REVIEW

More than just SCID—The phenotypic range of combined immunodeficiencies associated with mutations in the recombinase activating genes (RAG) 1 and 2

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Abstract Combined immunodeficiencies with impaired numbers and function of T- and B-cells can be attributed to defects in the recombinase activating genes (RAG). The products of these genes, the RAG1 and 2 proteins, are key players in the V(D)J recombination process leading to the assembly of antigen receptor genes. Complete RAG deficiency (RAGD) with no V(D)J (~1% recombination activity of wild type) is associated with classical SCID and absence of T- and B-cells. In RAGD with residual V(D)J activity (~1% recombination activity of wild type), several clinical and immunological subtypes have been described: classical SCID with skin inflammation and αβ T-cell expansion (classical Omenn syndrome), RAGD with skin inflammation and without T-cell expansion (incomplete Omenn syndrome), RAGD with γδ T-cell expansion and RAGD with granulomas. Engraftment of maternal T-cells can add to variation in phenotype. The potential role of epigenetic factors that influence the emergence of these phenotypes is discussed. Thorough assessment and interpretation of clinical and immunological findings will guide treatment modalities as intense as hematopoietic stem cell transplantation.

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Introduction

Severe combined immunodeficiency (SCID) has been the prototype of a primary immunodeficiency (ID) with a complete lack of adaptive immunity. Pathophysiologically, SCID can be subdivided into defects that render lymphocytes (but also other cell lineages) sensitive to abnormally increased apoptosis (reticular dysgenesis caused by adenylate kinase 2 (AK2), adenosine-deaminase-deficiency), defects of cytokine signaling (X-linked SCID, IL7-receptor-α-, JAK3-deficiency), defects in T-cell receptor (TCR) assembly and signaling (RAG1/2, DNAPKcs, Artemis and Cernunnos, CD3 defects) and by general T-cell signaling defects associated with Ca2+ release-activated Ca2+ channels (CRAC) and yet unclassified defects such as the deficiency of RNA component of mitochondrial RNA processing endoribonuclease (RMPR) also known as Cartilage-Hair-Hypoplasia [1].

In 1996, Klaus Schwarz and his group identified mutations in the recombinase-activating genes 1 and 2 (RAG1 and RAG2) as responsible for one of the classical SCID forms that lack T- and B-cells but have normal numbers of NK cells (T-B-NK) [2]. It is estimated that RAG1/2 accounts for approximately 50% of all patients with a T-B-NK+ SCID phenotype [3]. Whereas in the mid-90s RAG deficiency (RAGD) was thought to either lead to classical SCID or Omenn syndrome (OS), it has now become evident that the clinical spectrum is broader, including non-classical phenotypes of combined immunodeficiency, e.g. RAGD with expansion of γδ T-cells and RAGD characterized by granulomatous lesions in skin and other tissues [4-6].

These forms are often referred to as "leaky" or "atypical" SCID. In this review, we prefer to use the term RAG deficiency (RAGD). A systematic approach on how to classify RAGD and their clinical phenotypes is presented below.

Function of RAG

The adaptive immune system is characterized by its ability to form millions of antigen-specific receptors by the process of V(D)J-recombination. V, D and J stand for Variability Diversity and Joining gene segments in the genes encoding for the immunoglobulin, and T-cell receptor (TCR) chains. The ability to accomplish V(D)J recombination differentiates the adaptive from the innate immune system. V(D)J recombination not only serves as a tool for antigen-recognition, it is also essential for the development of lymphocytes (no V(D)J recombination, no mature lymphocytes). V(D)J recombination consists of several steps: First, RAG introduces a double-strand break in the DNA, creating two types of DNA ends (hairpin-sealed coding ends and blunt signal ends). In the next steps, coding DNA ends are opened and joined [7,8].

RAG1 and 2 are located on human chromosome 11p13, closely associated with each other and highly conserved in the jawed vertebrate immune system. They were discovered when Schatz and Baltimore stably transfected fibroblasts with a construct containing a selectable marker with a V(D)J recombination-dependent expression [9]. In fibroblasts (non-lymphoid-cells), no recombination took place. However, when genomic DNA fragments were stably cotransfected, recombination activity in some fibroblasts could be detected, depending on whether these fragments contained RAG1/2 or not [10]. Human RAG1 and RAG2 have 1043 and 527 amino acids respectively and their expression is lymphoid-specific. RAG1/2 biochemistry has been reviewed elsewhere [11]. With the molecular characterization of RAGD, it has become clear that both the RAG1/2 core and non-core regions are essential for normal V(D)J recombination [11,12].

In B lymphopoiesis, there are two waves of RAG expression, one during recombination of the immunoglobulin heavy chain and immunoglobulin light chain. Interestingly, a second wave of expression is associated with the editing of the IgM receptor on immature B-cells. This process of receptor-editing is induced by auto-antigens and if this second physiological RAG expression is missing, autoimmunity may be favored (Walter, unpublished work).

Clinical, immunophenotypical and histopathological classification of RAG deficiencies (RAGD)

For classification purposes, we differentiate between RAGD with no V(D)J recombination (<1% recombination activity of wild type) and RAGD with residual V(D)J recombination (>1% recombination activity of wild type). Both can occur with engraftment of maternal T-cells, but this is rare in hypomorphic RAGD. In RAGD with residual V(D)J recombination,
there appear to be several subtypes, depending on epigenetic and other factors (Fig. 1).

A very important determinant of the phenotype associated with any RAG mutation is the quantity of V(D)J activity resulting from this mutation. An altered RAG protein function can be evaluated in the so-called V(D)J recombination assay: Primary human dermal fibroblasts which lack RAG1/2 (but express all other known genes of the non-homologous end

**Figure 1** RAG deficiency with residual V(D)J recombination: From genotype to phenotype. AI, autoimmunity; BCR, B-cell receptor; HSM, hepatosplenomegaly; LN, lymph nodes; MFT, maternofetal transfusion; OS, Omenn syndrome; RAGD, RAG deficiency; TCR, T-cell receptor.

A systematic literature search in Pubmed, using the terms "RAG deficiency" and "RAG mutation" (limiting by "human", "1999–November 2009") was performed in order to obtain genotype/phenotype data. Of the 164 resulting articles, those that described clinical and immunological characteristics of patients, in addition to molecular genetics, were selected. Excluded were articles in which genetic or immunological characteristics of the patients were missing. This resulted in 17 articles with a total of 119 reported patients [4–6,12,15,17,24,26,32,41–48].

<table>
<thead>
<tr>
<th>Clinical phenotype</th>
<th>Patient number (n)</th>
<th>RAG1</th>
<th>RAG2</th>
<th>Homozygous</th>
<th>Compound heterozygous</th>
<th>Type of mutation</th>
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<td>RAGD with no V(D)J recombination</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Classical SCID</td>
<td>31</td>
<td>16</td>
<td>15</td>
<td>28</td>
<td>3</td>
<td>FS (4), NS (3), MS (24)</td>
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<td>7</td>
<td>1</td>
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<tr>
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<td>36</td>
<td>20</td>
<td>30</td>
<td>26</td>
<td>FS (12), NS (0), MS (44)</td>
</tr>
<tr>
<td>Incomplete OS</td>
<td>15</td>
<td>12</td>
<td>3</td>
<td>6</td>
<td>9</td>
<td>FS (2), NS (0), MS (13)</td>
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<tr>
<td>Expansion/predominance of γδ T-cells</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>FS (1), NS (2), MS (3)</td>
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<tr>
<td>Granulomatous inflammation</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>FS (0), NS (0), MS (3)</td>
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<td>Total</td>
<td>119</td>
<td>78</td>
<td>41</td>
<td>77</td>
<td>42</td>
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</table>

FS, frameshift; NS, nonsense; MFT, maternofetal transfusion; MS, missense; OS, Omenn syndrome; SCID, severe combined immunodeficiency; RAGD, RAG deficiency.

A literature review of genotype/phenotype correlation in patients with RAGD.
joining (NHEJ) pathway) are cotransfected with expression plasmids coding for wild-type or mutant RAG1 or RAG2 proteins and a reporter construct as read-out. This system allows for scoring of V(D)J recombination efficacy [2,13,14]. The location of mutations in either RAG1 vs. RAG2 or in core vs. non-core regions does not influence the phenotype [11,15]. Hypomorphic RAG mutations lead to combined immunodeficiency (CID). In two seminal papers, Anna Villa and collaborators showed that mutations with residual V(D)J activity can lead to very different immunological and clinical phenotypes [14,15]. Even within one family, the same genotype (e.g. A444V, R561H) may lead to different phenotypes presenting as either classical SCID or OS (Clinical description, see below) or SCID with expansion of γδ T-cells [4,5,16,17]. Genotype-phenotype correlation in patients with RAGD reported in the literature, is summarized in Table 1.

RAG deficiencies with no V(D)J recombination

Classical SCID

The age of onset is usually within the first weeks of life. Opportunistic infections involve mainly the lungs and intestines. Infections with fungi (Pneumocystis jirovecii, Candida sp. and Aspergillus sp.) and viruses (CMV, Parainfluenza, HSV1, VZV, RSV, Adenovirus) as well as bacterial infections (BCGitis, Pseudomonas) predominate. Unless transplanted with hematopoietic stem cells, classical SCID is most often fatal within the first year of life. For a detailed description of Classical SCID please refer to [3]. The immunophenotype is T−B−NK+. Virtually no B- and T-cells are produced, and there is profound lymphopenia analogous to other defects that affect V(D)J recombination (Artemis, DNA-Ligase IV, Cernunnos). Eosinophilia may be present in SCID [18]. There is virtually no immunoglobulin synthesis. Histopathologically, SCID patients have a very small thymus, which contains no thymocytes and lacks the classical corticomedullary distinction as well as Hassal’s corpuscles (Fig. 2B). The thymic epithelium itself is normal and is repopulated following hematopoietic stem-cell transplantation. The T-dependent areas of the spleen are depleted of lymphocytes and lymphoid tissues such as lymph nodes, tonsils, adenoids and Peyer’s patches are absent or underdeveloped.

Classical SCID with maternofetal transfusion

In the cohort reported by Müller, 15% of all SCID patients had signs of clinical and/or laboratory Graft-versus-host (GVH) reaction associated with maternofetal transfusion (MFT) [19]. The eczematous-erythematous rash may be confounded with atopic dermatitis. It affects palms and soles, which is very unusual for atopic dermatitis (Fig. 3). Other organs may be affected, most frequently liver and gut. Liver enzymes

Figure 2 Thymic biopsies from a normal individual and RAGD patients (kindly provided by Luigi Poliani, University of Brescia). Hematoxylin–eosin staining. All panels are 10× original magnification. (A) Representative section from a normal thymic sample showing a mature phenotype with normal compartmentalization of the cortex (c) and the medulla (m) and Hassall’s body formation. (B) Thymic biopsy from a representative RAG null patient showing profound modifications consisting of a pronounced high cellular lobular architecture surrounded by thin collagen septa with loss of cortico-medullary differentiation (CMD) and absence of Hassall’s bodies. Lobules contain immature thymocytes dispersed in a disorganized epithelial cell network. (C) Thymic biopsies from a patient with hypomorphic RAG2 gene mutation associated with Omenn syndrome and showing marked hypoplasia with lobules containing immature thymic epithelial cells with depleted lymphoid cell component. There is loss of CMD, even peripheral condensation of the epithelial cell component results in a pseudo-CMD.
Figure 3  Erythematous–eczematous rash of the palms in a child with RAGD, classical SCID and GVHD as a result of maternofetal T-cell transfusion.

may be elevated in case of liver involvement and eosinophilia/elevated IgE may be present. Single cases of nephritis and cholestasis due to GVHD have been described [19]. Severe neutropenia in SCID with MFT has been observed and found to be responsive to GCSF [20]. In RAGD with MFT, the immunophenotype is T+ B− NK+ with variable numbers of activated HLA DR+ CD45 RO+ T-cells. The histopathology (e.g. in skin and liver) shows cell mediated inflammatory reactions consistent with GVHD [19].

RAG deficiencies with residual V(D)J recombination

Classical Omenn syndrome (OS)

Similar to classical SCID, the age of onset is the first weeks of life, with signs of severe immunodeficiency. The leading sign is generalized and pronounced erythroderma usually developing shortly after birth. Secondary alopecia and loss of eyebrows are characteristic (Fig. 4). In contrast to classical SCID, patients with OS present with large lymph nodes and hepatosplenomegaly [21]. OS has most frequently been described with RAGD, with a T+ B− NK+ phenotype. OS may also occur in children diagnosed with mutations in the following genes: Artemis, DNA ligase 4, IL-2 receptor gamma chain, IL-7 receptor alpha chain, adenosine deaminase and following genes: Artemis, DNA ligase 4, IL-2 receptor gamma chain, IL-7 receptor alpha chain, adenosine deaminase and RMRP. OS has rarely been described in the context of RAG deficiencies with residual V(D)J activity. T-cells are autologous and not of maternal origin with exception of IgE, which commonly is markedly elevated. It has been postulated that there is a lack of Treg cells (CD4− CD25 high FOXP3+), which may control lymphocyte homeostasis [23,24]. Severe skin inflammation is attributed to autologous T-cells (for histopathology see Fig. 4D). Protein loss through skin and gut may favor edema and other metabolic disturbances [23,25].

In the thymus, there are few remnant lymphoid cells with absence of Hassal’s corpuscles and a loss of the normal architecture as depicted in Fig. 2C.

Incomplete OS

Patients with incomplete OS were first described by Anna Villa and collaborators in 2001 [15]. She originally described 11 patients with heterogeneous clinical and immunological characteristics: The range of presentation was from birth to 3.5 months of life. Four patients presented with protracted diarrhea, failure to thrive and erythroderma/skin rash. None had lymphadenopathy or splenomegaly, however 4 had hepatomegaly. The most frequent immunophenotype was T+ B− NK+; however, 4 patients had undetectable B-cells. Most patients presented without lymphocytosis, with the exception of 2 patients that showed an increased absolute lymphocyte count. Both B- and T-cells were present in low numbers and associated with residual V(D)J activity, T-cells were HLADR+ CD45RO+. Detailed histopathological analyses are lacking in this group of patients.

Expansion/predominance of γδ T-cells

These patients presented within their first year of life with complications of severe viral infections as well as autoimmune cytopenias with detection of autoantibodies against erythrocyte antigens, platelets and neutrophils. Most patients had suffered from CMV infection, a few developed EBV associated lymphoproliferation or genitopharyngeal ulcers due to HSV-1 [4,5,26]. The immunophenotype is T+ B− NK+ or T+ B− NK+. Lymphocyte numbers are low to normal. There may be eosinophilia. T-cells show a characteristic distribution with 70 to 90% γδ T-cells among all T-cells. γδ T-cells counts are up to 3200 cells/μl, in the case reported by Ehl up to 300 cells/μl. In the description by de Villartay, these γδ T-cells were also CD8+. Interestingly, immunoglobulins showed largely normal values for IgG, IgA, IgM and also IgE. Moreover, antigen-specific (CMV) IgG and IgM were detected in these patients. Apart from lymphoproliferation and ulcerative inflammation, no peculiar histopathology has been described.

Granulomatous inflammation

There is a surprisingly late age of onset in some of these patients: The index patient [6] presented at 3 years of age, with no infections in her medical history (Fig. 5). In this respect, RAGD with granulomatous inflammation is clearly different from all other RAG deficiencies which usually present in the first year of life. The immunophenotype is T+ B− NK+ with low for age lymphocyte-numbers (between 320 and 1554/μl). There is eosinophilia in some patients but IgE is not elevated. T-cells are clearly decreased for age but not absent (120–1057/μl) with an increased expression of HLA DR and CD45 RO. Maternal T-cells were not detected and there was no significant increase in γδ T-cells. The presence of CD4+ CD45 RA+ cells (1–27% of CD4) may reflect production of naïve T-cells by the thymus and residual V(D)J repertoire. Interestingly, spectratyping of the αβ T-cell receptors in two of the three patients revealed a diverse repertoire. This is in contrast to classical SCID with no T-cells or OS having cells with a very restricted, highly oligoclonal TCR repertoire. B-cell numbers were low for age (0–202/μl ) but not absent in most cases. Normal IgG levels and levels of specific antibodies (e.g. to tetanus toxoid) were documented, while there is selective IgA deficiency in some patients. The striking histopathological feature of this RAGD is the formation of epitheloid, non-caseating granulomas in diffe-
rent organs and tissues (e.g. skin, lungs, the tongue, adenoids, spleen). Granuloma formation has been studied in depth [27]. In the context of RAG deficiency, the immunopathogenesis of the granulomas is unclear. Granuloma formation is not specific for RAG as it has also been reported in a patient with an Artemis deficiency. For granuloma formation a functional TNF and TNF receptor axis is important, [28,29]. Interestingly, one member of the TNF superfamily, B-cell activating factor (BAFF), is increased not only in patients with RAGD and granulomas and in CVID but also in patients with Wegener’s Granulomatosis, where inflammation is characterized by the formation of granulomas [30,31], (Walter unpublished work).

Maternofetal transfusion
The coexistence of autologous host B-cells and maternal T-cells derived from MFT in a girl with mild immunodeficiency was described by Kumaki [32]. This patient presented at 4

Figure 4 Photographs and skin biopsy of a child with classical OS (kindly provided by Hagen Ott, Aachen). Panel A shows alopecia. Furthermore, ichthyosiform erythroderma with marked pachydermia (B) and onychodystrophy (C) could be observed. Hematoxylin–eosin staining of the patient’s paraffin-embedded skin biopsy (D). Beside epithelial hyperplasia, spongiosis, focal basal vacuolation and parakeratosis, an inflammatory infiltrate in the upper dermis mainly consisting of lymphocytes with few interspersed eosinophils is demonstrated. This child was successfully transplanted with cord blood (E) [25].
months of age both with lymphadenopathy and a diffuse papular scaly rash and recent infections (otitis, impetigo). There were raised IgM and IgE levels and hypereosinophilia (N 9000/μl). The clinical picture was a combination of signs of autoimmunity, incomplete OS and maternofetal engraftment. Of note, she was able to produce specific antibodies (anti HSV1 antibodies) and was followed on an outpatient basis for >6 years.

Genotype–phenotype correlation in RAG deficiency

Genetic or epigenetic factors (infections, engraftment of maternal T-cells) as well as iatrogenic factors may influence RAGD phenotype (Fig. 1).

Role of gene modifiers

RAGD is of autosomal recessive inheritance and often diagnosed in consanguineous families. Therefore, patients could be expected to be homozygous also for other gene variants that might influence the phenotype by favoring T-cell expansion, skewing of cytokine expression and/or development of granuloma. However, search for these possible modifier genes has been inconclusive so far. Using homozygosity mapping, de Villartay et al. investigated genotype alterations in 4 infants who presented with unusually severe CMV infections [5]. Homozygosity mapping allows the detection of both mutations in RAG and in modifier genes. This study could not identify any mutations other than RAG1. The three RAGD patients with granulomas described by us were from a non-consanguineous background, hence the role of modifier genes may be of less significance for the clinical phenotype. Mutations in the NOD-2 gene, which may lead to granulomatous colitis and Crohn’s disease were excluded [6].

Role of epigenetic factors

In patients with RAGD and residual T-cells, pathogens may induce T-cell expansion, which alters the histopathological picture and thus contributes to the phenotype. Infections have been postulated to trigger OS, e.g. OS has been associated with Parainfluenza-3 virus infections [33]. EBV may stimulate T-cell expansion in OS and induce lymphoproliferation and malignancy, e.g. one of the cases with RAGD and granulomas suffered from a lymphoma of the tonsil [4,6].

Figure 5  Clinical characteristics of the index patient with RAGD and granulomas [6]. Violet erythematous papulonodular lesions of the face and the legs (A), tonsilar EBV lymphoma (B). Skin biopsies taken from the right thigh of the index patient (C and D) showing diffuse lymphogranulomatous infiltrates in the dermis consisting of many histiocytes in poorly organized granulomas intermingled with many small slightly atypical lymphocytes. Blastic cells are rare. In the center of the granuloma, small foci with signs of apoptosis are found (hematoxylin–eosin stain).
In the four cases described by de Villartay, expansion of $\gamma \delta$ T-cells may have been caused by CMV antigens. The expansion consisted of clones with a TCR $\gamma \delta$ rearrangement reminiscent of skin or gut $\gamma \delta$ T-cells (not the physiologically predominant $V_{\gamma}9-V_{\delta}2$ T-cell population) \[5\]. Atypical mycobacteria may be the cause of the granulomatous lesions seen in some patients with RAGD and residual V(D)J. In the patients reported by us, it was not possible to detect mycobacteria from granulomas despite extensive screening by PCR or culture \[6\].

At present, there is no clear evidence that the massive lymphoproliferation seen in OS is induced by infections alone. In an experimental setting, OS can even be observed in mice held under pathogen-free conditions. It has been hypothesized that homeostatic proliferation can lead to immunodysregulation and an OS-like phenotype \[34\].

Defective pathogen clearance due to inefficient recognition and dysregulation of B- and T-cell responses in RAGD may favor autoantigen responses and autoimmunity (AI). In RAGD deficiencies with no V(D)J recombination (classical SCID) and most forms of RAGD with residual V(D)J recombination, children are referred for stem cell transplantation early in life and therefore rarely develop chronic AI. In somewhat milder forms of RAGD, AI and even development of malignancy are observed \[5,6\]. In RAGD, the most prevalent sign of AI is cytopenia due to autoantibodies against erythrocytes, platelets or granulocytes. AI in SCID has recently been reviewed \[35\] (Schuetz, unpublished work).

### Role of iatrogenic factors

Early recognition of a combined immunodeficiency will lead to institution of immunoglobulin substitution and use of anti-infective agents given therapeutically or prophylactically. This will have an effect on the severity and dissemination of infections. Inflammatory skin lesions may guide the physician to use immunosuppressive agents or chemotherapy. This may be necessary, but may also lead to deterioration of skin lesions as documented in the cohort of RAGD with granulomas \[6\].

### Perspectives

The “5” in SCID stands for Severe and SCID has been defined immunologically as complete absence of specific immune functions and clinically as a disease in infants that is lethal within the first year of life due to opportunistic infections, unless treated by hematopoietic stem cell transplantation \[36\]. With the detection and characterization of hypomorphic variants, it becomes clear that SCID causing mutations can result in surprisingly mild phenotypes that do not match the definition of SCID. The paradigm that severe forms of CID usually present in infancy or early childhood needs to be challenged. Molecular analysis of RAG and other genes associated with SCID will be of interest not only in infants but also in children and adults with unexplained autoimmunity, granulomatous disease and rare or unusually severe infections \[37\]. In adults, the French DEFI cohort recently defined a subset (n=28, 8.9%) of their 313 CVID patients as late-onset combined immunodeficiency LOCID. Of note, in this subset, granulomas and EBV associated lymphomas were more common than in CVID \[38\].

More research into the pathophysiology of RAGD is necessary. It is still unclear why the same mutation leads to classical SCID in one patient and to OS in the other. Modifier genes, epigenetic and iatrogenic factors may play a role, but the precise mechanisms leading to a certain phenotype remain to be elucidated. Investigating the different types of inflammation and their distribution (lymphocytic vs. granulomatous, skin vs. organs) may reveal that the interphase between the adaptive and innate immune systems may be central to understanding the variation in phenotypes, e.g. mediated by altered expression of BAFF and other cytokines/chemokines or altered TLR signaling \[39,40\], (Walter, unpublished work).

Finally, research on the outcome of RAGD with residual V(D)J recombination is needed. While the treatment of choice for severe RAGD (classical SCID) is transplantation with hematopoietic stem cells, the management is less clear in older children with residual T-cell function and chronic infections.

### Conclusions

RAGD patients with null mutations and no V(D)J recombination display a severe phenotype, i.e. classical SCID, whereas RAGD patients with hypomorphic mutations and residual V(D)J recombination may present with less severe phenotypes. In RAGD patients with residual V(D)J recombination, several subtypes can be differentiated: Classical OS, incomplete OS, combined immunodeficiency (CID) with expansion/predominance of $\gamma \delta$ T-cells or with granulomatous inflammation. Factors determining phenotype apart from RAG genotype are age at the first encounter of infections, other genetic (modifier genes) and epigenetic factors that have not yet been well characterized. These factors lead to very different clinical and immunological phenotypes, even on the background of the same mutation. For the clinical immunologist, it is very important to allocate a certain clinical and immunological phenotype (e.g. susceptibility to infections, malignancy, autoimmunity) to a mutation in a SCID associated gene, because only then the appropriate treatment for the immunodeficient patient will be considered.

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### References

Combined immunodeficiencies associated with RAG1/2 deficiency


