropathy	23	Acute liver failure
P4 (f)	10	Respiratory involvement	20	Relaps of disease following lung transplantation
P5 (f)	7	CVID; growth retardation	20	Septic shock
P6 (m)	8	Evans Syndrom	23	Uk; following colectomy for severe enteropathy
P7 (f)	12	Enteropathy; respiratory involvement	24	septic embolism (MRSA infection)
P8 (m)	10	ALPS-like phenotype; cytopenia	21	Septic multiorgan failure
P9 (f)	26	Lymphoma; cytopenia	53	Lymphoma
P10 (f)	9	Evans' syndrome; neurological involvement	40	Sepsis (GI perforation)
P11 (m)	10	Enteropathy	35	Bacterial sepsis
P12 (m)	10	Lymphadenopathy; cytopenia	15	Post HSCT, GvHD
P13 (m)	2	Enteropathy; type 1 diabetes	22	Post HSCT, metabolic ketoacidosis
P14 (m)	6	Lymphoma; cytopenia	22	Lymphoma
P15 (f)	1	Cytopenia; respiratory/neurologic involvement	14	Post HSCT, GvHD
P16 (f)	40	CVID; respiratory involvement	73	HCV infection
P17 (f)	uk	Lymphoma, endocrinological involvement	60	Lymphoma

However, 17 patients (almost 20%) have died at an average age of 23 years

HSCT in patients with *CTLA4* mutations

	Age	Reason for HSCT	HLA-match/ Donor	Conditioning	Outcome
P 1	15	cITP; widespread lymphoid hyperplasia	10/10; MUD; PBSC	Alem, Flu, Mel	Death due to GvHD
P 2	20	Cytopenia; bronhciectasis; enteropathy (TPN-dependent)	10/10; MUD; PBSC	Alem, Flu, Mel	Died 2.5 years after due to diabetic ketoazidosis
P 3	14	Immunodeficiency; CMV infection	9/10; MUD; PBSC	Thio, Flu, Treo	Death due to GvHD
P 4	16	Cytopenia; arthritis; lymphadenopathy	PBSC	Alem, Flu, Treo	Alive and well 6.1 years
P 5	10	Enteropathy; Cytopenia		Alem, Flu, Mel	Alive and well 10.5 years
P 6	16	Cytopenia; lymphoprolife		m, Flu, Treo	Alive and well 5.6 years
P 7	51	Hodgkin lymphoma; HLH		Flu, Bu	Alive and well 100 days follow-up
P 8	13	Cytopenia; autoimmune-e		o, Flu, Treo, ATG	Alive and well 10 months follow-up
P 9	20	Cytopenia; hemolysis, vasculitis paraplegy		Thio, Flu, Treo	Alive and well 10 months follow-up
P 10	17	Hodgkin lymphoma; enteropathy	10/10; MUD; BM	Alem, Flu, Treo, Thio	Alive and well 12 months follow-up
P11	14	Recurrent infections, enteropathy	7/8; MMUD; BM	Flu, Mel, TBI 3 Gy	Alive and well 7 months follow-up
P12	14	Enteropathy, , respiratory disease	10/10; MUD; BM	Alem, Flu, Bu	Alive and well 90 days follow-up



Centrum für Chronische Immundefizienz

UNIVERSITÄTS KLINIKUM FREIBURG

- Start
- Patients
- Physicians
- Scientists

CTLA-4 Deficiency

Welcome!

The goal of this website is to inform patients and their relatives, physicians and scientists about the molecular basis of and the clinical manifestations that can result from CTLA-4 deficiency. Should you have any additional questions you are very welcome to contact us at any time.

For Patients | For Physicians | For Scientists

CTLA-4 deficiency (cytotoxic T-lymphocyte-associated protein-4 deficiency), which results from a germline mutation in the *CTLA4* gene, can cause an immune defect- and immune dysregulation syndrome in mutation carriers. The inheritance pattern is autosomal-dominant, which means that the chance to inherit the mutation is 50%. Both the penetrance and the expressivity are reduced, therefore not all mutation carriers show symptoms, and the severity of the clinical manifestations can vary in individual patients. Patients often develop a syndrome that

Contact Info

Center for Chronic Immunodeficiency
FREIBURG UNIVERSITY MEDICAL CENTER
Breisacher Strasse 117
79106 Freiburg im Breisgau
Germany

Tel. +49 761 270-77732

Fax. +49 761 270-77744

E-Mail | Website

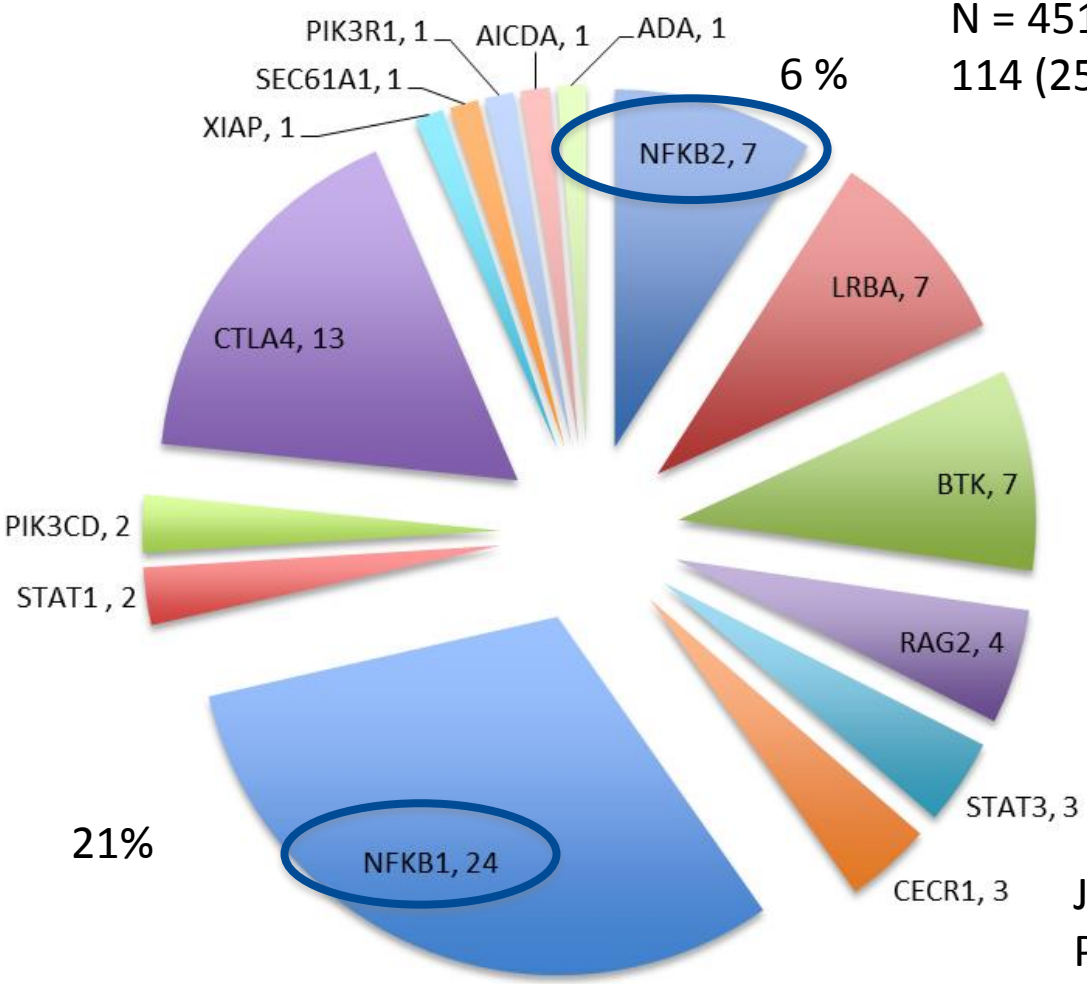
Outpatient Clinic

Marion Klima
Tel. +49 761 270-37500

Heike Spitznagel
Tel. +49 761 270-77580

www.ctla4-deficiency.org
www.ctla4-defizienz.de

Monogenetic Causes for Hypogamma-/Agammaglobulinemia



N = 451 patients
114 (25%) with clear mutation

Jessica Rojas et al.,
Poster Discussion ED05
Tuesday, 13:10

Clinical Phenotype of NFkB1 Mutations

- Heterogeneous clinical presentations…

ARTICLE

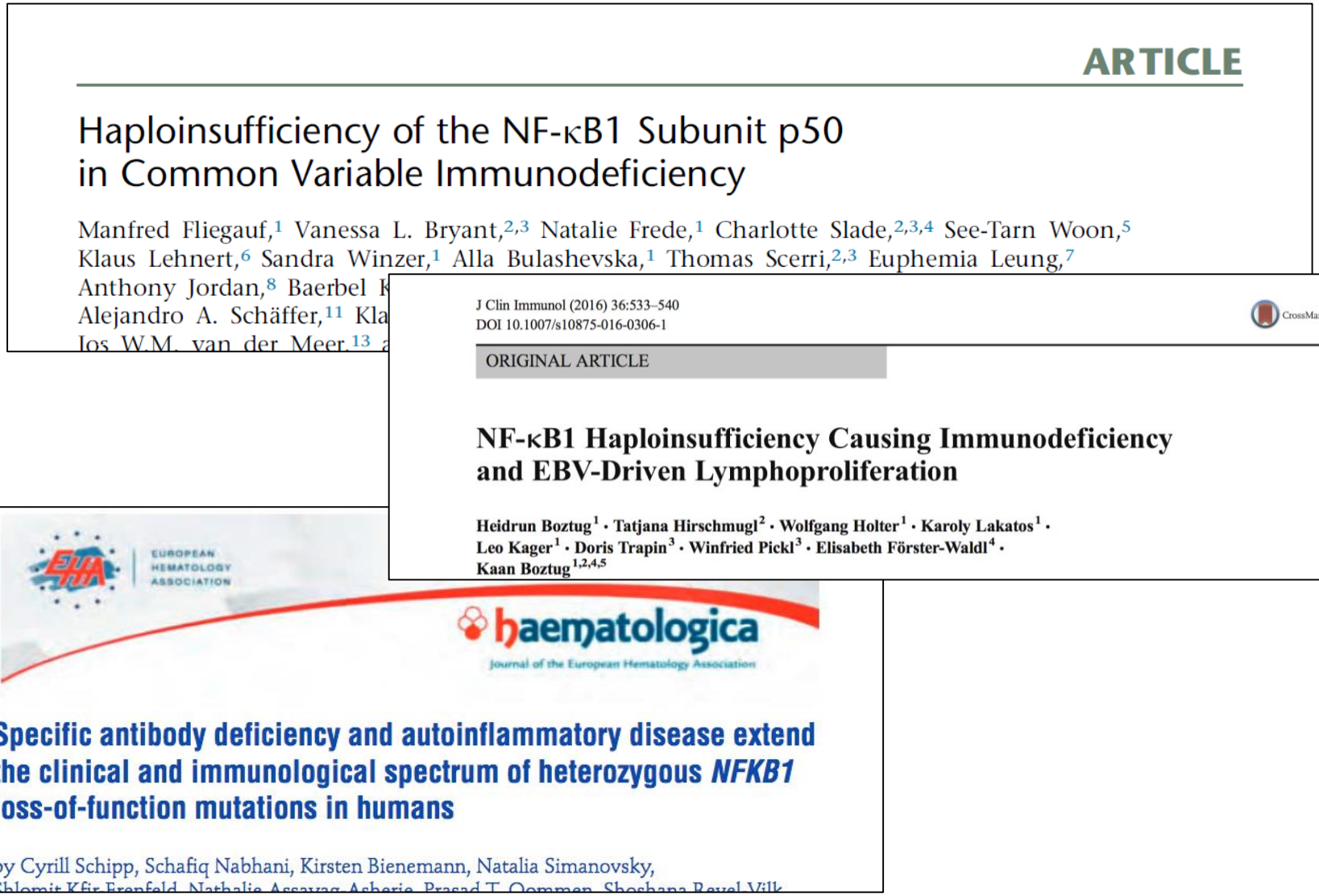
Haploinsufficiency of the NF-κB1 Subunit p50 in Common Variable Immunodeficiency

Manfred Fliegauf,[1] Vanessa L. Bryant,[2,3] Natalie Frede,[1] Charlotte Slade,[2,3,4] See-Tarn Woon,[5] Klaus Lehnert,[6] Sandra Winzer,[1] Alla Bulashevska,[1] Thomas Scerri,[2,3] Euphemia Leung,[7] Anthony Jordan,[8] Baerbel Keller,[1] Esther de Vries,[9] Hongzhi Cao,[10] Fang Yang,[10] Alejandro A. Schäffer,[11] Klaus Warnatz,[1] Peter Browett,[7] Jo Douglass,[2,4,12] Rohan V. Ameratunga,[5] Jos W.M. van der Meer,[13] and Bodo Grimbacher[1,14,*]

Fliegauf M et al, Am J Hum Genet, 2015; Boztug H et al, J Clin Immunol, 2016;
Schipp C et al, Haematologica, 2016

Clinical Phenotype of NFkB1 Mutations

- Heterogeneous clinical presentations…



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J Clin Immunol (2016) 36:533–540
DOI 10.1007/s10875-016-0306-1

ORIGINAL ARTICLE

NF-κB1 Haploinsufficiency Causing Immunodeficiency and EBV-Driven Lymphoproliferation

Heidrun Boztug[1] · Tatjana Hirschmugl[2] · Wolfgang Holter[1] · Karoly Lakatos[1] · Leo Kager[1] · Doris Trapin[3] · Winfried Pickl[3] · Elisabeth Förster-Waldl[4] · Kaan Boztug[1,2,4,5]

EUROPEAN HEMATOLOGY ASSOCIATION

haematologica

Journal of the European Hematology Association

Specific antibody deficiency and autoinflammatory disease extend the clinical and immunological spectrum of heterozygous *NFKB1* loss-of-function mutations in humans

by Cyrill Schipp, Schafiq Nabhani, Kirsten Bienemann, Natalia Simanovsky, Shlomit Kfir-Erenfeld, Nathalie Assayag-Asherie, Prasad T. Oommen, Shoshana Revel-Vilk

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Haploinsufficiency of the NF-κB1 Subunit p50 in Common Variable Immunodeficiency

CVID

Manfred Fliegauf,[1] Vanessa L. Bryant,[2,3] Natalie Frede,[1] Charlotte Slade,[2,3,4] See-Tarn Woon,[5] Klaus Lehnert,[6] Sandra Winzer,[1] Alla Bulashevska,[1] Thomas Scerri,[2,3] Euphemia Leung,[7] Anthony Jordan,[8] Baerbel K… Alejandro A. Schäffer,[11] Kla… Jos W.M. van der Meer,[13] a…

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Combined Immunodeficiency

Heidrun Boztug[1] · Tatjana Hirschmugl[2] · Wolfgang Holter[1] · Karoly Lakatos[1] · Leo Kager[1] · Doris Trapin[3] · Winfried Pickl[3] · Elisabeth Förster-Waldl[4] · Kaan Boztug[1,2,4,5]

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by Cyrill Schipp, Schafiq Nabhani, Kirsten Bienemann, Natalia Simanovsky, Shlomit Kfir-Erenfeld, Nathalie Assayag-Asherie, Prasad T. Oommen, Shoshana Revel-Vilk

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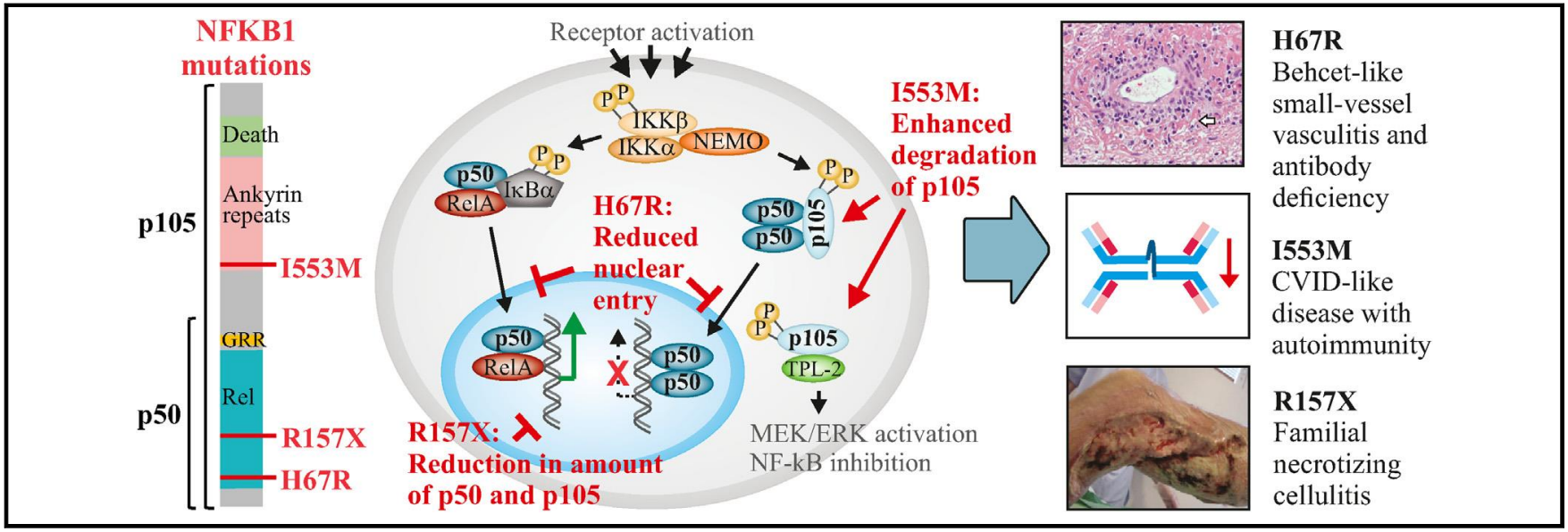
Extended phenotype of NFkB mutations

Meri Kaustio, MSc,[a]* Emma Haapaniemi, MD, PhD,[b,c]* Helka Göös, MSc,[d]* Timo Hautala, MD, PhD,[e] Giljun Park, PhD,[f] Jaana Syrjänen, MD, PhD,[g] Elisabet Einarsdottir, PhD,[b,c,t] Biswajyoti Sahu, PhD,[h] Sanna Kilpinen, MD, PhD,[i] Samuli Rounioja, MD, PhD,[j,k] Christopher L. Fogarty, MSc,[b,l,m] Virpi Glumoff, PhD,[n] Petri Kulmala, MD, PhD,[n,o] Shintaro Katayama, PhD,[c] Fitsum Tamene, MSc,[d] Luca Trotta, MSc,[a] Ekaterina Morgunova, PhD,[c] Kaarel Krjutškov, PhD,[b,c,p] Katariina Nurmi, PhD,[q] Kari Eklund, MD, PhD,[q] Anssi Lagerstedt, MD, PhD,[j] Merja Helminen, MD, PhD,[k] Timi Martelius, MD, PhD,[r] Satu Mustjoki, MD, PhD,[f,s] Jussi Taipale, PhD,[c] Janna Saarela, MD, PhD,[a]‡ Juha Kere, MD, PhD,[b,c,t]‡ Markku Varjosalo, PhD,[d]‡ and Mikko Seppänen, MD, PhD[r,u]‡

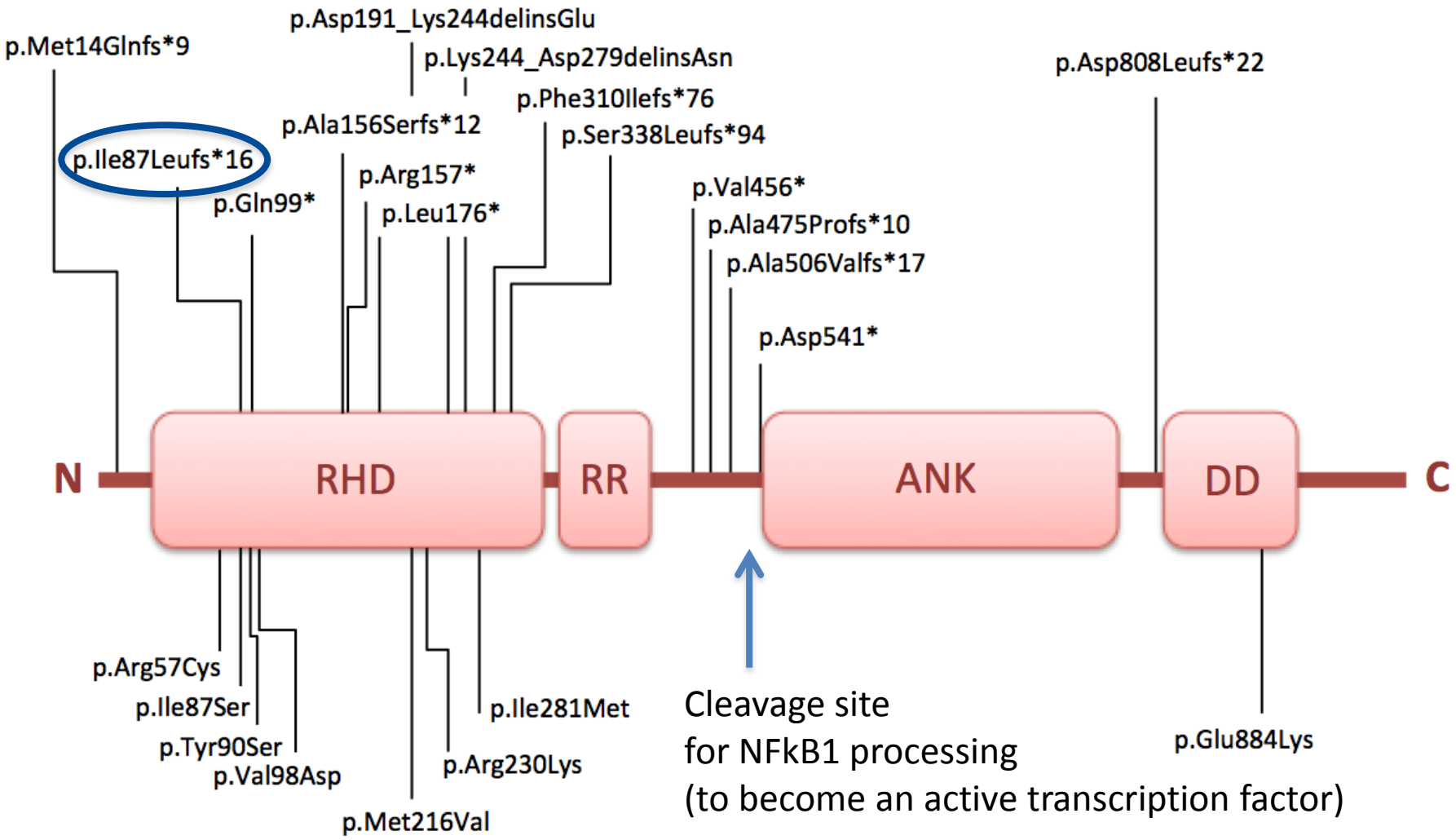
Helsinki, Oulu, Tampere, and Jyväskylä, Finland; Stockholm, Sweden; and Tartu, Estonia

GRAPHICAL ABSTRACT



NFkB1 Mutations

- Autosomal dominant inheritance



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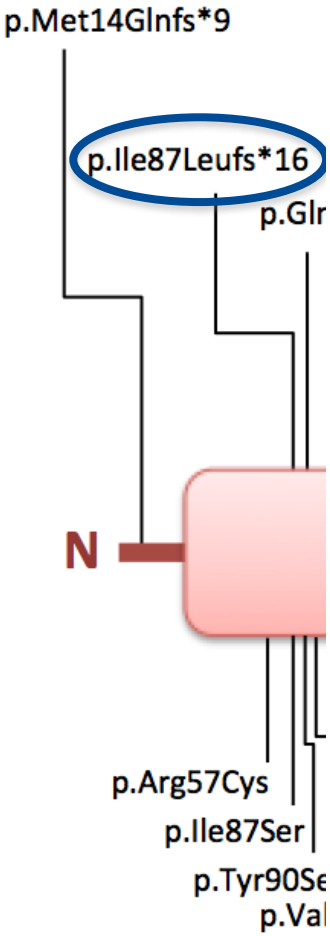

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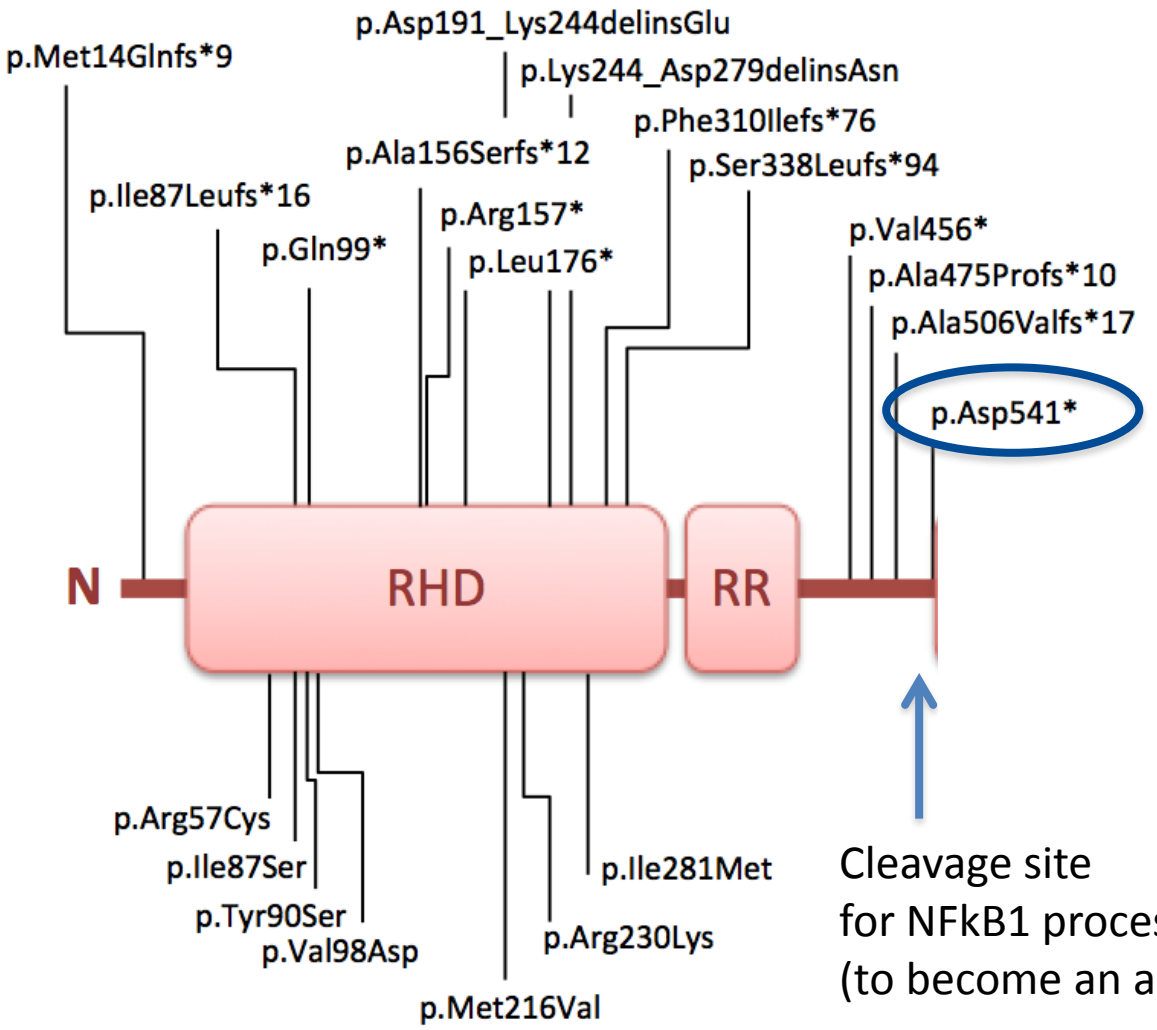
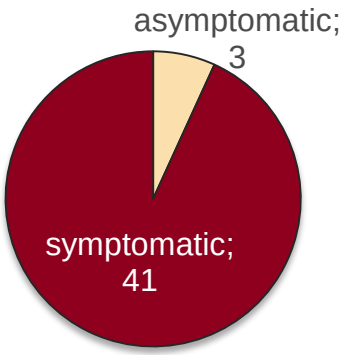


Cleavage site
for NFkB1 processing
(to become an active transcription factor)

Clinical manifestations

- 44 patients with NFkB1 mutations



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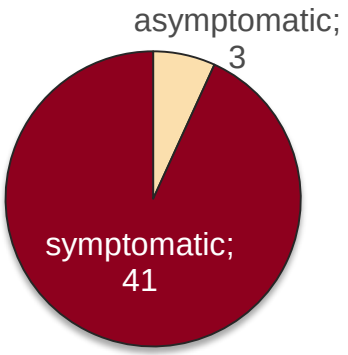
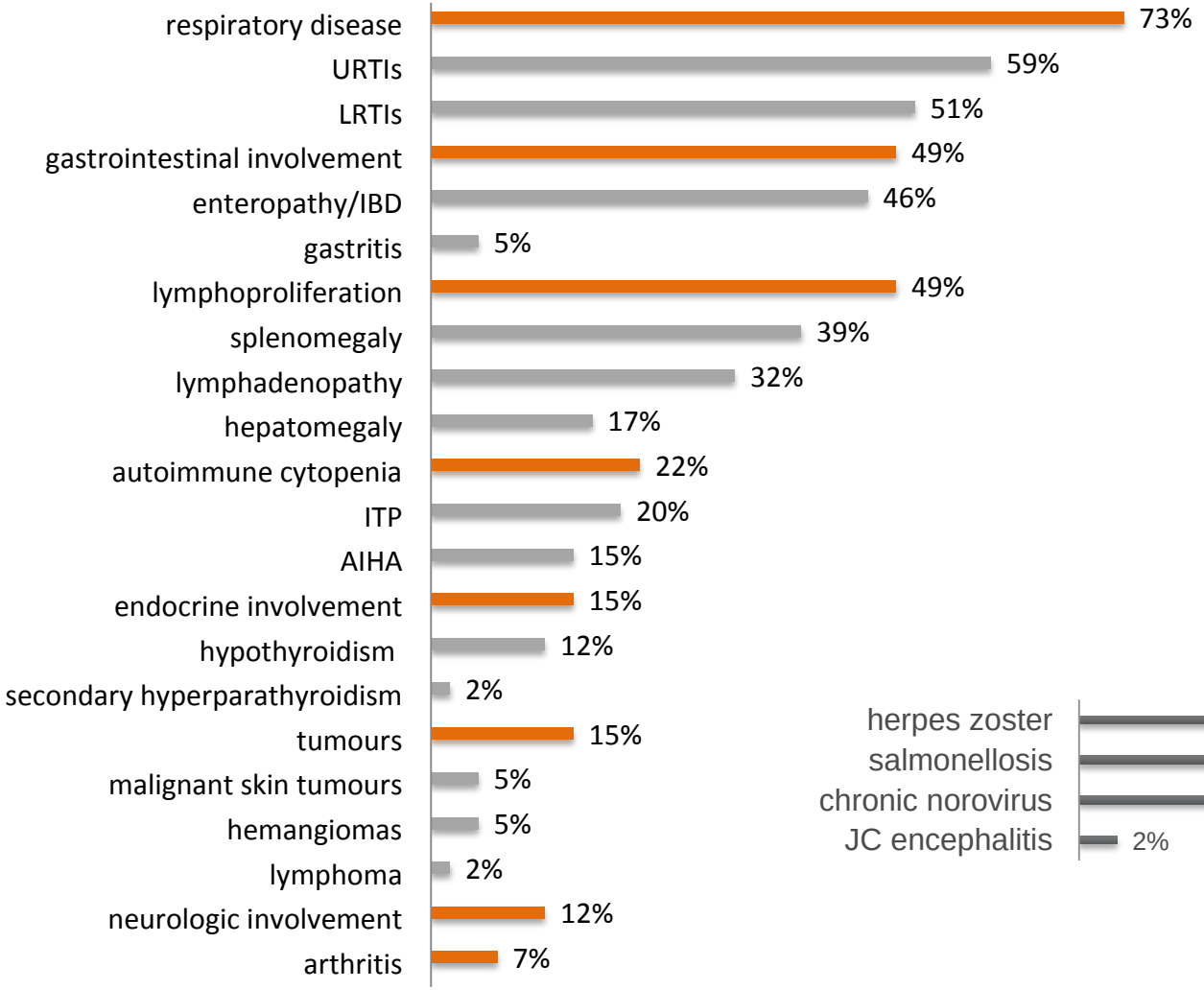
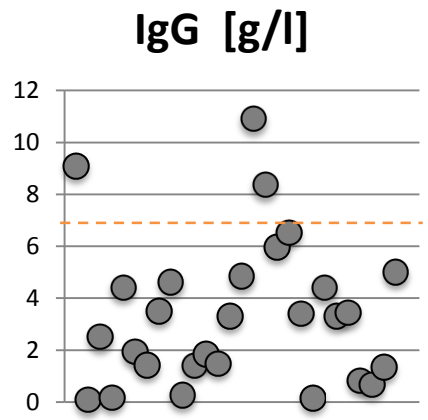
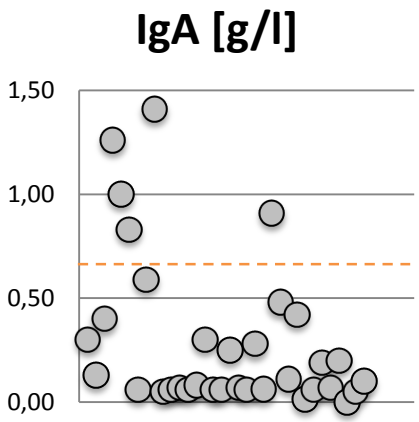


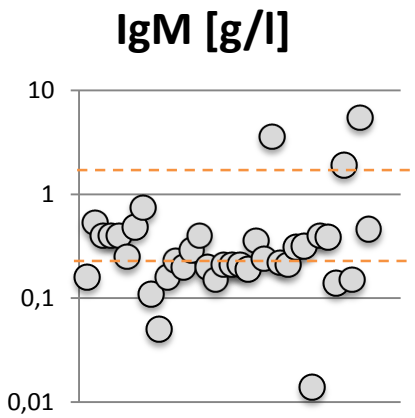


Clinical manifestations

- Laboratory values

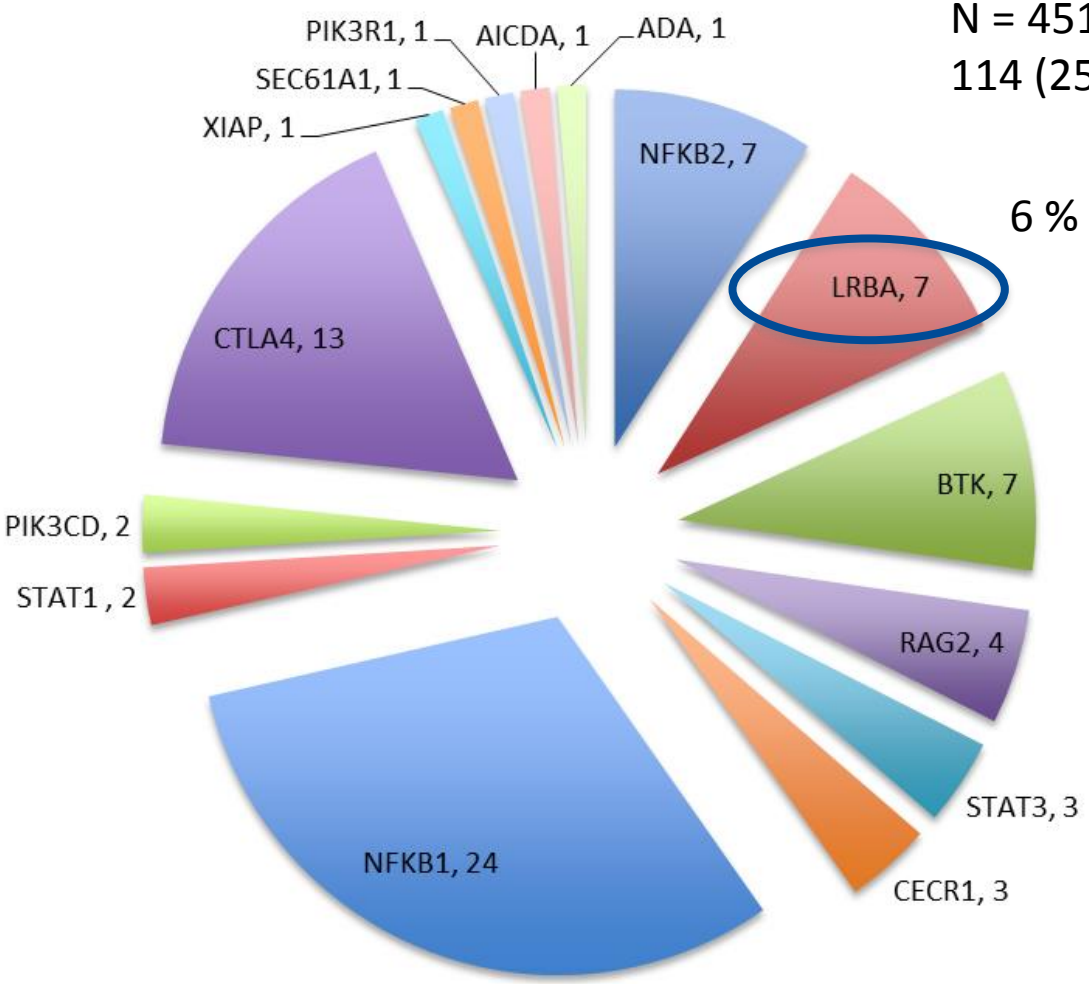






- 83% of patients have hypogammaglobulinemia
- 26 patients diagnosed with CVID, 2 with HIGM
- 2/3 of all patients on immunoglobulin replacement therapy

Monogenetic Causes for Hypogamma-/Agammaglobulinemia

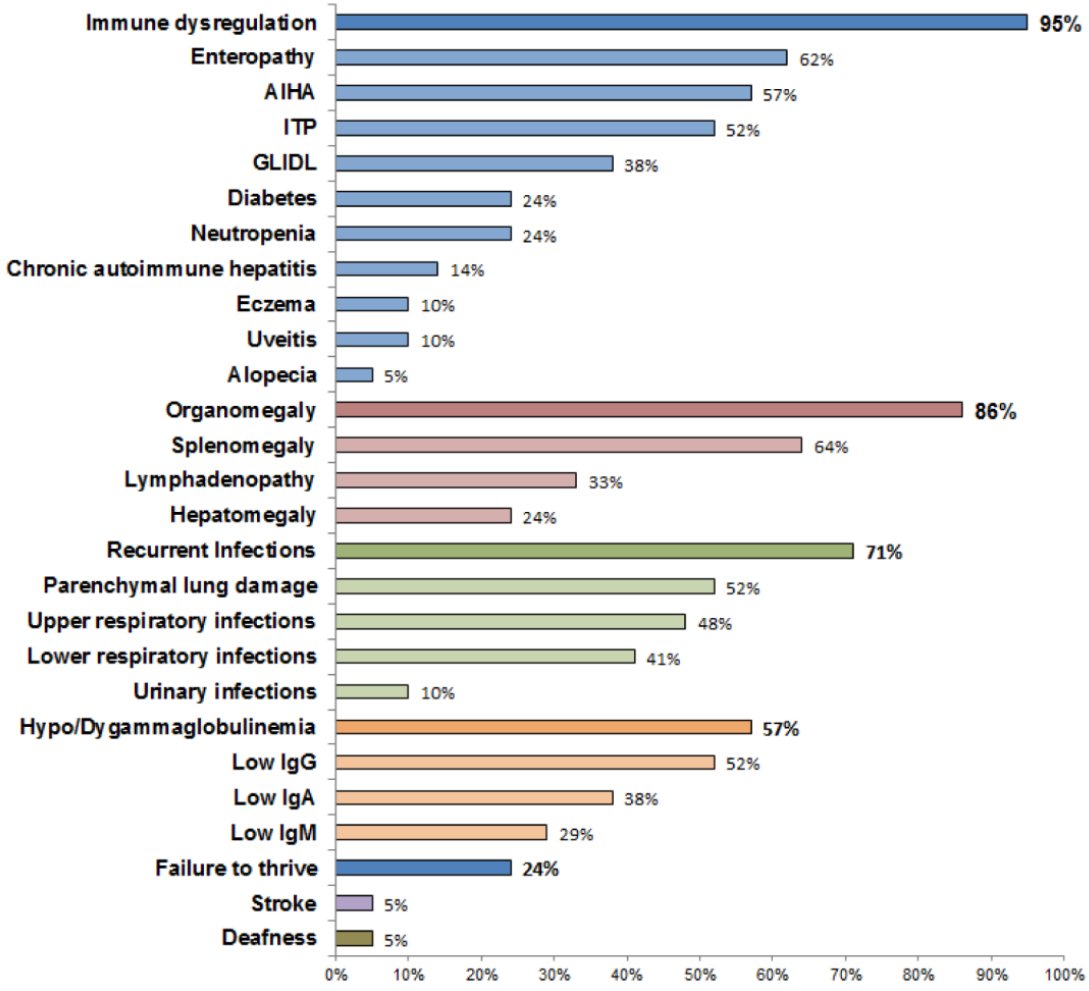


N = 451 patients
114 (25%) with clear mutation

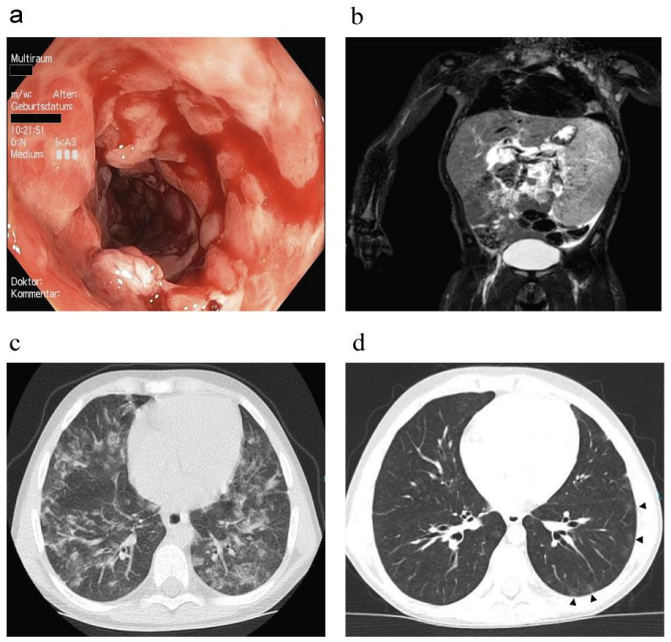
6 %

LRBA deficiency: PID and immune dysregulation syndrome

Caused by biallelic mutations in *LRBA* with loss of protein expression



Laura Gamez
Tuesday, 12 noon
Pentland Suite



Gámez-Díaz et al. JACI 2016

Biology of CTLA4 recycling

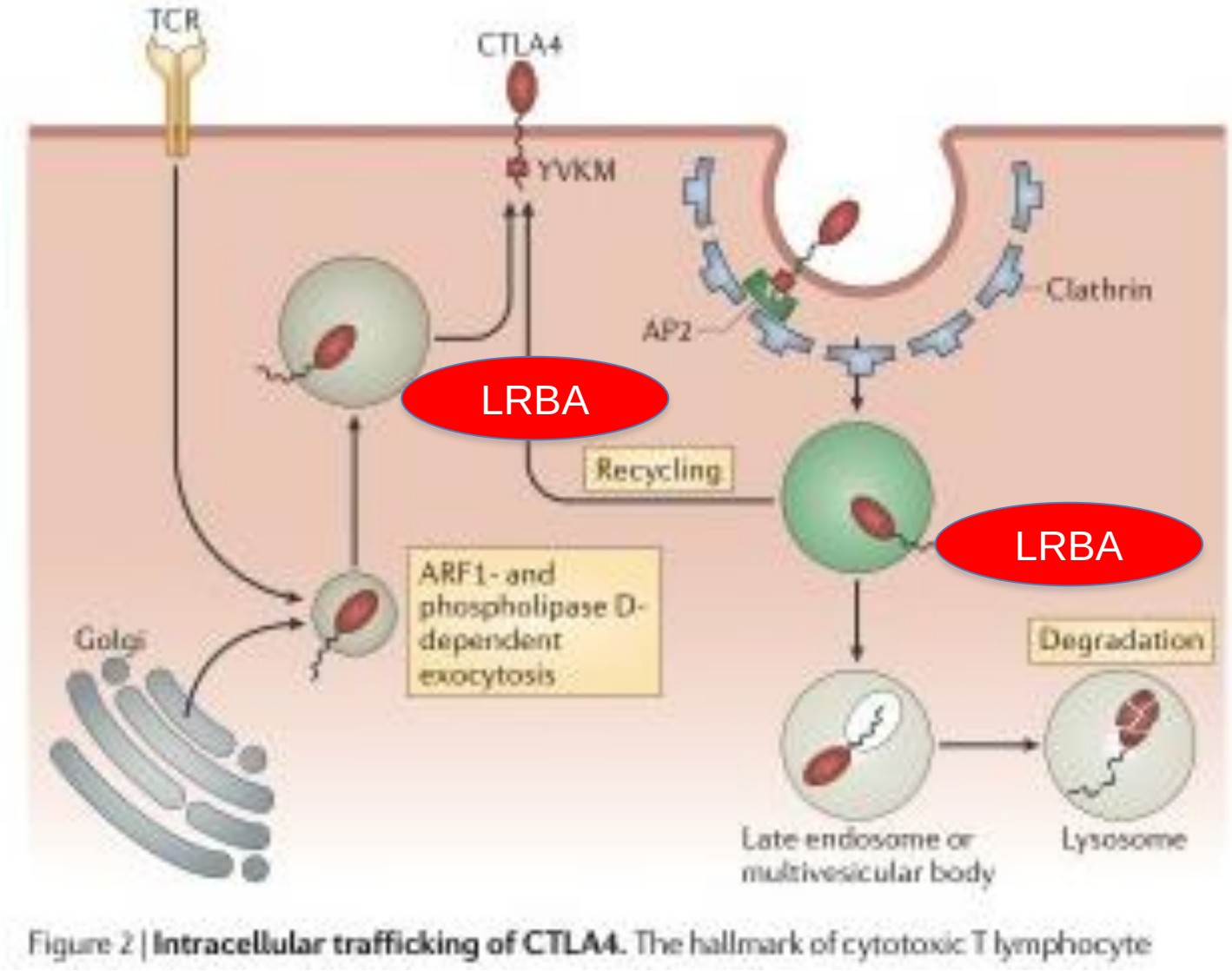
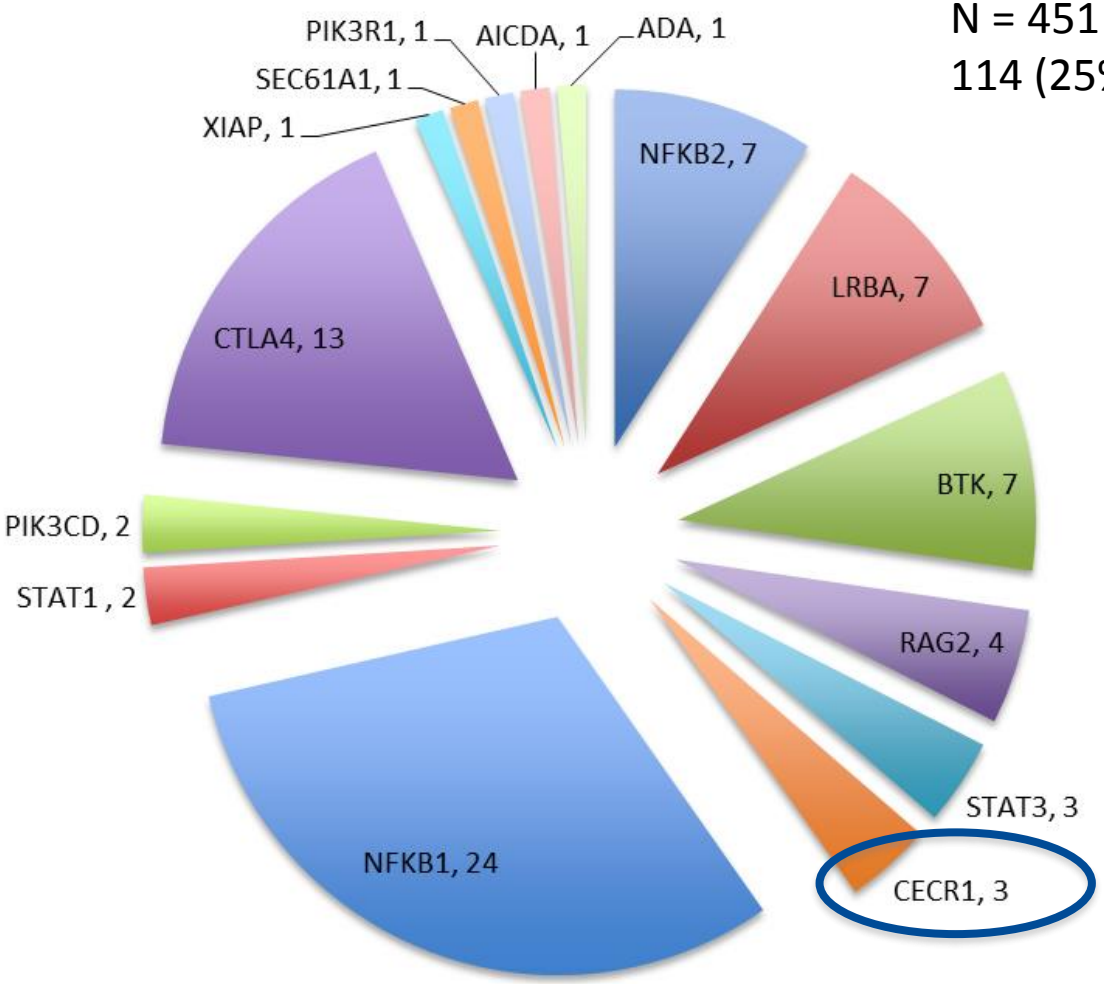


Figure 2 | **Intracellular trafficking of CTLA4.** The hallmark of cytotoxic T lymphocyte

Sansom and Walker, Nat Rev Immunol. 2011_11:852-63.

Monogenetic Causes for Hypogamma-/Agammaglobulinemia



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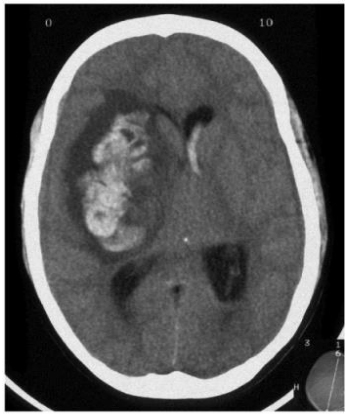
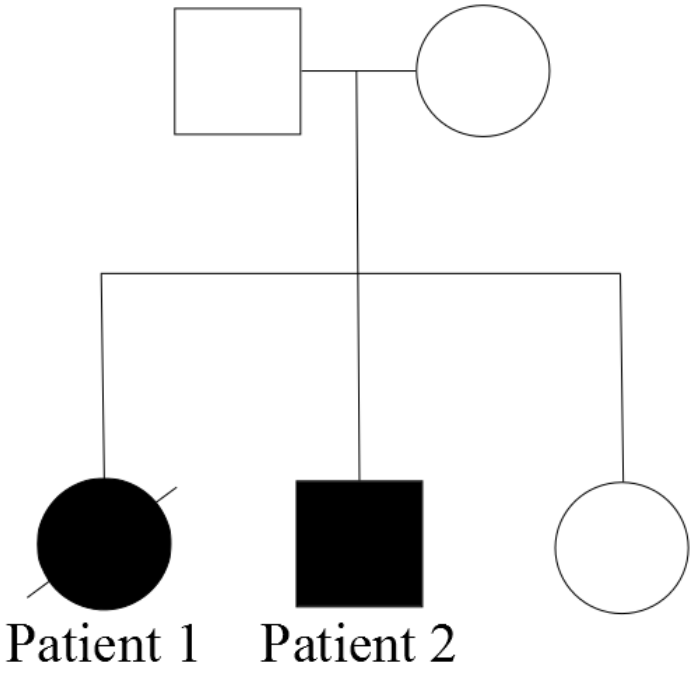
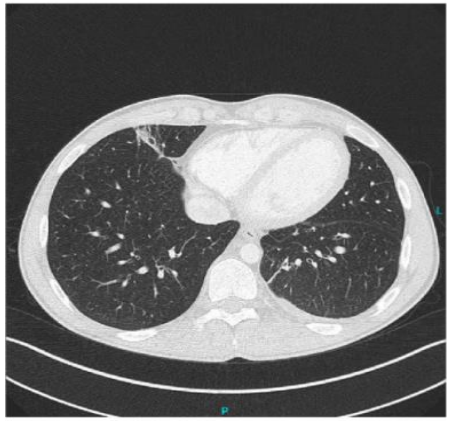
Case report 1

Monogenic cause of immunodeficiency and immune dysregulation…







Female patient SLE-like

- hypogammaglobulinemia
  - SLE-like phenotype: polyarthritis, splenomegaly, vasculitis of the skin, kidney involvement, leukopenia, microcytic hypochromic anemia
- renal failure → nephrectomy at age 13
- died of a cerebral bleeding at age 17

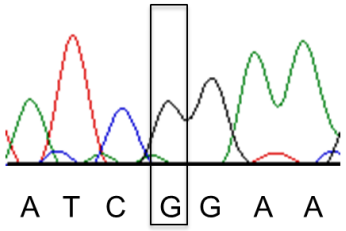
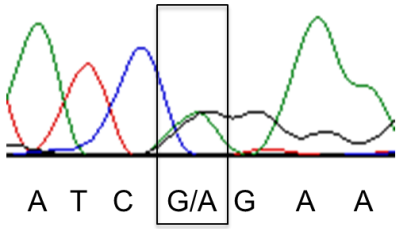
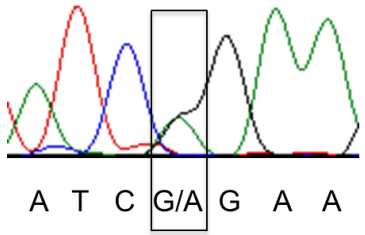
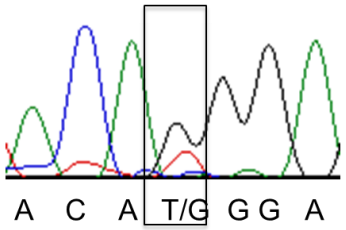
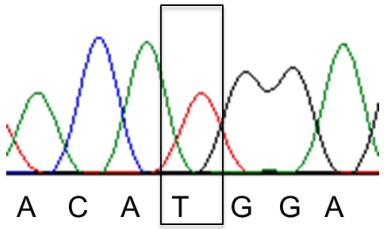
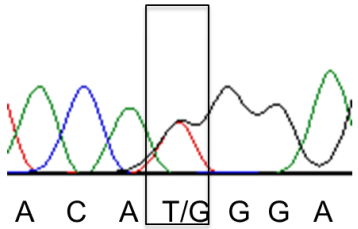
Male patient CVID

- hypogammaglobulinemia
- recurrent respiratory infections
- chronic diarrhea
- Lymphoproliferation

Johanna Schepp et al., *JoCI*, Feb. 2016

… we found a compund heterozygous mutation in *CECR1* …

Genetic Analysis

Mutation	mother	father	patient
Family A Chr22:17687997C>T R169Q			
Chr22:17684478A>C M243R			

Johanna Schepp et al., *JoCI,* Feb. 2016

CECR1 encodes for ADA2.

Identification of ADA-2 deficiency (DADA2) in 2014:

N Engl J Med. 2014 Mar 6;370(10):911-20. doi: 10.1056/NEJMoa1307361. Epub 2014 Feb 19.

Early-onset stroke and vasculopathy associated with mutations in ADA2.

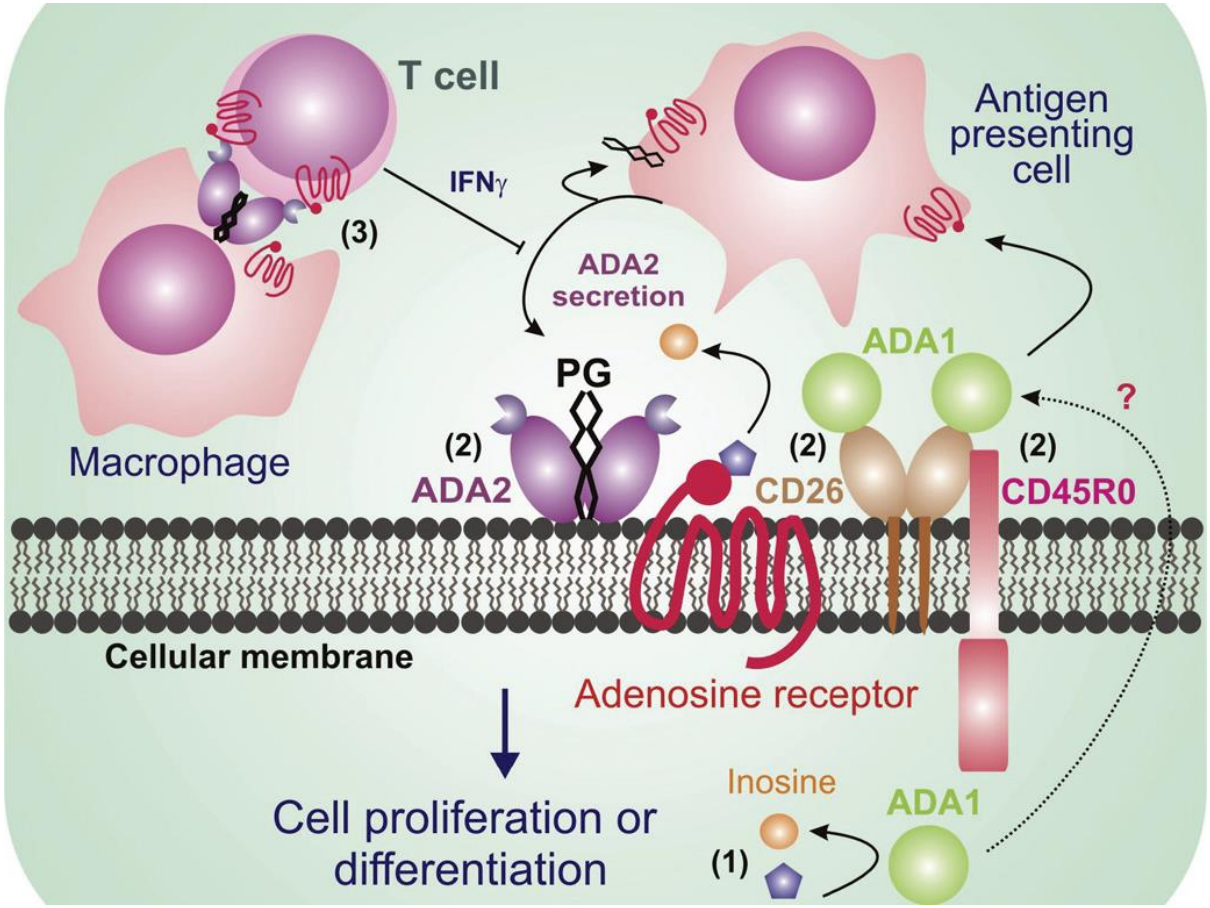
Zhou Q[1], Yang D, Ombrello AK, Zavialov AV, Toro C, Zavialov AV, Stone DL, Chae JJ, Rosenzweig SD, Bishop K, Barron KS, Kuehn HS, Hoffmann P, Negro A, Tsai WL, Cowen EW, Pei W, Milner JD, Silvin C, Heller T, Chin DT, Patronas NJ, Barber JS, Lee CC, Wood GM, Ling A, Kelly SJ, Kleiner DE, Mullikin JC, Ganson NJ, Kong HH, Hambleton S, Candotti F, Quezado MM, Calvo KR, Alao H, Barham BK, Jones A, Meschia JF, Worrall BB, Kasner SE, Rich SS, Goldbach-Mansky R, Abinun M, Chalom E, Gotte AC, Punaro M, Pascual V, Verbsky JW, Torgerson TR, Singer NG, Gershon TR, Ozen S, Karadag O, Fleisher TA, Remmers EF, Burgess SM, Moir SL, Gadina M, Sood R, Hershfield MS, Boehm M, Kastner DL, Aksentijevich I.

N Engl J Med. 2014 Mar 6;370(10):921-31. doi: 10.1056/NEJMoa1307362. Epub 2014 Feb 19.

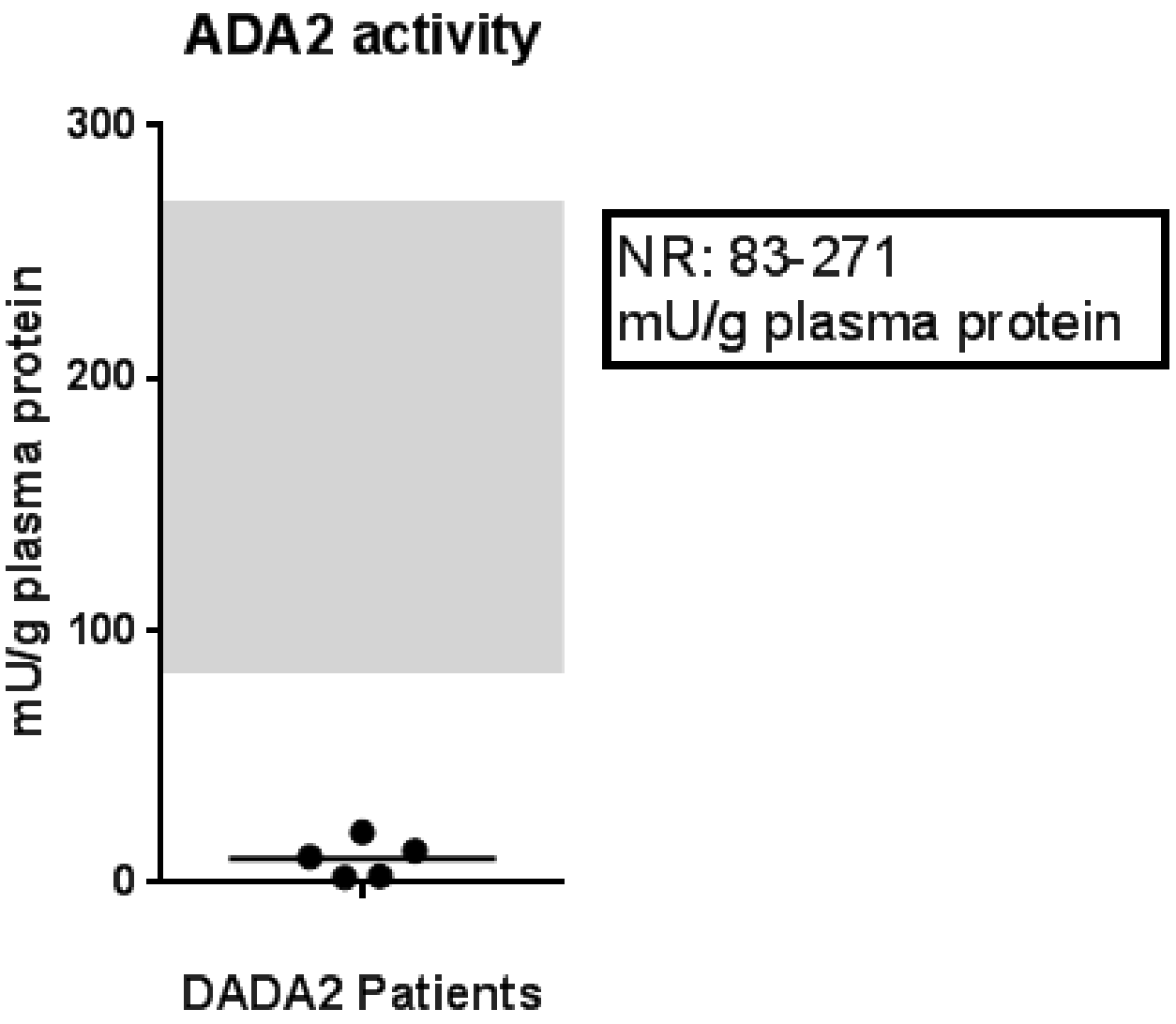
Mutant adenosine deaminase 2 in a polyarteritis nodosa vasculopathy.

Navon Elkan P[1], Pierce SB, Segel R, Walsh T, Barash J, Padeh S, Zlotogorski A, Berkun Y, Press JJ, Mukamel M, Voth I, Hashkes PJ, Harel L, Hoffer V, Ling E, Yalcinkaya F, Kasapcopur O, Lee MK, Klevit RE, Renbaum P, Weinberg-Shukron A, Sener EF, Schormair B, Zeligson S, Marek-Yagel D, Strom TM, Shohat M, Singer A, Rubinow A, Pras E, Winkelmann J, Tekin M, Anikster Y, King MC, Levy-Lahad E.

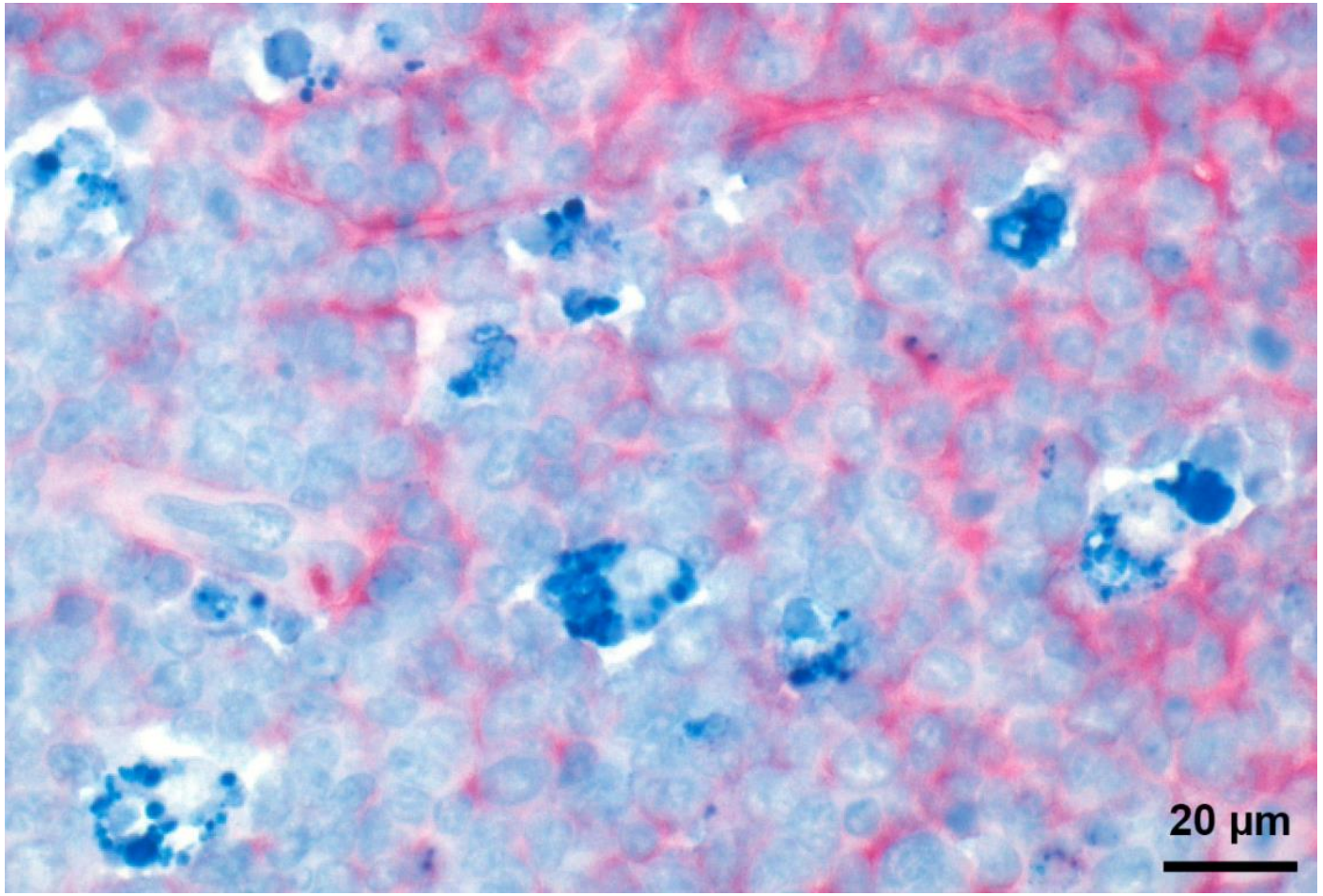
ADA2: Current State-of-Art



Zavialov A.V. et al Leukoc. Biol. 88(2):279-90


ADA2 activity
300
200
100
0
mU/g plasma protein
NR: 83-271
mU/g plasma protein
DADA2 Patients

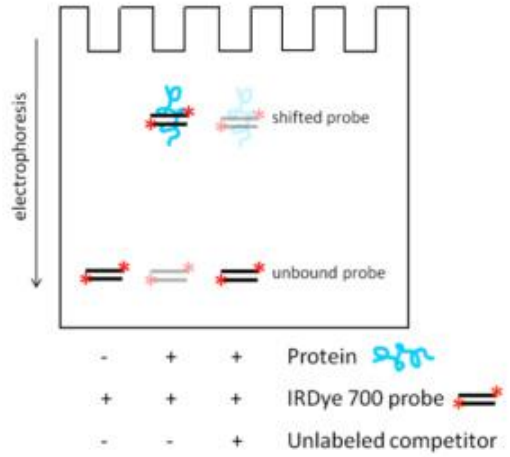
ADA2 staining in tissue macrophages resembles phagolysosomes

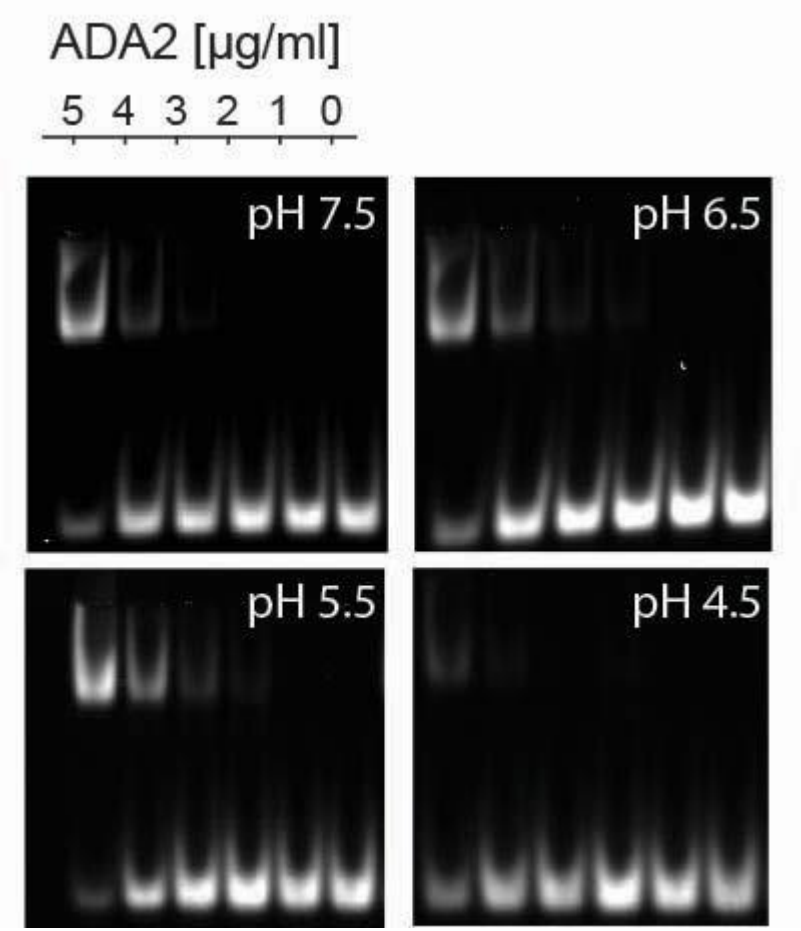


ADA2 binds viral ssRNA and degrades RNA at an acid pH

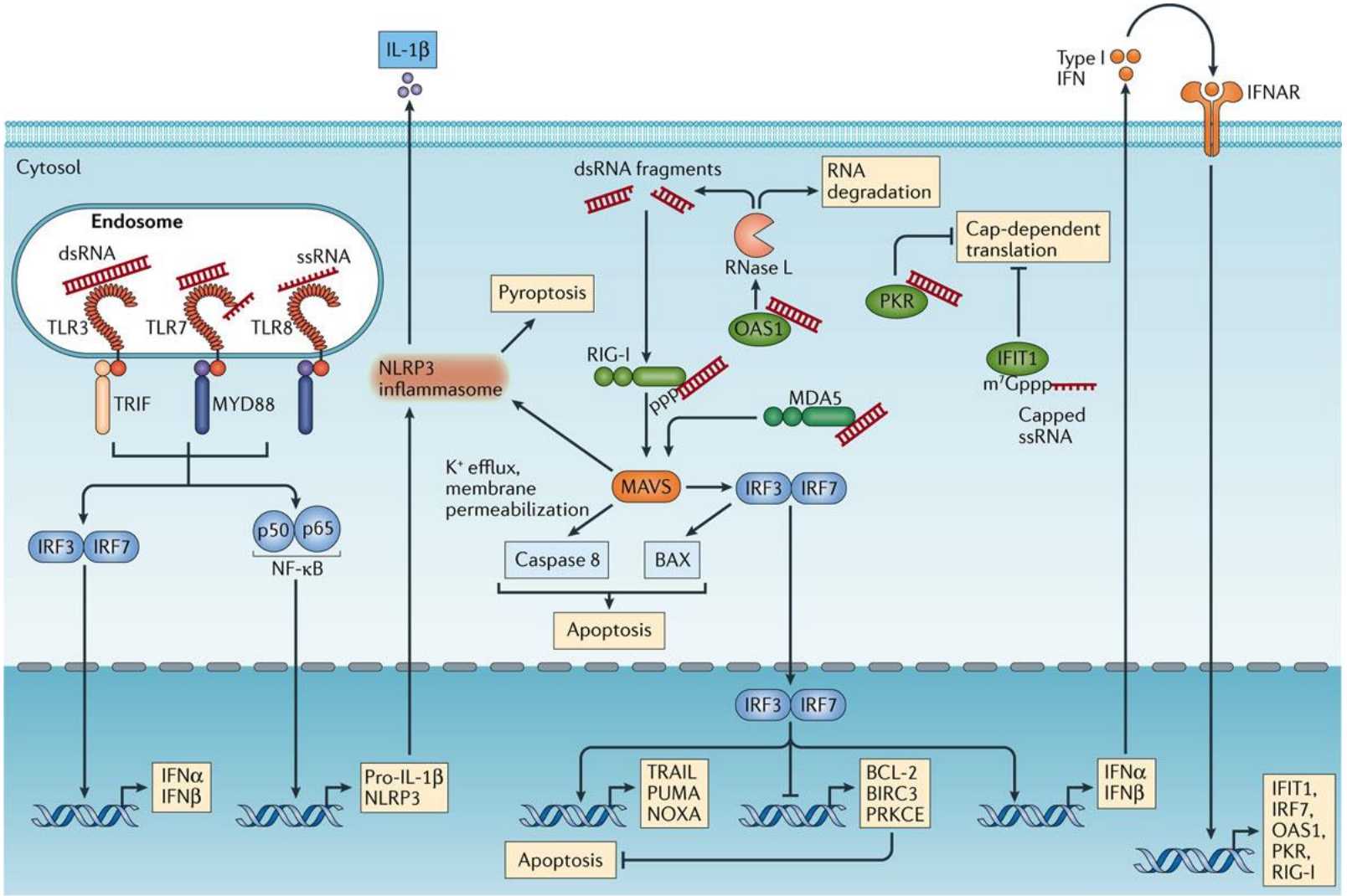
Human recombinant ADA2 incubated with IRD_Dye_ssRNA (47 pairs ssRNA coding for the segment 6 of influenza virus) and run on polyacrylamide gel

EMSA: Electrophoretic mobility shift assay



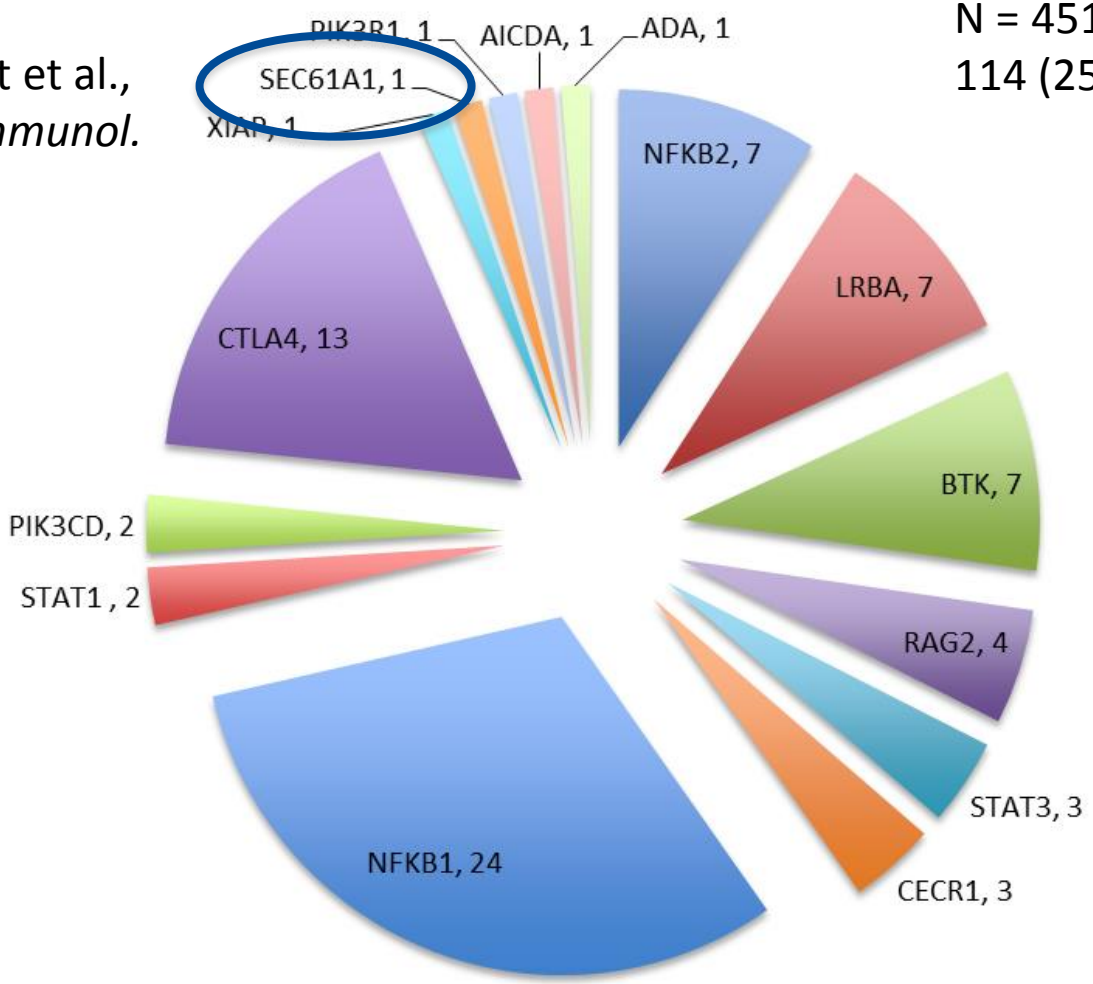


Lysosomes are operation-centers of non-self nucleic acid sensing



Monogenetic Causes for Hypogamma-/Agammaglobulinemia

Desirée Schubert et al.,
J. Allergy Clin. Immunol.
in print

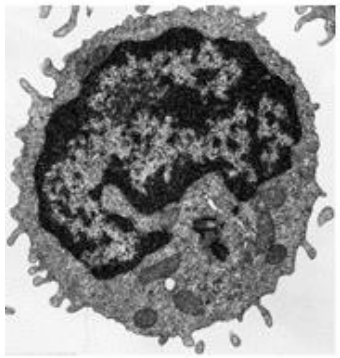


N = 451 patients
114 (25%) with clear mutation

B cells terminally differentiate into antibody-producing plasma cells

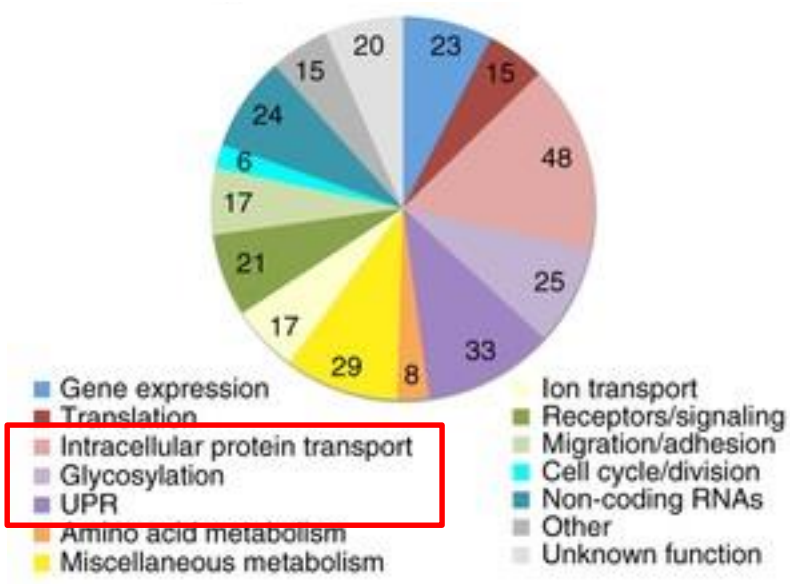
- Shi et al. Nature Immunology (2015) Transcriptional profiling of mouse B cell terminal differentiation defines a signature for antibody-secreting plasma cells.

Resting B cell



FoB
MZB
B1B

300 genes define a plasma cell signature

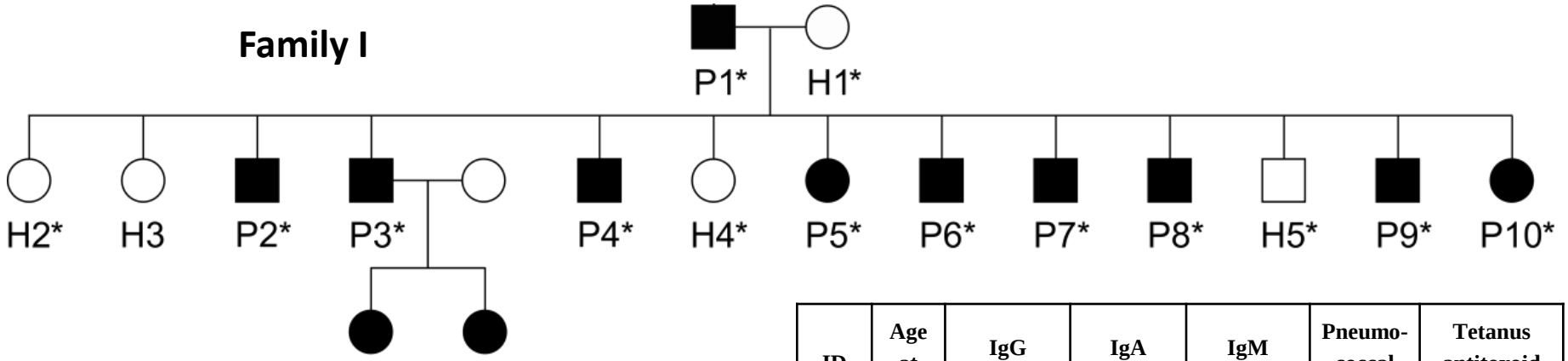


mouse, mRNA, sorted cells, ex vivo

Plasma cell

splPB
splPC
bmPC

A family with an autosomal dominant primary antibody deficiency

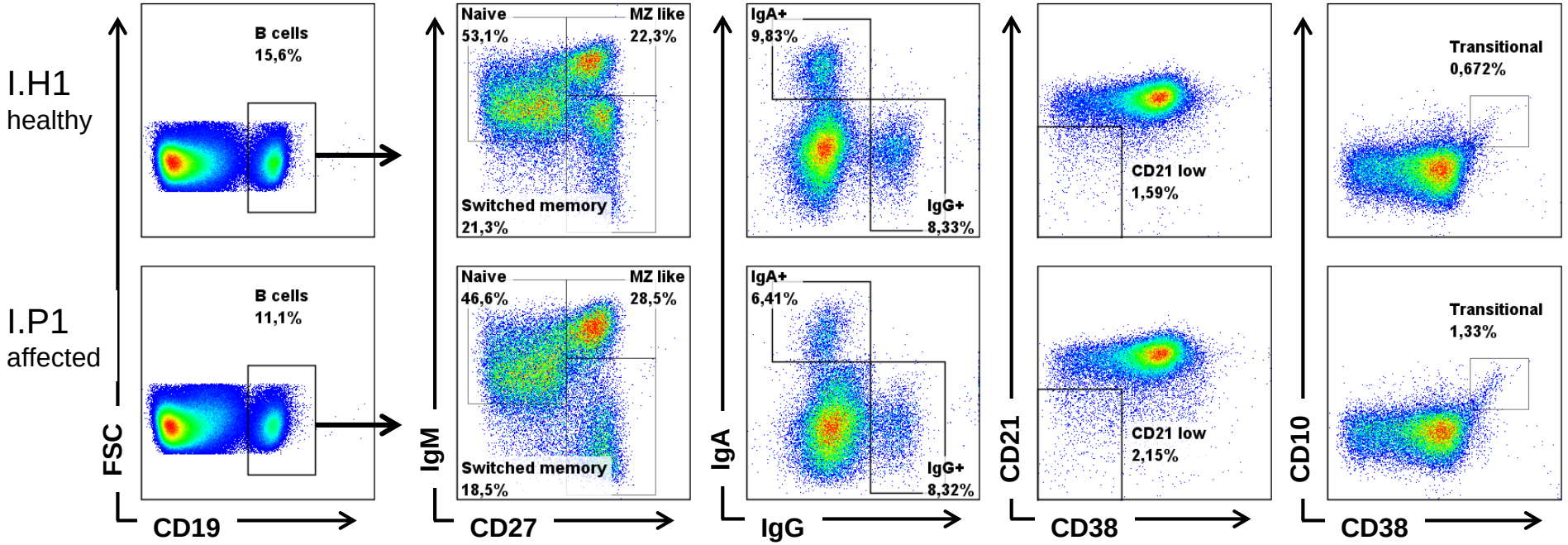


- autosomal dominant inheritance
- Early disease onset (1st year of life)
- Diagnosis: common variable immunodeficiency (CVID)
  - Antibody deficiency of IgG, IgA and IgM
  - Infections only (mainly respiratory tract incl. bronchitis, pneumonia, otitis media)
  - Reduced or absent vaccination responses to polysaccharide antigen and protein antigen (tetanus)
- Successfully treated with immunoglobulin replacement therapy and antibiotics

ID	Age at exam	IgG (mg/l)	IgA (mg/l)	IgM (mg/l)	Pneumo-coccal response	Tetanus antitoxoid IgG (IU/ml)
I.P1	45	504 (700-1600)	39 (70-400)	33 (40-230)	No response	1.34 Protective
I.P3	14	430 (549-1584)	23 (61-348)	9 (23-259)	No response	1.16 Protective
I.P4	16	693 (549-1584)	58 (61-348)	14 (23-259)	No response	n.a.
I.P6	11	634 (698-1560)	22 (53-204)	22 (31-179)	No response	n.a.
I.P7	9	517 (572-1474)	37 (34-305)	27 (31-208)	No response	n.a.
I.P8	6	421 (504-1464)	31 (27-195)	12 (24-210)	No response	0.16 Intermediate
I.P9	2	345 (453-916)	9 (20-100)	16 (19-146)	No response	0.34 Intermediate
I.P5	13	468 (759-1549)	27 (58-358)	26 (35-239)	No response	0.29 Intermediate
I.P10	9	159 (572-1474)	<7 (34-305)	<10 (31-208)	No response	<0.1 Undetected

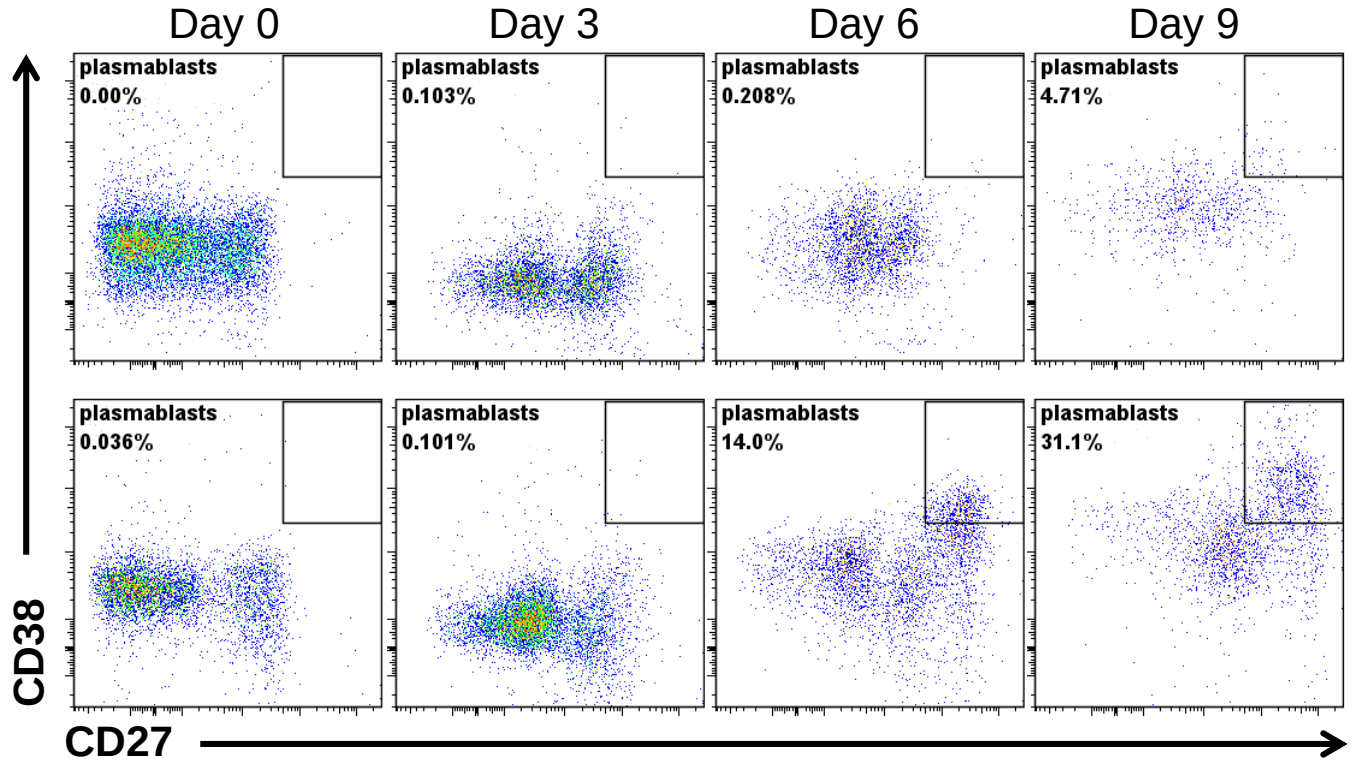
Patients have normal subpopulations of peripheral B cells

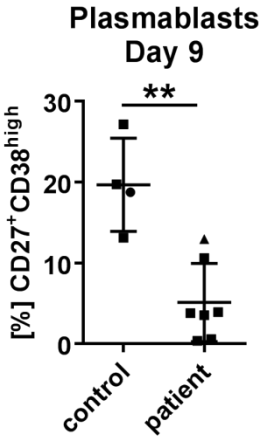
- B cells from peripheral bood were analyzed by flow cytometry

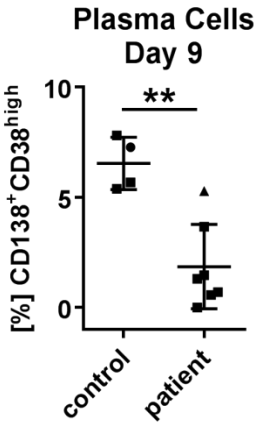


Plasmablast differentiation is reduced upon *in vitro* stimulation

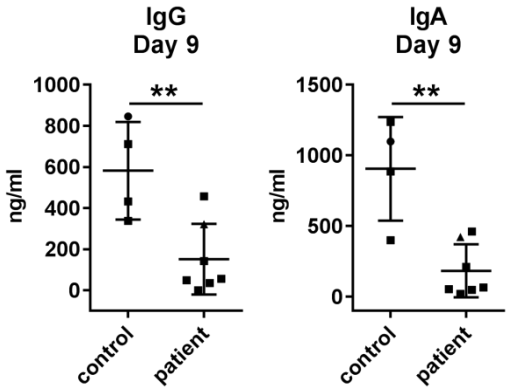
- Stimulation of isolated primary B cells with αIgM, Baff and CpG for nine days







Ig secretion:



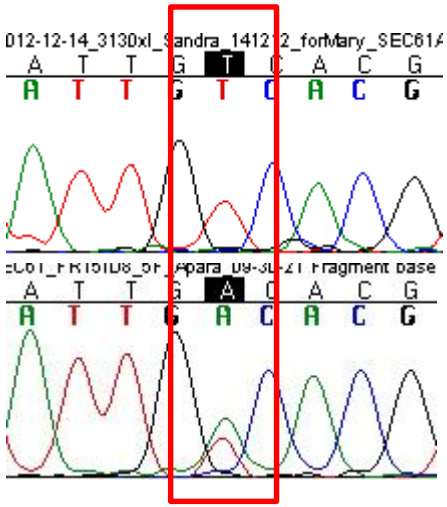
Identification of a novel heterozygous missense mutation in *SEC61A1*

- Whole exome sequencing was performed in 4 affected and 2 healthy subjects

g. 5102T>A
c.254T>A
p.V85D

control

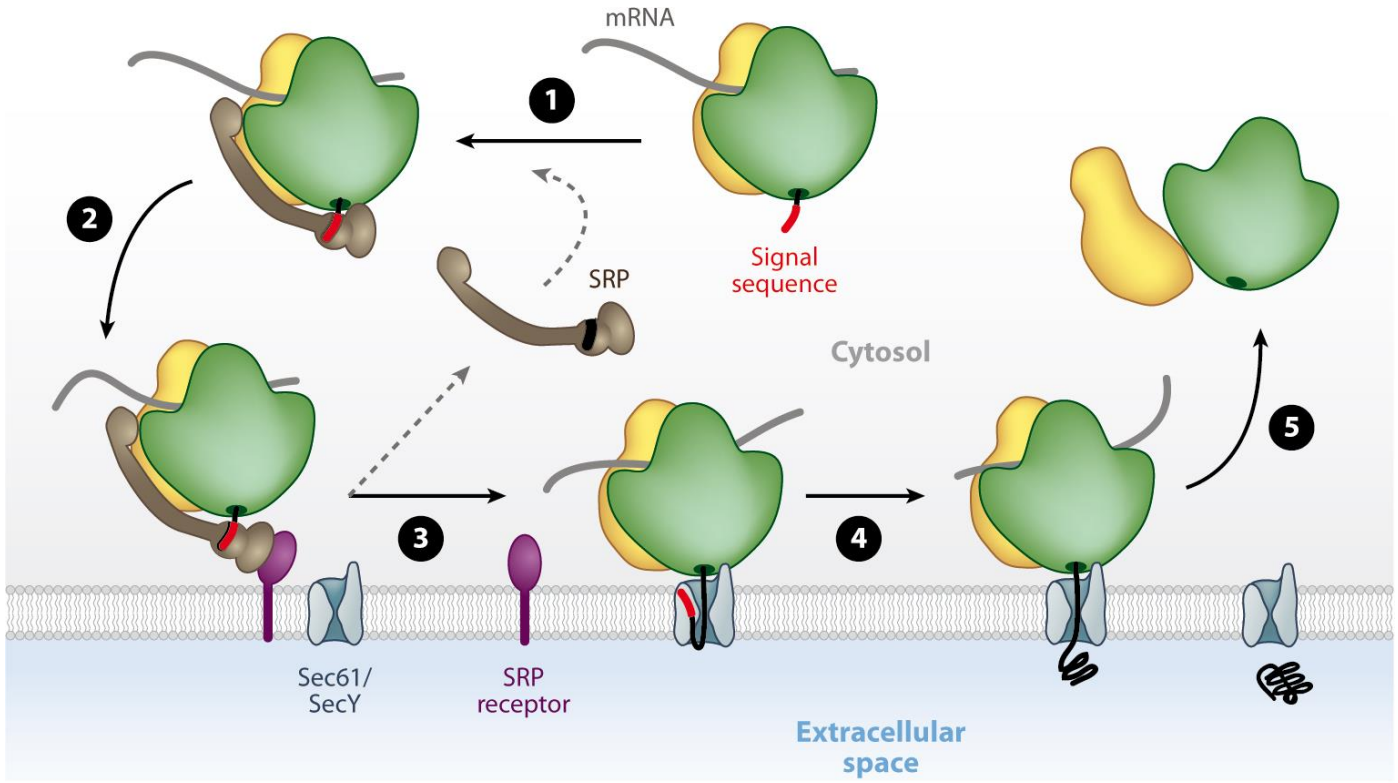
patient



SIFT score: 0.000
Provean score: -6.26
MutationTaster score: 4.15

Protein Acc.	Organism	SEC61A1 p.V85
NP_037468.1	Homo sapiens	NRGTL MELGISPIVTSGLIMQLLAGAK
NP_058602.1	Mus musculus	NRGTL MELGISPIVTSGLIMQLLAGAK
NP_954865.1	Rattus norvegicus	NRGTL MELGISPIVTSGLIMQLLAGAK
NP_001003315.1	Canis lupus familaris	NRGTL MELGISPIVTSGLIMQLLAGAK
NP_001035594.1	Bos taurus	NRGTL MELGISPIVTSGLIMQLLAGAK
NP_001080244.1	Xenopus laevis	NRGTL MELGISPIVTSGLIMQLLAGAK
NP_595226.1	S. pombe	NRGTL MELGISPIVTSSMLVQLLVGSQ
XP_958835.1	Neurospora crassa	NRGTL MELGITPI ISSGMVFQLLAGTH
XP_710932.1	Candida albicans	NRGTL MELGISPIVSSGMLFQLLQGTK
NP_013482.1	S. cerevisiae	NRGTL LELGVSPIITSSMIFQFLQGTQ
NP_986143.1	Ashbya gossypii	NRGTLMELGVSPIITSSMIFQFLQGTQ

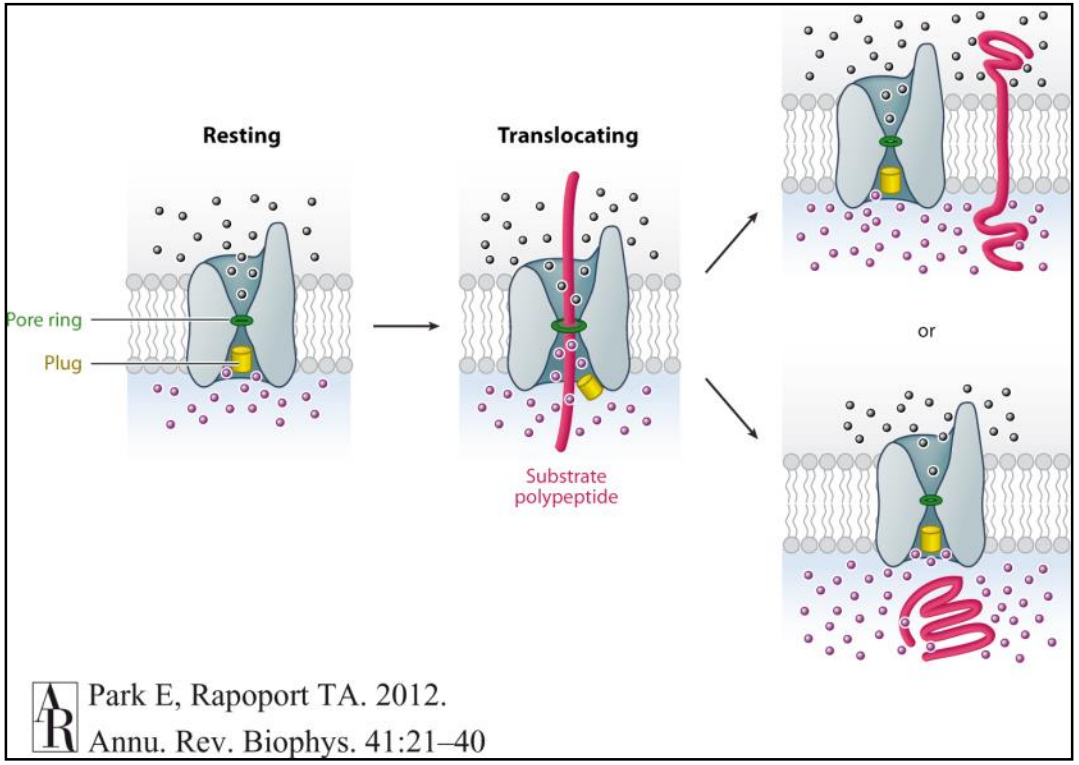
The Sec61 complex - an ubiquitously expressed and essential protein transporter in the ER membrane



Park E, Rapoport TA. 2012.
Annu. Rev. Biophys. 41:21–40

The p.V85D mutation disrupts the highly conserved pore ring of SEC61A1

- The Sec61 comlex passively acts as a calcium leakage channel

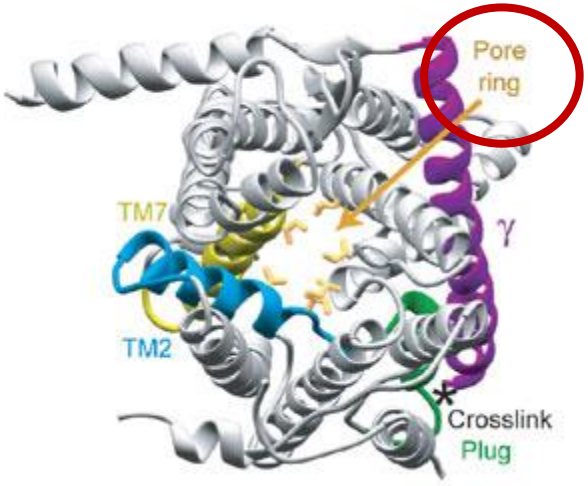


SEC61A1-V85D

Valine

↓

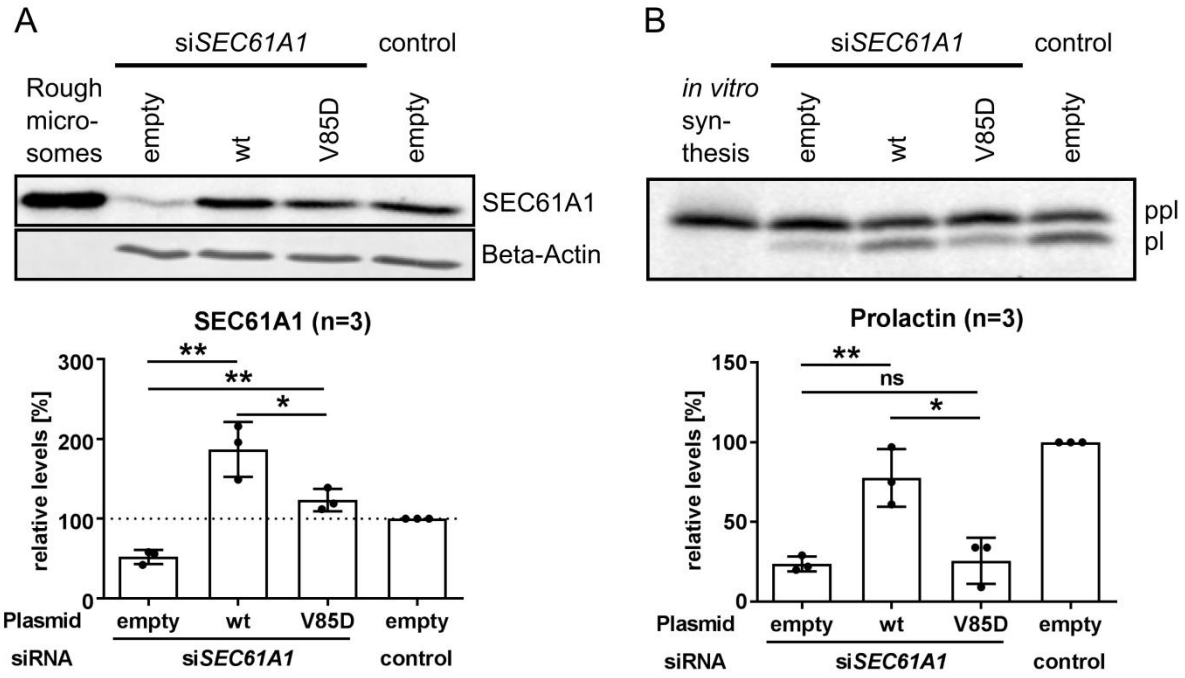
Aspartic acid



Van den Berg B et al. Nature 2004 Jan 1;427(6969):36-44

Impaired cotranslational protein transport by SEC61A1-V85D

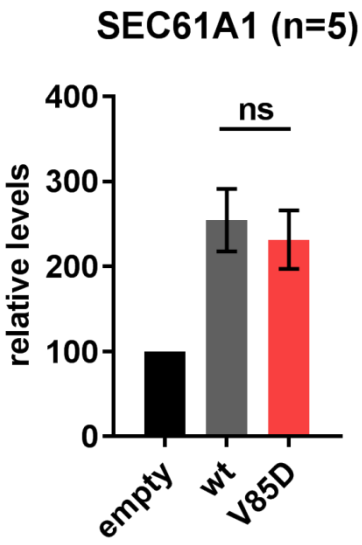
•Cotranslational transport of pre-prolactin was assessed in semi-permeabilized HeLa cells expressing *SEC61A1-V85D*

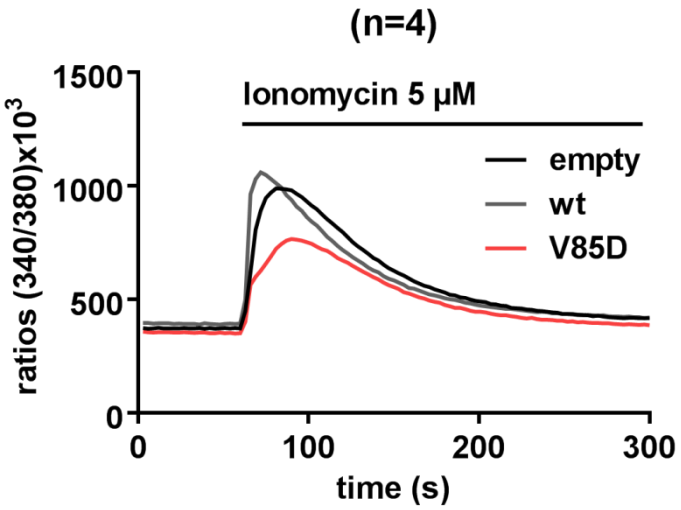


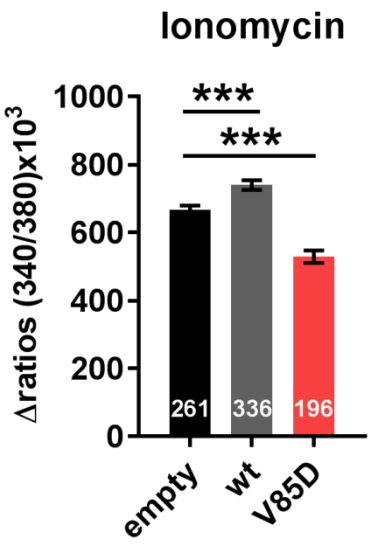
Experiments conducted by Sarah Haßdenteufel, AG Zimmermann, Saarland University Homburg

Dissipation of the ER/cytosol calcium gradient

- Live cell calcium imaging in HeLa cells overexpressing *SEC61A1-V85D*



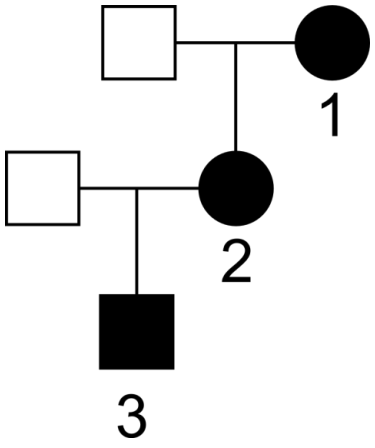




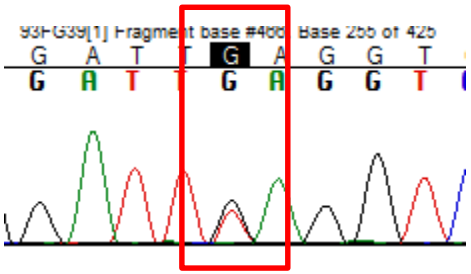
Experiments conducted by Marie-Christine Klein, AG Zimmermann, Saarland University Homburg

A heterozygous nonsense mutation in *SEC61A1* in a young patient with antibody deficiency

Family II



g.15946G>T
c.1325G>T
p.E381*



- Index patient (II.P3) from Hamburg, Germany
  - Hypogammaglobulinemia since birth
  - Since then (7 years) treated with IvIG
- Mother (II.P2) is HIV+, no further information
- Grandmother (II.P1) suffered from recurrent otitis in childhood and had an abdominal abscess as a young woman. Nowadays, she is completely fine and has normal Ig levels (IgG 8,61 g/L, IgA 2,34 g/L, IgM 0,64 g/L, IgE 208 kU/L)

Acknowledgements

A big thank you especially to all patients, physicians and healthy controls

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Faculty include:
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Professor Bodo Grimbacher: Consultant Immunologist, CCI, Freiburg, Germany
Professor Sophie Hambleton: Consultant in Paediatric Immunology, GNCH and Newcastle University
Dr Andy Gennery: Consultant in Paediatric Immunology, GNCH and Newcastle University
Professor Adrian Thrasher: Consultant Paediatric Immunologist, GOSH and ICH
Dr Siobhan Burns: Consultant Immunologist, Royal Free Hospital and UCL
Dr Matt Buckland: Consultant Immunologist, Royal Free Hospital and GOSH

Organising and Scientific committee:
Dr Siobhan Burns (Chair)
Professor Sophie Hambleton

Fall 1

Vor einer geplanten Fensterungsoperation werden bei einer 22-jährigen Frau mit rekurrierender Sinusitis folgende Blutwerte erhoben:

- **Normales Blutbild**
- **Normaler Urin und Elektrolyte, normale Gerinnung,**
- **normale Nieren- und Leberwerte**
  - **IgG 6.5g/ L (>7g/ L)**
  - **IgA <0.1g/ L (>0.7g/ L)**
  - **IgM 0.6g/ L (>0.4g/ L)**

Fragen:

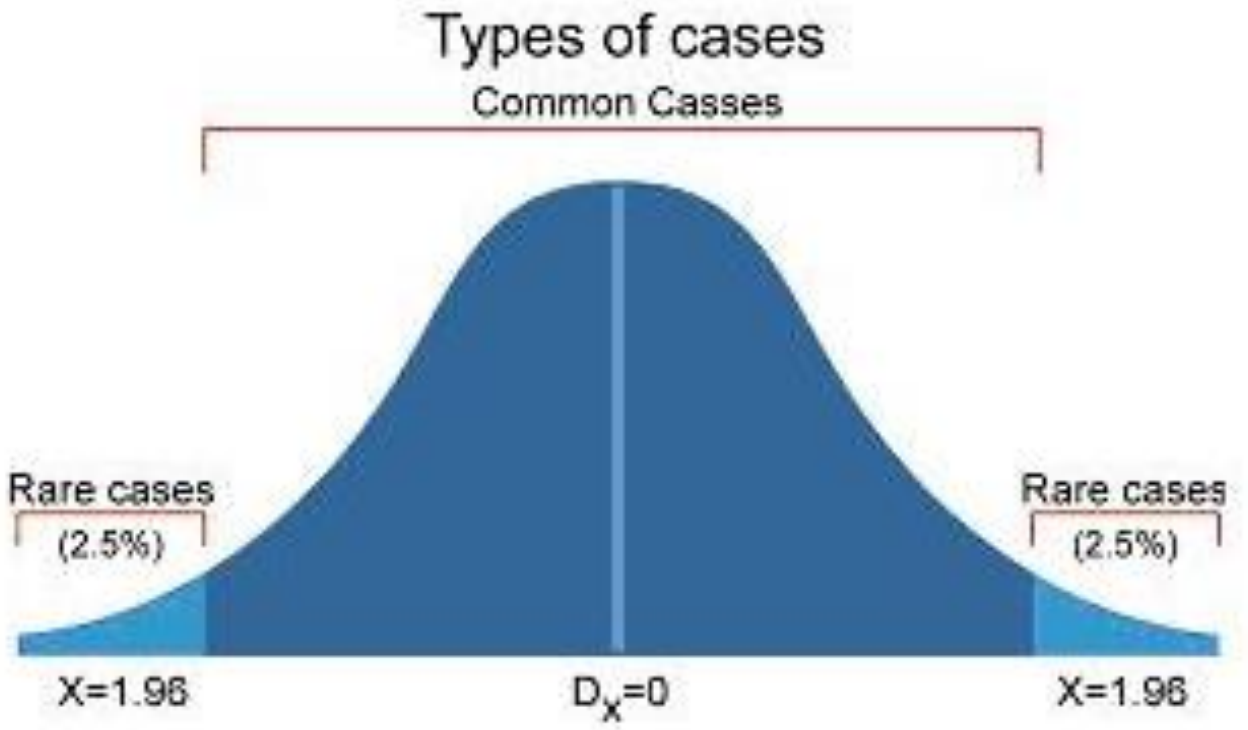
a) Was ist die Diagnose?

b) Muss ich Laborwerte wiederholen?

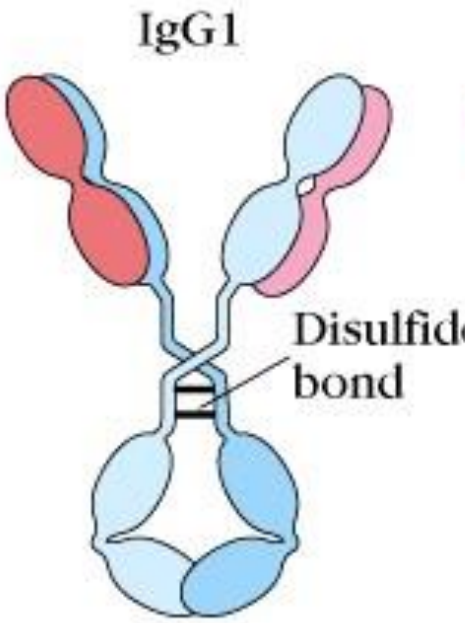
c) Kann operiert werden?

Fall 1

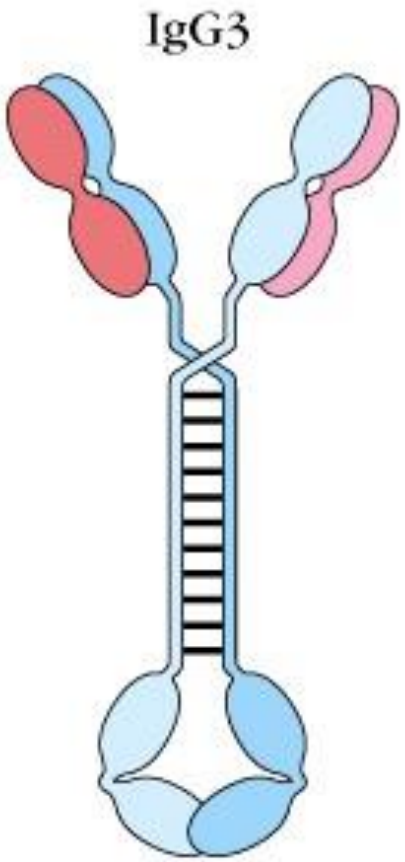
- IgG von 6,5 g/L könnte eine Variante der Norm sein.



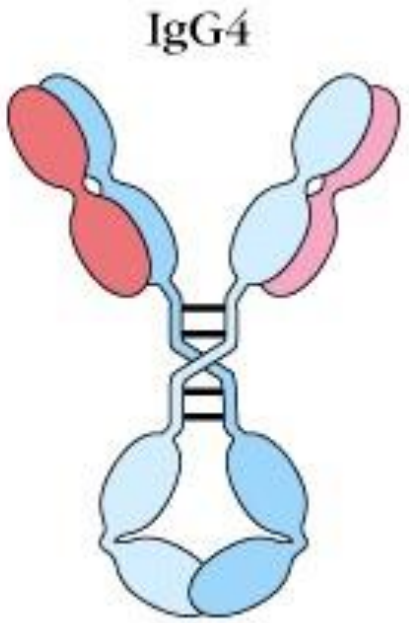
Kann es sich um einen IgG Subklassendefekt handeln?



75% des Gesamt-IgG



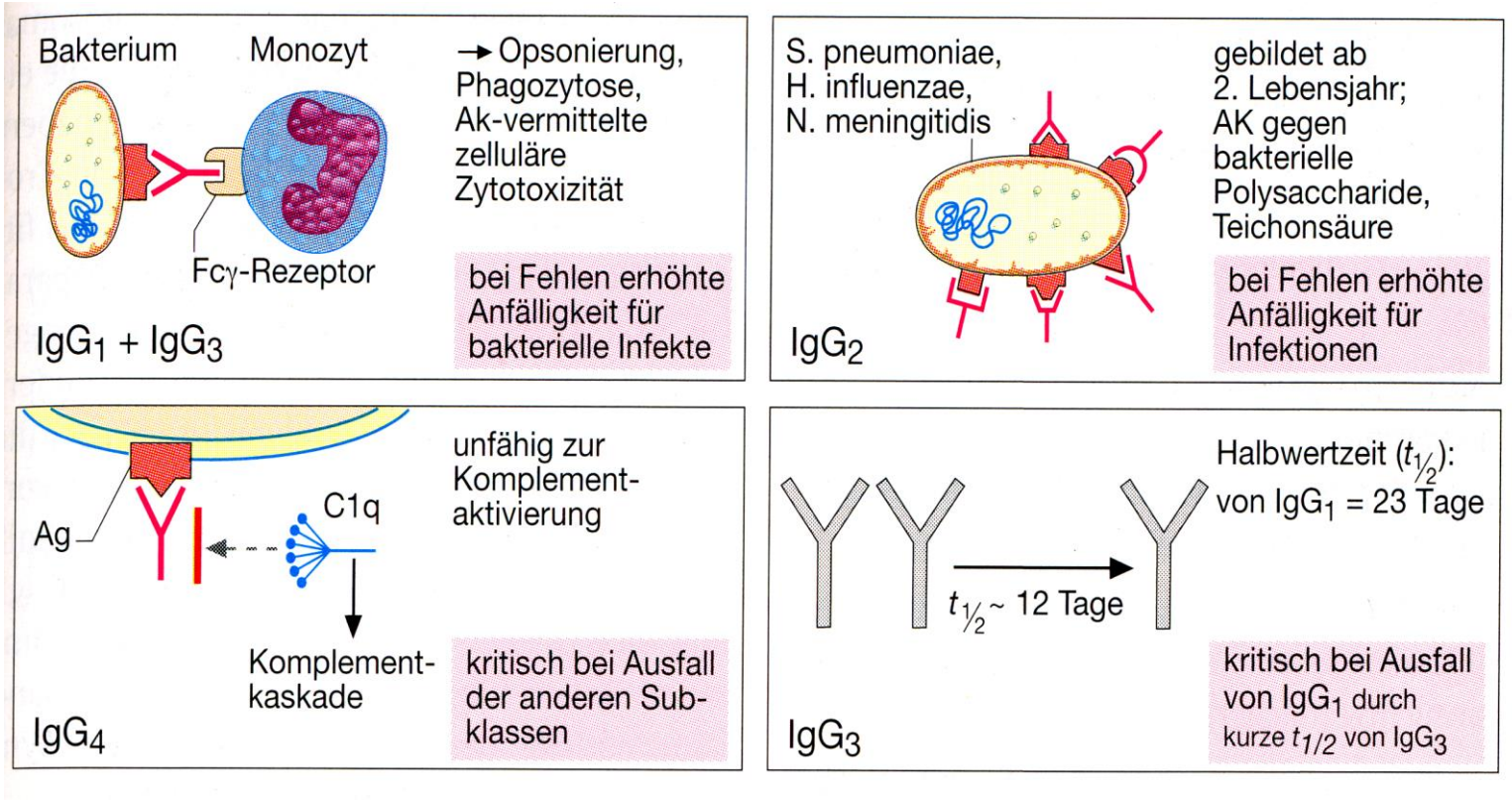
<5%, hat nur kurze Halbwertszeit



Fehlt bei 8% der Bevölkerung

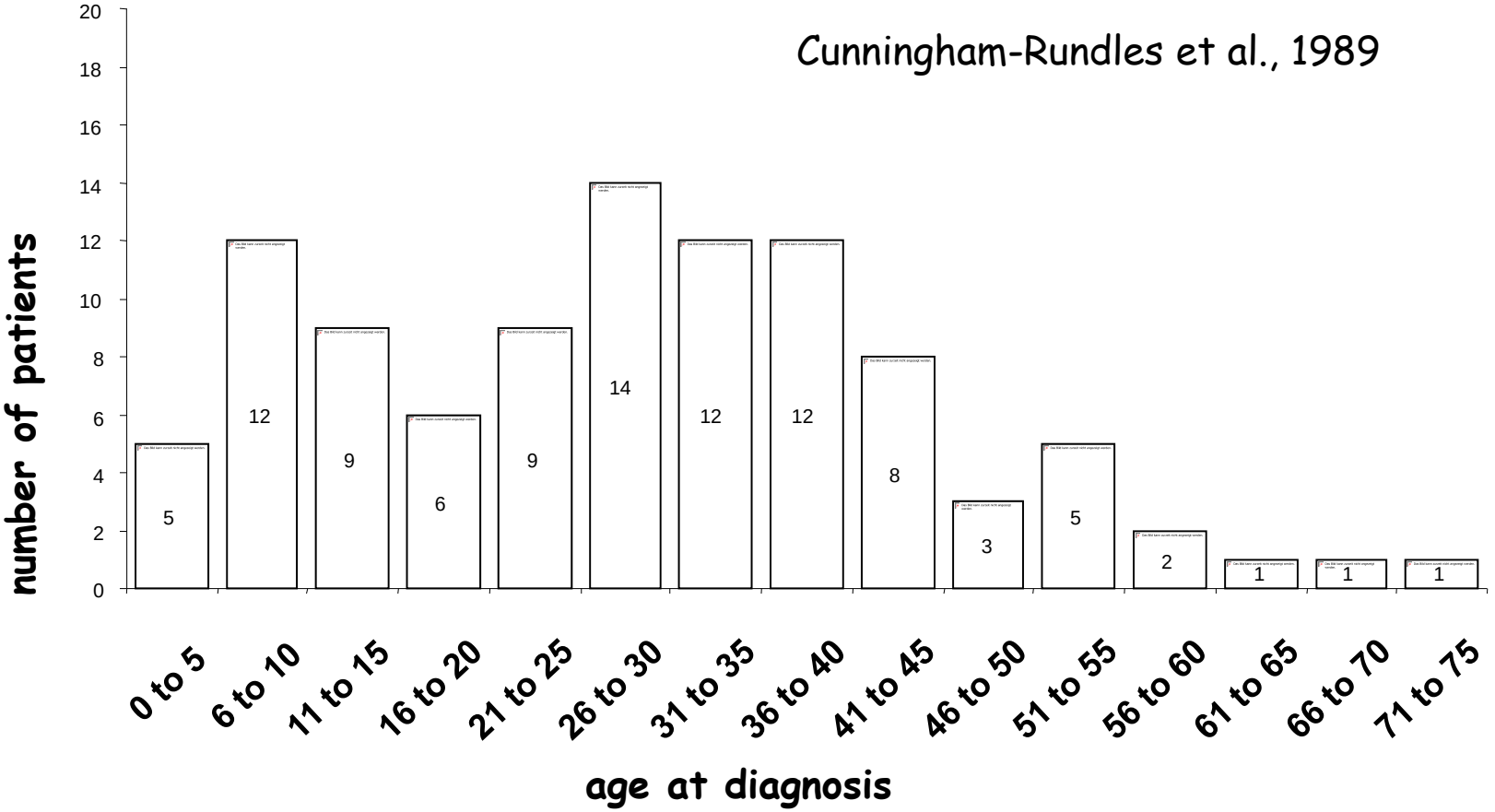
Fall 1

- Die Patientin könnte einen IgG2 Subklasendefekt haben



Case

Die Patientin könnte einen CVID entwickeln



Fall 1

Vorgeschlagenes Procedere:

1. Wdh. der Immunoglobulin Bestimmung
2. Bestimmung der IgG Subklassen
3. Messung spezifischer IgG Antikörper Titer
   e.g. Tetanus, Pneumococcus, Hib
4. Impfen, wenn niedrig
5. Kontrolle der Impfantwort
6. Follow-up der Patientin (mit IgG und
   Infektionshäufigkeit) in jährlichem Abstand

Case Presentation

Chief presenting features

- 22 year-old woman
- Rash over both shins and easy bruising, otherwise well
- Examination reveals petechial rash, no lymphadenopathy, and no skin or joint abnormality

Previous medical history

- Normal childhood growth and development
- Fully vaccinated (UK vaccination schedule)
- Three episodes of otitis media in the last 2 months; slow response to prescribed oral antibiotics
- Family history is unremarkable.

Initial laboratory findings

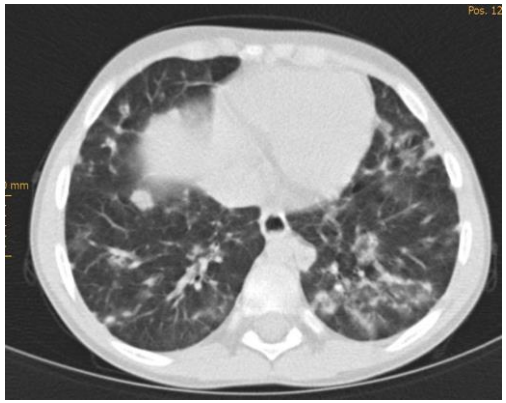
Full blood count		
	Mean level	**Normal range[a]**
Platelets (x 10^9/L)	**22**	150 – 400
White blood cell count (x10^9/L)	10.3	3.5 – 11.0
Neutrophils (x10^9/L)	8.0	2.0 – 8.0
Lymphocytes (x10^9/L)	1.01	1.0 – 3.5
Haemoglobin (g/L)	120	115 – 160
Red blood cell count (x10^{12}/L)	4.3	3.9 – 5.4

[a]Age and gender dependent

Possible diagnoses

- **A** Idiopathic thrombocytopenic purpura (ITP)
- **B** Thrombocytopenia secondary to haematological malignancy
- **C** ITP associated with underlying systemic disease
- **D** Human immunodeficiency virus (HIV)
- **E** Drug-induced thrombocytopenia

Results of additional diagnostic testing



- **Blood film and bone marrow examination:**

  Normal platelet precursors but peripheral thrombocytopenia.

  No evidence of malignant infiltration of the bone marrow

  Granulocyte and erythrocyte development was also normal.

- **CT examination:** abnormal

  No evidence of lymphadenopathy or splenomegaly

  But nodularity within the lung parenchyma with small areas of non-specific ground glass change were noted.

- **Serum and urine electrophoresis:**

  Urine electrophoresis was normal with no evidence of Bence jones protein or nephrosis. However, **serum electrophoresis** showed reduction in the gamma region with normal alpha and beta regions.

Measurement of blood immunoglobulins			
	IgG	**IgA**	**IgM**
Mean levels (g/L)	**1.2**	**<0.1**	**<0.1**
Normal range	7.0–16.0	0.7–4.0	0.4–2.3

- **Specific antibody titres to tetanus and diphtheria were absent.**
- Results of HIV and ANA tests were normal.

Refined likely diagnoses

A **Idiopathic thrombocytopenia** alone would not explain the hypogammaglobulinaemia.

B **Thrombocytopenia secondary to haematological malignancy**
There is no evidence, either clinically or on blood and bone marrow exam, for an underlying malignancy.

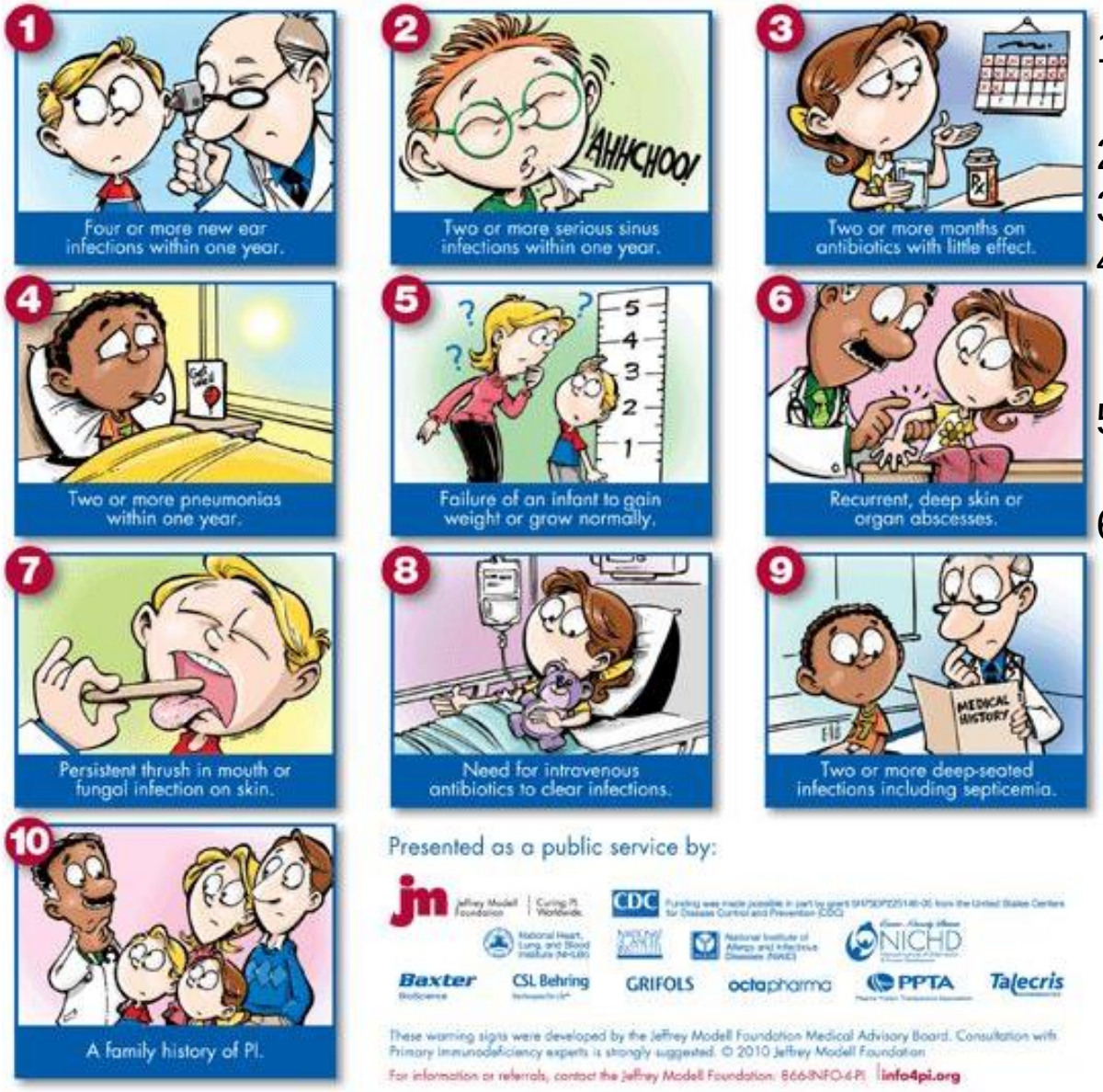
C **ITP associated with underlying common variable immunodeficiency (CVID)**
The most likely diagnosis, given the profoundly low antibody levels, is CVID with associated ITP.

D **HIV** was excluded with negative PCR.

E **Drug-induced thrombocytopenia** was excluded by the drug history.

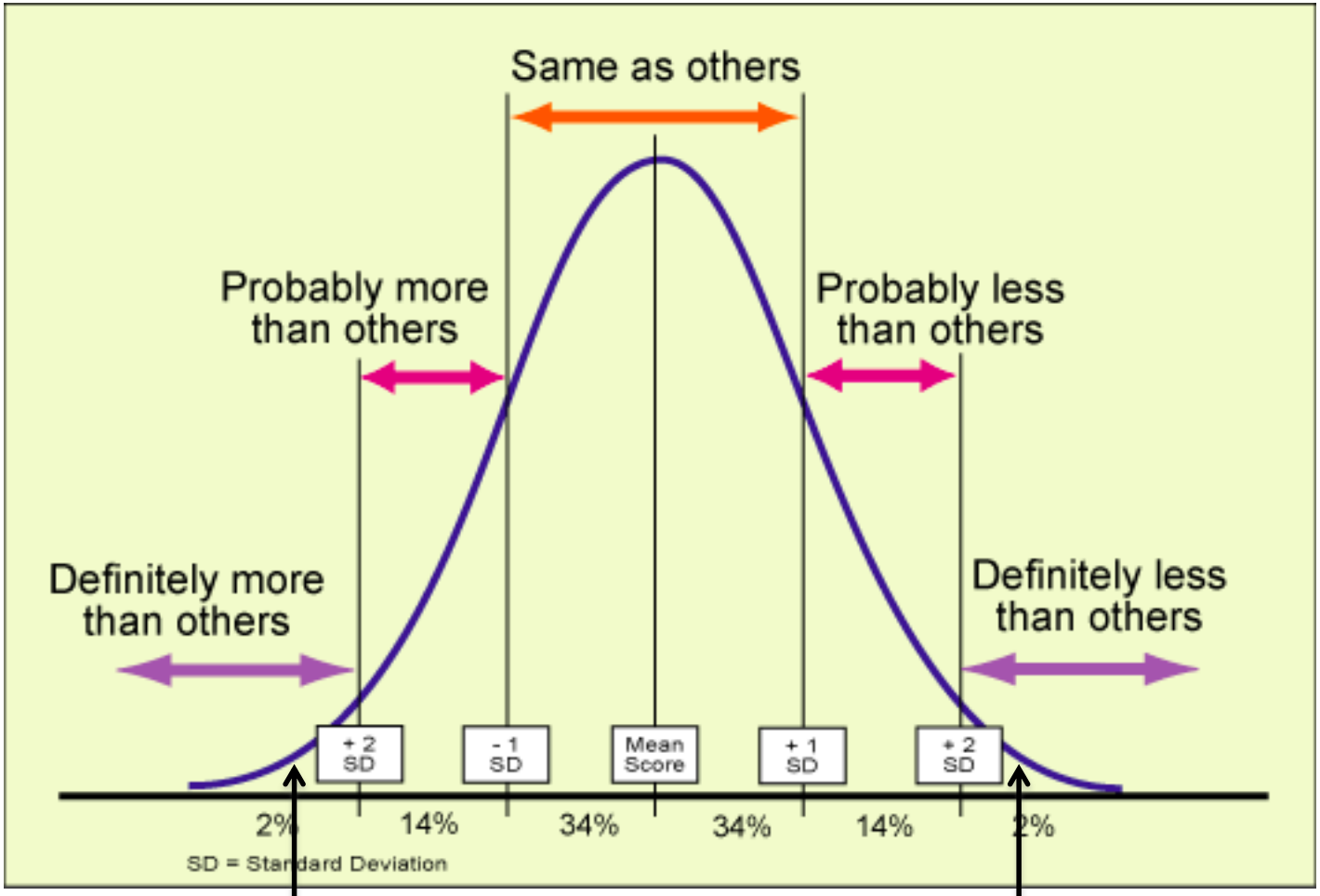
F **Sarcoidosis** normally presents with hilar lymphadenopathy and normal or raised immunoglobulins, although, on rare occasions levels can be reduced. Non-specific nodular change within the lung fields shows a classical peribronchial distribution.

Die 10 Warnzeichen für Immundefekte



1. > 3 Infekte mit Antibiotika pro Jahr
2. B-Symptomatik
3. Rekurrierende Pneumonie
4. Rekurrierende Weichteil-infektionen (Abszesse der inneren Organe)
5. Besondere Erreger (atypisch/opportunist.)
6. Positive Familienanamnese

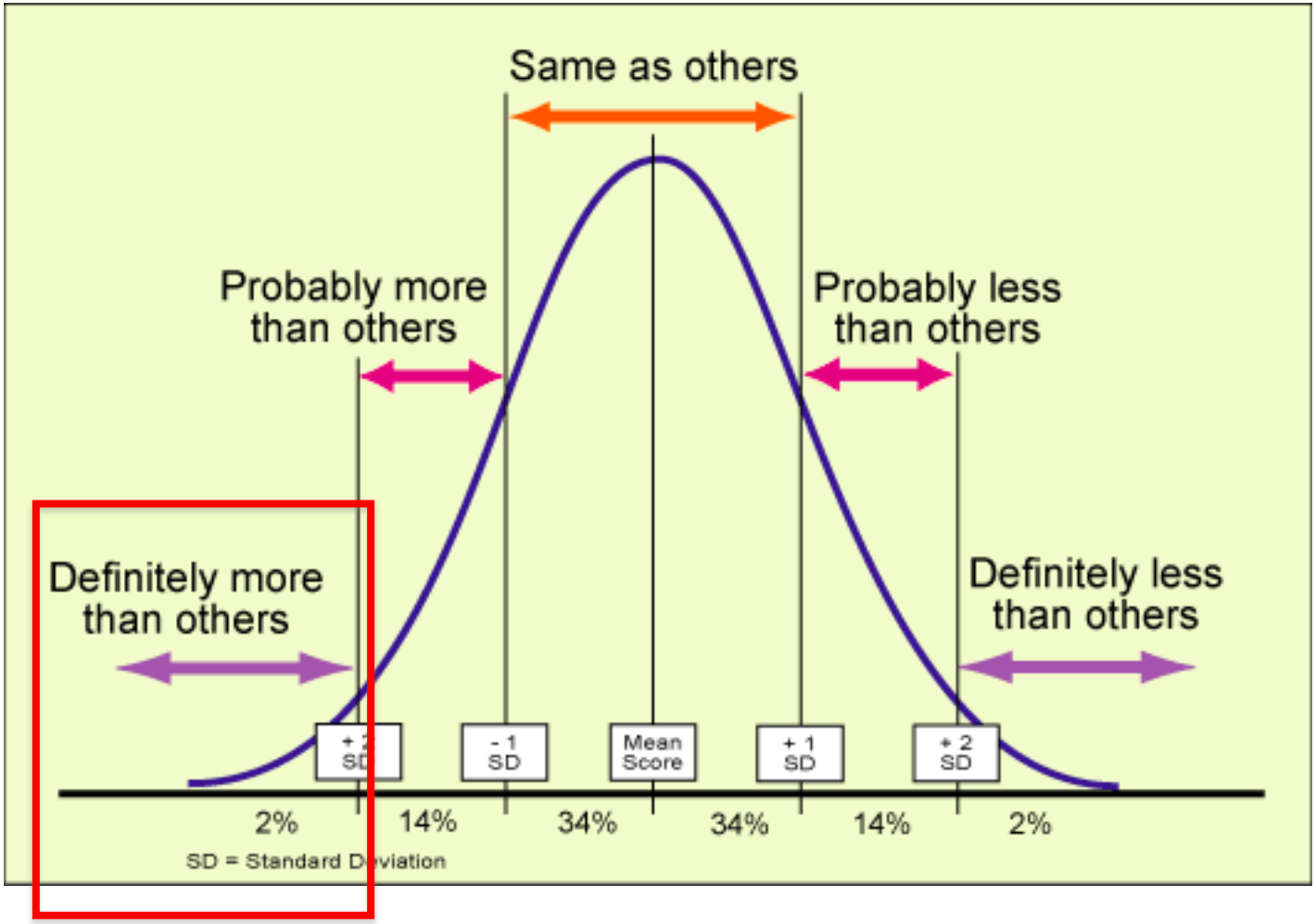
Die Fitness deines Immunsystems



SCID patient

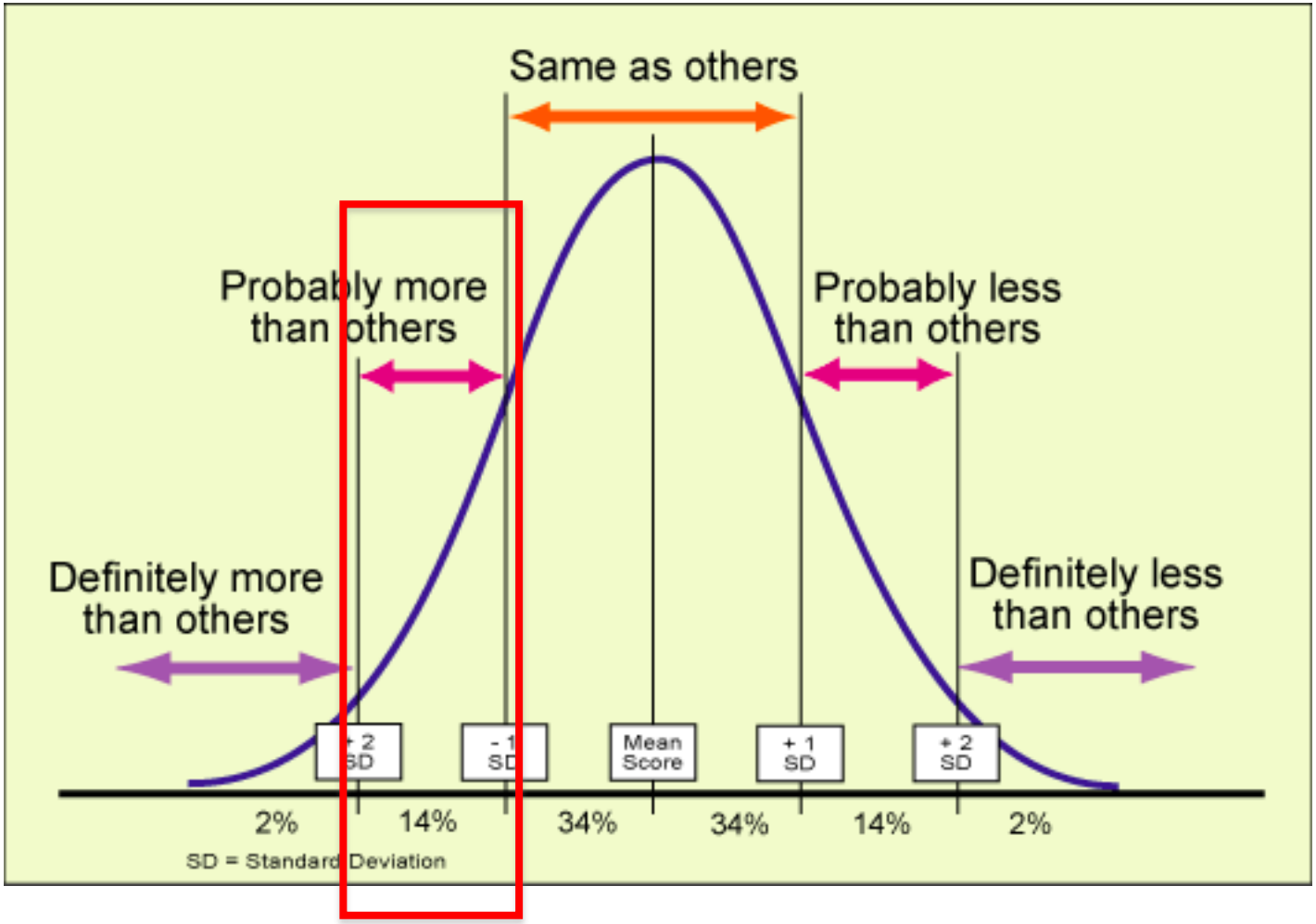
90yr-old, never ill

Die Fitness deines Immunsystems



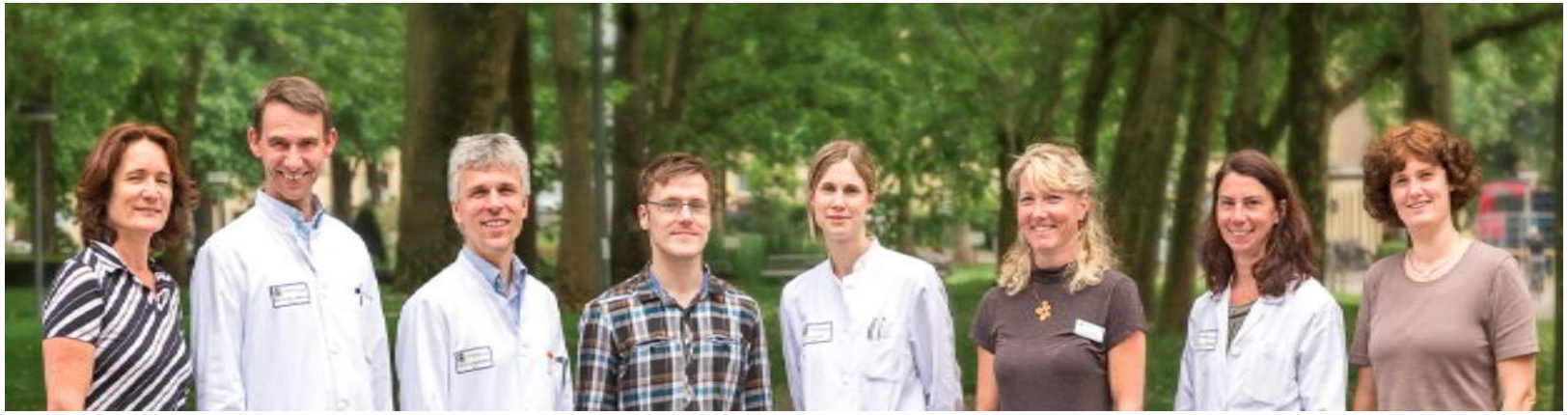
Diagnostizierbar durch die aktuellen Tests

Die Fitness deines Immunsystems



Nicht eindeutig diagnostizierbar durch die aktuellen Tests

Center for Chronic Immunodeficiency
CCI, Freiburg, Germany



bodo.grimbacher@uniklinik-freiburg.de

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