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Identification of Patients with *RAG* Mutations Previously Diagnosed with Common Variable Immunodeficiency Disorders

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Abstract

Purpose—Combined immunodeficiency (CID) presents a unique challenge to clinicians. Two patients presented with the prior clinical diagnosis of common variable immunodeficiency (CVID) disorder marked by an early age of presentation, opportunistic infections, and persistent lymphopenia. Due to the presence of atypical clinical features, next generation sequencing was applied documenting RAG deficiency in both patients.

Methods—Two different genetic analysis techniques were applied in these patients including whole exome sequencing in one patient and the use of a gene panel designed to target genes known to cause primary immunodeficiency disorders (PIDD) in a second patient. Sanger dideoxy sequencing was used to confirm *RAG1* mutations in both patients.

Results—Two young adults with a history of recurrent bacterial sinopulmonary infections, viral infections, and autoimmune disease as well as progressive hypogammaglobulinemia, abnormal antibody responses, lymphopenia and a prior diagnosis of CVID disorder were evaluated. Compound heterozygous mutations in *RAG1* (1) c256_257delAA, p86VfsX32 and (2) c1835A>G, pH612R were documented in one patient. Compound heterozygous mutations in *RAG1* (1) c.1566G>T, p.W522C and (2) c.2689C>T, p. R897X) were documented in a second patient post-mortem following a fatal opportunistic infection.

Conclusion—Astute clinical judgment in the evaluation of patients with PIDD is necessary. Atypical clinical findings such as early onset, granulomatous disease, or opportunistic infections should support the consideration of atypical forms of late onset CID secondary to RAG deficiency. Next generation sequencing approaches provide powerful tools in the investigation of these patients and may expedite definitive treatments.

Keywords

RAG1; RAG deficiency; primary immunodeficiency; severe combined immune deficiency; common variable immunodeficiency disorder; exome sequencing; gene panel

Introduction

Combined immunodeficiency (CID) presents a diagnostic challenge for clinicians [1]. Next generation sequencing has supported the recent discovery of a large variety of single-gene abnormalities associated with primary immunodeficiency disorders (PIDD) [2]. We describe the presence of hypomorphic *RAG* mutations in two patients with the clinical diagnosis of a common variable immunodeficiency (CVID) disorder. Next generation sequencing has also supported an increase in our understanding of the breadth of phenotypes associated with *RAG* mutations in humans [3], which has continued to expand beyond the classic phenotype of severe combined immunodeficiency (SCID) [4]. Based on the estimated prevalence of pathogenic homozygous or compound heterozygous *RAG1/2* variants (1:6000 in individuals of European descent), next generation sequencing will continue to support this expansion in genotypic as well as phenotypic heterogeneity of RAG deficiency [5]. Atypical clinical features such as early age of presentation, opportunistic infections, and granulomatous disease should alert the astute clinician to the possibility of a diagnosis of late onset CID secondary to RAG deficiency. These cases highlight the importance of considering SCID-associated genes, such as RAG deficiency among patients presenting with atypical features in the context of PIDD. The application of next generation sequencing to provide an accurate diagnosis in these challenging cases is also discussed.

Case Report 1

A previously healthy 3 year-old Caucasian female was referred to CHOC Children's Hospital, Orange, California, with immune thrombocytopenia purpura. She was treated with intravenous immunoglobulin and Rh(D), but her response was poor prompting further evaluation. Quantitative immunoglobulin levels were unremarkable (IgG 839 mg/dL, IgM 70 mg/dL, IgA 23 mg/dL) following administration of intravenous immunoglobulin. The past medical history was otherwise non-contributory. A bone marrow examination was normal. Transient use of prednisone resulted in normalization of her platelet count.

By 5 years of age she developed recurrent sinopulmonary infections and hepatosplenomegaly. A chest CT demonstrated diffuse interstitial infiltrates; infection was excluded. Laboratory evaluation (Fig. 1) demonstrated low quantitative immunoglobulins (IgG 390 mg/dL, IgM 38 mg/dL, IgA <6 mg/dL), lymphopenia, and a normal serum IgE (<1 IU/mL). An absolute T cell count of 808/uL (normal range: 714–2266/uL) was documented. Poor antibody responses (tetanus toxoid, *Haemophilus influenzae* Type b, Hepatitis B) were

noted. Abnormal mitogen and antigen T cell proliferation responses were noted. The following mitogen responses were documented: a phytohemagglutinin (PHA) (1:25) stimulation index (SI) of 40, a PHA (1:125) SI of 120, a PHA (1:625) SI of 1, a pokeweed mitogen SI of 146, and a concanavalin A SI of 106. The following antigen specific responses were documented: a tetanus SI of 2, and a *Candida* SI of 1. Based on the clinical history of recurrent sinopulmonary infections in combination with hypogammaglobulinemia and poor antibody responses, a diagnosis of a CVID disorder was considered. She was started on intravenous immunoglobulin replacement.

At 7 years of age the patient developed worsening respiratory distress prompting a lung biopsy that revealed granulomatous-lymphocytic interstitial lung disease (Fig. 2) including features consistent with granulomatous disease, lymphocytic interstitial pneumonitis, and follicular bronchiolitis. The differential diagnosis of granulomatous-lymphocytic interstitial lung disease was considered including infectious causes (e.g., tuberculosis, histoplasmosis) and non-infectious causes (e.g., histiocytic disorders, vasculitis, lymphoma, sarcoidosis). Pulmonary function assessment demonstrated changes consistent with mild restrictive disease (FEV1 71 % predicted and FVC 75 % predicted). She was treated with corticosteroids and infliximab, which resulted in radiographic improvement. Pulmonary function assessment also demonstrated improvement (FEV1 93 % predicted and FVC 92 % predicted). Serial imaging showed no further progression of granulomatous-lymphocytic interstitial lung disease and adenopathy. At 16 years of age she has a documented history of recurrent sinopulmonary infections (*Penicillium*, *Corynebacterium propinquum*, and *Pseudomonas aeruginosa*), viral infections (shingles), and recurrent autoimmune disease (autoimmune cytopenias, vitiligo). She is alive and well and currently being considered as a candidate for unrelated donor hematopoietic cell transplantation. Anti-cytokine antibody screening was negative except for anti-TNF α antibody, which was likely present secondary to ongoing infliximab treatment.

Whole exome sequencing identified compound heterozygous mutations in *RAG1*. Sanger sequencing confirmed the presence of the compound heterozygous *RAG1* mutations c.256_257delAA, p.K86VfsX33 and c.1835A>G, p.H612R. The c.256_257delAA, p.86VfsX32 mutation was present in the mother and the c.1835A>G, p.H612R was present in the father. In order to assess RAG1 activity for the mutant alleles, Abl-*Rag1*^{-/-} pro-B cells containing a green fluorescent protein cassette flanked by recombination signal sequences was infected with retroviral vector expressing each *RAG1* mutant. RAG activity was quantified by flow cytometry. The c.256_257delAA, p.K86VfsX32 mutation reduced RAG1 activity to 2.7 % of wild-type levels in Abl-*Rag1*^{-/-} pro-B cells. The p.K86VfsX32 mutation also has been described previously as a pathogenic [6]. The c.1835A>G mutation is predicted to affect splicing, which could not be examined in Abl-*Rag1*^{-/-} pro-B system; however, a patient homozygous for the c.1835A>G mutation has been reported with a SCID phenotype [1].

Case Report 2

A 23 year-old Caucasian female was referred to University of Utah Medical Center in Salt Lake City, Utah for evaluation and treatment of a PIDD. Her clinical history was notable for

delayed separation of the umbilical cord and omphalitis in infancy. She developed pneumonia at 1 year of age and continued to suffer from chronic upper and lower respiratory tract infections. At 3 years of age an absence of IgA and a negative *Haemophilus influenzae B* titer was noted; however, a response to tetanus was protective after vaccination. At 5 years of age she had severe varicella infection.

At 14 years of age, laboratory studies demonstrated decreased IgG and IgM. A diagnosis of a CVID disorder was considered and she was started on intravenous immunoglobulin replacement therapy. She had low B cells, mildly decreased NK cells and a skewed T cell compartment with a relative increase of CD4+CD45RO+ T cells (Online Table 1). She had autoimmune features including vitiligo and occasional cytopenias. At age 24 the patient first developed chronic diarrhea with Norovirus, which led to marked weight loss. At 27 year of age the patient was recruited to a study of a cohort of 26 cases of patients with a clinical diagnosis of a CVID disorder to screen for genetic causes. Six months later, she developed *Aspergillus* pneumonia, which was rapidly fatal.

Genetic analysis was completed postmortem using a gene panel designed to target genes known to cause primary antibody or combined immunodeficiencies. We identified three variants. One heterozygous variant (c.191G>T, p.P21R) in *TNFRSF13C* encoding BAFF-Receptor and compound heterozygous mutations (c.1566G>T, p.W522C and c.2689C>T, p.R897X) in *RAG1* (Online Figure 1). Heterozygous p.P21R has been reported to increase the susceptibility to CVID disorders by decreasing BAFF-R expression and oligomerization on the cell surface, but the P21R allele was common (6.7 %) in the control population [7] therefore unlikely to be the cause of the severe phenotype of the patient. The RAG1 R897X substitution has been reported both in classic [4], and atypical forms of SCID [8]; R897X is a complete loss of function mutation [8]. RAG1 W522C substitution has been reported at least three times [5, 9, 10]; W522C results in a 50–60 % loss of recombinase activity in vitro [5, 10]. Further immunological evaluation could not be accomplished since compound heterozygous *RAG1* mutations were not identified until after the patient died.

Discussion

The wide phenotypic spectrum of SCID, including late onset CID is now being appreciated to a greater extent [11]. Recombinase-related disorders including RAG deficiency provide an excellent example of this phenotypic heterogeneity [3]. Reports of hypomorphic *RAG* mutations preserving higher RAG activity level (averaging >30 %) are associated with the generation of near normal T and B cell counts and risk for CID with immune dysregulation including granulomatous disease and/or autoimmunity [3]. Moreover, immune dysregulation marked by granulomatous disease and autoimmunity are seen across a wide spectrum of PIDD and may relate to abnormalities in T and B cell development as well as associated abnormalities in the cellular and/or cytokine environment [12]. The population frequency of pathogenic homozygous or compound heterozygous *RAG1/2* variants is estimated to be about 1:6000 in individuals of European descent [5], thus we expect to find cases of partial RAG-deficiency more frequently in the era of next generation sequencing techniques such as gene panels and exome sequencing.

Both patients presented here had a diagnosis of a CVID disorder characterized by childhood-onset and clinically-defined, progressive CID that was complicated by severe infections and autoimmunity. Several case reports have highlighted that hypomorphic *RAG* mutations display diverse phenotypes, such as atypical SCID with a predominance of TCR $\gamma\delta^+$ T cells following systemic cytomegalovirus infection [13–15], and combined immunodeficiency with granulomatous disease with or without autoimmunity (CID-G/AI) [10, 16, 17]. The CID-G/AI phenotype is associated with unique clinical features resembling granulomatosis with polyangiitis as well as complications of midline destruction or chronic sterile multifocal osteomyelitis. Moreover, T cell lymphopenia without overt manifestations of autoimmunity has also been associated with pathogenic *RAG* mutations [18, 19].

Atypical and/or progressively worsening clinical course marked by opportunistic infections and early-onset granulomatous lung disease may be consistent with a CVID disorder, but should raise concern for the astute clinician. In fact, estimates suggest that autoimmunity and lymphopenia are found in approximately one-third of patients with CVID disorders [20]. We tested 26 random CVID disorder patients using the gene panel as noted (Online Supplement). Based on this data we can say that the frequency of *RAG* deficiency is 1/26; however, this estimate may be higher if patients are selected based on the presence of features consistent with CID. Identification of these cases should promote increased awareness of the phenotypic heterogeneity of atypical forms of SCID including those associated with hypomorphic *RAG* mutations. Among patients with a clinical and immunological phenotype suggestive of CID some phenotypic overlap with CVID disorders may be appreciated; however, novel techniques such as next generation sequencing approaches should be considered in order to provide a prompt and accurate diagnosis. As an example, a recent report documents a 14 year old with a diagnosis of a CVID disorder marked by granuloma formation, lymphoma, and autoimmune neutropenia that was discovered to have homozygous *RAG1* mutations [21].

Astute clinical evaluation is vital in the evaluation of PIDD patients. Atypical clinical and laboratory manifestation should prompt clinicians to consider alternative diagnoses which can be confirmed via the application of next generation sequencing tools. Next generation sequencing techniques have been instrumental in the diagnosis of a variety of novel PIDD [2, 22]. Moreover, the use of these techniques has supported a change in our understanding of the landscape of phenotypic heterogeneity among patients with genetically defined PIDD. We anticipate that the judicious use of tools such as next generation sequencing will continue to support our ability to elucidate the causative single gene defects in many of these challenging clinical cases. Early identification of etiologies such as *RAG* deficiency can expedite therapeutic interventions including hematopoietic cell transplantation and may prevent escalation of autoimmune disease and fatal infections.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Immunologic evaluation (Case Report 1) demonstrating lymphopenia and hypogammaglobulinemia. The *x* axis depicts the patient age in years and the *y* axis depicts the serum IgG level (mg/dL), IgA (mg/dL), IgM level (mg/dL), IgE level (IU/mL), absolute eosinophil count (cells/uL), and absolute lymphocyte counts (cells/uL). The grey zone is the normal age adjusted reference range.

Fig. 2.
a Granulomatous-lymphocytic interstitial lung disease as documented by chest CT imaging and lung biopsy in Case Report 1. Chest CT imaging documenting **a** interstitial infiltrates and multiple pulmonary nodules bilaterally **b** improvement in interstitial infiltrates and multiple pulmonary nodules bilaterally following infliximab. Lung biopsy results demonstrating **c** epithelioid granulomas with giant cells **d** germinal center formation and a **e** T cell predominance