La genetica al servizio della clinica nelle immunodeficienze primitive

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SPECIAL ARTICLE

PRIMARY IMMUNODEFICIENCIES

Report of a World Health Organization Committee

### TABLE II
Classification of Primary Immunodeficiency Disorders

<table>
<thead>
<tr>
<th>Type</th>
<th>Suggested Cellular Defect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B-cells</td>
</tr>
<tr>
<td>Infantile X-linked agammaglobulinemia</td>
<td>+</td>
</tr>
<tr>
<td>Selective immunoglobulin deficiency (IgA)</td>
<td>+</td>
</tr>
<tr>
<td>Transient hypogammaglobulinemia of infancy</td>
<td>+</td>
</tr>
<tr>
<td>X-linked immunodeficiency with hyper-IgM</td>
<td>+</td>
</tr>
<tr>
<td>Thymic hypoplasia (pharyngeal pouch syndrome, DiGeorge's syndrome)</td>
<td>+</td>
</tr>
<tr>
<td>Episodic lymphopenia with lymphocytopenia</td>
<td>+</td>
</tr>
<tr>
<td>Immunodeficiency with normal or without hyperimmunglobulinemia</td>
<td>+</td>
</tr>
<tr>
<td>Immunodeficiency with ataxia-telangiectasia</td>
<td>+</td>
</tr>
<tr>
<td>Immunodeficiency with thrombocytopenia and eczema</td>
<td>+</td>
</tr>
<tr>
<td>(Wiskott-Aldrich syndrome)</td>
<td></td>
</tr>
<tr>
<td>Immunodeficiency with thymoma</td>
<td>+</td>
</tr>
<tr>
<td>Immunodeficiency with short-limbed dwarfism</td>
<td>+</td>
</tr>
<tr>
<td>(Gatti, et al., 1969; Lux, et al., 1970)</td>
<td></td>
</tr>
<tr>
<td>Immunodeficiency with generalized hematopoietic hypoplasia</td>
<td>+</td>
</tr>
<tr>
<td>Severe combined immunodeficiency</td>
<td></td>
</tr>
<tr>
<td>(a) autosomal recessive</td>
<td>+</td>
</tr>
<tr>
<td>(b) X-linked</td>
<td>+</td>
</tr>
<tr>
<td>(c) sporadic</td>
<td>+</td>
</tr>
<tr>
<td>Variable immunodeficiency (common, largely unclassified)</td>
<td>+</td>
</tr>
</tbody>
</table>


• Incidence ~ 1:10.000 – 1:100.000
  • Rare disease... but most frequent symptomatic PID in adults!

• Mendelian Inheritance ~5-25%
  • However until 2012 only rare monogenic forms: ICOS, BAFF-R, CD19, CD20, CD81
  • Some common genetic factors (TACI/TNFRSF13B and MSH5 polymorphisms)

• Bimodal peak of incidence (first decade and third decade)
  • Long diagnostic delay! (~4-10 years)

ESID/PAGID 1999, CVID:
• Decrease of IgG (at least 2 SD below the mean for age) and a marked decrease in at least one of the isotypes IgM or IgA,
• Onset of immunodeficiency at greater than 2 years of age
• Absent isohemagglutinins and/or poor response to vaccines
• Defined causes of hypogammaglobulinemia have been excluded according to a list of differential diagnosis

[...] The number of potential distinct entities within this group is still unknown, and the diagnosis remains one of exclusion. Monogenic forms have been described, but polygenic inheritance is likely in most. Despite the fact that several monogenic defects underlying apparent CVID have been defined, because of the rarity of each defect and the lack in most cases of significant impact on management, as well as the cost of testing, genetic studies are not considered appropriate for routine use in patients with CVID at this time.
Heterogeneous Disease

33-80% “infection-only phenotype”
Vs
20-67% “complicated phenotype”

Neoplastic
Hodgkin and non-Hodgkin Lymphoma
Cancer
The importance of being complicated

’70: survival 12 years after diagnosis = <30%
’90: survival 20 years after diagnosis = 64-67% (vs general population 92-94%)

33-80% “infection-only phenotype”

Vs

20-67% “complicated phenotype”

The long road to phenotyping

The EUROclass trial: defining subgroups in common variable immunodeficiency

Claudia Wehr,1 Teemu Kivioja,2 Christian Schmitt,3 Berne Ferry,4 Torsten Witte,5 Efrem Eren,6 Marcela Vikova,7 Manuel Hernandez,8 Drahomira Detkova,8 Philip R. Bos,9 Gonke Poerksen,10 Horst von Bernuth,10 Ulrich Baumann,11 Sigune Goldacker,1 Sylvia Gutenberger,1 Michael Schlesier,1 Florence Bergeron-van der Cruyssen,3 Magali Le Garff,3 Patrice Debré,3 Roland Jacobs,5 John Jones,4 Elizabeth Bateman,4 Jiri Litzman,7 P. Martin van Hagen,9 Alessandro Plebani,12 Reinhold E. Schmidt,5 Vojtech Thon,7 Isabella Quinti,13 Teresa Espanol,8 A. David Webster,6 Helen Chapel,4 Mauno Vihinen,2,14 Eric Oksenhendler,3 Hans Hartmut Peter,1 and Klaus Warnatz1

<table>
<thead>
<tr>
<th>Nearly absent B cells (&lt;1%)</th>
<th>Includes all patients with severe defects in B-cell differentiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low switched memory B cells (&lt;2%)</td>
<td>Indicates a defective germinal center development similar to</td>
</tr>
<tr>
<td>CD27+IgM−IgD−</td>
<td>• ICOS deficiency · CD40L deficiency</td>
</tr>
<tr>
<td>Increased risk:</td>
<td></td>
</tr>
<tr>
<td>Splenomegaly</td>
<td></td>
</tr>
<tr>
<td>Granulomatous disease</td>
<td></td>
</tr>
<tr>
<td>Expansion of transitional B cells (&gt;9%) CD38hiIgMhi</td>
<td>Associated with lymphadenopathy</td>
</tr>
<tr>
<td>Expansion of CD21low B cells (&gt;10%)</td>
<td>Associated with splenomegaly</td>
</tr>
</tbody>
</table>
Late-Onset Combined Immune Deficiency: A Subset of Common Variable Immunodeficiency with Severe T Cell Defect

Marion Malphettes,1,2 Laurence Gérard,1 Maryvonne Carmagnat,3 Gaël Mouillot,4 Nicolas Vince,5 David Bouthoul,6 Alice Béreznè,7 Raphaëlle Nove-Josserand,8 Vincent Lemoin,9 Laurent Tetu,10 Jean-François Viallard,11 Bernard Bonnotte11,12 Michel Pavic,13 Julien Haroche,13 Claire Larroche,13 Jean-Claude Brouet,13 Jean-Paul Fermand,13 Claire Rabian,13 Claire Fieschi,13 and Eric Oksenhendler13 for the DEFI Study Group*

Table 2. Comparison of Patients with Common Variable Immunodeficiency (CVID) and Patients with Late-Onset Combined Immune Deficiency (LOCID)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients with CVID (n = 285)</th>
<th>Patients with LOCID (n = 28)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of male patients</td>
<td>119</td>
<td>15</td>
<td>NS</td>
</tr>
<tr>
<td>No. of female patients</td>
<td>166</td>
<td>13</td>
<td>NS</td>
</tr>
<tr>
<td>% of patients with known consanguinity</td>
<td>8</td>
<td>29</td>
<td>.004</td>
</tr>
<tr>
<td>% of patients with a familial case</td>
<td>22</td>
<td>18</td>
<td>NS</td>
</tr>
<tr>
<td>Median age at onset, years</td>
<td>19</td>
<td>19</td>
<td>NS</td>
</tr>
<tr>
<td>Median age at evaluation, years</td>
<td>46</td>
<td>43</td>
<td>NS</td>
</tr>
<tr>
<td>% of patients with respiratory tract infection</td>
<td>82</td>
<td>86</td>
<td>NS</td>
</tr>
<tr>
<td>% of patients with GI tract disease</td>
<td>42</td>
<td>75</td>
<td>.001</td>
</tr>
<tr>
<td>% of patients with splenomegaly</td>
<td>31</td>
<td>64</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>% of patients with lymphoid hyperplasia</td>
<td>26</td>
<td>29</td>
<td>NS</td>
</tr>
<tr>
<td>% of patients with granuloma</td>
<td>11</td>
<td>43</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>% of patients with autoimmune cytopenia</td>
<td>18</td>
<td>18</td>
<td>NS</td>
</tr>
<tr>
<td>% of patients with lymphoma</td>
<td>4</td>
<td>29</td>
<td>.001</td>
</tr>
<tr>
<td>Median serum IgG level, g/L</td>
<td>2.3</td>
<td>2.8</td>
<td>NS</td>
</tr>
<tr>
<td>Median serum IgA level, g/L</td>
<td>0.19</td>
<td>0.25</td>
<td>NS</td>
</tr>
<tr>
<td>Median serum IgM level, g/L</td>
<td>0.22</td>
<td>0.25</td>
<td>NS</td>
</tr>
</tbody>
</table>

NOTE: GI, gastrointestinal; NS, not significant; OI, opportunistic infection.
ESID registry CVID criteria

At least one of the following:

- increased susceptibility to infection
- autoimmune manifestations
- granulomatous disease
- unexplained polyclonal lymphoproliferation
- affected family member with antibody deficiency

AND marked decrease of IgG and marked decrease of IgA with or without low IgM levels (measured at least twice; <2SD of the normal levels for their age);

AND at least one of the following:

- poor antibody response to vaccines (and/or absent isohaemagglutinins); i.e. absence of protective levels despite vaccination where defined
- low switched memory B cells (<70% of age-related normal value)

AND secondary causes of hypogammaglobulinaemia have been excluded (see separate list)

AND diagnosis is established after the 4th year of life (but symptoms may be present before)

AND no evidence of profound T-cell deficiency, defined as 2 out of the following (y=year of life):

- CD4 numbers/microliter: 2-6y <300, 6-12y <250, >12y <200
- % naive CD4: 2-6y <25%, 6-16y <20%, >16y <10%
- T cell proliferation absent
…e la genetica...?

- Mutazioni o polimorfismi relativamente comuni con scarso significato diagnostico o prognostico (TACI)
- Mutazioni rarissime

- Ricerca con studi di singole famiglie, mediante geni candidati, alterazioni immunologiche evidenti o con studi omozigosità e altri approcci complessi e costosi.
- Nella pratica metodiche di sequenziamento “tradizionali”: lente e costose
Sanger Sequencing

dideoxynucleotides

capillary electrophoresis tube

larger fragments

smaller fragments

detector

laser
Next Generation Sequencing
Sanger sequencing = ~2400$/Mbp

Various NGS platforms = 10 to 0.05$/Mbp
<table>
<thead>
<tr>
<th>Relative costs compared to WES (2015)</th>
<th>Targeted panel</th>
<th>WES (60X coverage)</th>
<th>WGS (60X coverage)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Depends on size of gene panel</td>
<td>1</td>
<td>~3x as expensive as WES</td>
</tr>
</tbody>
</table>

| Gene panel coverage |                                      | 100%, when complemented with Sanger sequencing | 97.5% | >97.5% |

| Analysis of new disease genes |                                      | +     | +     |

| CNV-calling                   |                                      | Depends on size of gene panel | > 3 exons | all |

| Intronic variants (>30bp from splice-site) |                                      | -    | -     | +    |

| Incidental findings |                                      | -    | +     | +    |
Expanding and merging of disease phenotypes

DCLREC1C / ARTEMIS
JAK3
AK2
RAG1 and RAG2

SCID
CID
LOCID
CVID

DCLRE1C (ARTEMIS) mutations causing phenotypes ranging from atypical severe combined immunodeficiency to mere antibody deficiency.
Volk T' et al.

Common Variable Immunodeficiency or Late-Onset Combined Immunodeficiency: A New Hypomorphic JAK3 Patient and Review of the Literature.
Abolhassani H, Cheraghi T, Rezaei N, Aghamohammadi A, Hammarström L.

A NOVEL MUTATION IN AK2 RESULTS IN COMMON VARIABLE IMMUNODEFICIENCY
R. Hoyos et al. Poster ESID6-0116 Barcelona 2016
RAG deficiency


**Unrelated Hematopoietic Cell Transplantation in a Patient with Combined Immunodeficiency with Granulomatous Disease and Autoimmunity Secondary to RAG Deficiency.** John T¹, et al.
Tumors, DNA repair and radiosensitivity

Malignancies are the major cause of death in patients with adult onset CVID

Kaplan-Meier curves for patients with CVID. (1) Cumulative; (2) without cancers; and (3) with cancer complications.

Tumors, DNA repair and radiosensitivity

Altered spectrum of somatic hypermutation in common variable immunodeficiency disease characteristic of defective repair of mutations.
Duvvuri B¹, Duvvuri VR, Grigull J, Martin A, Pan-Hammarström Q, Wu GE, Larijani M.

Unique DNA repair gene variations and potential associations with the primary antibody deficiency syndromes IgAD and CVID.
Offer SM¹, Pan-Hammarström Q, Hammarström L, Harris RS.

MLH1, MSH2, MSH5, NBS1, RAD50
Common variable immunodeficiency caused by FANC mutations

Y. Sekinaka et al. Poster ESID6-0818 Barcelona 2016

2 Patients presenting with adult onset CVID
skewed CD45RO+ memory T-cells, absent TRECs and sjKRECs
No anemia, thrombocytopenia or neutropenia
WES ➔ FANCA and FANCE mutations

Common variable immunodeficiency syndrome associated with BRCA2 mutation

R. Romano et al. Poster ESID6-0540 Barcelona 2016

The patient was born to first-degree consanguineous parents, with history of cancer and mental retardation. Diagnosis of CVID was made at 9 years old of age. Moreover, the patient showed typical symptoms of Fanconi Anemia-like syndrome, such as short stature, mental and developmental delay
Tumors, DNA repair and radiosensitivity

Implicazioni nella gestione clinica:
• Uso di radiazioni ionizzanti ?
• Uso di chemioterapici alchilanti ?

Implicazioni prognostiche ed etiche:
• Screening per tumori più stretto ?
• Rischio di tumori nella famiglia ?
Deficiency of Adenosine Deaminase 2 Causes Antibody Deficiency.

Schepp J¹, et al.

Deficiency of ADA2

- Familial Panarteritis Nodosa
- Sneddon syndrome
- Livedo racemosa

Rapid and sustained effect of anti-TNF treatment in patients with ADA2 deficiency

Immunedysregulation, Autoimmunity and Lymphoproliferation

CTLA-4 and LRBA
Immunedysregulation, Autoimmunity and Lymphoproliferation

**CTLA-4 deficiency or haploinsufficiency**

- AD with incomplete penetrance

<table>
<thead>
<tr>
<th>Clinical manifestations</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea/Enteropathy</td>
<td>11/14 (78%)</td>
</tr>
<tr>
<td>Hypogammaglobulinemia</td>
<td>10/13 (76%)</td>
</tr>
<tr>
<td>Granulomatous lymphocytic interstitial lung disease</td>
<td>8/12 (66%)</td>
</tr>
<tr>
<td>Respiratory infections*</td>
<td>8/14 (57%)</td>
</tr>
<tr>
<td>Organ infiltration (bone marrow, kidney, brain, liver)</td>
<td>7/14 (50%)</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>6/12 (50%)</td>
</tr>
<tr>
<td>Autoimmune thrombocytopenia</td>
<td>5/14 (35%)</td>
</tr>
<tr>
<td>Autoimmune hemolytic anemia</td>
<td>4/14 (28%)</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td>4/14 (28%)</td>
</tr>
<tr>
<td>Psoriasis/other skin diseases*</td>
<td>3/14 (21%)</td>
</tr>
<tr>
<td>Autoimmune thyroiditis</td>
<td>2/13 (15%)</td>
</tr>
<tr>
<td>Autoimmune arthritis</td>
<td>2/14 (14%)</td>
</tr>
<tr>
<td>Solid cancer</td>
<td>1/14 (7%)</td>
</tr>
</tbody>
</table>

**LRBA deficiency**

- Autosomal recessive

---

*These clinical manifestations differ from CTLA-4 deficiency.
Immunedysregulation, Autoimmunity and Lymphoproliferation

**CTLA-4 and LRBA deficiency**

---

**Assessment of CTLA-4 Deficiency-Related Autoimmune Choroidopathy Response to Abatacept.**  
Shields CL¹, et al.

**AUTOIMMUNE DISEASE.** Patients with LRBA deficiency show CTLA4 loss and immune dysregulation responsive to abatacept therapy.  
Lo B¹, et al.
Immunedysregulation, Autoimmunity and Lymphoproliferation

Activated PI3Kδ Syndrome (APDS)

Phosphoinositide 3-kinase δ gene mutation predisposes to respiratory infection and airway damage.
Angulo I¹, et al.

Heterozygous splice mutation in PIK3R1 causes human immunodeficiency with lymphoproliferation due to dominant activation of PI3K.
Lucas CL¹, et al.

Phosphatase and tensin homolog (PTEN) mutation can cause activated phosphatidylinositol 3-kinase δ syndrome-like immunodeficiency.
Tsujita Y¹, et al.
Immunedysregulation, Autoimmunity and Lymphoproliferation

Activated PI3Kδ Syndrome (APDS)

- Age (<4 years)
- Respiratory tract infections
- Bronchiectasis
- Cervical/dental abscess
- EBV/CMV infections/viremia
- Extensive warts/Molluscum
- Lymphadenopathies
- Mucosal lymphoid aggregates
- (Hepato-) splenomegaly
- Gastrointestinal symptoms
- Autoimmune Cytopenias
- Arthritis
- Developmental delay
- Failure to thrive/poor growth
- Microcephaly
- Lymphoma/malignancy
- Deceased
- Hypogammaglobulinemia
- Hyper IgM
- Low CD3+/CD4+ lymphocytes
- Low B cell numbers
- Low switched B memory cells
- Immunoglobulin substitution
- Antibiotic prophylaxis
- Immunosuppressive therapy
- HSCT
Immunedysregulation, Autoimmunity and Lymphoproliferation

Activated PI3Kδ Syndrome

RTK  BCR  GPCR

SF1126  SAR245408  Buparlisib  Copanlisib  Idelalisib

Perifosine  MK-2206

Everolimus  Temsirolimus

mTORC1  mTORC2

mTOR  mLST8  Raptor  mTOR  mLST8  Rictor

4E-BP1  S6K1

CARD11  BCL10  MALT1  NFκB
Genetic studies to investigate monogenic forms of CVID or for disease-modifying polymorphisms are not generally required for diagnosis and management in most of the patients, especially those who present with infections only without immune dysregulation, autoimmunity, malignancy, or other complications. In these latter groups of patients, however, single gene defects may be amenable to specific therapies (eg, stem cell therapy) and molecular genetic diagnosis should be considered when possible.
Molecular screening of 415 patients with antibody deficiency

120 candidate genes, analyzed by panel sequencing or WES

<table>
<thead>
<tr>
<th>Gene</th>
<th>Variants</th>
</tr>
</thead>
<tbody>
<tr>
<td>BTK</td>
<td>IVS7+2 (A→G); L528Wfs<em>2; K300</em>; R13*; V219Lfs<em>10, Y344Wfs</em>57, Q140*</td>
</tr>
<tr>
<td>CECR1</td>
<td>IVS2+1; C167Y; R169Q; P251L; H112Q;</td>
</tr>
<tr>
<td>CTLA4</td>
<td>IVS1+1(G→T); R70W; T124P; C85Y; R75W; I116T; P136L; E94*, Q76*; etc</td>
</tr>
<tr>
<td>LRBA</td>
<td>E946*; Q474*; S348*; N1217Sfs<em>7; S2446</em>; Q1715*; S1885*; L156S; R182*</td>
</tr>
<tr>
<td>NFKB1</td>
<td>A475Pfs<em>10; V456</em>; IVS11+1 (G→C); IVS9+2 (T→G); D541*; IVS5-2; R156*; Q98* , IVS9+2; etc</td>
</tr>
<tr>
<td>NFKB2</td>
<td>D854Efs<em>31; IVS14+1 (G→T); S866Cfs</em>19, R853*</td>
</tr>
<tr>
<td>PIK3R1</td>
<td>IVS5+1</td>
</tr>
<tr>
<td>RAG2</td>
<td>G35A; G451A; D177H</td>
</tr>
<tr>
<td>STAT3</td>
<td>R152W</td>
</tr>
<tr>
<td>AICDA</td>
<td>R190*; C147*</td>
</tr>
</tbody>
</table>

N=415 patients, 96 monogenetic hits (23%)
Genetic Diagnosis Using Whole Exome Sequencing in Common Variable Immunodeficiency

WES was performed on 50 subjects diagnosed with CVID who had at least one of the following criteria: early onset, autoimmune/inflammatory manifestations, low B lymphocytes, and/or familial history of hypogammaglobulinemia. We identified 17 probable disease-causing mutations in 15 patients (30%).

WES combined with analysis of PID-associated genes is a cost-effective approach to identify disease-causing mutations in CVID patients with severe phenotypes and was successful in 30% of our cohort. As targeted therapeutics are becoming the mainstay of treatment for non-infectious manifestations in CVID, this approach will improve management of patients with more severe phenotypes.
Exome and genome sequencing for inborn errors of immunity.

Meyts I, Casanova JL et al.

[...] Whole-exome sequencing (WES) is presently the most cost-effective approach for research and diagnostics, although whole-genome sequencing offers several advantages.

The scientific or diagnostic challenge consists in selecting 1 or 2 candidate variants among thousands of NGS calls.

[...] Subsequent functional validation of the disease-causing effect of the candidate variant is critical. Even the most up-to-date dry lab cannot clinch this validation without a seasoned wet lab. [...] Finding the needle in the haystack takes patience, prudence, and discernment.
Riassumendo:

Nei pazienti con CVID con:
- **Esordio precoce e/o familiarità** per PID, neoplasie o autoimmunità
- **Complicata** da autoimmunità, infiammazione, linfoproliferazione, neoplasie

La ricerca di una causa genetica è raccomandabile in quanto:
- Può cambiare la **diagnosi** (CVID mimics)
- Può cambiare la **gestione** clinica (radiosensibilità, screening per tumori)
- Può cambiare la **prognosi** (forme con progressione a LOCID)
- Può cambiare la **terapia** (HSCT, terapie biologiche mirate)
- Può fornire informazioni sulla **ereditabilità** e counseling genetico per i familiari

Il miglior rapporto costo/performance è fornito dal **whole exome sequencing**
- Permette attualmente la diagnosi in ~20-50% dei pazienti
- Prevedibile aumento della sensibilità grazie all’approccio unbiased, al continuo riconoscimento di nuove varianti, al miglioramento dei software di analisi (es. CNVs) e dei database di riferimento.
- Necessaria grande **attenzione nella identificazione delle varianti** (coerenza clinica, Sanger di conferma, studi funzionali)
In conclusione....

**Pillars of PID diagnosis**

1. History
2. Examination
3. Immune function
4. Molecular genetics
In conclusione....
Vi ringrazio per l’attenzione...

Azienda Ospedaliero Universitaria di Careggi

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I tecnici del laboratorio di Immunoallergologia
Autoinflammation


Cold urticaria, immunodeficiency, and autoimmunity related to PLCG2 deletions. Ombrello MJ¹, et al.

**PLCG2-associated Antibody deficiency and Immune Dysregulation**

<table>
<thead>
<tr>
<th>Clinical Manifestation</th>
<th>Frequency no./total no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cold urticaria</td>
<td>27/27 (100)</td>
</tr>
<tr>
<td>Recurrent sinopulmonary infection</td>
<td>12/27 (44)</td>
</tr>
<tr>
<td>Antibody deficiency*</td>
<td>15/20 (75)</td>
</tr>
<tr>
<td>Common variable immunodeficiency</td>
<td>3/27 (11)</td>
</tr>
<tr>
<td>Symptomatic autoimmune disease†</td>
<td>7/27 (26)</td>
</tr>
<tr>
<td>Positive test for antinuclear antibodies‡</td>
<td>13/21 (62)</td>
</tr>
<tr>
<td>Symptomatic allergic disease</td>
<td>15/27 (56)</td>
</tr>
</tbody>
</table>
Early-onset lymphoproliferation and autoimmunity caused by germline STAT3 gain-of-function mutations. Milner JD¹, et al.

**Key Points**
- Germline gain-of-function mutations in STAT3 lead to lymphoproliferation and autoimmunity with prominent cytopenias.
- Mutations in STAT3 cause altered regulatory T cells and cytokine signaling.

**Clinical manifestations:**
- **STAT3 LOF = AD-HIES**
  - Mucocutaneous infections (S. aureus and C. albicans)
  - Pneumonia (S. aureus and S. pneumoniae), pneumatoceles
  - Dermatitis
  - Connective tissue abnormalities

- **STAT3 GOF**
  - ALPS-like
  - IPEX-like
  - STAT5b-deficiency-like
  - Various organ autoimmunity
  - Repeated infections
  - Immune deficiency: hypo-Ig, reduced switched memory B cells
Expanding and merging of disease phenotypes

Atypical familial hemophagocytic lymphohistiocytosis due to mutations in UNC13D and STXBP2 overlaps with primary immunodeficiency diseases.
Rohr J¹, et al.

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Alterations of the X-linked lymphoproliferative disease gene SH2D1A in common variable immunodeficiency syndrome.
Morra M¹, et al.

X-Linked Lymphoproliferative Syndrome and Common Variable Immunodeficiency May Not Be Differentiated by SH2D1A and XIAP/BIRC4 Genes Sequence Analysis.
Gulez N¹, et al.
Expanding and merging of disease phenotypes

Assessment of male CVID patients for mutations in the Btk gene: how many have been misdiagnosed?

Weston SA, Prasad ML, Mullighan CG, Chapel H, Benson EM.
Next Generation Sequencing

1 Library Preparation

Input DNA → Fragmentation → Adaptor ligation

Next Generation Sequencing

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Four main DNA sequencing methods used by NGS systems

1. Pyrosequencing
2. Sequencing by synthesis
3. Sequencing by ligation
4. Ion semiconductor sequencing
Common Variable Immunodeficiency and Circulating $T_{FH}$

Ana Coraglia,¹ Nora Galassi,² Diego S. Fernández Romero,³ M. Cecilia Juri,³ Marta Felippo,² Alejandro Malbrán,³ and María M. E. de Bracco¹,²

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Low Circulating Natural Killer Cell Counts are Associated With Severe Disease in Patients With Common Variable Immunodeficiency

Mikael Ebbo a,b,c,d, Laurence Gérard e,f, Sabrina Carpentier g, Frédéric Vély a,b,c,h, Sophie Cypowyj a,b,c, Catherine Farnarier h, Nicolas Vince i, Marion Malphettes e,i, Claire Fieschi e,i, Eric Oksenhendler e, Nicolas Schleinitz a,b,c,d, Eric Vivier a,b,c,g,* for the DEFI Study Group ¹:

Expansion of inflammatory innate lymphoid cells in patients with common variable immune deficiency.