Bodo Grimbacher
Angeborene Immundefekte, 2. Oktober 2017, Stuttgart
Categories of Primary Immunodeficiencies

"early-onset" (<= 15 years), "late-onset" (> 15 years)

source: ESID registry: www.esid.org
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

Schubert et al. 2014 Nature Medicine 20;1410–1416
The clinical phenotype is characterized by T-cell-mediated inflammation, lymphoproliferation, and hypogammaglobulinemia.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Percentage of &quot;Affected&quot; Mutation Carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypogammaglobulinemia</td>
<td>84%</td>
</tr>
<tr>
<td>Lymphoproliferation</td>
<td>73%</td>
</tr>
<tr>
<td>Respiratory tract involvement</td>
<td>67%</td>
</tr>
<tr>
<td>Gastrointestinal involvement</td>
<td>60%</td>
</tr>
<tr>
<td>Hematological involvement</td>
<td>59%</td>
</tr>
<tr>
<td>Skin involvement</td>
<td>55%</td>
</tr>
<tr>
<td>Clinically reactivated/apparent infections</td>
<td>53%</td>
</tr>
<tr>
<td>Endocrinological involvement</td>
<td>34%</td>
</tr>
<tr>
<td>Neurological involvement</td>
<td>29%</td>
</tr>
<tr>
<td>Growth retardation</td>
<td>14%</td>
</tr>
<tr>
<td>Arthritis</td>
<td>14%</td>
</tr>
<tr>
<td>Liver involvement</td>
<td>13%</td>
</tr>
<tr>
<td>Kidney involvement</td>
<td>13%</td>
</tr>
<tr>
<td>Malignancy</td>
<td>11%</td>
</tr>
</tbody>
</table>
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

130 mutation carriers with 42 different mutations

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

Transmembrane domain (exon3): 6 mutations

<table>
<thead>
<tr>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.2T&gt;C; p.? §</td>
</tr>
<tr>
<td>c.25_26 insulation ACAAGGCTCAGCTG; p.N14Ffs*5 §</td>
</tr>
<tr>
<td>c.34C&gt;T; p.Q12*</td>
</tr>
<tr>
<td>c.76_77InsT; p.F28Sfs*40 §</td>
</tr>
<tr>
<td>c.94_101delInsTTCCTTCATCA; p.P32Ffs*29</td>
</tr>
<tr>
<td>c.105C&gt;A; p.C35* §</td>
</tr>
<tr>
<td>c.109+1G&gt;T §</td>
</tr>
<tr>
<td>c.534C&gt;G, p.S178R</td>
</tr>
<tr>
<td>c.530_543del; p.F179Cfs*29</td>
</tr>
<tr>
<td>c.529T&gt;G; p.Y177D §</td>
</tr>
<tr>
<td>c.529_530InsA; p.Y177Fs*</td>
</tr>
<tr>
<td>c.518G&gt;A; p.G173E §</td>
</tr>
<tr>
<td>c.494G&gt;A; p.W165*</td>
</tr>
</tbody>
</table>

§, have previously been described

<em>CTLA4</em> Haploinsufficiency Presenting Gastric Cancer. 
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...

...presenting symptoms...

87

„affected“

...without need for special medical care/treatment...

43

„unaffected“
Hypogammaglobulinemia is most common symptom in affected and unaffected mutation carriers whereas lymphoproliferation occurs primarily in the affected individuals.
Previously unaffected CTLA4 insufficiency
Necrotizing fasciitis, *S. pyogenes* cultured
**Immunological phenotype**

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

![Graphs showing absolute lymphocytes, CD3+, and CD19+ B cells](image)
CTLA-4 expression in FoxP3+ Treg cells

MFI CTLA-4

CTLA-4+/+  CTLA-4+-  CTLA-4+-  CTLA-4+-

unaffected  affected

*****  *  

1000000

100000

10000

1000

100 100 100
**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 missense 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 involvement 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

<table>
<thead>
<tr>
<th>Drug</th>
<th>Total patients</th>
<th>Good response</th>
<th>Positive effects</th>
<th>Transient effect</th>
<th>Side effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rituximab</td>
<td>25</td>
<td>15</td>
<td>Cytopenia (3), GI symptoms (1)</td>
<td>0</td>
<td>B cell loss in general</td>
</tr>
<tr>
<td>Sirolimus</td>
<td>12</td>
<td>7</td>
<td>GI symptoms (3), splenomegaly (2), cytopenia (2), CMV load (1), lymphadenopathy (1)</td>
<td>1</td>
<td>Raising CMV load (1), maintenance of lymphopenia (1), infections (1), sepsis (1)</td>
</tr>
<tr>
<td>Abatacept/Belatacept</td>
<td>13</td>
<td>11</td>
<td>GI symptoms (5), respiratory symptoms (2), lymphadenopathy (1), dropping of sIL2 receptor (1)</td>
<td>1</td>
<td>EBV reactivation (2) → HLH (1)</td>
</tr>
<tr>
<td>Patient (sex)</td>
<td>Age of onset (y)</td>
<td>Primary manifestations (first symptom)</td>
<td>Age of death (y)</td>
<td>Primary cause of death</td>
<td></td>
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<td>--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
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<td>-----------------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>P1 (m)</td>
<td>12</td>
<td>CVID; lung disease; <strong>neurological involvement</strong>; enteropathy</td>
<td>34</td>
<td>Wasting enteropathy and lung disease</td>
<td></td>
</tr>
<tr>
<td>P2 (f)</td>
<td>17</td>
<td>CVID; <strong>respiratory involvement</strong></td>
<td>37</td>
<td>Relaps of disease following lung transplantation</td>
<td></td>
</tr>
<tr>
<td>P3 (m)</td>
<td>15</td>
<td>Enteropathy</td>
<td>23</td>
<td>Acute liver failure</td>
<td></td>
</tr>
<tr>
<td>P4 (f)</td>
<td>10</td>
<td>Respiratory involvement</td>
<td>16</td>
<td>Relaps of disease following lung transplantation</td>
<td></td>
</tr>
<tr>
<td>P5 (f)</td>
<td>7</td>
<td>CVID; <strong>growth retardation</strong></td>
<td>24</td>
<td>Septic shock</td>
<td></td>
</tr>
<tr>
<td>P6 (m)</td>
<td>8</td>
<td>Evans Syndrom</td>
<td>23</td>
<td>Uk; following colectomy for severe enteropathy</td>
<td></td>
</tr>
<tr>
<td>P7 (f)</td>
<td>12</td>
<td>Enteropathy; <strong>respiratory involvement</strong></td>
<td>24</td>
<td><strong>septic embolism (MRSA infection)</strong></td>
<td></td>
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<tr>
<td>P8 (m)</td>
<td>10</td>
<td>ALPS-like phenotype; <strong>cytopenia</strong></td>
<td>21</td>
<td>Septic multiorgan failure</td>
<td></td>
</tr>
<tr>
<td>P9 (f)</td>
<td>26</td>
<td>Lymphoma; <strong>cytopenia</strong></td>
<td>53</td>
<td>Lymphoma</td>
<td></td>
</tr>
<tr>
<td>P10 (f)</td>
<td>26</td>
<td>Evans syndrome; <strong>neurological involvement</strong></td>
<td>40</td>
<td>Sepsis (GI perforation)</td>
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<tr>
<td>P11 (m)</td>
<td>10</td>
<td>Enteropathy</td>
<td>35</td>
<td>Bacterial sepsis</td>
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<tr>
<td>P12 (m)</td>
<td>10</td>
<td>Lymphadenopathy; <strong>cytopenia</strong></td>
<td>15</td>
<td>Post HSCT, GvHD</td>
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<tr>
<td>P13 (m)</td>
<td>2</td>
<td>Enteropathy; <strong>type 1 diabetes</strong></td>
<td>22</td>
<td>Post HSCT, metabolic ketoacidosis</td>
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<tr>
<td>P14 (m)</td>
<td>6</td>
<td>Lymphoma; <strong>cytopenia</strong></td>
<td>22</td>
<td>Lymphoma</td>
<td></td>
</tr>
<tr>
<td>P15 (f)</td>
<td>1</td>
<td>Cytopenia; respiratory/neurologic involvement</td>
<td>14</td>
<td>Post HSCT, GvHD</td>
<td></td>
</tr>
<tr>
<td>P16 (f)</td>
<td>40</td>
<td>CVID; <strong>respiratory involvement</strong></td>
<td>73</td>
<td>HCV infection</td>
<td></td>
</tr>
<tr>
<td>P17 (f)</td>
<td>uk</td>
<td>Lymphoma, <strong>endocrinological involvement</strong></td>
<td>60</td>
<td>Lymphoma</td>
<td></td>
</tr>
</tbody>
</table>

However, 17 patients (almost 20%) have died at an average age of 23 years.
<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Reason for HSCT</th>
<th>HLA-match/ Donor</th>
<th>Conditioning</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>P 1</td>
<td>15</td>
<td>cITP; widespread lymphoid hyperplasia</td>
<td>10/10; MUD; PBSC</td>
<td>Alem, Flu, Mel</td>
<td>Death due to GvHD</td>
</tr>
<tr>
<td>P 2</td>
<td>20</td>
<td>Cytopenia; bronchiectasis; enteropathy (TPN-dependent)</td>
<td>10/10; MUD; PBSC</td>
<td>Alem, Flu, Mel</td>
<td>Died 2.5 years after due to diabetic ketoazidosis</td>
</tr>
<tr>
<td>P 3</td>
<td>14</td>
<td>Immunodeficiency; CMV infection</td>
<td>9/10; MUD; PBSC</td>
<td>Thio, Flu, Treo</td>
<td>Death due to GvHD</td>
</tr>
<tr>
<td>P 4</td>
<td>16</td>
<td>Cytopenia; arthritis; lymphadenopathy</td>
<td>10/10; MUD; PBSC</td>
<td>Alem, Flu, Treo</td>
<td>Alive and well 6.1 years</td>
</tr>
<tr>
<td>P 5</td>
<td>10</td>
<td>Enteropathy; Cytopenia</td>
<td>9/10; MUD; BM</td>
<td>Alem, Flu, Mel</td>
<td>Alive and well 10.5 years</td>
</tr>
<tr>
<td>P 6</td>
<td>16</td>
<td>Cytopenia; lymphoproliferation</td>
<td>9/10; MUD; BM</td>
<td>Thio, Flu, Bu</td>
<td>Alive and well 5.6 years</td>
</tr>
<tr>
<td>P 7</td>
<td>51</td>
<td>Hodgkin lymphoma; HLH</td>
<td>10/10; MUD; BM</td>
<td>Thio, Flu, Bu</td>
<td>Alive and well 100 days follow-up</td>
</tr>
<tr>
<td>P 8</td>
<td>13</td>
<td>Cytopenia; autoimmune-encephalitis</td>
<td>10/10; MUD; BM</td>
<td>Thio, Flu, Treo, ATG</td>
<td>Alive and well 10 months follow-up</td>
</tr>
<tr>
<td>P 9</td>
<td>20</td>
<td>Cytopenia; hemolysis, vasculitis, paraplegy</td>
<td>10/10; MUD; BM</td>
<td>Thio, Flu, Treo</td>
<td>Alive and well 10 months follow-up</td>
</tr>
<tr>
<td>P 10</td>
<td>17</td>
<td>Hodgkin lymphoma; enteropathy</td>
<td>10/10; MUD; BM</td>
<td>Alem, Flu, Treo, Thio</td>
<td>Alive and well 12 months follow-up</td>
</tr>
<tr>
<td>P 11</td>
<td>14</td>
<td>Recurrent infections, enteropathy</td>
<td>7/8; MMUD; BM</td>
<td>Flu, Mel, TBI 3 Gy</td>
<td>Alive and well 7 months follow-up</td>
</tr>
<tr>
<td>P 12</td>
<td>14</td>
<td>Enteropathy, respiratory disease</td>
<td>10/10; MUD; BM</td>
<td>Alem, Flu, Bu</td>
<td>Alive and well 90 days follow-up</td>
</tr>
</tbody>
</table>
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.

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 includes antibody deficiency, recurrent respiratory infections,
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...

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 Grimbacher1,14,*

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DOI 10.1007/s10875-016-0306-1

**ORIGINAL ARTICLE**

**NF-κB1 Haploinsufficiency Causing Immunodeficiency and EBV-Driven Lymphoproliferation**

Heidrun Boztug1 · Tatjana Hirschmugl2 · Wolfgang Holter1 · Karoly Lakatos1 · Leo Kager1 · Doris Trapin3 · Winfried Pickl3 · Elisabeth Förster-Waldl4 · Kaan Boztug1,2,4,5

**Specific antibody deficiency and autoinflammatory disease extend the clinical and immunological spectrum of heterozygous NFκB1 loss-of-function mutations in humans**

by Cyrill Schipp, Schafiq Nabhani, Kirsten Bienemann, Natalia Simanovsky, Chiara Kiefermann, Nathalia Aguar, Adaxios, Freiburg T. Cosman, Sheikha Royal Villa

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Extended phenotype of NFkβ 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 Rouonioja, 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,f 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

H67R
Behcet-like small-vessel vasculitis and antibody deficiency

I553M
CVID-like disease with autoimmunity

R157X
Familial necrotizing cellulitis

NFκB1 mutations

Death
Ankyrin repeats
I553M
R157X
H67R
GRR
Rel
p50
RelA
p50
IKKβ
IKKa
NEMO

p105

p50

p50

p50

p105

p105

p105

Pp
Pp

Pp

Pp

Pp

H67R: Reduced nuclear entry
R157X: Reduction in amount of p50 and p105
I553M: Enhanced degradation of p105

MEK/ERK activation
NF-κB inhibition

[Diagram of NFκB pathway]
NFkB1 Mutations

- Autosomal dominant inheritance

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

- Autosomal dominant inheritance
NFkB1 Mutations

- Autosomal dominant inheritance

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

- 44 patients with NFkB1 mutations

- 3 asymptomatic
- 41 symptomatic
Clinical manifestations

- 44 patients with NFkB1 mutations

- Respiratory disease: 73%
  - URTIs: 59%
  - LRTIs: 51%
- Gastrointestinal involvement: 49%
  - Enteropathy/IBD: 46%
  - Gastritis: 5%
- Lymphoproliferation: 49%
  - Splenomegaly: 39%
  - Lymphadenopathy: 32%
  - Hepatomegaly: 17%
- Autoimmune cytopenia: 22%
  - ITP: 20%
  - AIHA: 15%
- Endocrine involvement: 15%
  - Hypothyroidism: 12%
- Secondary hyperparathyroidism: 2%
- Tumours: 15%
  - Malignant skin tumours: 5%
  - Hemangiomas: 5%
  - Lymphoma: 2%
- Neurologic involvement: 12%
- Arthritis: 7%
- Herpes zoster: 10%
- Chronic norovirus: 7%
- Salmonellosis: 7%
- JC encephalitis: 2%
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
LRBA deficiency: PID and immune dysregulation syndrome

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

<table>
<thead>
<tr>
<th>Condition</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune dysregulation</td>
<td>95%</td>
</tr>
<tr>
<td>Enteropathy</td>
<td>62%</td>
</tr>
<tr>
<td>AIHA</td>
<td>57%</td>
</tr>
<tr>
<td>ITP</td>
<td>52%</td>
</tr>
<tr>
<td>GLIDL</td>
<td>38%</td>
</tr>
<tr>
<td>Diabetes</td>
<td>24%</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>24%</td>
</tr>
<tr>
<td>Chronic autoimmune hepatitis</td>
<td>14%</td>
</tr>
<tr>
<td>Eczema</td>
<td>10%</td>
</tr>
<tr>
<td>Uveitis</td>
<td>10%</td>
</tr>
<tr>
<td>Alopecia</td>
<td>5%</td>
</tr>
<tr>
<td>Organomegaly</td>
<td>86%</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>64%</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td>33%</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>24%</td>
</tr>
<tr>
<td>Recurrent Infections</td>
<td>71%</td>
</tr>
<tr>
<td>Parenchymal lung damage</td>
<td>52%</td>
</tr>
<tr>
<td>Upper respiratory infections</td>
<td>48%</td>
</tr>
<tr>
<td>Lower respiratory infections</td>
<td>41%</td>
</tr>
<tr>
<td>Urinary infections</td>
<td>10%</td>
</tr>
<tr>
<td>Hypo/Dy gammaglobulinemia</td>
<td>57%</td>
</tr>
<tr>
<td>Low IgG</td>
<td>52%</td>
</tr>
<tr>
<td>Low IgA</td>
<td>38%</td>
</tr>
<tr>
<td>Low IgM</td>
<td>29%</td>
</tr>
<tr>
<td>Failure to thrive</td>
<td>24%</td>
</tr>
<tr>
<td>Stroke</td>
<td>5%</td>
</tr>
<tr>
<td>Deafness</td>
<td>5%</td>
</tr>
</tbody>
</table>

Laura Gamez  
Tuesday, 12 noon  
Pentland Suite
Biology of CTLA4 recycling

Monogenetic Causes for Hypogamma-/Agammaglobulinemia

N = 451 patients
114 (25%) with clear mutation
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 compound heterozygous mutation in \textit{CECR1} ... 

**Genetic Analysis**

<table>
<thead>
<tr>
<th>Mutation</th>
<th>mother</th>
<th>father</th>
<th>patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chr22:17687997C&gt;T R169Q</td>
<td><img src="image1" alt="Graph" /></td>
<td><img src="image2" alt="Graph" /></td>
<td><img src="image3" alt="Graph" /></td>
</tr>
<tr>
<td>Chr22:17684478A&gt;C M243R</td>
<td><img src="image4" alt="Graph" /></td>
<td><img src="image5" alt="Graph" /></td>
<td><img src="image6" alt="Graph" /></td>
</tr>
</tbody>
</table>
CECR1 encodes for ADA2.

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

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


**Mutant adenosine deaminase 2 in a polyarteritis nodosa vasculopathy.**

ADA2: Current State-of-Art

ADA2 activity

NR: 83-271 mU/g plasma protein
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 Hypogammagamma-/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


Resting B cell

- FoB
- MZB
- B1B

Plasma cell

- splPB
- splPC
- bmPC

300 genes define a plasma cell signature

Gene expression
Translation
- Intracellular protein transport
- Glycosylation
- UPR
- Amino acid metabolism
- Miscellaneous metabolism

mouse, mRNA, sorted cells, ex vivo
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

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

- B cells from peripheral blood 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

**Day 0**  
**Day 3**  
**Day 6**  
**Day 9**  

**Plasmablasts**

- **Day 0**: 0.00%
- **Day 3**: 0.103%
- **Day 6**: 0.208%
- **Day 9**: 4.71%

**Ig secretion:**

**Day 9**

- **IgG**: 1000 ng/ml (control), 800 ng/ml (patient)
- **IgA**: 1500 ng/ml (control), 1200 ng/ml (patient)

52 · 2. Oktober 2017
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**

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

**Variant Details:**

<table>
<thead>
<tr>
<th>Protein Acc.</th>
<th>Organism</th>
<th>SEC61A1 p.V85</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP_037468.1</td>
<td>Homo sapiens</td>
<td>NRGTL MELGISPIVTSSLIMQLLAGAK</td>
</tr>
<tr>
<td>NP_058602.1</td>
<td>Mus musculus</td>
<td>NRGTL MELGISPIVTSSLIMQLLAGAK</td>
</tr>
<tr>
<td>NP_954865.1</td>
<td>Rattus norvegicus</td>
<td>NRGTL MELGISPIVTSSLIMQLLAGAK</td>
</tr>
<tr>
<td>NP_001003315.1</td>
<td>Canis lupus familiaris</td>
<td>NRGTL MELGISPIVTSSLIMQLLAGAK</td>
</tr>
<tr>
<td>NP_001035594.1</td>
<td>Bos taurus</td>
<td>NRGTL MELGISPIVTSSLIMQLLAGAK</td>
</tr>
<tr>
<td>NP_001080244.1</td>
<td>Xenopus laevis</td>
<td>NRGTL MELGISPIVTSSLIMQLLAGAK</td>
</tr>
<tr>
<td>NP_595226.1</td>
<td>S. pombe</td>
<td>NRGTL MELGISPIVTSSLIMQLLAGAK</td>
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<tr>
<td>XP_958835.1</td>
<td>Neurospora crassa</td>
<td>NRGTL MELGISPIVTSSLIMQLLAGAK</td>
</tr>
<tr>
<td>XP_710932.1</td>
<td>Candida albicans</td>
<td>NRGTL MELGISPIVTSSLIMQLLAGAK</td>
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<tr>
<td>NP_013482.1</td>
<td>S. cerevisiae</td>
<td>NRGTL MELGISPIVTSSLIMQLLAGAK</td>
</tr>
<tr>
<td>NP_986143.1</td>
<td>Ashbya gossypii</td>
<td>NRGTL MELGISPIVTSSLIMQLLAGAK</td>
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</tbody>
</table>
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 complex passively acts as a calcium leakage channel

SEC61A1-V85D

Valine

Aspartic acid


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

- 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

CCI Freiburg
Annemarie Gabrysch
Charlotte Schwab
Natalie Frede
Desirée Schubert
Katrin Hübscher
Alla Bulashevska
Maximilian Seidl
Annette Schmitt-Gräff
Ulrich Salzer
Klaus Warnatz
Florian Emmerich
Carsten Speckmann
Christian Klemann
Stephan Ehl

Sevilla, Spain
Peter Olbrich
José Manuel Lucena
Olaf Neth

Tokyo, Japan
Akihiro Hoshino
Hirokazu Kanegane
Kohsuke Imai

Montevideo, Uruguay
Virginia Patiño

Regensburg, Germany
Daniel Wolff
Sebastian Klobuch

Oslo, Norway
Ingunn Dybedal
Kjetil Taskén

Newcastle upon Tyne, UK
Elizabeth McDermott
Su Bunn
Mary Slatter
Sophie Hambleton
Peter Arkwright
Andrew Cant

Prague, Czech Republic
Lenka Petruzlkova
Zdenek Sumnik
Anna Sediva

Boston, USA
Alan Leichtner
Maya DeGroote
Richard Blumberg
Scott Snapper
Craig Platt
Talal Chatila
Raif Geha
Janet Chou

Dresden, Germany
Sebastian Zeissig

London, UK
Saranjith Seneviratne
Jamanda Haddock
David Sansom

Zurich, Switzerland
Jana Pachlupnik Schmid
Antonios Kolios

Hiroshima, Japan
Satoshi Okada
Masao Kobayashi
Seiichi Hayakawa

München, Germany
Fabian Hauck
Michael H. Albert

Brescia, Italy
Vassilios Lougaris
Alessandro Plebani

Athens, Greece
Maria Kanariou

Heidelberg, Germany
Thomas Giese
Hanns-Martin Lorenz

Cincinnati, USA
Zeynep Yesim Kucuk

Montréal, Canada
Hugo Chapdelaine

Brno, Czech Republic
Tomas Freiberger
Jiri Litzman

Prague, Czech Republic
Veronika Kandrová
Eva Froňková

New Haven, USA
Christina Price

Jena, Germany
Monika Kurzai

Ulm, Germany
Ansgar Schulz

Melbourne, Australia
Gary Unglik

Nijmegen, The Netherlands
Frank van de Veerdonk

Krefeld, Germany
Tim Niehues
Gregor Dückers

Barcelona, Spain
Laia Alsina
Ferran Casals
Angela Deyà-Martinez

New York, USA
Lisa Giulino-Roth
Olivier Elemento

Basel, Switzerland
Mike Recher

Liège, Belgium
Michel Moutschen

Pennsylvania, USA
Kathleen E. Sullivan

Federal Ministry of Education and Research
Acknowledgements NFkB1

- Center for Chronic Immunodeficiency (CCI), Freiburg
  - Natalie Frede
  - Manfred Fliegauf
  - Alla Bulashevska
  - Jessica Rojas
  - Katrin Hübscher

- Tiziana Lorenzini (Brescia, Italy)
- Alessandro Plebani (Brescia, Italy)
- Polina Stepansky (Jerusalem, Israel)
- Marielle van Gijn (Nijmegen, Netherlands)
- Ute Fischer (Düsseldorf, Germany)
- Lennart Hammarström (Stockholm, Sweden)
Thank you for your attention!

• Special thanks goes to all physicians, patients and healthy controls

University Medical Center Freiburg
Desirée Schubert
Janine Kemming
Johannes Kühn
Manfred Fliegauf
Sandra Winzer
Alla Bulashevska
Linlin Yang
Michele Proietti
Andrés Caballero-Oteyza
Hermann Eibel
Marta Rizzi
Reinhard Voll
Stephan Rusch
Yong Li
Anna Köttgen

Saarland University, Homburg
Richard Zimmermann
Adolfo Cavalié
Marie-Christine Klein
Sarah Haßdenteufel
Nico Schäuble

Max-Planck Institute of Biochemistry, Martinsried
Stefan Pfeffer

NIH, Bethesda
Alejandro Schäffer

University of California, San Francisco
Jennifer M. Puck

University Medical Centre Hamburg
Robin Kobbe

University Hospitals Regional Hospitals Richmond Heights
Amy Marks, Brian P. Peppers, Robert W. Hostoffer

Science and Technology Department, BGI-Shenzhen
Hongzhi Cao
Fang Yang

IRB Bellinzona
Roger Geiger
Advances in Primary Immunodeficiency

19th – 21st February 2018
at Windsor Park
A Winter School run by the
UCL Centre for Immunodeficiency

Faculty include:
Professor Luigi Notarangelo: NIH, Bethesda, USA
Professor Bodo Grimbacher: Consultant Immunologist, CCI, Freiburg, Germany
Professor Sophie Hambleton: Consultant in Paediatric Immunology, GNCH and Newcastle University
Dr Andy Gernery: 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 rekurringender 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

Disulfid bond

<5%, hat nur kurze Halbwertszeit

Fehlt bei 8% der Bevölkerung
Fall 1

- Die Patientin könnte einen IgG2-Subklasendefekt haben.
Die Patientin könnte einen CVID entwickeln

Cunningham-Rundles et al., 1989
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

шение
- 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

<table>
<thead>
<tr>
<th>Full blood count</th>
<th>Mean level</th>
<th>Normal range(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelets ((x\ 10^9/L))</td>
<td>22</td>
<td>150 – 400</td>
</tr>
<tr>
<td>White blood cell count ((x10^9/L))</td>
<td>10.3</td>
<td>3.5 – 11.0</td>
</tr>
<tr>
<td>Neutrophils ((x10^9/L))</td>
<td>8.0</td>
<td>2.0 – 8.0</td>
</tr>
<tr>
<td>Lymphocytes ((x10^9/L))</td>
<td>1.01</td>
<td>1.0 – 3.5</td>
</tr>
<tr>
<td>Haemoglobin ((g/L))</td>
<td>120</td>
<td>115 – 160</td>
</tr>
<tr>
<td>Red blood cell count ((x10^{12}/L))</td>
<td>4.3</td>
<td>3.9 – 5.4</td>
</tr>
</tbody>
</table>

\(^a\)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.

<table>
<thead>
<tr>
<th>Measurement of blood immunoglobulins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Mean levels (g/L)</td>
</tr>
<tr>
<td>Normal range</td>
</tr>
</tbody>
</table>

- 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 Weichteilinfektionen (Abszesse der inneren Organe)
5. Besondere Erreger (atypisch/opportunist.)
6. Positive Familienanamnese
Die Fitness deines Immunsystems

SCID patient

90yr-old, never ill

SD = Standard Deviation
Diagnostizierbar durch die aktuellen Tests

Die Fitness deines Immunsystems
Die Fitness deines Immunsystems

Nicht eindeutig diagnostizierbar durch die aktuellen Tests
Center for Chronic Immunodeficiency
CCI, Freiburg, Germany

bodo.grimbacher@uniklinik-freiburg.de

www.uniklinik-freiburg.de/cci