Novel mutations and diverse clinical phenotypes in recombinase-activating gene 1 deficiency

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Abstract

Background: Severe combined immunodeficiency is within a heterogeneous group of inherited defects throughout the development of T- and/or B-lymphocytes. Mutations in recombinase-activating genes 1 or 2 (RAG1/2) represent approximately 10% of all SCID cases. RAG1/2 are essential for V(D)J rearrangement of the B- and T-cell receptors.

Objectives: The aim of this study was to review clinical, immunological and molecular findings of Turkish SCID patients with RAG1 defects and to draw attention to novel mutations, genotype-phenotype correlations and the high rate of BCG infections within this group.

Methods: Eleven patients (F/M: 6/5) were included. Molecular, immunological and clinical data were evaluated.

Results: Five patients were classified as T-B-NK + SCID, four patients as T + B-NK + SCID (two of these patients were diagnosed as classical Omenn syndrome) and two patients as T + B + NK + SCID with respect to clinical presentations and immunological data. Mean age of the whole study group, mean age at onset of symptoms and mean age at diagnosis were: 33.0 ± 42.8, 3.1 ± 3.3 and 10.4 ± 13.5 months, respectively. Consanguinity rate was 54%. Some novel mutations were found in RAG1 gene in addition to previously reported mutations. Genotype-phenotype correlation was not significantly apparent in most of the cases. BCG infection was observed in 36.4% of patients (two BCG-osis and two BCG-itis).

Conclusion: Epigenetic factors such as compound genetic defects, enviromental factors, and exposure to recurrent infections may modify phenotypical characteristics of RAG deficiencies. Inoculation of live vaccines such as BCG should be postponed until primary immunodeficiency disease is excluded with appropriate screening tests in suspected cases.

Keywords: Recombinase-activating genes, Immunodeficiency, BCG
and absence of T and B cells. It is estimated that RAG1/2 is involved in approximately 50% of all patients with T-B-NK + SCID phenotype [10]. In RAGD with residual V(D)J activity (> 1% recombination activity of wild type), several clinical and immunological subtypes have been described; such as RAGD 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 [2,3,11]. B cell levels and immunoglobulin concentrations are almost normal or only slightly reduced in patients with hypomorphic mutations [12]. In recent studies, autoimmune cytopenia and resistant CMV infection are reported in hypomorphic RAG mutated patients [12-14]. These patients usually present with severe and life-threatening bacterial, viral and fungal infections in early infancy regardless of SCID type and the underlying genetic defect. Some patients with RAG deficiency may present later in life as combined immunodeficiency with granuloma formation [15]. Patients with SCID also experience opportunistic infections of mycobacterium species and complications after vaccination with Bacille Calmette-Guerin (BCG).

The aim of this study was to review clinical, immunological and molecular findings of Turkish SCID patients with RAG1 defects admitted to a Pediatric Immunology Department in the western region of the country and to draw attention to novel mutations, genotype-phenotype correlations and high frequency of BCG infections.

**Patients and methods**

**Patients**

Eleven patients with the diagnosis of SCID in respect to severe upper and lower respiratory tract infections from 2002 to 2010 in Ege University Faculty of Medicine, Department of Pediatric Immunology, Izmir, Turkey; were reviewed in this study. An evaluation sheet was used to summarize demographic information of patients including name, gender, date of birth, age of onset of symptoms, clinical symptoms, age at diagnosis, family history and consanguinity, previous history of medications and vaccination, and laboratory and molecular data. The patients were diagnosed and classified according to clinical and laboratory criteria of SCID reported by IUIS Expert Committee on Primary Immunodeficiencies [16].

All patients were screened for mutations in RAG1, RAG2 gene. Ten age-matched, healthy people (mean age: 27.4 ± 2.1 years) served as controls for the analyses of genetic data. This study was approved by the Ethics Committee at Ege University, and an informed consent was obtained for each participating patient.

**Cellular and immunological assays**

Complete blood count with peripheral blood smear evaluation, serum immunoglobulins by nephelometry (Dade Behring BNII Nephelometer Analyzer, Germany), antibody response to previous vaccines, and lymphocyte phenotyping (T, B, and NK cells) by flow cytometry (FACSCalibur, Becton-Dickinson, USA) were the standard laboratory investigations performed for our patients.

**Clinical samples and DNA isolation**

Peripheral blood samples were obtained from the patients by venipuncture. Genomic DNA was then extracted from the blood samples using standard techniques [17].

**Mutations analyses at the RAG-1 and RAG-2 loci**

Because of the restricted expression of RAG-1 and RAG-2 genes and the fact that the coding region of each gene is contained in a single exon, coding sequences were amplified from genomic DNA. Primers were designed for the amplification of the RAG genes based on the sequences reported in databases (RAG-1, M29474; RAG-2, M94633). RAG gene sequence information was obtained as previously specified [18]. Alternatively, the RAG-1 gene was amplified in 2 segments (94-1852 and 1781-3262), and the RAG-2 gene was amplified in one segment (1201-2922) with the following primers: RAG-1-90 F, CTG AGC AAG GTA CCT CAG C; RAG-1-1852 R, GCC TTC CAA GAT GTC TTCTTC; RAG-1-1781 F, GCAAAG AGG TTC CGC TAT GA; RAG-1-3262 R, CAT AAG TGG TTG CCC TAC TT; RAG-2-1201 F, ATG TCT CTG CAG ATG GTA AC; RAG-2-2922 R, CTG GCC CTT AAT TCA TGT AAC. Sequencing was performed directly on the PCR products purified from the gel with the Thermosequenase kit (Amersham Pharmacia Biotech UK, Buckinghamshire, United Kingdom). For patients who were compound heterozygotes, mutations were confirmed either by restriction analysis or by analysis of several clones from PCR amplification products cloned in TA vector (Invitrogen) and sequenced by the dideoxynucleotide chain termination method using the Sequenase kit (USB), as previously described [18].

SPSS was used for statistical analyses (Windows Version 15.0, SPSS Inc., Chicago, IL).

**Results**

Eleven patients (F/M: 6/5) with SCID or Omenn Syndrome who had RAG mutations were analyzed. Their demographic findings are listed in Table 1. Among them, two have been previously reported [14]. Five patients were classified as T-B-NK + SCID, four patients as T + B-NK + SCID (two of these patients were
diagnosed as classical Omenn syndrome) and two patients as T + B + NK + SCID with respect to clinical presentations (Table 1) and immunological data (Table 2). Mean age of the whole study group, mean age at onset of symptoms and mean age at diagnosis were; 33.0 ± 42.8, 3.1 ± 3.3 and 10.4 ± 13.5 months, respectively. Consanguinity was present in 6 (54%) of 11 patients and all of them were second degree relatives. Positive family history for immunodeficiency was determined in five patients (45%).

Absolute lymphocyte counts and lymphocyte subset distributions in the RAGD patients are shown in Table 2. Lymphopenia was documented in 36% of all patients (in 80% of T-B-NK + SCID patients, and 25% of T + B-NK + SCID patients). A high variability in the total lymphocyte count was documented in these groups. Data on the expression of activation markers (HLA-DR) was obtained for the subgroups of RAGD SCID patients. In particular, the T + B-NK + group showed increased percentages of HLA-DR + cells (100%). Two groups (T + B- and T-B+) of patients with RAG1 defects had very low proportions of CD19 cells. The highest NK cell percentages were found in patients with T-B- SCID (73.2%). Eosinophilia, a well-known feature of Omenn Syndrome, was observed in 2/4 of the T + B-patients group, in 1/5 of T-B- patients and 1/2 of T + B + patients. None of the patients have been shown to produce specific antibodies against vaccines, such as tetanus toxoid, *Haemophilus Influenzae* type B or hepatitis B.

**Genotype-phenotype correlation**

We investigated the mutations in RAG1 and RAG 2 genes in SCID patients and the control group (Table 2) and compared them with the clinical phenotypes. The following findings were detected in 11 SCID patients:

### Two novel mutations in four patients

Homozygous mutation of *p.R394Q/p.R394Q* was found in three patients of the T-B- group (patient 1- 2-5). Two of these patients and their parents (no. 1 and 5) were cousins. Lymphopenia, hypogammaglobulinemia, early onset of disease symptoms were significant in these three patients.

Another mutation which was not reported before, in T-B- SCID group, was *p.R776Q, 3047- 3049 del GCC* (patient 3). This female patient had chronic diarrhea beginning from the neonatal period. The other clinical findings were hepatomegaly, splenomegaly, dermatitis, anal fissure and failure to thrive. Direct Coombs test was positive. In addition to chronic pulmonary infection, lymphadenopathy was observed both in computerized tomography of the thorax and the axillary area. *Mycobacterium bovinum* was detected in her sputum and she began to receive antimycobacterial therapy after the diagnosis of BCG infection.

### Two previously reported mutations in two patients

Homozygous mutation of *Q248X* in our Omenn Syndrome patient (case no.9) has been reported before in T-B- NK + SCID and Omenn Syndrome cases. Similar to the previously reported cases, *P85fs32X (del A256/A257)* mutation was found in one (case no.11) of our leaky SCID patients. This case had refractory CMV infection, autoimmunity and elevated gamma-delta T cells.

### Amino acid changes: Mutations or polymorphisms? (Five patients)

*p.H249R* amino acid change (homozygous) was detected in a T-B-NK + patient (no.4), who was admitted on 40/365 day because of pulmonary infection and moniliasis and underwent BMT when he was three months old.

*p.H249R* amino acid change (heterozygous) was observed in two of our cases (patients 8 and 10), one

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**Table 1 Demographic and clinical findings of patients with RAG1 defects**

| SCID type   | n | Gender (f/m) | Mean Age | Mean age at onset of symptom months | Mean age at diagnosis | consanguinity (n) | Positive autoimmunity (n) | CMV infect (n) | BCG infect (n) | BMT (n) | Follow up |
|-------------|---|--------------|----------|-----------------------------------|-----------------------|--------------------|--------------------------|---------------|---------------|---------|-----------|
| T-B- NK+    | 5 | 3/2          | 10.4 ± 9.4 | 2.6 ± 1.7                         | 5.9 ± 4.7             | 3                  | 1                        | 0             | 1             | 3       | 4 1 0     |
| T+B- NK+    | 2 | 1/1          | 8.5 ± 9.1  | 0.6 ± 0.5                         | 1.5 ± 1.4             | 0                  | 1                        | 0             | 1             | 0       | 2 0 0     |
| Other phenotype | 1/1 | 98 ± 72.1 | 7.0 ± 7.0 | 33 ± 21.2 | 1 | 0 | 0 1 0 0 2 0 0 |
| T+B+ NK+    | 2 | 1/1          | 49 ± 4.2   | 2.7 ± 0.3                         | 8.0 ± 2.8             | 2                  | 1                        | 2             | 1             | 2       | 0 1 1     |
| Total       | 11| 6/5          | 33 ± 42.8  | 3.1 ± 3.3                         | 10.4 ± 13.5           | 6                  | 3                        | 2             | 4             | 5       | 6 4 1     |

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| T-B-NK+ (n = 5) | T + B-NK+ (n = 4) | Omenn n(2) | T + B + NK+ (n = 2) |
|----------------|------------------|-------------|-------------------|
| Patient 1     | Patient 2        | Patient 3   | Patient 4        | Patient 5 | Patient 6 | Patient 7 | Patient 8 | Patient 9 | Patient 10 | Patient 11 |
| IgG (mg/dl)   | 371              | 546         | 546              | 517       | 312       | 1372      | 1840      | 1080      | 662        | 159        | 1590       |
| IgA (mg/dl)   | < 6              | < 6         | < 6              | < 6       | 22        | 219       | 49        | < 6       | 8          | 52         |
| IgM (mg/dl)   | < 17             | < 17        | < 17             | < 17      | 222       | 120       | 179       | < 17      | 52         | 75         |
| WBC           | 7800             | 8220        | 532              | 8280      | 6780      | 5900      | 8740      | 24700     | 19200      | 15500      | 11000      |
| Abs.lymph. h./mm³ | 1028          | 657         | 180              | 3120      | 650       | 3835      | 1748      | 9386      | 6144       | 7630       | 5500       |
| CD3+ %        | 10               | 0           | 0                | 0         | 0         | 56        | 78        | 92        | 78         | 59         | 51         |
| CD3 + CD 4+ % | 0                | 0           | 0                | 0         | 0         | 11        | 17        | 19        | 33         | 37         | 9          |
| CD3 + CD 8+ % | 10               | 0           | 0                | 0         | 0         | 50        | 55        | 60        | 47         | 19         | 39         |
| CD19+ %       | 0                | 0           | 0                | 0         | 0         | 1         | 0         | 0         | 30         | 36         |
| NK + %        | 75               | 43          | 90               | 78        | 63        | 43        | 12        | 2         | 11         | 7          | 16         |
| HLA DR + T cell % | 10                | 58          | 0                | 0         | 0         | 45        | 67        | 85        | 73         | 12         | 37         |

The most common symptoms were pneumonia (n = 10), chronic diarrhea (n = 6), infections plus dermatitis (n = 3), infections plus moniliasis (n = 3) or all of these (n = 3). The respiratory tract diseases were the most frequent infection type. Lymphadenopathy and hepatosplenomegaly were observed in 36% and 90% of RAGD patients, respectively. Autoimmunity was recorded in three patients. CMV infection was detected in two patients with T + B + NK + phenotype.

Table 2 Immunological and genetic findings of patients with RAG1 defects

The molecular analyses revealed a diversity of mutations, including homozygous and compound heterozygous mutations. For instance, Patient 1 demonstrated a homozygous mutation (p.R394Q) in the RAG1 gene, which is associated with a higher risk of disease. Patient 2 showed a compound heterozygous mutation (p.R394Q/p.K820R), indicating a more complex genetic background. The genetic variants identified in Patients 3 to 11 included a range of mutations, polymorphisms, and deletions, each with implications for disease causation and clinical presentation.
with Omenn Syndrome and one with T + B + SCID. The Omenn Syndrome case had all the classical clinical and laboratory findings. He had early-onset generalized erythroderma, failure to thrive, protracted diarrhea, hepatosplenomegaly, lymphadenopathy, and eosinophilia. This case had developed left axillary lymphadenopathy six weeks after BCG infection. *Mycobacterium bovis* was isolated from the lymph node (BCGitis).

The other case with T + B + SCID had recurrent pulmonary infections, splenomegaly and CMV infection. She received the BCG vaccine before the diagnosis of SCID and developed bilateral axillary lymphadenopathy three months after vaccination. Granulomatous lymphadenitis was observed in lymph node biopsy and *Mycobacterium bovis* was isolated (BCGitis). Analysis of the parents of these two patients revealed that the mothers also had the same findings.

p.H249R/p.K820R amino acid changes (compound heterozygous) were observed in two patients of T + B-group. The male case (patient 7) had diarrhea, recurrent bronchiolitis and autoantibodies. Several nodules were observed on his shoulder and gluteal area when he was three years old. *Mycobacterium Bovinum* was isolated both from biopsy of nodules and from bronchoalveolar lavage fluid (BCGosis). The female case (patient 6) had recurrent pulmonary infections, diarrhea, hepatomegaly, splenomegaly, and bronchiectasis. She is now 13 years old and receiving regular intravenous immunoglobulin therapy.

Mutations or amino acid changes have not been observed in the healthy control group. There was no mutation in RAG 2 gene in either the patients’ or control groups.

**RAG1base and RAG2base mutation databases**

All reported RAG-1 mutations were recorded into databases called RAG1base and RAG2base. These databases were constructed according to http://www.uta.fi/imt/bioinfo/RAG1base and/RAG2base.

**Mortality and survival**

Five patients underwent bone marrow (BMT) or umbilical cord stem cell transplantation. These patients received bone marrow from sibling (n = 1), haploidentical mother (n = 2) (Patient 11 had 2 BMTs), haploidentical father (n = 1) and matched unrelated (n = 2) donors. Mean follow-up duration after transplantation was 9.0 ± 8.9 months. Two patients deceased after transplantation and 4 patients deceased before transplantation during follow-up. Engraftment was not achieved in two cases. One of them was a T + B + NK + leaky SCID male patient and the donor was his mother, and the other patient was a T-B-NK + patient and the donor was his brother. One patient (T + B + NK + leaky SCID female patient) has fully recovered after BMT. Two patients are currently being followed by prophylactic IVIG replacements and they are waiting for the results of matched unrelated donor screening.

**Discussion**

Mutations in RAG1 or RAG2 represent approximately 10% of all SCID cases and most of them are T-B-NK + SCID [16]. For our patients, the frequencies of T-B-NK +, T + B-NK + and T + B + NK + SCID phenotype were 45%, 36% and 18%, respectively. All of our patients fulfilled previously defined clinical criteria for SCID. Consanguinity (54%) and family history for immunodeficiency (45%) were very high within our patient group. A high rate of consanguineous marriages (21%) is a social and health problem in Turkey as a result of some traditional concepts, especially in eastern regions. Female/male ratio was found to be 1.2. Because of these findings and the effect of high consanguinity rate, RAGD is previously reported to be inherited in an autosomal recessive pattern [18-20].

To date, at least 55 different hypomorphic RAG mutations have been described; however, clear correlation between the type of mutations and clinical presentations has not been observed. This may be due to specific mutations or other genetic factors, or epigenetic mechanisms. The mutations identified, both in RAG1 and RAG2 genes; can either be severe, leading to null alleles, or mild, leading to hypomorphic alleles that can still maintain a residual enzymatic activity of RAG1/2 proteins. Null mutants typically predominate in classical T-B-SCID, with no productive rearrangement of the T cell receptor (TCR) or B-cell receptor (BCR); while missense mutations predominate in Omenn syndrome and leaky SCID [21,22]. The same mutations in different individuals usually lead to similar phenotypes. There are also a few cases in which the same mutation gives rise to a different clinical presentation. This suggests that yet unknown factors may play a role in determining the clinical picture and outcome [21-25].

The hypomorphic mutation, P85fs32X (del A256/A257) (old nomenclature is k242fsX246 del A368/A369), demonstrated in our eleventh patient, was previously reported by Villa A et al [13]. In another report by de Villartay et al, four unrelated patients, with the same hypomorphic RAG1 mutations were presented [26]. The immunological phenotype of Villartay et al’s patients consisted of a restricted T cell repertoire, with TCRβ lymphopenia, but markedly high levels of TCRγT cells. Severe, disseminated CMV infection and autoimmune blood cell manifestations shaped their clinical phenotype. Our patient with the same hypomorphic mutation had almost the same clinical and laboratory
findings, showing a clear correlation between genotype and phenotype as these 4 patients.

Sobacchi et al [2] have reported 24 RAGD cases and 16 novel mutations in 2006. One of the mutations in this report was a p.Q248X homozygous mutation observed in a T-B-SCID case. Our patient (no.9) with the same mutation had the characteristic findings of Omenn Syndrome. Asai et al have recently reported (2011) 4 missense and 1 nonsense mutations in RAG1 of three SCID children [27]. An atypical SCID case with maternal T cell engraftment carried a homozygous novel E770K mutation [27]. One case with atypical Omenn syndrome was compound heterozygous bearing novel R142X and R396H mutations [27]. The last case with γδ T- CMV infection was a compound heterozygote bearing novel R474C and L732F mutations in RAG1 [27]. None of our cases was carrying these mutations.

Pico-Knijnenburg et al [28] have reported a family with three siblings who were identified by early onset of infections, pneumonia, sepsis, generalized dermatitis, malnutrition and diarrhea. There was a strong reduction of T and B cells and a complete block in precursor B cell differentiation in bone marrow that showed a V(D)J recombination defect [28]. In all siblings, a heterozygous mutation in RAG1 was identified (c2571A > G; p.Cys328Tyr) [28]. They thought that, in case of heterozygous mutations of RAG1 gene, there might be another defect on the second allele, which was not picked up with routine methods such as PCR and sequencing of the coding region. In her cohort of patients, Villa A also has observed Omenn cases carrying mutations on only one allele and no mutations in the promoter regions or in other genes involved in VDJ recombination (personal communication). According to her, a careful single nucleotide polymorphisms (SNPs) analysis would also be useful to clarify these patients’ defects.

H249R (one homozygous and two heterozygous patients in our study) and K820R (two compound heterozygous patients) variants have been observed in our patient group, but not in our small healthy control group. Notarangelo LD (Boston Children’s Hospital, USA) has found several normal subjects with these variants and he thought that they could not be disease-causing-polymorphisms, (personal communication). Our five patients presented with classical signs, symptoms and laboratory findings of SCID and our control group for the molecular analyses included ten people only. Thus, we suggest that a future study disclosing the functional activity of human RAG mutants would tell us if these variants are disease causing or polymorphisms. In addition, analyses of RAG1 gene in at least one-hundred healthy Turkish people will lead us to know more about the disease activity of these variations.

High frequency of BCG infection in our study also needs to be discussed. Yeganeh et al reported that complicated Bacillus Calmette-Guérin (BCG) vaccination was documented in 18 of 40 SCID cases (45%) following the routine vaccination at birth [29]. In another study, incidence of severe adverse reactions after BCG vaccination was reported as 0.0182 cases per 100,000 vaccinations in Japanese children. Among 39 cases with adverse reactions, 13 had been diagnosed to have some types of primary immunodeficiency. Four of them had SCID [30]. Sadeghi-Shanbestari M et al [31] have reported that seven of their eleven disseminated BCG infection and primary immunodeficiency patients were SCID cases. From these seven SCID and BCG infection cases, four patients had T-B-NK + SCID; probably RAG genes were involved [31]. The same authors have also reported a novel RAG2 mutation in a patient with SCID and BCG disease [32]. Four of our 11 RAGD cases (36.4%) had BCG disease (2 BCG-itis and 2 BCG-osis) and had to receive anti-mycobacterial therapy. The clinical findings and management of the patients who had documented BCG infection were worse than the rest of our group.

We reach to several conclusions from this review of our RAGD patients:

Epigenetic factors such as compound genetic defects, environmental factors and exposure to infectious agents may modify phenotypical characteristics of RAGD. Homozygous mutation of p.R394Q/p.R394Q and p.R776Q, 3047-3049 del GCC mutations are novel and they are causing serious T-B-NK + SCID.

It is very important to increase the awareness of pediatricians for early diagnosis and treatment of these patients with RAG defects.

Inoculation of live vaccines such as BCG should be postponed in suspected cases for primary immunodeficiency disease, until appropriate screening tests exclude this diagnosis. Therefore, knowledge of previous cases with immunodeficiency within the same family is beneficial for early diagnosis and decision for postponing BCG in such patients.

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Authors’ contributions
AB carried out the molecular genetic studies. NG and NEK reviewed the data and wrote the paper. NK and GA analyzed the data and edited the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.
References

1. Ehl S, Schwarz K, Enders A, Duffner U, Pannicke U, Kühn J, Mascart F, Schmitt-Graeff A, Niemeyer C, Fischer P: A variant of SCID with specific immune responses and predominance of T+ T cells. J Clin Invest 2005, 115:3140-8.

2. Sobacchi C, Marrella V, Rucci F, Vezzoni P, Villa A: RAG-dependent primary immunodeficiencies. Hum Mutat 2006, 27:1174-84.

3. Niehues T, Perez-Becker R, Schuetz C: More than just SCID: The phenotypic range of combined immunodeficiencies associated with mutations in the recombination activating genes (RAG) 1 and 2. Clin Immunol 2010, 133:183-92.

4. Pessach I, Walter J, Notarangelo LD: Recent advances in primary immunodeficiencies: identification of novel genetic defects and unanticipated phenotypes. Pediatr Res 2009, 65:3R-12R.

5. Fischer A: Have we seen the last variant of severe combined immunodeficiency? N Engl J Med 2003, 349:1799-92.

6. Schatz DG, Obermair MA, Baltimore D: The VDJ recombination activating gene, RAG-1. Cell 1989, 59:1033-48.

7. Oettinger MA, Schatz DG, Baltimore D: RAG-1 and RAG-2, adjacent genes that synergistically activate VDJ recombination. Science 1990, 248:1517-22.

8. Ohm-Laursen L, Nielsen C, Fisker N, Lillevang ST, Barington T: Molecular analysis of T-B-NK + severe combined immunodeficiency and aberrant DNA rearrangements due to a hypomorphic RAG1 mutation. J Med Genet 2009, 46:711-16.

9. Schwarz K, Gauss GH, Ludwig L, Pannicke U, Li Z, Lindner D, Friedrich W, Seger RA, Hansen-Hagge TE, Desendio S, Lieber MR, Bartram CR: RAG mutations in human B-cell-negative SCID. Science 1996, 274:97-10.

10. Buckley RH, Schiff RI, Schiff SE, Markert ML, Williams LW, Harville TO, Roberts JS, Puck AM: Human severe combined immunodeficiency: genetic, phenotypic, and functional diversity in one hundred eight Normal B Cells Despite Partial RAG1 Deficiency and Atypical SCID/ Omenn Syndrome. J Clin Immunol 2008, 28:588-92.

11. Sanger R, Chaffey S, English J, Pett B, Delle: Identification of novel genetic defects and clinical phenotypes in recombinase-activating gene 1 deficiency. Pediatr Allergy Immunol 2007, 18:225-30.

12. Pasick S, Dujovic S, Ristic G, Slavkovic B: Recombinase-activating gene 1 immunodeficiency: different immunologic phenotypes in three siblings. Acta Pediatr 2009, 98:602-64.

13. Villa A, Santagata S, Bozzi F, Giliani S, Frattini A, Imberti L, Gatta LB, Ochs HD, Schwarz K, Notarangelo LD, Vezzoni P, Spano-Poulou J, Perez-Becker R, Schuetz C, Fischer A, de Villartay JP: Severe combined immunodeficiency and aberrant DNA rearrangements due to a hypomorphic RAG1 mutation. Blood 2009, 113:2955-75.

14. Altuglu I, Kutukculer N: Functional, phenotypic and constitutional diversities in Omenn syndrome and severe combined immune deficiency. Pediatr Transplantation 2009, 13:244-50.

15. de Villartay JP, Lim A, Moussa H, Dupont S, Dechante-Menille J, Courau-Maito E, Gougeon ML, Lemaineque C, Eidsenreich C, Jouanguy E, Abel B, Casanova JL, Fischer A, Le Deist F: A novel immunodeficiency associated with hypomorphic RAG1 mutations and CMV infection. J Clin Invest 2005, 115:3291-95.

16. Asai E, Wada T, Sakaiakara Y, Toga A, Toma T, Shimizu T, Nampoothiri S, Imai K, Nonoyama S, Morio T, Muramatsu H, Kamachi Y, Obara O, Yachie A: Analysis of mutations and recombination activity in RAG-deficient patients. Clin Immunol 2011, 138:172-7.

17. Pico-Krijnenburg I, Jisper H, Pac M, Turlot J, Hartwig N, Sanders E, Tesican I, Bernatowicka E, van Dongen J, van der Burg M, Wolfs-Kusniercz B: Can heterozygous RAG1 mutations be disease-causing? Xth Meeting of the European Society for Immunodeficiencies (ESID) 2010, 203-4. Istanbul, abstract book.

18. Veganeh M, Hedarzade M, Pourpak Z, Parvaneh N, Rezaei N, Gharagouzioz M, Movahedi M, Shabestari MS, Mannishi S, Aghamohammadi A, Amin M: Severe combined immunodeficiency: a cohort of 40 patients. Pediatr Allergy Immunol 2008, 19:303-6.

19. Toida I, Nakata S: Severe adverse reactions after vaccination with Japanese BCG vaccine: a review. Kekkaku 2007, 82:829-34.

20. Sadeghi-Shanbestari M, Ansarin K, Maljaei SH, Rafeey M, Pezeshki Z, Sadeghi-Shanbestari M, Vesal S, Jabbarpour-Bonyadi M, de Villartay JP: Severe adverse reactions after vaccination with the Japanese BCG vaccine: a review. Kekkaku 2007, 82:829-34.

21. Aghamohammadi A, Amin M: Severe combined immunodeficiency: a cohort of 40 patients. Pediatr Allergy Immunol 2008, 19:303-6.

22. Vignali D, Schier A, de Villartay JP: Novel mutations and diverse clinical phenotypes in recombinase-activating gene 1 deficiency. Italian Journal of Pediatrics 2012 38:8.