

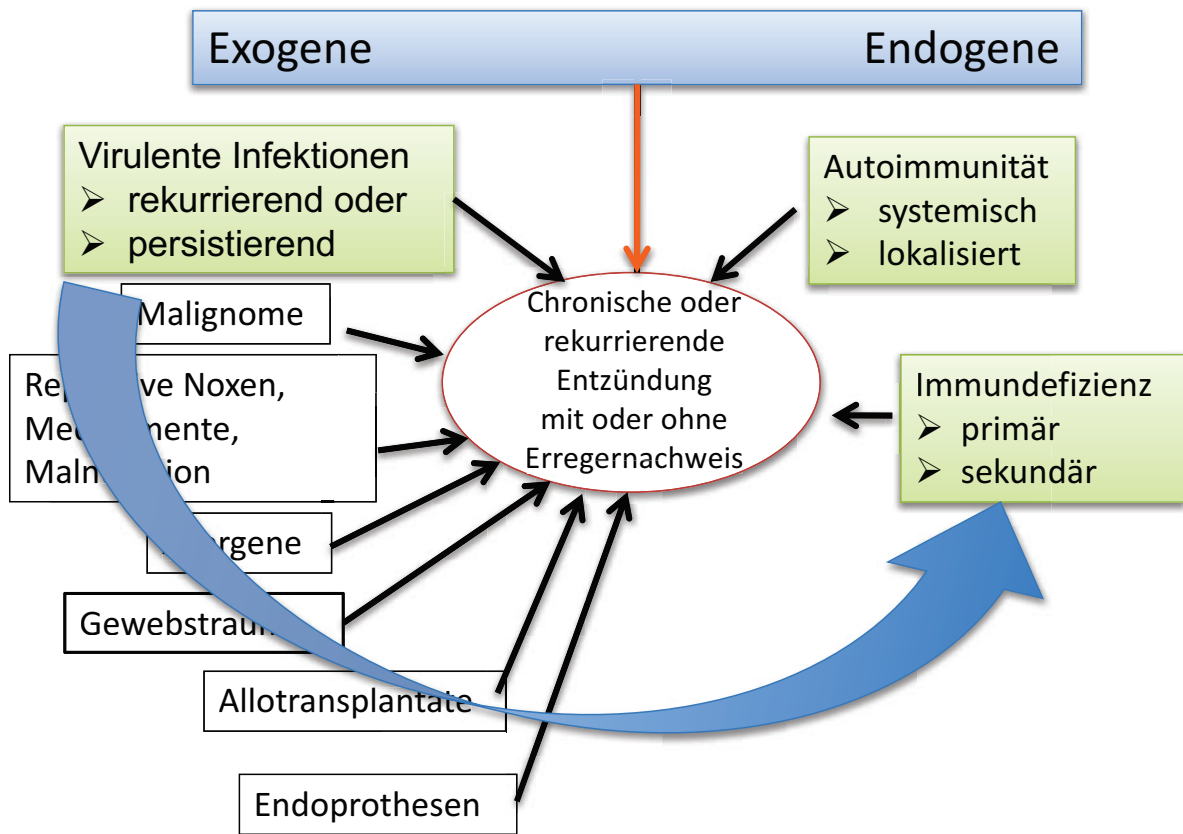
# Normale Infektanfälligkeit und Immundefizienz beim Erwachsenen

Hans-Hartmut Peter  
DSAI Seminar 4.11.17  
Mainz

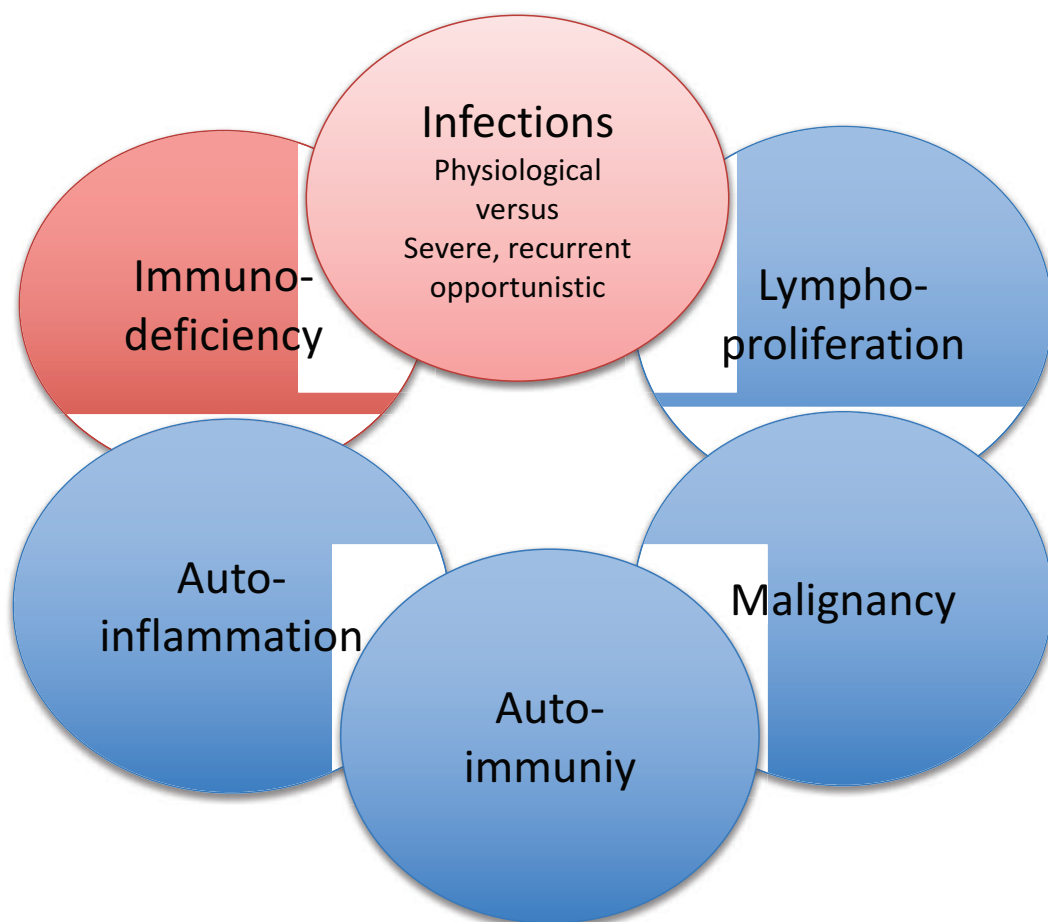




# Ursachen für ein dereguliertes Immunsystem



# Phänotypen eines deregulierten Immunsystems



## Unterschiede zwischen physiologischer und pathologischer Infektanfälligkeit

(V. Wahn, S. Ehl, T. Niehues . Allergologie, 26: 456-459, 2003)

Eigenschaften der Infektionen	Normale/Physiologische Infektanfälligkeit	Pathologische Infektanfälligkeit
Häufigkeit	< 8 Minor-Infektionen/Jahr bis zum Kleinkindesalter, danach seltener	>8 Infektionen/Jahr bis zum Kleinkindesalter und darüber hinaus
Schweregrad	Leicht, Minor-Infektionen	z.T. schwer, Major-Infektionen*
Verlauf	akut	Chronisch, rezidivierend
Residuen	nein	ja
Rezidiv mit demselben Erreger	nein	ja
Opportunistische Infektionen (Pneumocystis J., Mykobakterien, Candida, CMV , Toxoplasmose u.a)	nein	ja

\*Pneumonie, Sepsis, Meningitis, Zellulitis, Osteomyelitis, septische Arthritis, tiefe viscerale Abszesse (nicht cervikale Lymphknoten)



# Epidemiologische Querschnittsstudie zur Häufigkeit von Atemwegsinfekten (AWIS) in der Allgemeinbevölkerung (18-70Jahre) (Nieters et al 2015)

## ➤ AWIS Fragebogen

- **Häufigkeit/Dauer von RTI (letzte 2 J.)**
- **Schwerwiegende Infekte (jemals)**
- **Antibiotika Verschreibung (letzte 2 J.)**
- **Krankenhaus wg Infekten (jemals)**
- **Selbsteinschätzung bzgl. Infektneigung und Behinderung im Alltag**
- OP an Immunorganen
- Chronische Erkrankungen
- Allergien
- Medikamente
- Derzeitiges u. früheres Rauchen
- Kontakt zu Kindern
- Body mass index
- Demographische Daten

- Informations-Brief
- Einverständnis

The image displays three pages of the 'Atemwegsinfektanfälligkeitstudie (AWIS)' questionnaire. The first page (top) includes a header with the study name and a date field. It contains several numbered questions (1-10) regarding general health, hospitalizations, and antibiotic use. The second page (middle) continues with questions (11-14) about chronic conditions, allergies, and current/past smoking. The third page (bottom) includes questions (15-18) about self-perceived infection susceptibility and daily limitations, followed by a section for 'Einverständniserklärung' (informed consent) and a signature line.

# Airwayinfection Susceptibility study (AWIS)

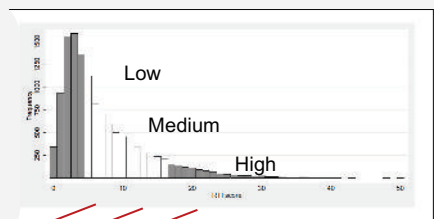
## Phase 1

- Population-based cross-sectional study
- Screening questionnaire to identify infection risk groups



Respiratory tract infection (RTI) score to identify groups of

- High risk
- Medium risk
- Low risk



## Phase 2

2012  
2013  
2014  
2015  
2016  
2017

Repeated Infection diaries

AWIS PLUS (n=1.631)

## Phase 3

AWIS Cohort  
N=1002

### Questionnaire Infection diaries

- Saliva

### Visit of CCI study center

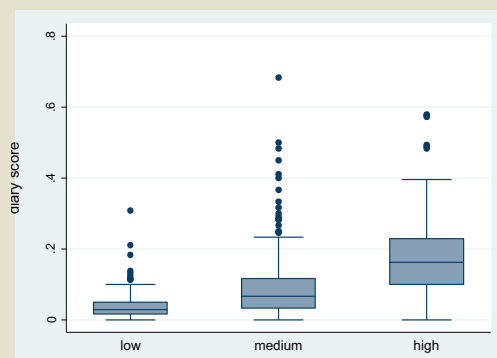
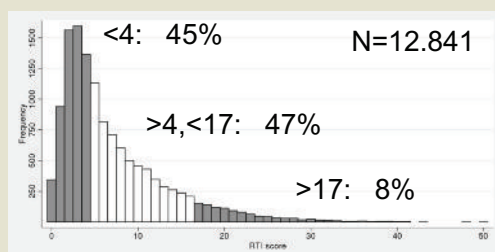
- Blood, urine
- Nasal and mouth swabs
- Interview, questionnaire
- Physical examination
- Infection diaries
- Spirometry

### Lab analyses

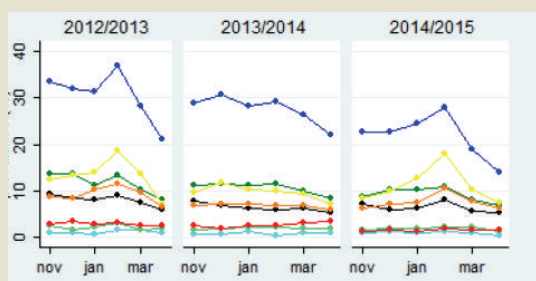
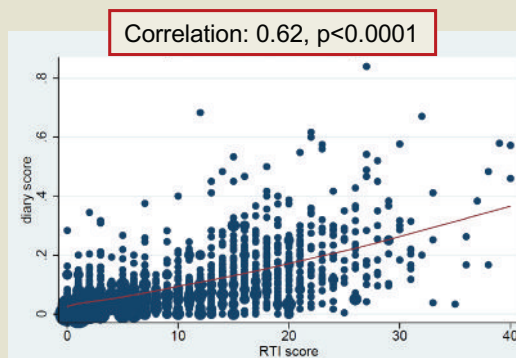
- Vitamin D, other biomarkers
- Differential blood count
- Total IgGs, IgA, IgM, IgE
- Immunophenotypes
- Pneumococcal IgG

# Atemwegsinfektanfälligkeit in der Normalbevölkerung (18-70J)

- Baseline RTI score ist ein guter Prädiktor für den prospektive Tagebuch Score



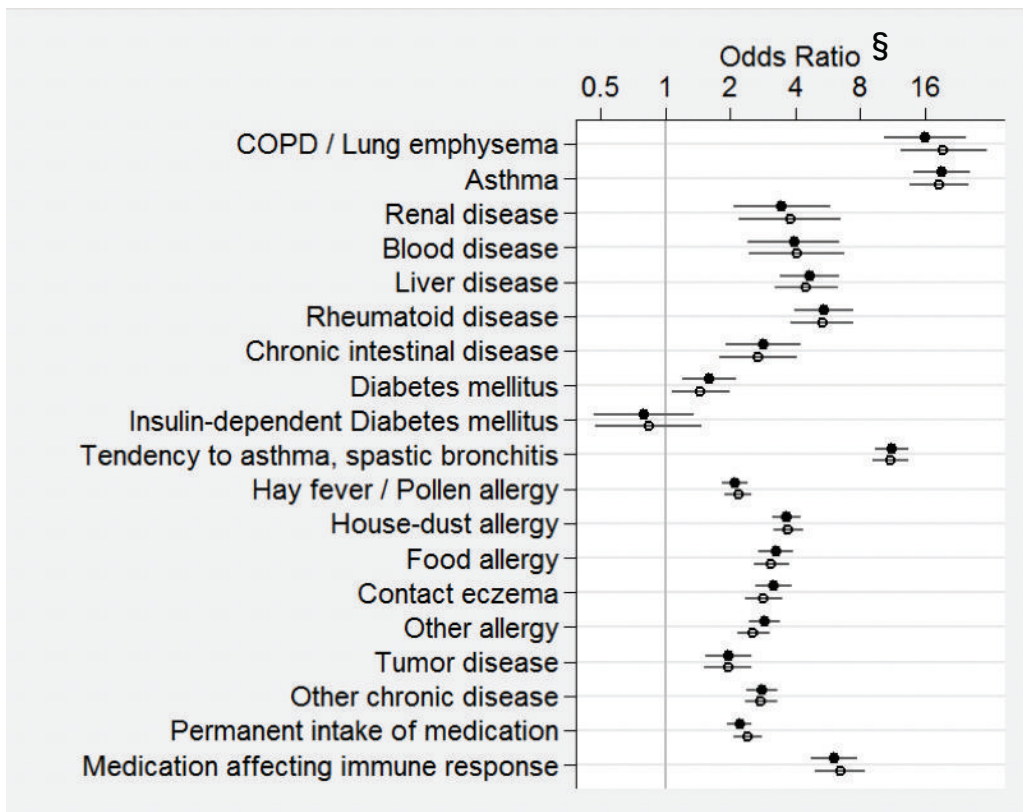
RTI-score: three risk groups



Cumulative prospective RTIs: Diary score

# Risiko für eine Association von Komorbiditäten mit hohen im Vergleich zu niedrigen RTI Gruppen

Nieters A et al *Int J Resp Med.* 2(1): 6-13, 2017



Adjusted: ○  
Unadjusted: ●

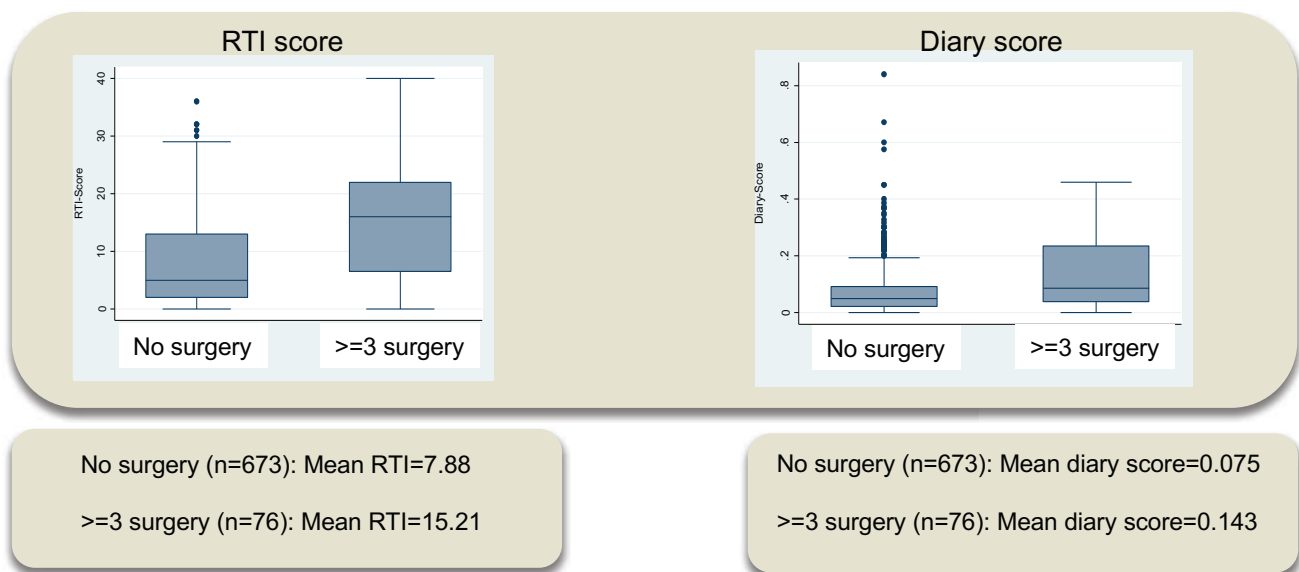
Adjustment variables:

- Age
- Gender
- Education level
- BMI
- smoking

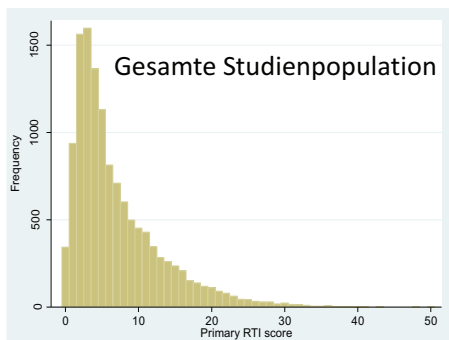
§ Odds Ratios mit 95% CI

## Effekte von Operationen an Organen des Immunsystems mit zurückliegenden und zukünftigen RTIs

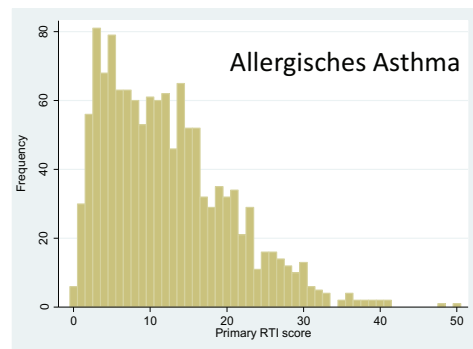
- Frühere Tonsilectomie, Polypectomie, NNH-Sinus-Op, Appendectomie, Splenectomie, Thymectomy, korrelieren mit erhöhten RTIs; besonders deutlich bei kumulativen operativen Eingriffen.
- Die stärksten Effekte zeigen Appendectomie, gefolgt von NNH-Sinus-Op
- Eine berichtete Besserung nach Ops korreliert signifikant mit weniger RTIs, aber die Raten sind immer noch höher als in Probanden ohne entsprechende Ops.



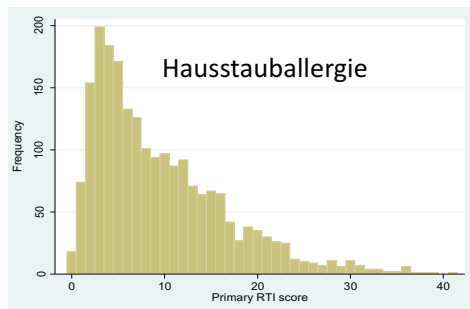
# Einfluss von Allergien auf die Atemwegsinfektanfälligkeit in der Allgemeinbevölkerung (AWIS Studie: MD Thesis von S. Wolfrum, 2017)



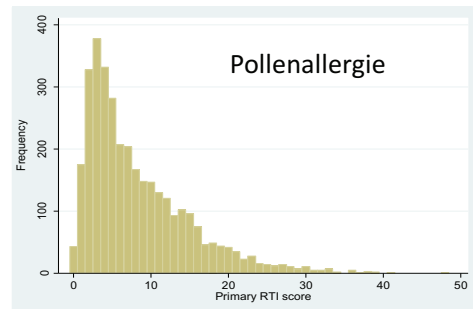
Scoreverteilung - Gesamte Studienpopulation  
Mittlerer RTI-Score: 7.02 KI:6,91 - 7.13



Scoreverteilung - Allergisches Asthma  
Mittlerer RTI - Score: 12.23 KI: 11.79 - 12.68

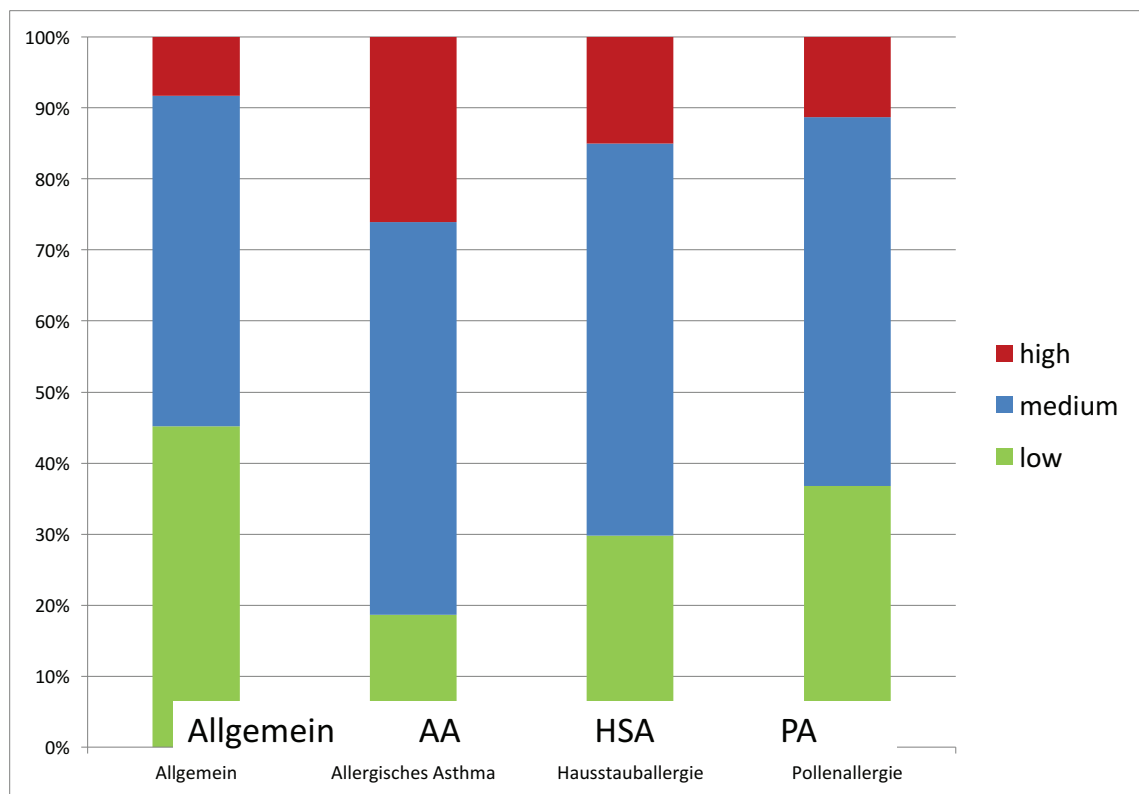


Scoreverteilung - Hausstauballergie  
Mittlerer RTI - Score: 9,44 KI:9,13 - 9,74



Scoreverteilung - Pollenallergie  
Mittlerer RTI - Score: 8,29 KI: 8,06 - 8,51

# Verteilung in den RTI-Score Kategorien



## Kernaussagen zu Risiken für eine erhöhte Atemwegsinfektanfälligkeit (AWI)

- Der RTI-Basis-Score erfragt Atemwegsinfekte retrospektiv (2 J); die Daten korrelieren signifikant mit den prospektiven Tagebuch-Scores (3 J)
- Für Geschlecht, Alter, Bildungsstand, Übergewicht und Rauchen werden die RTI-Scores adjustiert
- Diverse **Komorbiditäten** beeinflussen die AWI erheblich. Besonders **Allergisches Asthma** und **COPD** bedingen ein **erhöhtes Risiko für AWI**.
- Frühere **operative Eingriffe** an Organen des Immunsystems erhöhen das RTI Risiko..



## Warnhinweise für Immundefizienz

Kinder	Erwachsene
<p><b>Pathologische Infektanfälligkeit:</b>            Erreger, Lokalisation, protrahierter Verlauf, Intensität, Häufigkeit.            Faustregel für Erwachsene: &gt;3 Infekte pro Jahr, länger als 3-4 Wo Dauer</p>	
<p><b>Immunodysregulation:</b>            Granulome, Autoimmunity, rez. Fieber, Ekzem, Lymphoproliferation, chronische Diarrhoe, gehäuftes Auftreten von Malignomen</p>	
<b>Gedeihstörung</b>	<b>Gewichtsverlust</b> (oft mit Diarrhoe)
<p><b>Auffällige Familienanamnese:</b>            Konsanguinität, Immundefekte oder pathologische Infektanfälligkeit in Familie bekannt, Impfkomplicationen, Kinder früh an Infekten verstorben</p>	
<p><b>Basis-Labor:</b>            Blutbild mit Diff-BB, Lymphopenie, Neutropenie, Hypogammaglobulinämie, CRP, VitD3, Virustiter (EBV,CMV,HIV u.a.)</p>	
<p><b>Spezialambulanz mit erweitertem Immunlabor:</b>            IgG-Subklassen, Impfantwort (Diph, Tet., Pneumokokken), Complement (CH50,C3,C4) Lymphozyten-Panel, Zytotoxizität, Proliferation, Zytokine, Genetic.</p>	

## Differentialdiagnose\* der ID im Erwachsenenalter

### Primary ID

- **Antibody deficiency:** CVID, XLA, HIM, subclass deficiency, sIgA deficiency, specific AD
- **Combined ID / syndromes of Immunodysregulation:** atypical SCID, TCR signaling defects, Profound CID, LateOnset CID
- **Other complex PID:** WAS,, DiGeorge, AT, HIES
- **Hemophagocytic syndromes**

### Secodary ID

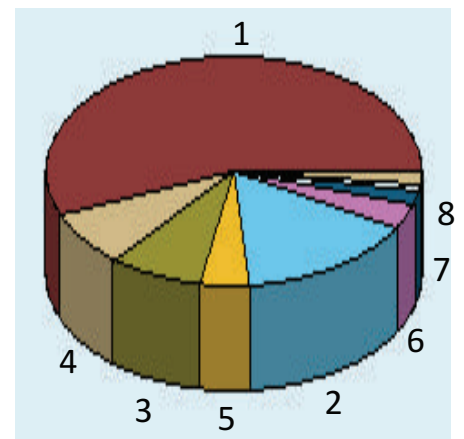
- Lymphoid Malignancies: CLL, Myeloma, NHL, Good's S.
- Prolonged cytotoxic and immunosuppressive therapy (notably Cyc +RTX)
- Post-splenectomy syndrome
- Post-HSCT
- Post solid organ transplant
- HIV
- Renal or enteral protein loss

Clinical immunologists, rheumatologists, infectiologists, hematologists, dermatologists, neurologists ..

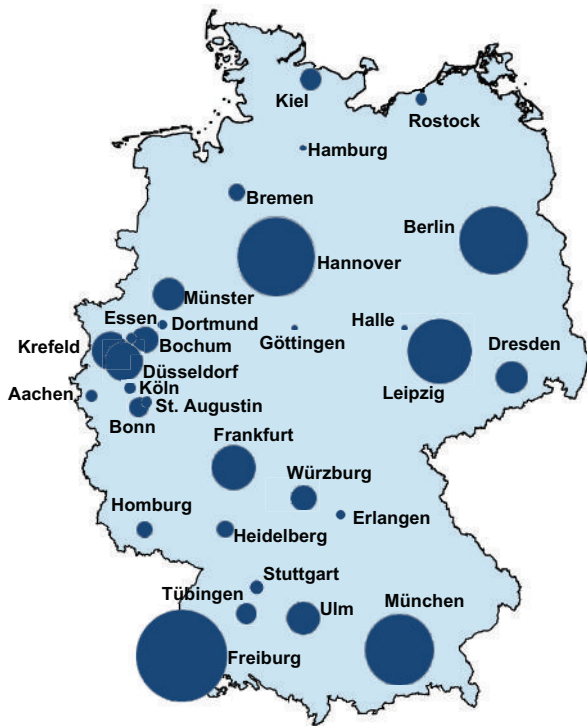
# European PID Registry in 2014:

19,355 Patienten

	Diagnosis – IUIS-groups	n	%
1	Predominant antibody disorders	10,966	56.66
2	Other well defined PID (WAS,AT, DG)	2,693	13.91
3	Defects of phagocyte number&function	1,689	8.73
4	Predominant T cell deficiencies	1,445	7.47
5	Complement deficiency	946	4.89
6	Immunodysregulatory syndromes (HLH), Interferonopathien)	753	3.89
7	Autoinflammatory syndromes	398	2.06
8	Defects of innate immunity	193	1.00
9	Unclassified PIDs	272	1.41
	<b>Total number of patients</b>	<b>19,355</b>	<b>100.0</b>

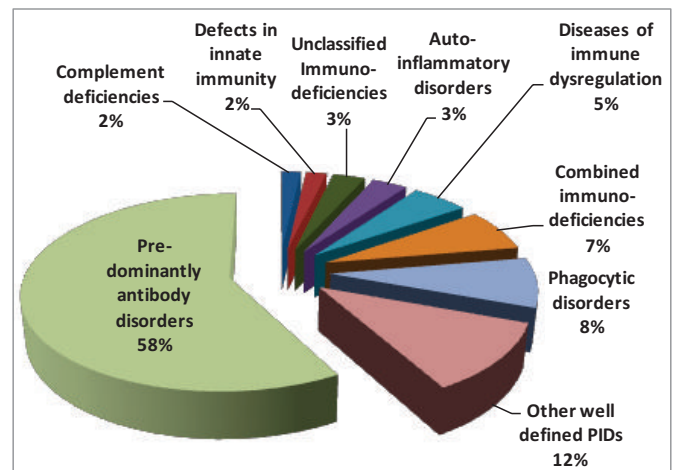


# German PID NET : Centers – Entering Data - Overview



The size of the circles is proportional to the number of registered patients.

Germany: PID-NET Registry (N=2230)



## First PID-Presenting Symptoms in 2230 pts

Symptoms	No pts	Percent (%)
Infections	1421	63
Immune dysregulation	446	20
Syndromal manifestations	167	7
Malignancy	15	1
Other	218	10
<b>Without clinical symptoms</b>	<b>76</b>	<b>3,4</b>
<b>Laboratory anomalies only</b>	<b>55*</b>	<b>2,4</b>

\*Among the clinical asymptomatic patients 55 pts presented with Lab anomalies: 30 hypogammaglobulinemia, 6 neutropenie, 2 thrombocytopenie 10 other.

## PID Patients and HSCT in Germany

PID	Patienten	Patienten mit HSCT
Severe combined immunodeficiency (SCID)	71	61
Chronic granulomatous disease (CGD)	108	50
Combined immunodeficiency (CID)	57	18
Wiskott-Aldrich syndrome (WAS)	23	16
Familial hemophagocytic lymphohistiocytosis syndromes (FHLH)	17	15
CSR defects and Hyper IgM (HIGM) syndromes	27	7
Hyper IgE syndrome (HIES)	49	7
X-linked lymphoproliferative syndrome (XLP)	25	7
Congenital neutropenia	25	5
Atypical Severe Combined Immunodeficiency (Atypical SCID)	6	4
FOXP3 deficiency (IPEX)	4	4
Unclassified phagocytic disorders	9	4
Chediak Higashi syndrome	4	3
<b>Common variable immunodeficiency disorders (CVID)</b>	<b>646</b>	<b>3</b>
Dyskeratosis congenita	6	3
Omenn syndrome	4	3
Unclassified immunodeficiencies	41	3
Cartilage hair hypoplasia	3	2
Defects with susceptibility to mycobacterial infection (MSMD)	5	2
Griscelli syndrome, type 2	2	2
HLA class II deficiency	3	2
Leukocyte adhesion deficiency (LAD)	2	2
Nijmegen breakage syndrome (NBS1)	7	2
Unclassified disorders of immune dysregulation	17	2
Unclassified syndromic immunodeficiencies	14	2
X-linked thrombocytopenia with mutations in WASP	3	2
Agammaglobulinemia	99	1
Ataxia telangiectasia (ATM)	56	1
Defects of TLR/NFkappa-B signalling	8	1
<b>Unclassified antibody deficiency</b>	<b>223</b>	<b>1</b>
<b>Total</b>	<b>1564</b>	<b>235</b>

## Primäre Antikörpermangel Syndrome (PAD)

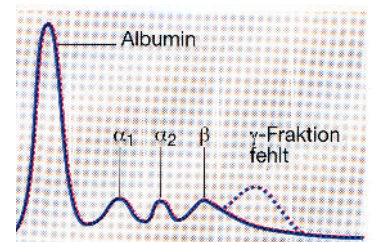
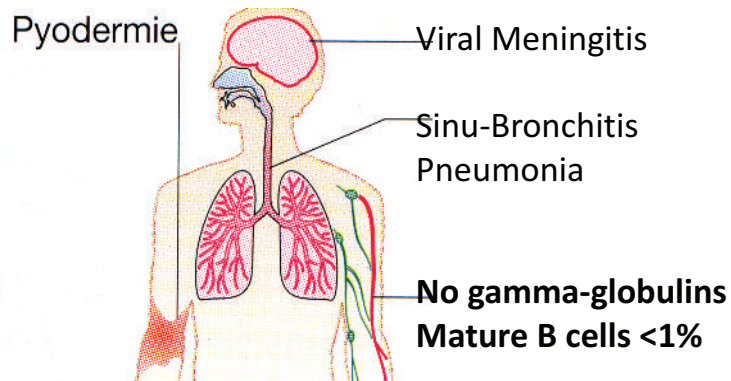
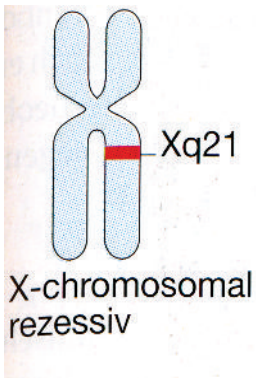
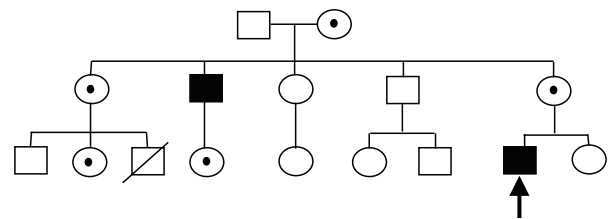
- |    |  |       |
|----|--|-------|
| 1. | Agammaglobulinaemien: XLA (M.Bruton), AR                 | (10%) |
| 2. | Hypogammaglobulinaemien:                                 | (90%) |
|    | Common variable Immunodeficiency (CVID)                  |       |
|    | Hyper-IgM-Syndrome (CD40L, CD40, Nemo, AID, UNG, PMS2)   |       |
|    | Selective IgA-Defizienz                                  |       |
|    | IgG-Subclassen Defizienz                                 |       |
|    | Kappa-Leichtketten Defizienz                             |       |
|    | Selective Antikörper Defizienz (SAD)                     |       |
|    | Transitorische Hypogammaglobulinemi des Kleinkindes      |       |
| 3. | Good Syndrom (Thymom, wenig B und NK Zellen, Hypo-gamma) |       |

# X-Linked Agammaglobulinemia (XLA, M.Bruton)



Bruton OC (1952). "Agammaglobulinemia".  
Pediatrics 9 (6): 722–8.

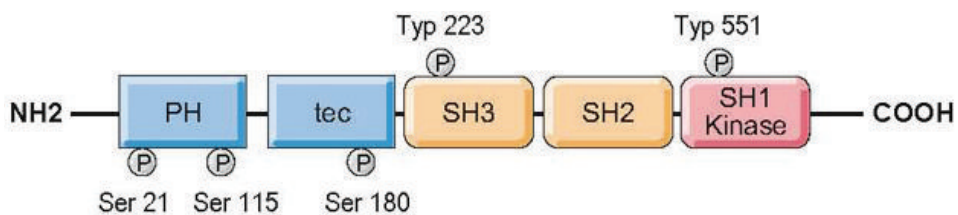
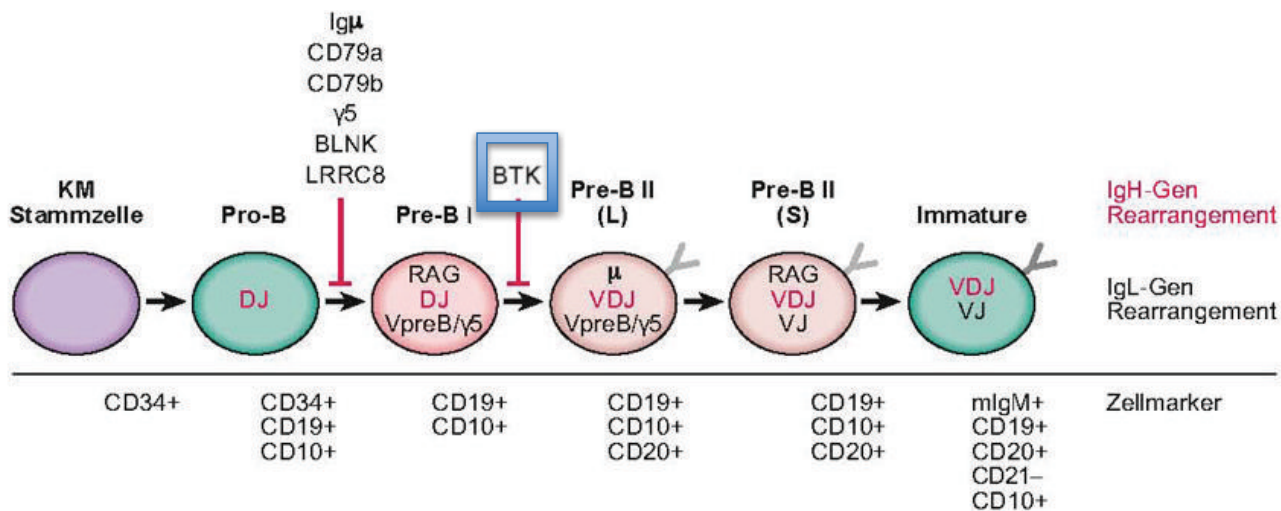
Only boys affected  
Mothers are conductors





# Mutationen im *BTK* Gen verursachen XLA

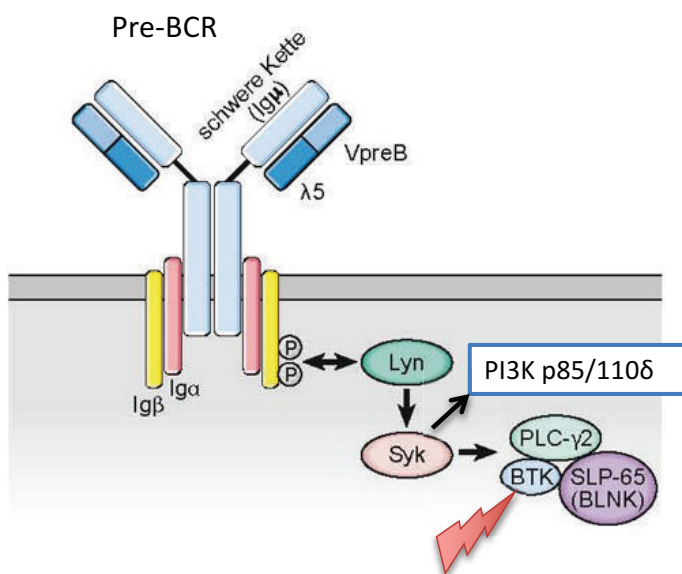
(Vetrie et al 1993, Tsukada et al 1993)



# Gestörte Pre-B Zellrezeptorfunktion bei Agammaglobulinämie

(U. Salzer in "Klinische Immunologie" 2012, Elsevier)

## XLA = *BTK* deficiency



## Autosomal recessive Formen (kommen auch bei Mädchen vor)

1. Cμ heavy chain deficiency
2. λ5 deficiency
3. Igα deficiency
4. Igβ deficiency
5. BLNK deficiency
6. LRRC8 deficiency
7. PI3K p85 deficiency)

# Common Variable Immunodeficiency Disorders (CVID)

Die häufigste Form des pAMS im Erwachsenenalter

**Definition:** Heterogener, primärer Antikörpermangel  
8-10% sind genetisch definiert

**Diagnose Kriterien unter ([www.esid.org](http://www.esid.org)):**

1. Hypogammaglobulinemie von IgG, IgA u/o IgM (2 SD < mean)
2. Beginn der Immunodefizienz jenseits des 4. Lebensjahres
3. Schlechte Impfantwort und Fehlen von Isoagglutininen

# CVID Fallberichte

Fall 1

unkompliziertes CVID Syndrome  
“infections only”

## Fall 1: Vorgeschichte: Mann \*1950

- Von Kindheit an vermehrte sinubrochiale Infektanfälligkeit
- Während der Adoleszenz **mehrfach NNH Operationen**
- Mit 18 Jahr Kur wegen **chronischer Sinusitis**
- Im weiteren Verlauf ca. **6 Pneumonien bis 2000**
- 2004 **Splenomegalie** festgestellt u. zunehmende Lymphopenie
- 9/2007 Hautveränderungen i.S. eines Sweet Syndromes an Händen u. Extremitäten, Besserung mit Dapson u. Steroide.
- Seit 9/2008 Durchfall ohne Erregernachweis, C-Gastritis
- Seit 2/2009 Myrarthralgien unklarer Genese, wieder besser
- 4/2009 Vorstellung zur Abklärung u. Therapie der Infektanfälligkeit

## Untersuchungsbefund 4/2009

- 58-jähriger Patient in gutem AZ, EZ (71 kg, 168 cm). Keine Oedeme
- Unauffällige Hautfarbe und Turgor, aktuell noch leichtgradige Pigmentstörungen nach Sweet-Syndrom.
- 
- Blutdruck 160/90 mmHg, Herzfrequenz 76/Min., regelmäßig,
- Cor, Pulmo, Abdomen unauffällig, Milz nicht sicher tastbar, Lymphknotenstatus oB
- Neurologischer Status, Psyche und Bewegungsapparat normal

# Routinelabor

- BSG 5/10 mm n.W..
- Leukozyten 10,6 Tsd/ $\mu$ l; PMN 88, **Ly 7**, Mono 3, Eos 0, Baso 0; Thrombozyten 182 Tsd/ $\mu$ l;
- Erythrozyten 4,46 Mio/ $\mu$ l; Hämoglobin 13,7 g/dl; Hämatokrit 40,6 %; MCV 91,0 fl; MCH (HbE) 30,7 pg; MCHC 33,7 g/dl;
- Natrium 143 mmol/l; Kalium 3,8 mmol/l; Calcium 2,20 mmol/l; Eisen 57  $\mu$ g/dl; Ferritin 120 ng/ml; Harnstoff 34 mg/dl; Serum-Kreatinin 0,88 mg/dl;
- LDH 240 U/l; CK 93 U/l; GPT (ALT) 37 U/l; Gamma-GT 28 U/l; **TSH 0,23  $\mu$ U/ml ↓**; **25-OH Vit. D3 10 ng/ml ↓**
- **Urin:** Leuko neg; spezifisches Gewicht 1,010 g/ml; Nitrit neg; pH 8; Eiweiß neg;

# Immunlabor

- IgG **<0.07 g/l** (7-16 g/l)
- IgA **< 0.06 g/l** (0.7 -4 g/l)
- IgM **<0.19 g/l** (0.4 -2.3 g/l)
- IgE **< 4 E/ml** (10-100 E/ml)
- kappa fr.Leichtketten (Serum) **<0,540 mg/l;**
- lambda fr.Leichtketten (Serum) **<0,720 mg/l;**
- löslicher Interleukin 2 Rezep. **1048 U/ml;**
- Kein Paraprotein in Serum u. Urin
- CH50, C3d, CRP normal
- **Kein Impfschutz für Tetanus und Pneumokokken**



# Lymphozyten-Typisierung

## T-Zellen

- Lymphozyten 889/ul (8.4%)
- CD3+ 792/ul
- CD4+ 217/ul
- CD8+ 549/ul
- CD4/CD8 0,40

## B-Zellen

- CD19+ 71/ul (5%)
- Naïve B-Zellen 84%
- IgM Memory 15%
- IgG Memory 0%
- IgA Memory 0%
- Plasmablasten 0%

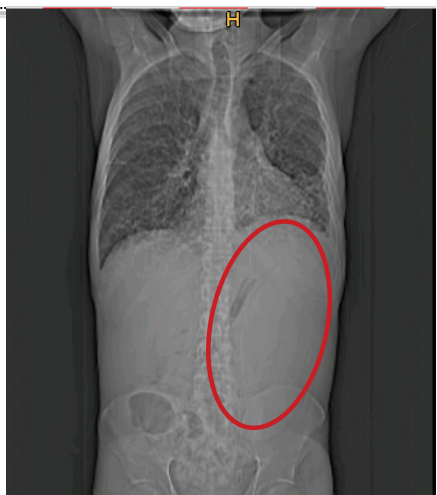
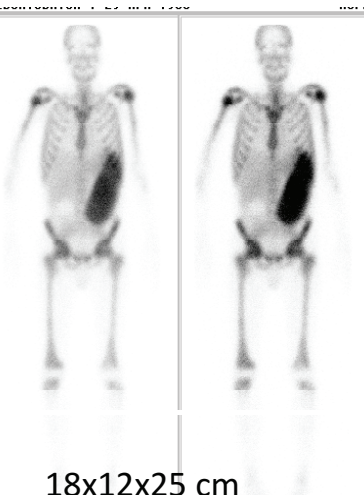
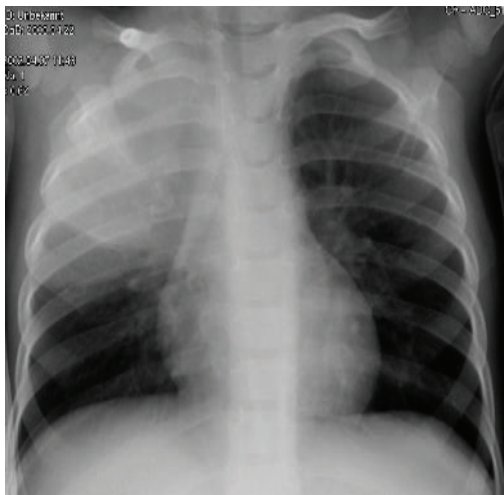
# CVID Fallberichte

Fall 2

Kompliziertes CVID Syndrom  
RTI, lymphoproliferation, Evans syndrome,  
Colitis, sarcoid-like granulomas

## Fall 2: Mann\* 1968

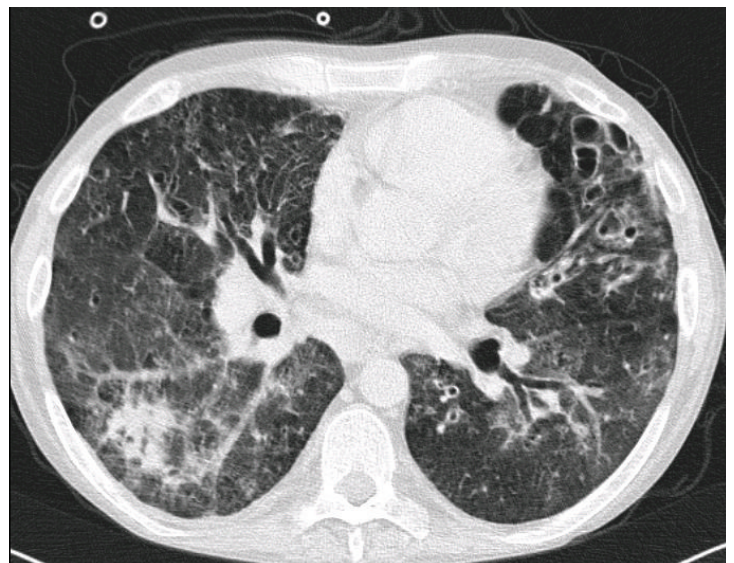
- 1989 Wiederholte ORTI und erste Pneumonie  
Schwere Hypogammaglobulinemie  
Erstdiagnose : CVID
- 1993: Komplikationen: ITP + AIHA (Evans Syndrom), Splenomegalie
- 1999: Distale Colitis ulcerosa
- 2001: Splenectomie



## Fall 2: Mann\* 1968

2002 Bronchiektasen und  
Interstitial lung disease  
Pulmonale Hypertonie

2003 Gelistet für HSCT



## Fall 2: Diagnose und Behandlung

### **Typische kompliziertes CVID Syndrom**

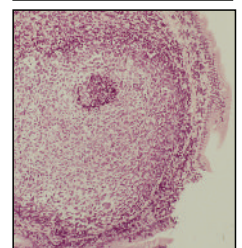
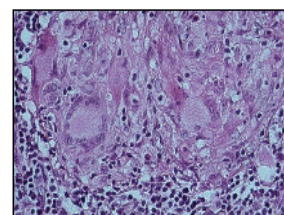
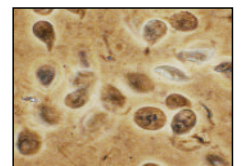
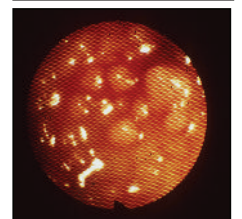
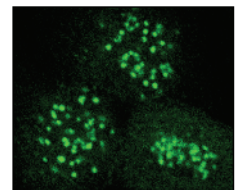
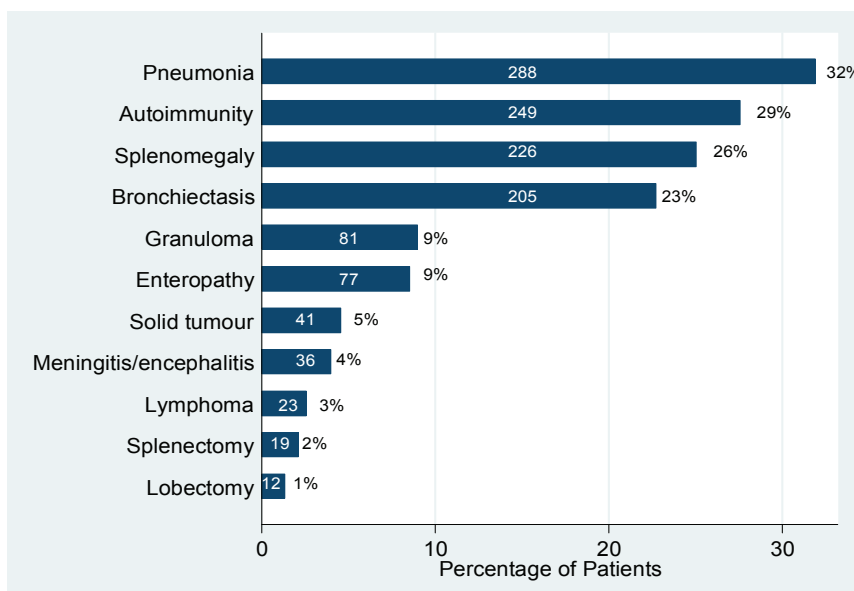
- Autoimmunität (AIHA, ITP)
- Lymphoproliferation / Splenomegalie
- Colitis ulcerosa/ Interstitial lung disease
- Oligoklonale CD8+ Lymphocytose,
- Recurrente CMV Reactivierung

### **Behandlung:**

- Seit 1989 regelmäßige IVIg Substitution
- Intermittierend Pred 5-10mg/d
- 2001 Splenectomie, Listung für allogene HSCT,
- Tod durch Pneumonie während Spendersuche

# Epidemiologie und klinischer Phenotyp des CVID Syndromes

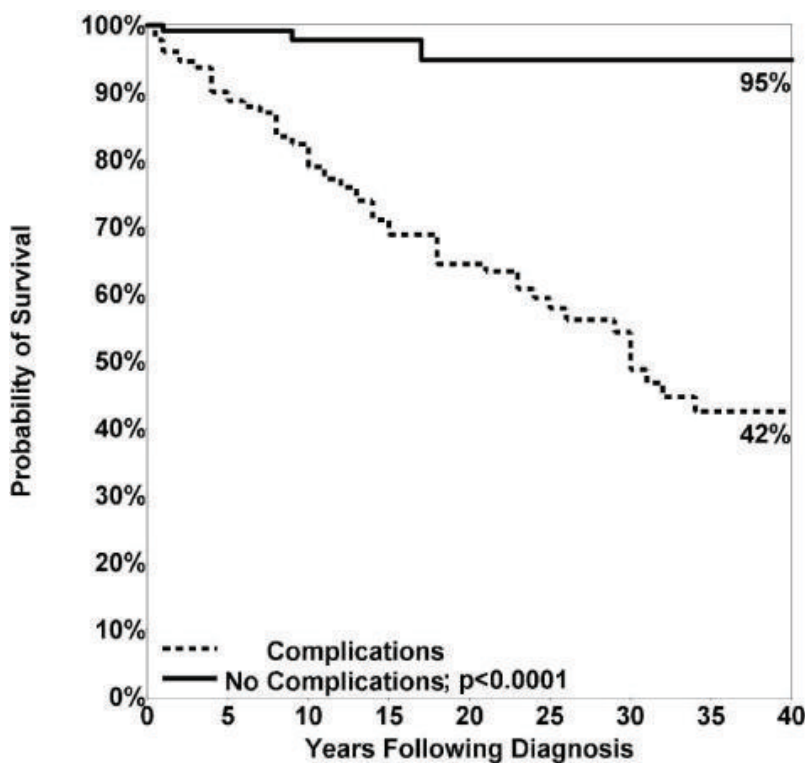
ESID Registry 2014 (n=832)



Gathmann B et al JACI (2014) 134:116-126

# Überleben bei unkompliziertem u. kompliziertem CVID

(ES Resnick et al 2011)



Infections only

Infections plus complications

Complications	HR	p
Any	10.96	<0.0001
GI disease	2.78	<0.004
Liver disease	2.48	<0.003
Lymphoprolif.	2.44	<0.01
Chronic lung dis	2.06	<0.01
Malabsorption	2.06	<0.022

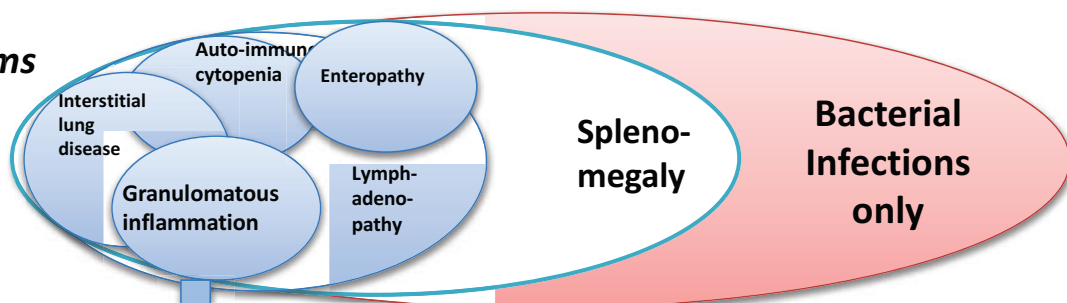
# CVID – common variable immunodeficiency

- **Diagnosis**

- hypogammaglobulinemia (low IgG + low IgA or IgM)
- poor response to vaccination
- exclusion of secondary hypogammaglobulinemia
- exclusion of profound T cell defect

- **Symptoms**

CVIDc ≈  
smB-21<sup>low</sup>  
elevated



- **Treatment**

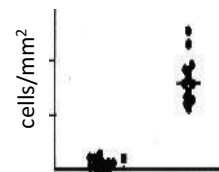
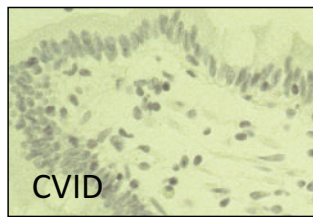
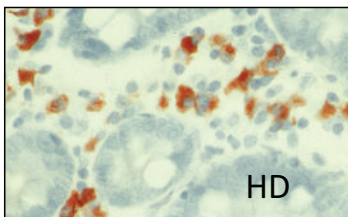
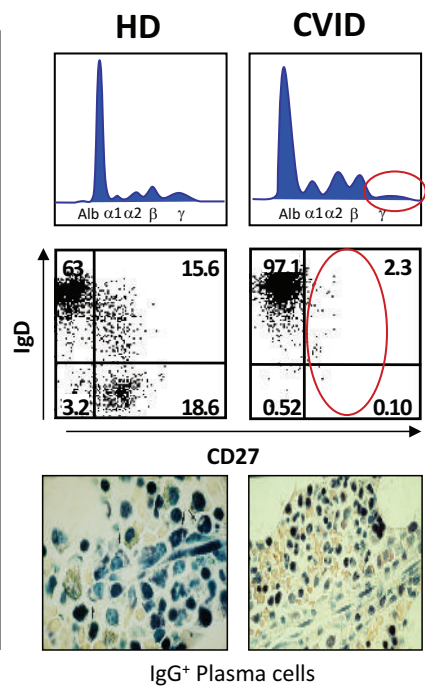
Regular IVIG or SCIG therapy, antibiotics if necessary

- +
- Immunosuppression (Pred. CyA, Rapa ?)
  - HSCT?



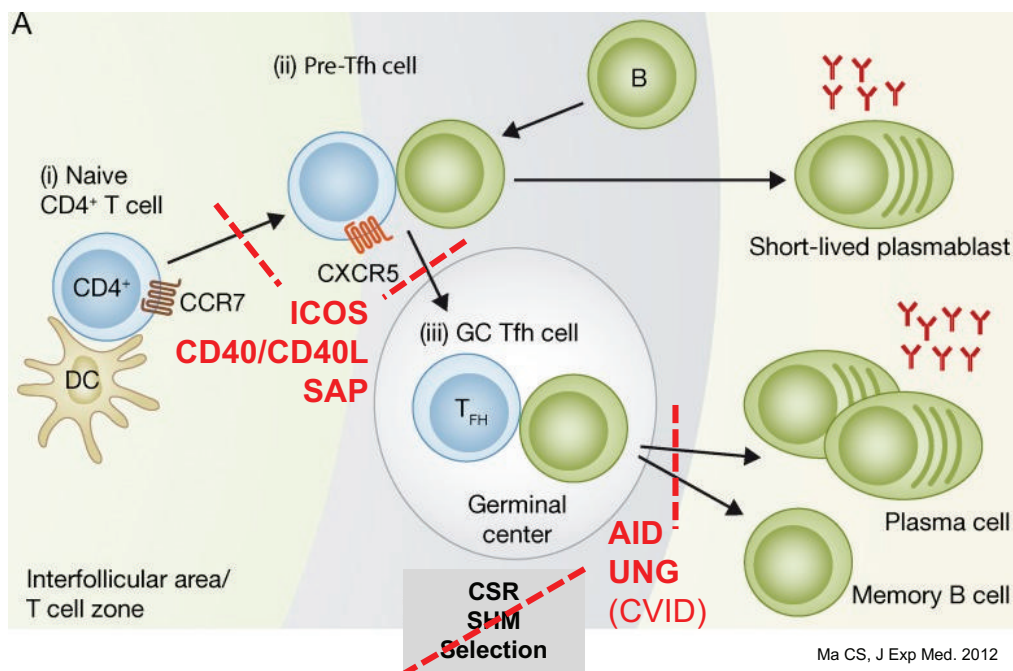
# Immunological Phenotype of CVID

- Hypogammaglobulinemia, antibody deficiency  
Failure to respond to vaccines
- CD19<sup>+</sup> B cell numbers normal or reduced
- CD19<sup>+</sup> CD27<sup>+</sup> switched memory B cells reduced in 80% of patients (Warnatz et al 2002)
- CD21<sup>low</sup> B cells increased (Rakhmanov M 2009)
- No long-lived plasma cells in bone marrow (Artus et al 1995, Gomes et al 2011)
- Absence of IgA<sup>+</sup> plasma cells in intestinal lamina propria (Armbruster et al 1994)
- Naive CD4 T cells reduced (Giovannetti A et al 2007)

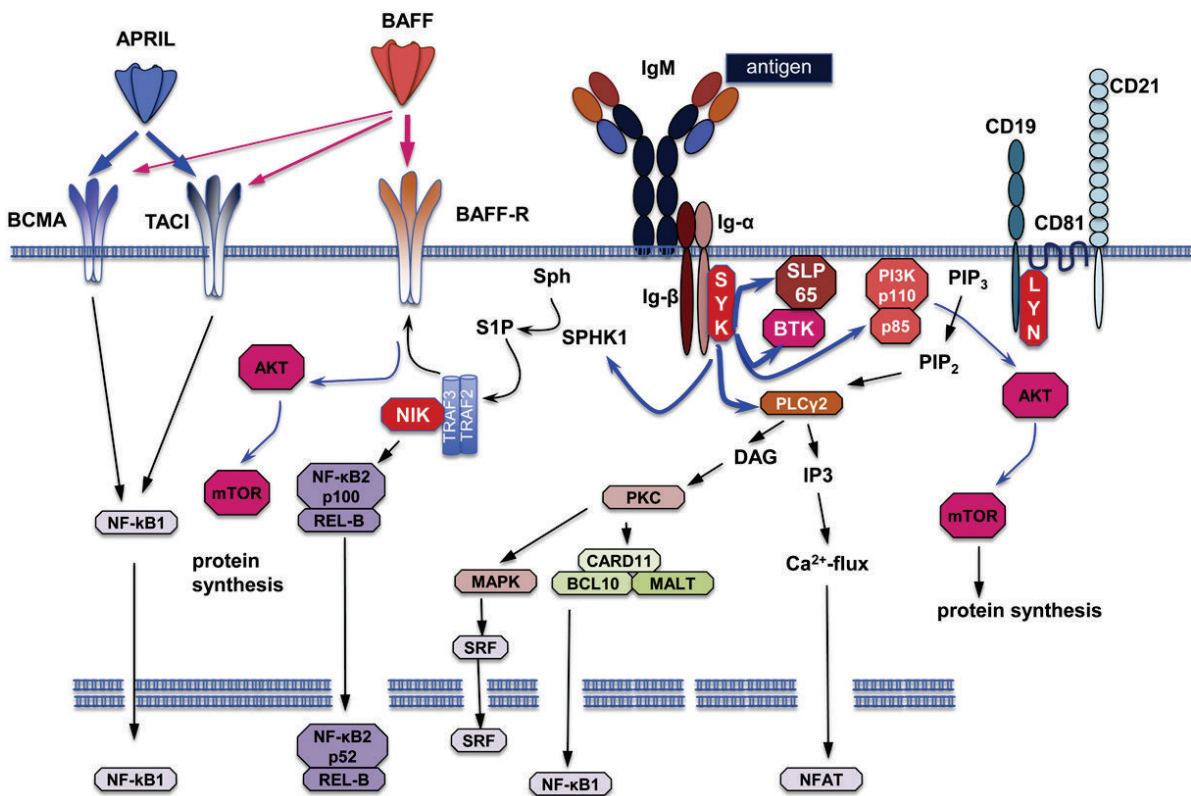


HH Peter

## T follicular helper cells (TFH) are essential for adaptive humoral immunity



# Signal-Transduktion in B-Zellen



Pieper et al, *JACI* 2013

# Attempts at deciphering genetics of CVID

(modified Park et al 2012, Salzer et al 2012, Orange et al 2011)

1995-2000

## Early genome wide linkage family studies:

IgAD1 locus in HLA region -> IgAD/CVID

Chromosome 16q, 4q -> autosomal dominant CVID

2010

## DNA repair variations:

MSH2, RAD50, RAD52, MLH1, NBS1, MSH5, others

2002-2015

## Monogenic defects important for B and T cell development and function

*ICOS*, *CD19*, *CD20*, *CD21*, *CD81*, *BAFF-R*, *TACI*, *LRBA*, *PI3KD*, *Card 11*, *CD27*, *SBDS*,  
*CTLA4*, *PLCγ2*, *PKCδ*, *LAT*, *NFKB1*, *NKκB2 a.o.*

2012-2015

## Next generation sequencing (NGS)

Whole exome sequencing (WES)

Whole genome sequencing (WGS)

# Targeted Panel Re-Sequencing

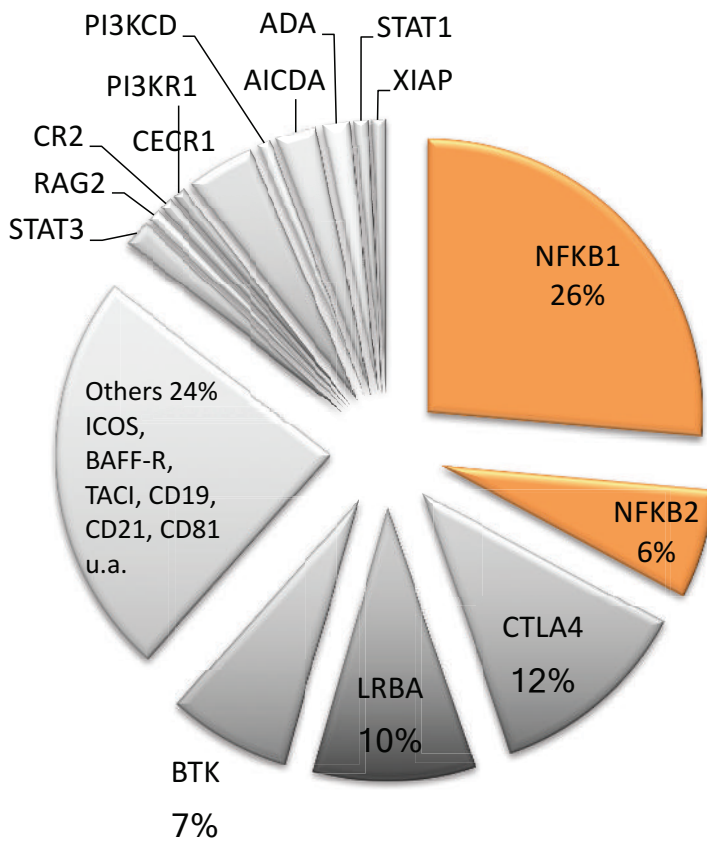
120 candidate genes associated with CVID/ agammaglobulinemia

ADA	CD79A	GATA2	KCNC4	P2RX7	RPTOR	TNFRSF10A
AICDA	CD79B	GRAP	KCNN4	PDCD1	SEC61A1	TNFRSF13B
AKT1	CD80	GRB2	KIDINS220	PDCD1LG2	SEC61A2	TNFRSF13C
AKT3	CD81	HDAC4	LRBA	PIK3AP1	SEC61G	TNFRSF17
APCS	CD86	HSPA5	LRR32	PIK3CD	SH2D1A	TNFRSF18
BCL2L1	CDX1	<b>ICOS</b>	LRRK2	PIK3R1	SH3KBP1	TNFRSF4
BCL6	CECR1	ICOSLG	MLH1	PRDM1	SOCS1	TNFSF10
BLNK	CLEC16A	IGHM	MS4A1	PRKCD	STAT1	TNFSF13
BTK	CORO1B	IGLL1	MSH2	PTEN	STAT3	TNFSF13B
CASP8	CR2	IKBKB	MSH5	PTPN1	STK11	USP8
CCL5	<b>CTLA4</b>	IKBKE	MTA3	PTPN6	TCF3	VAV1
CCR6	CXCL12	IKBKG	NBN	RAD50	TFRC	VAV2
CD19	CXCR4	IKZF1	NFKB1	RAG1	TGFB1	WNT5A
CD27	CXCR5	IL21	NFKB2	RAG2	TGFB2	XCL1
CD274	DCLRE1C	IL21R	NFKBIA	RELA	TGFB3	XIAP
CD28	DUSP2	IL4I1	NLRP12	RORA	TMEM173	
CD40	FCGRT	IRF2BP2	NOTCH1	RPS6KB2	TNFAIP1	
CD40LG	FOXP3	IRF4	NOTCH2	+ 7 undisclosed candidate genes		

AG Grimbacher 2016

# Targeted Re-Sequencing

- Analysis of 120 candidate genes



Gene	Mutation
BTK	IVS7+2 (A-->G); L528Wfs*2; K300*; R13*; V219Lfs*10, Y344Wfs*57, Q140*
CECR1	IVS2+1; C167Y; R169Q; P251L; H112Q;
CTLA4	IVS1+1(G-->T); R70W; T124P; C85Y; R75W; I116T; P136L; E94*, Q76*; etc
LRBA	E946*; Q474*; S348*; N1217Sfs*7; S2446*; Q1715*; S1885*; L156S; R182*
NFKB1	A475Pfs*10; V456*; IVS11+1 (G-->C); IVS9+2 (T-->G); D541*; IVS5-2; R156*, IVS9+2; etc
NFKB2	D854Efs*31; IVS14+1 (G-->T); S866Cfs*19, R853*
PIK3R1	IVS5+1
RAG2	G35A; G451A; D177H
STAT3	R152W
AICDA	C147*

## CVID, agammaglobulinemia (n=554)

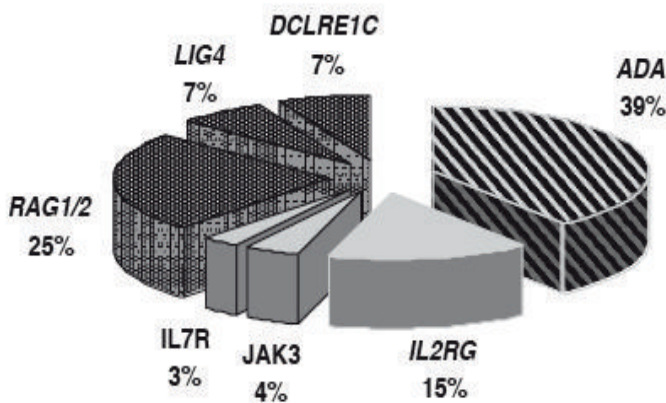
Identification of monogenic defects  
in approx.20% of patients

Bodo Grimbacher et al 2016

# Combined Immunodeficiency (CID)

## A. Atypical hypomorphic SCID

(Felgentreff K et al 2011)



## B. Complex syndromal CID:

WAS, DiGeorge, Hyper-IgE, AT, MHC II

## C. Impaired T cell activation

## D. Profound CID (LOCID)

CD3 T cell lymphopenia

Low naïve CD4 T cells

Lymphoproliferation to PHA ↓

γδ T cells ↑

IgE sometimes elevated

Specific antibodies partially pos

Trec-test usually normal

New gene defects are emerging:

PI3KD, PLCy2, LRBA, CTLA4,

PKCD, LAT ...

# Phosphatidylinositol-3 Kinase (PI3K) p85, p110 $\delta$ Mutations

Conley et al *J Exp Med.* 2012

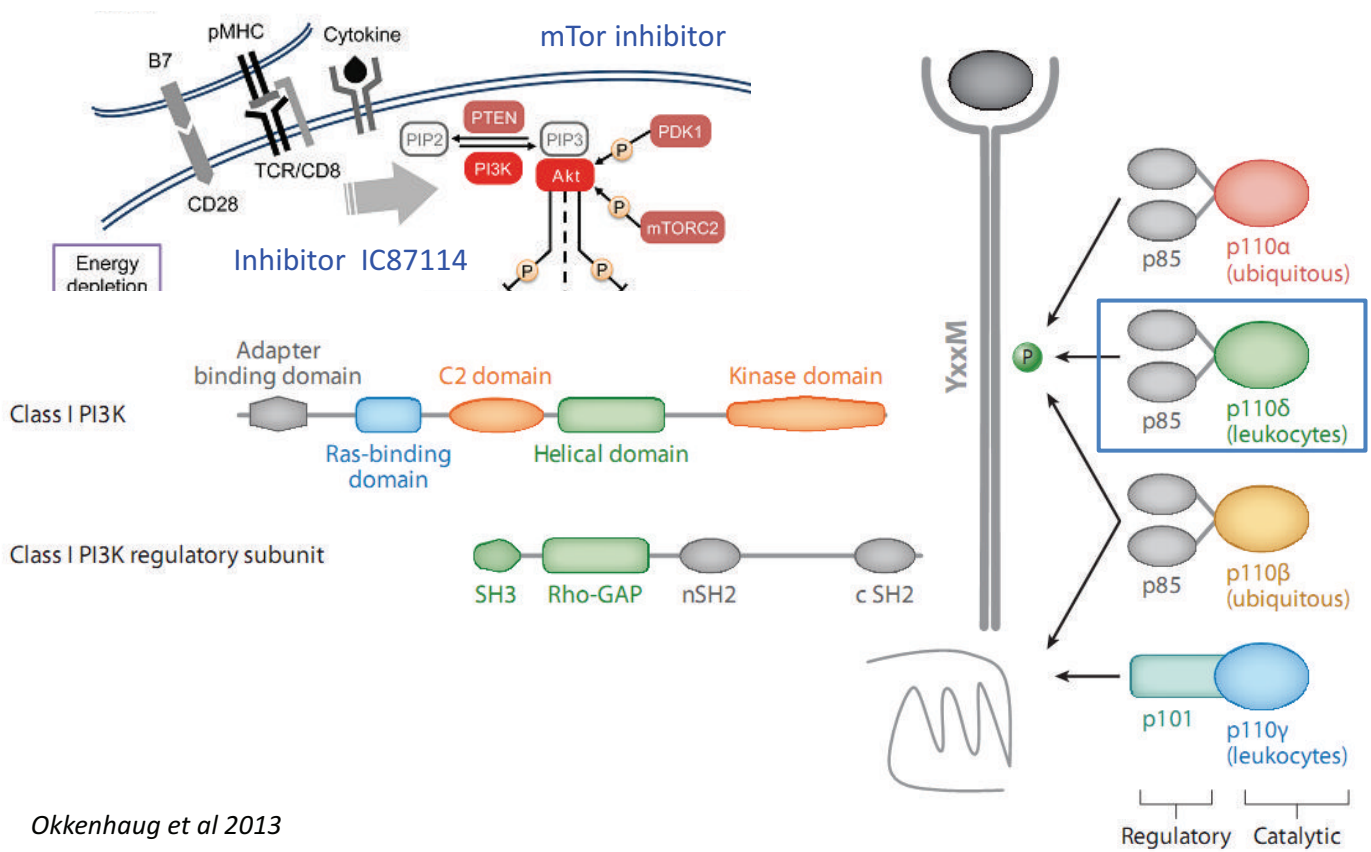
Deau et al *J Clin Invest.* 2014

Angulo et al *Science* 2013

Lucas et al *Nat Immunol* 2014



# PI3K: catalytic p110 and regulatory p85 subunit form heterodimers



Okkenhaug et al 2013

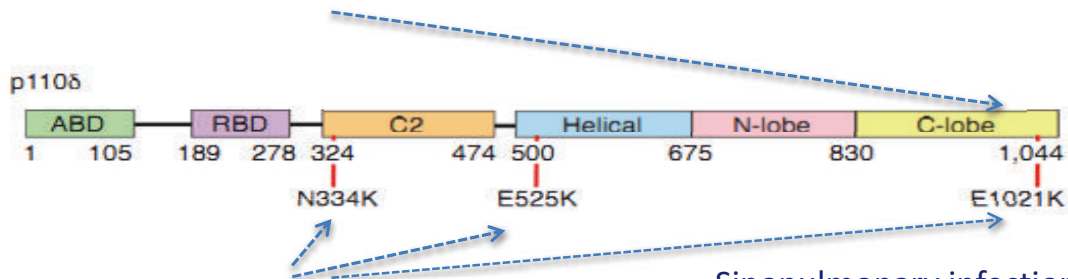
# *PIK3CD* encodes the catalytic subunit p110 $\delta$ of PI3K $\delta$ : Several germline GOF mutations cause „APDS 1“

**Phosphoinositide 3-Kinase  $\delta$  Gene**  
Mutation predisposes to respiratory  
infection and airway damage.

I Angulo, O Vadas, F Garcon ... S Nejentsev  
Science 2013. 15; 342: 866-71.

17x: c.3061G>A  $\rightarrow$  **E1021K** in the C-lobe

Immunodeficiency  
Airway infection  
Lymphopenia (AICD)  
IgM  $\uparrow$ , IgG2  $\downarrow$   
Vaccine response  $\downarrow$



**Dominant-activating germline mutations in Phosphoinositide 3-Kinase p110 $\delta$  cause T cell senescence and Human immunodeficiency.**

CL Lucas, HS Kuehn, F Zhao ... G Uzel  
Nat Immunol 2014; 15: 88-97

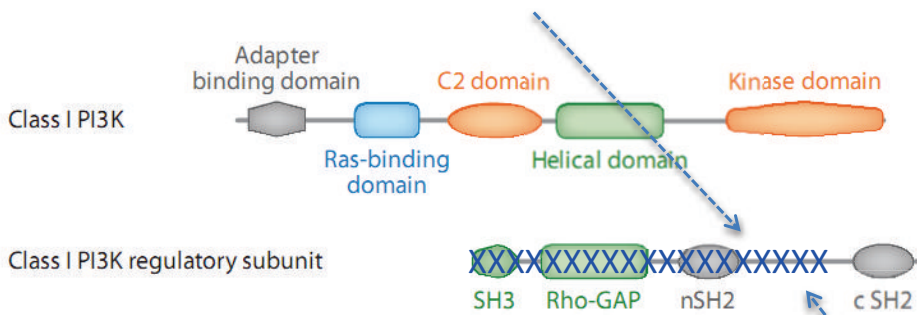
1xN334K, 7xE525K, 6x E1021K

Sinopulmonary infections  
Immunoodeficiency  
Lymphadenopathie  
Nodular lymphoid hyperplasia  
EBV,CMV viremia  
CD4 T cell lymphopenia, naive  $\downarrow$   
Senescent T cells  $\uparrow$

*PIK3R1* encodes p85 $\alpha$  regulatory subunit:  
so far 3 germline GOF mutations cause “APDS 2”

Deau M-C, Heurtier L .... Kracker S.  
**A human immunodeficiency caused by mutations in the *PIK3R1* gene.**  
*J Clin Invest.* 2014;124(9):3923–3928.  
*Aberrant splicing* → *loss of exon 10: ↑ p110 $\delta$*

Dysgamma/Hypo-  
Gammaglobulinemia  
Sinopulmonary infections  
Lymphoproliferation  
Expansion of  
Senescent CD8 cells



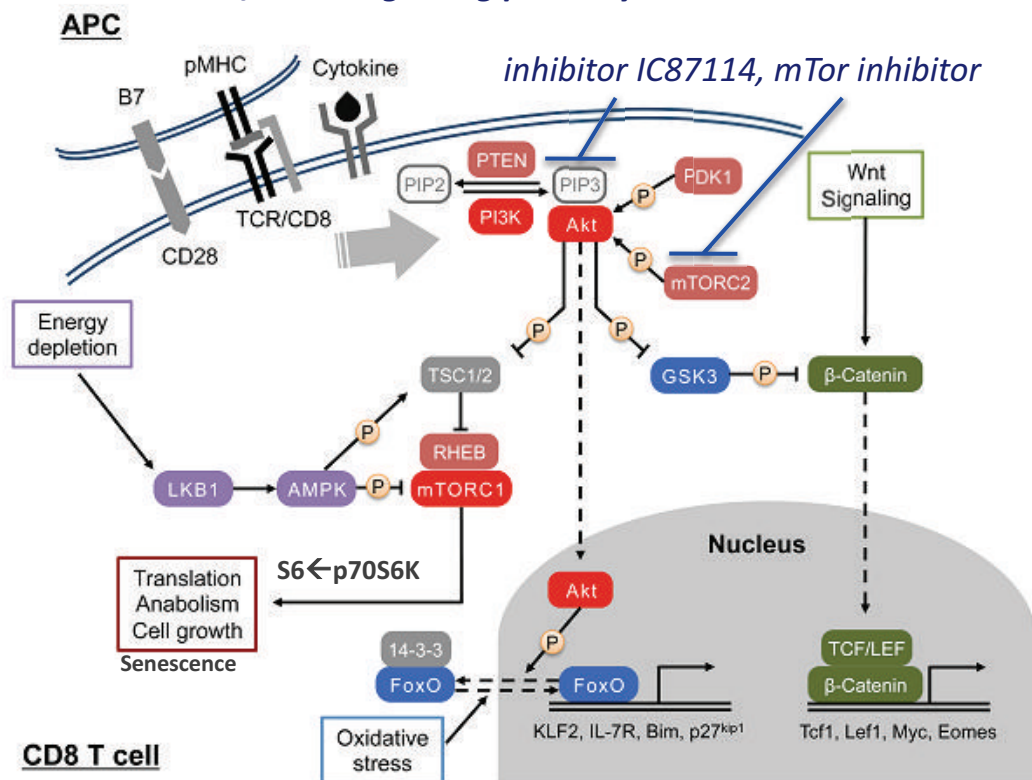
Lucas CL, Zhang Y ..Lenardo MJ  
**Heterozygous splice mutation in *PIK3R1* causes human immunodeficiency with lymphoproliferation due to dominant activation of PI3K.**  
*J Exp Med.* 2014 15;211: 2537-47.

*Splice site mutation in p85* →  
*Loss of AS 434-475 in the inter SH2 domain* ↑ p110 $\delta$

Conley ME, Dobbs AK ... Murray PJ.  
**Agammaglobulinemia and absent B lineage cells in a patient lacking the p85 $\alpha$  subunit of PI3K.**  
*J Exp Med.* 2012;209(3):463–470

# Treatment attempts of *PIK3CD* germline mutations

## *PI3K* activates *Akt/mTOR* signaling pathway



Adapted from Kim and Suresh 2013

# CTLA-4 Deficiency

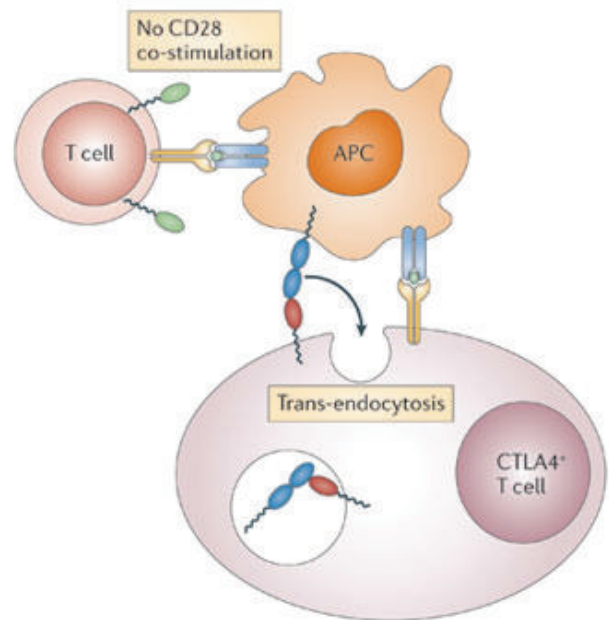
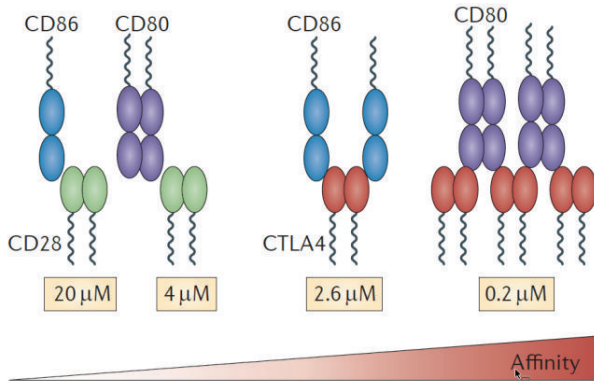
Schubert et al Nat Med. 2014

Kuehn et al Science. 2014

# CTLA-4 – an essential inhibitory receptor on Tregs

CTLA-4 captures its ligands CD80/86 from the surface of antigen presenting cells

Schubert D, Bode C ... Gribmacher B  
**Autosomal dominant immune dysregulation syndrome in humans with *CTLA4* mutations**  
 Nat Med. 2014; 20(12):1410-6  
*Heterozygous non-sense mutation in exon1*



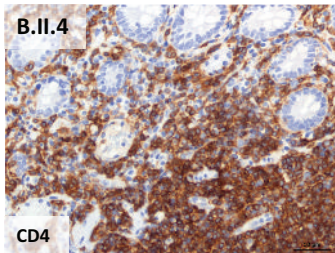
Nature Reviews | Immunology

Kuehn HS, Ouyand W ...Uzel G  
**Immune dysregulation in human subjects with heterozygous germline mutations in *CTLA4***  
 Science. 2014 ;345(6204):1623-7.  
*Heterozygous mutations in *CTLA4**

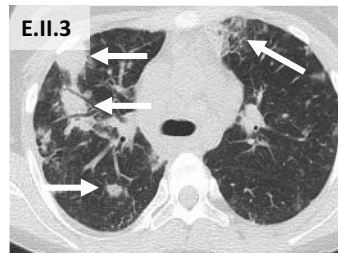
# Clinical manifestations

CTLA-4 deficient patients suffer from massive lymphocytic organ infiltrations

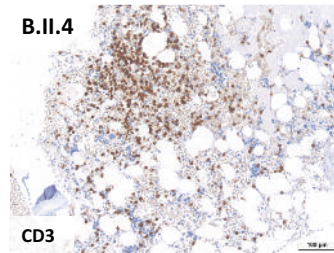
Duodenum



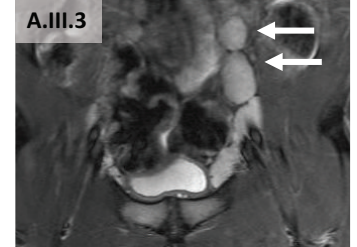
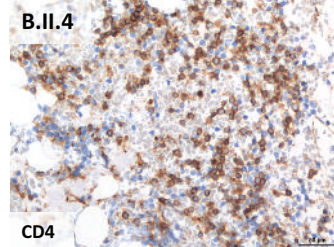
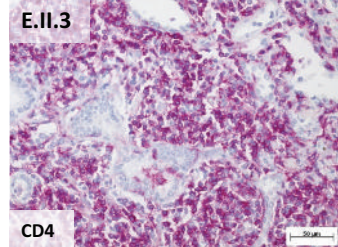
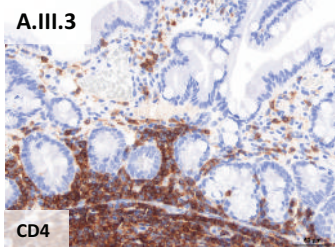
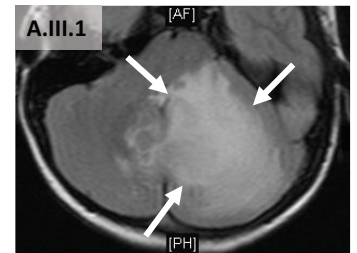
Lung



Bone marrow



Cerebellum



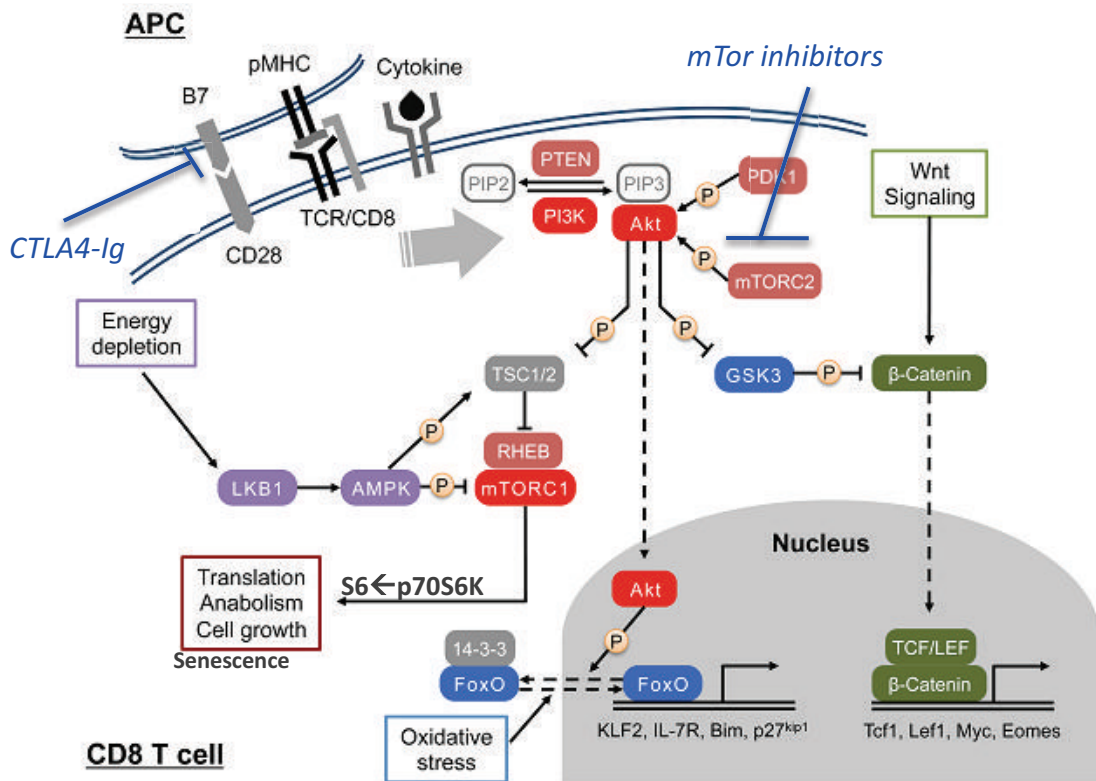
Hypogammaglobulinemia  
Recurrent infections  
Multiple autoimmune phenomena  
Non-functional Tregs, low B cells shift to loC21

Lymph nodes



# Treatment attempts in CTLA4 deficiency

*Relative lack of CTLA-4 favors enhanced CD28 signaling*



Adapted from Kim and Suresh 2013



# Zusammenfassung I

**In der CVID Kohorte finden sich B und T Zell intrinsische Defekte als Ursache für Antikörpermangel und Immunodysregulation**

- Am häufigsten finden sich monogene Störungen des **BCR**, seiner **Co-Rezeptoren** und nachgeschalteten Signalkaskaden:
- Ig $\alpha$ ,  $\beta$ , BTK, CD19, CD20, CD21, TACI, Baff-R, CD40/CD40L u.a.
- NF $\kappa$ B-Mutanten sind häufig und wichtig in der B Zell-Homeostase
- Komplexe CVID Formen mit Zeichen der kombinierten B und T-Zelldefizienz finden sich bei PI3K $\delta$ -GOF (APDS), PLC $\gamma$ 2-GOF, PRKC $\delta$  (LOF), LRBA-Mutanten. Sie führen zu einem CID mit Antikörpermangel, Lymphoproliferation u. Auto-Antikörper-Bildung. mTor Inhibitoren können bei diesen Störungen u.U. therapeutisch helfen

# Zusammenfassung II

Ein zunehmend dysfunktionelles T Zell Kompartiment findet sich bei Mutationen in T –Zell-spezifischen Genen:

CVID • **ICOS Defizienz:** gestörte folliculäre Th Funktion, fehlendes B Zell Gedächtnis

CID

- **CTLA-4 Defizienz:** funktioneller Treg Defect, Lymphoproliferation, Hypo-gammaglobulinämie. Behandlung mit CTLA-4-Fc (Abatacept, Belatacept) kann bei diesen Patienten temporär helfen.

SCID

- **LAT Defizienz:** persistierende CMV/EBV- Infekte, Lymphknoten- und Milzschwellung, AIHA, ITP, progressive Hypogammaglobulinämie

# Acknowledgments



**Prof. S. Ehl (Medical Director of CCI)**  
**Prof. B. Grimbacher (Scientific Director of CCI)**  
**PD Alexandra Nieters (Epidemiology Unit CCI)**

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**AG Eibel**

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M. Rizzi

**AG U.Salzer**

**Inst. of Pathology UK Fr**  
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**Immune Diagnostics:**

M. Schlesier R.Draeger F. Hässler

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I. Quinti + Lab L. Hammarstrom + Lab  
A.Plebani + Lab a.o.m.

**Sponsors:** DFG SFB620, EU FP7, BMBF

***And especially all our patients!***



# Proteine kinase C $\delta$ (PKCD) Deficiency

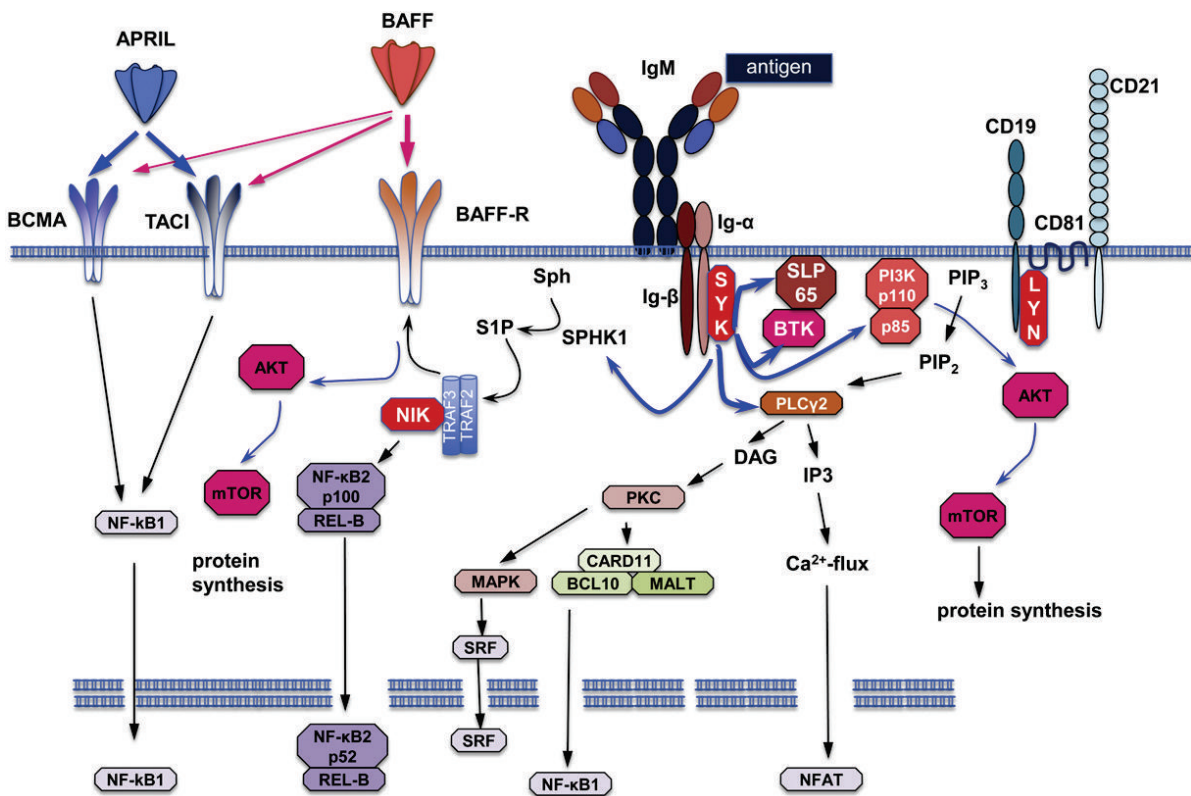
Ombrello et al N Engl J Med. 2012

Kuehn et al Blood. 2013

Belot et al Arthritis & Rheum. 2013

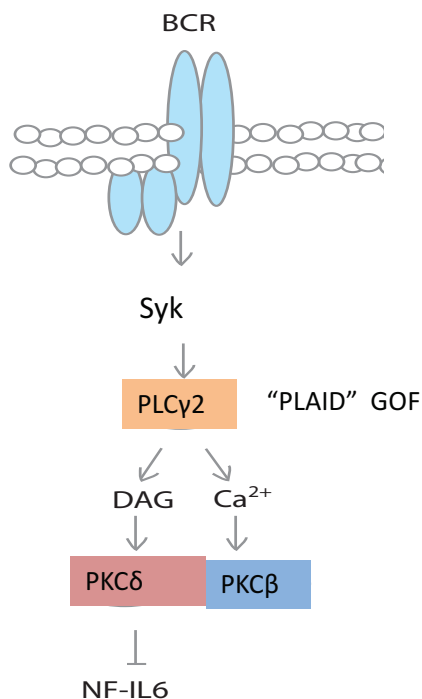
Salzer et al Blood 2013

## Signal-Transduktion in B-Zellen



Pieper et al, *JACI* 2013

# PLC $\gamma$ 2 dependent signaling and deficiency



adapted from Guo *et al*, Curr Opin Immunol, 2004

Ombrello MJ, Remmers EF, SunG .. Milner JD. **Cold urticaria, immunodeficiency and autoantibody related to *PLCG2* deletions.** N Engl J Med. 2012; 366(4): 330–338.

*Heterozygous deletions in *PLCG2* of exon 19 in 2 families and exon 20-22 in one family*  
 → *impairment of autoinhibitory domain and*  
 → *transcripts with constitutive PLC $\gamma$ 2 activity.*

**Table 1. Summary of the Clinical Manifestations of Phospholipase C $\gamma$ 2-Associated Antibody Deficiency and Immune Dysregulation in the Subjects.**

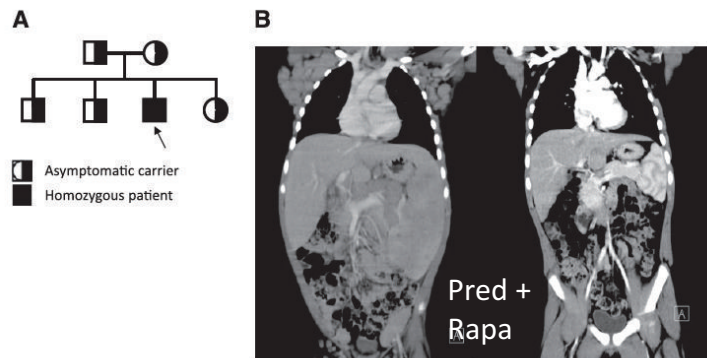
Clinical Manifestation	Frequency
	no./total no. (%)
Cold urticaria	27/27 (100)
Recurrent sinopulmonary infection	12/27 (44)
Antibody deficiency*	15/20 (75)
Common variable immunodeficiency	3/27 (11)
Symptomatic autoimmune disease†	7/27 (26)
Positive test for antinuclear antibodies‡	13/21 (62)
Symptomatic allergic disease	15/27 (56)

## Protein Kinase CD (PKC $\delta$ ) deficiency in humans

Kuehn HS, Niemela JE ... Oliveira JB

**Loss of function of the protein kinase C $\delta$  (PKC $\delta$ ) causes a B cell lymphoproliferation syndrome in humans.**

Blood. 2013;121(16):3117-3125



### Key Points

- Mutations in *PKCD* cause a syndrome characterized by chronic benign lymphadenopathy, NK dysfunction and pos. autoantibodies.
- PKCD deficiency disrupts control of B cell proliferation and apoptosis and affects NK cell cytolytic activity.

- 3 siblings with loss of function mutation in *PKCD*
- Altered B cell phenotype
- PKC $\delta$  involved in deletion of autoreactive B cells → resistance to BCR and Ca<sup>++</sup> dependent apoptosis → high B cell proliferation
- ANA+, glomerulonephritis, skin disease

Belot A, Kasher PR ... Bonnefoy N.

**Protein kinase C $\delta$  deficiency causes mendelian systemic lupus erythematosus with B cell defective apoptosis and hyperproliferation**

Arthritis & Rheumatism. 2013, 65: 2161–2171

# Examples of Autoimmunity associated with distinct monogenic mutations

Phospholipase  $\gamma$ 2 (PLCG2) GOF mutation

(Ombrello et al N Engl J Med. 2012)

Protein kinase C  $\delta$  (PKCD) LOF mutation

(Kuehn et al Blood. 2013, Belot et al Arthritis & Rheum. 2013, Salzer et al Blood 2013)

LAT deficiency,

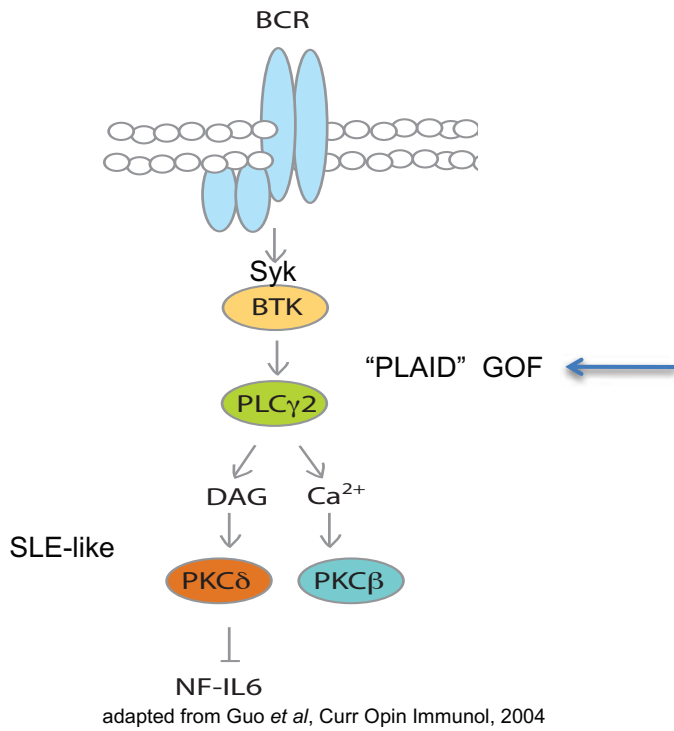
(Keller et al JEM 2016)



# PLC $\gamma$ 2 dependent signaling and deficiency

Ombrello MJ, Remmers EF, SunG .. Milner JD.  
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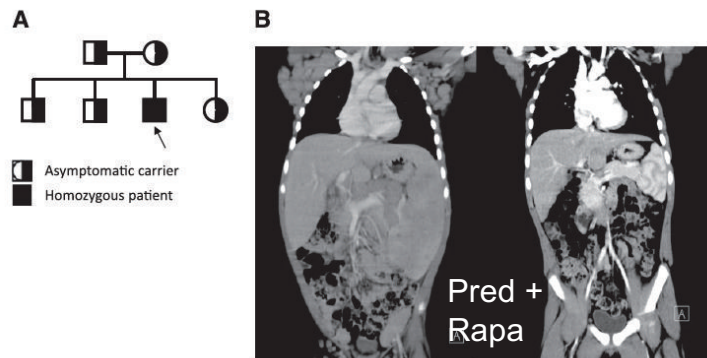
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Belot A, Kasher PR ... Bonnefoy N.

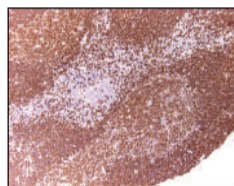
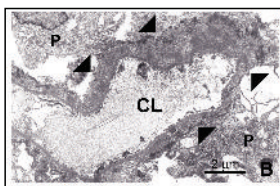
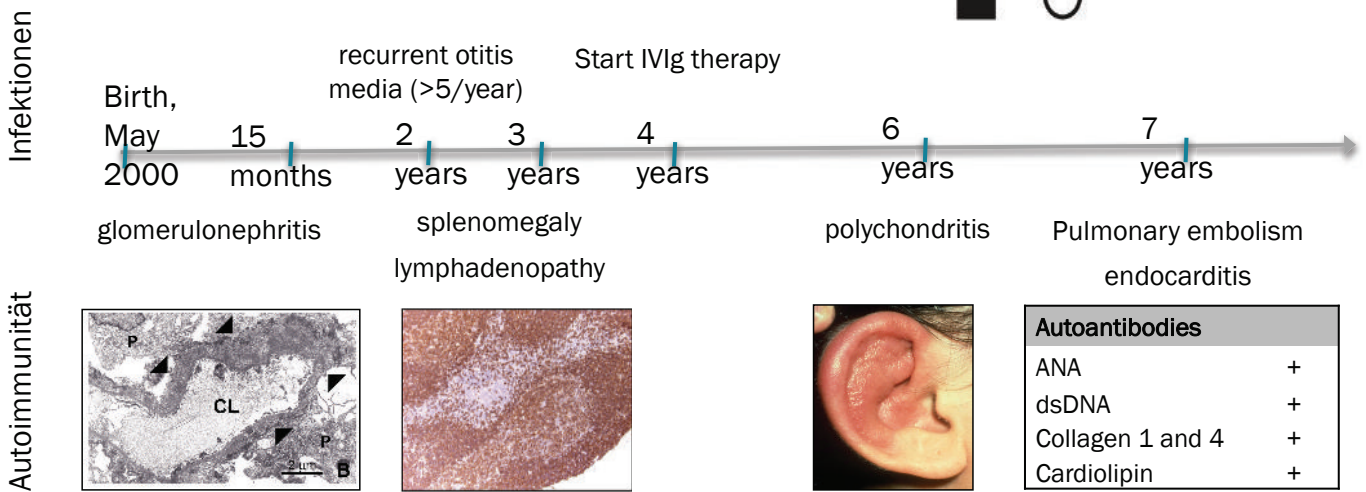
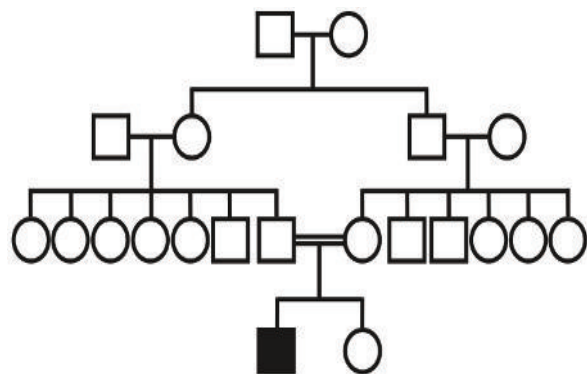
### Protein kinase C $\delta$ deficiency causes mendelian systemic lupus erythematosus with B cell defective apoptosis and hyperproliferation

Arthritis & Rheumatism. 2013, 65: 2161–2171

- 3 siblings with loss of function mutation in *PKCD*
- Altered B cell phenotype
- PKC $\delta$  involved in deletion of autoreactive B cells → resistance to BCR and Ca<sup>++</sup> dependent apoptosis → high B cell proliferation
- ANA+, glomerulonephritis, skin disease

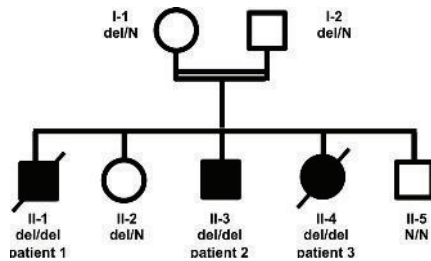
# A new systemic SLE-like disorder due PKC $\delta$ deficiency

Salzer, E., E. Santos-Valente, S. Klaver, ...  
 K Boztug **B-cell deficiency and severe autoimmunity caused by deficiency of protein kinase C delta.**  
*Blood* 2013; 112-3116

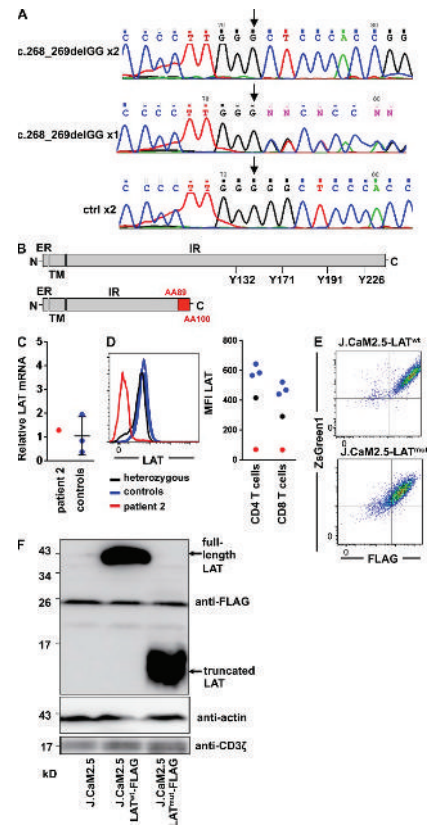


## Early onset combined immunodeficiency and autoimmunity in patients with loss of-function mutation in *LAT* (*linker for activation of T cells*)

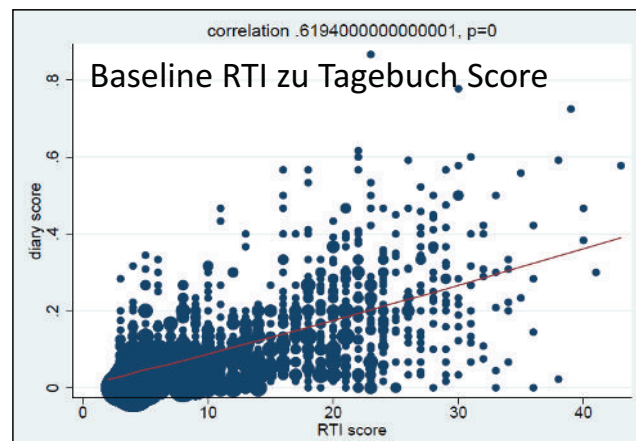
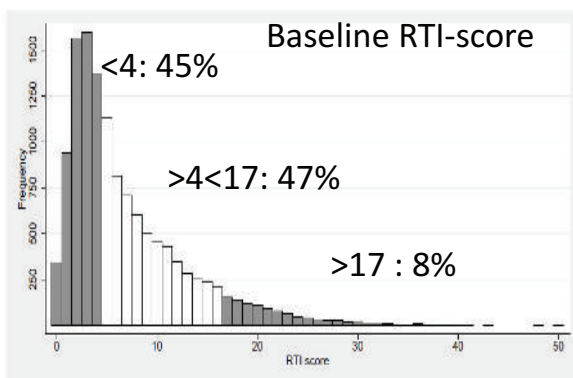
Keller B ... Warnatz K, Stepinsky P et al J.Exp. Med. 2016; 213:1185-1199



	5 mo	6 mo	10 mo	
Age of presentation	5 mo	6 mo	10 mo	
Infections	CMV, EBV Pneumonia + viremia	Congenital toxoplasmo- sis, CMV pneumonia	CMV viremia Recurrent pneumonia, GI infections	
Autoimmunity	Coombs + AIHA, ITP, ANP	Coombs+ AIHA, ITP	Anti- ADAMTS13+ MHA	
Lympho- proliferation	Lymphnodes, Spleen	LN, Spleen	LN, Spleen	
Lung	Chronic LD Bronchiect.	Chronic LD Bronchiect.	none	
Serum-Ig	Progressive Hypo-γ	Progressive Hypo-γ	Hyper-γ at 2y	
Outcome	Died at 9y due to CMV	Alive, 13 mo after ASCT	Died at 2y due to MHA	



# AWIS: RTI-Score und Tagebuch-Score



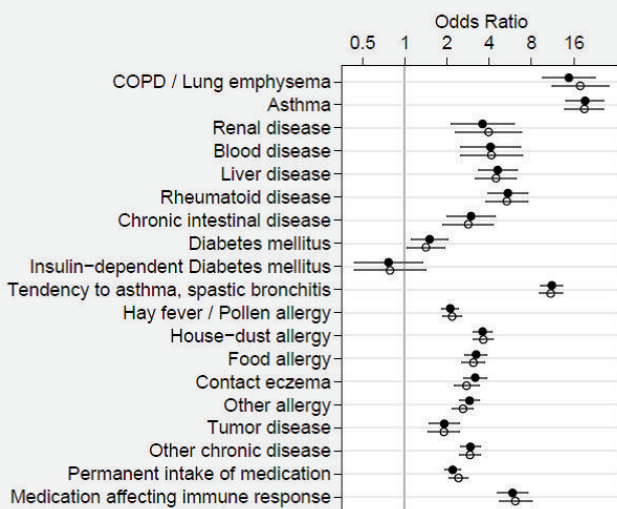
***Tagebuch Score ist nach 3 Jahren signifikant erhöht u.a. bei***

- hohem baseline RTI score
- Übergewicht und Fettsucht
- Rauchen
- Z.n. Operationen an Organen des Immunsystems
- Erhöhtem subjektiven Stresserleben
- Diverse Komorbiditäten vor allem Allergien und COPD

## Co-morbidities:

- Co-morbidities associated with high RTI score, particularly asthma and COPD

### Analysis of cross-sectional data\*



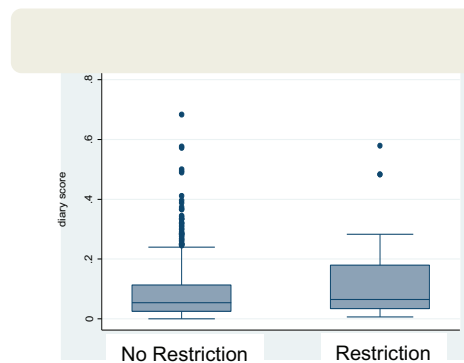
\*Adjusted for age, gender, education level, BMI, smoking (open circles)

MD thesis: Stefan Wolfrum

### Analysis of LUFU data

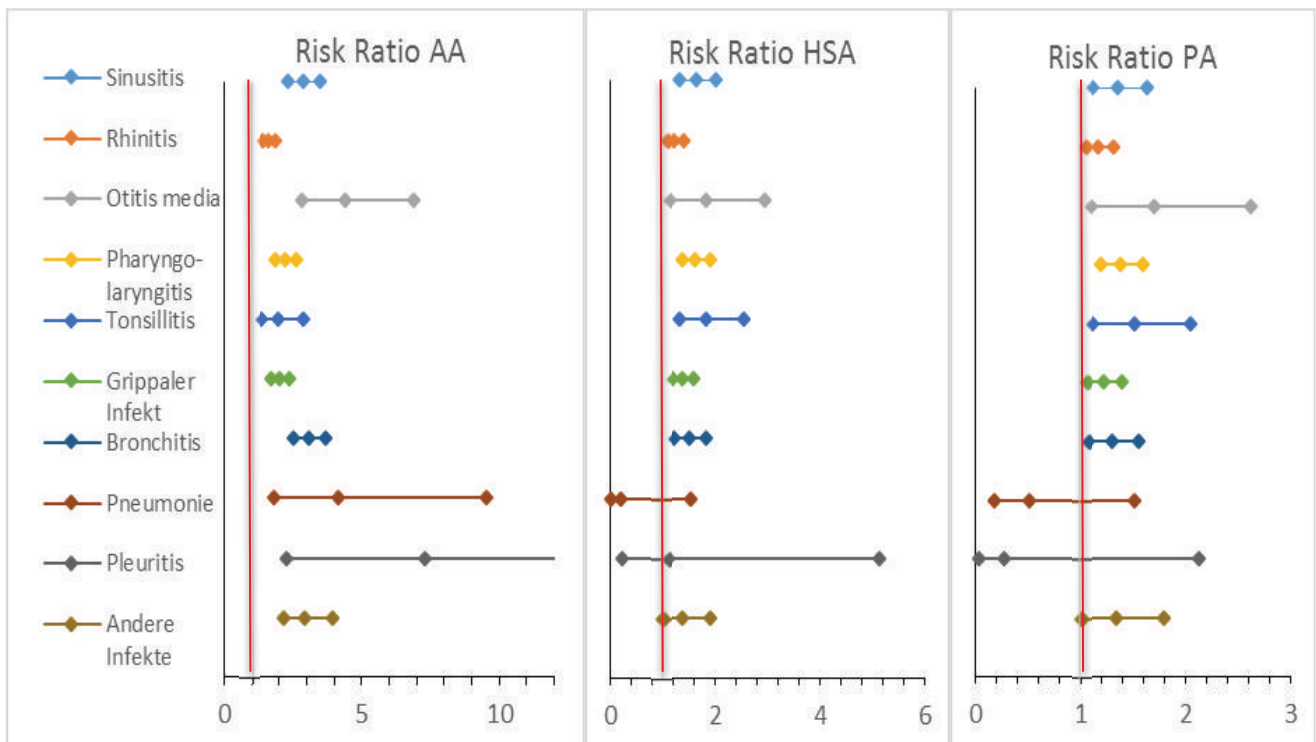
Obstruction not associated with future RTIs

Restriction significantly associated with future RTIs

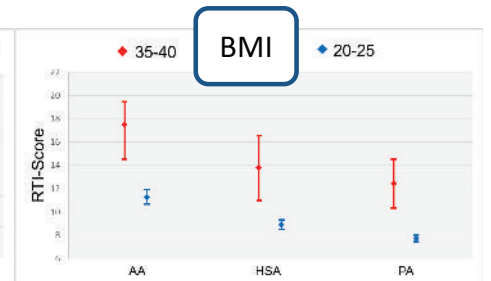
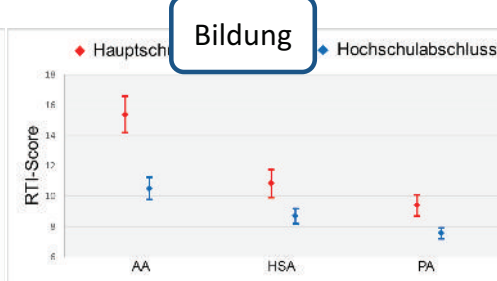
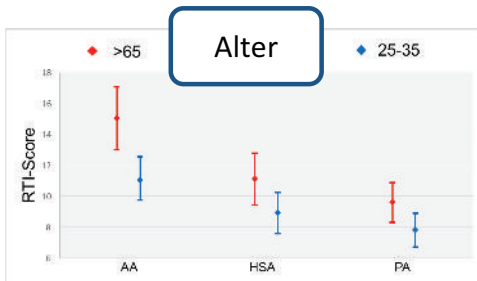
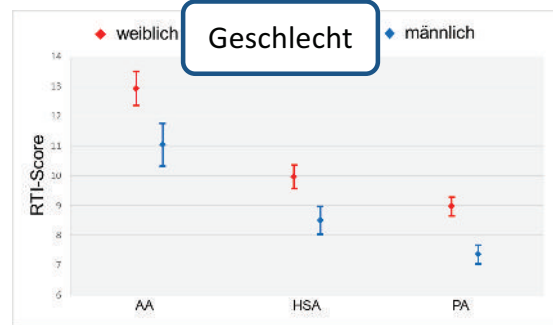
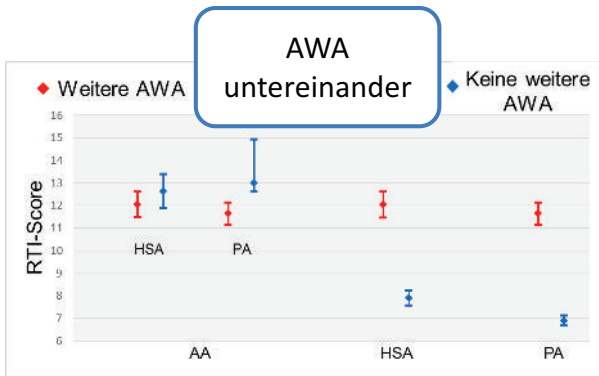


MD thesis: Jan Rosenbaum

## Risk Ratios - Atemwegsallergien und Infektionsvariablen



# Einflussfaktoren I





# Einflussfaktoren II

