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Adult onset myopathy in a patient with hypomorphic RAG2 mutations and combined immune deficiency

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To the Editor:

Severe loss of function mutations in recombination activating genes (*RAG1* and *RAG2*), required for the initial phase of V(D)J recombination in T cell and B cell receptors, are associated with a T-B-NK+ form of severe combined immunodeficiency (SCID) [1].

However, hypomorphic mutations in the same *RAG* genes can lead to atypical (or “leaky”) forms of SCID, which may present with a broad spectrum of phenotypes[1-4]. These include Omenn syndrome, which presents in infancy, and is characterized by erythroderma, eosinophilia, elevated IgE level, lymphadenopathy and hepatosplenomegaly. Often milder and with later onset, a range of immune dysregulation syndromes can be seen. These can be

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associated with autoantibodies against self-antigens and cytokines[1,5,6], as well as defects in T cell and B cell tolerance[1]. Some patients present with a combined immunodeficiency phenotype with granulomatous disease and autoimmunity, including autoimmune cytopenias, vitiligo, myasthenia gravis and psoriasis[1-3,5].

Patients with hypomorphic mutations in SCID-associated genes may appear to have common variable immune deficiency (CVID). Of note, a sizeable proportion of patients with CVID present with autoimmunity (approximately 30%) and/or granulomatous disease (approximately 10%)[7]. While there can be significant overlap in laboratory and clinical manifestation of CVID and hypomorphic *RAG* mutations, features such as opportunistic infections, significant T and B cell defects, the presence of autoantibodies, including those targeting cytokines and causing autoimmunity, should prompt investigation for an underlying monogenic defect, which could include hypomorphic *RAG* deficiency. We found two cases of *RAG* mutation in the literature that were uncovered in patients previously diagnosed with CVID who developed opportunistic infections as well as autoimmunity (vitiligo and autoimmune cytopenias) [1,8]) as well as two cases of *RAG* mutation in patients with selective antibody deficiency with impaired production of antibody against bacterial polysaccharide[9].

We present a man previously diagnosed with CVID, who developed progressive muscle weakness and atrophy in adulthood, and was found to have pathogenic hypomorphic *RAG2* mutations. This is to our knowledge the first case of *RAG2*-related myopathy, while there was a prior case of very early onset *RAG1*-related myopathy [4]. The study was approved by the Institutional Review Board at the National Institutes of Health (protocol 93-I-0119).

The patient is a 36-year-old man with a history of recurrent sinus and lung infections, bronchiectasis, onychomycosis, recurrent cutaneous herpes simplex virus (HSV), warts, psoriasis and splenomegaly. He presented to the University of Pennsylvania Allergy & Immunology clinic for evaluation of 2.5 years of progressive muscle weakness and atrophy, in the absence of a family history of myopathy. He was a 34-week-old premature infant and was otherwise relatively healthy as a child with the exception of severe eczema (which resolved), cutaneous warts and transient partial alopecia capitis, treated with phototherapy. He began to experience recurrent infections as an adult, including left knee infection and Methicillin Resistant *Staphylococcus Aureus* (MRSA) infection of the right shoulder. Five episodes of pneumonia required three hospitalizations over four years.

At 31 years and during his second hospitalization for pneumonia immunology evaluation showed low IgA and IgM, low anti-pneumococcal titers, low B and T cells and low-normal NK cells (Supp. Table 1). Chest CT demonstrated bronchiectasis and bronchoalveolar lavage demonstrated *Acinetobacter*, *Neisseria* and alpha hemolytic streptococcus. He had normal alpha-1 antitrypsin and cystic fibrosis testing. He was diagnosed with CVID and he started intravenous immunoglobulin (IVIG) replacement therapy. He had good response with significant reduction in sinopulmonary infections.

At 33 years he noticed weakness in his left leg which progressed to bilateral leg weakness and a 14lb weight loss. He had muscle atrophy with creatine kinase (as high as 1121 U/L)

and aldolase (as high as 22.1 U/L). Neurologic evaluation identified extensive bilateral muscle wasting in upper and lower extremities with scapular winging. There were no associated sensory findings. At 35 years a right bicep muscle biopsy demonstrated prominent perivascular inflammation and areas of atrophic fibers associated with fibrosis and ectatic capillaries and mild, active myonecrosis. The inflammatory infiltrate was generally comprised of T cells (Figure 1). Specifically, most inflammatory cells were CD3+, CD4+ T cells. Few CD8+ T cells and few B cells (CD20+) were seen.

Absence of dysferlin staining initially raised concern for a dysferlinopathy. A limb-girdle muscular dystrophy genetic panel reported two heterozygous variants of unknown significance in *TTN* and *SYNE2*, but no abnormalities in dysferlin. Genetic testing for facioscapulohumeral muscular dystrophy and a SNP microarray were also unremarkable. He was evaluated by Rheumatology due to concern for inflammatory myopathy. His evaluation was negative for autoimmune myositis. HCV, HBV and HIV antibodies were negative and respective viral polymerase chain reactions (PCR) were undetectable; JC virus PCR was positive in urine, but negative in blood and bone marrow.

At 36 years he started 60mg prednisone daily for a month with a long taper and IVIG was increased to 2g/kg monthly (from 0.5g/kg). His trial of high dose prednisone led to some improvement in extremity strength. Prednisone was weaned while methotrexate was started with a goal dose of 25mg per week and prednisone was ultimately weaned off. Unfortunately, his muscle weakness worsened and continued to progress, with development of limping and difficulty sustaining physical activity.

In the setting of myopathy, recurrent infections (including sinopulmonary and cutaneous warts and HSV requiring chronic suppression), re-evaluation of his immunophenotype was undertaken (Supp. Table 1). Low T and B cells and low normal NK cells were consistent with his prior results and lymphocyte proliferation studies were low-normal to antigen and low for mitogen stimulation (PHA and ConA both less than 10% of control). TCRVb spectratyping was essentially normal (Supp. Table 2A). Based on his immunophenotype and myopathy, whole exome sequencing (WES) trio analysis was performed (GeneDx). This revealed compound heterozygous likely pathogenic variants in the *RAG2* gene (paternally inherited c.518 A>G, p.N173S and maternally inherited c.1309 G>A, p.E437K) which are consistent with hypomorphic RAG deficiency. Both variants have been identified in a heterozygous state with a minor allele frequency of (0.000008 and .000016 respectively) in the ExAC database (Exome Aggregation Consortium, Cambridge, MA). The p.Asn173Ser is a conservative amino acid substitution that occurs at a position that is highly conserved through evolution and in silico models predict that it is damaging to protein structure. The second variant is a non-conservative amino acid substitution which is likely to impact secondary structure as these residues differ in polarity, size and charge. A missense variant in a nearby residue is reported in association with Omenn syndrome. These variants are likely to be pathogenic given they are found in *trans* in a patient with phenotype consistent with a RAG2-related immunodeficiency (including elevated CD45RO+ T cells and T cell lymphopenia) and the variants are extremely rare in population databases in the heterozygous state and absent in the homozygous state.

He was referred to the National Institutes of Health (NIH) for evaluation of a possible link between his immunodeficiency and myopathy, as well as management options including immunosuppression and allogeneic hematopoietic stem cell transplantation. Repeat electromyogram, nerve conduction studies and screening cardiac MRI were all normal. He had a positive IgM anti-myosin antibody but was otherwise autoantibody negative based on microarray analysis (data not shown). B cell depletion with anti-CD20 chimeric monoclonal antibody therapy (rituximab 1000mg/dose; 2 doses separated by 2 weeks) showed initial mild improvement in muscle strength, but then subsequent worsening of his muscle weakness. His muscle biopsy pathology was reviewed again and confirmed the rejection of the hypothesis of inclusion body myopathy as well as confirmed of the presence of T cell infiltrates, abnormal muscular fiber morphology and mild fibrosis (Figure 1).

Mycophenolate mofetil was initiated with some improvement in muscle enzymes. Based on his continued severe symptoms and known *RAG2* mutations, the patient underwent reduced intensity conditioning (with pentostatin, low-dose cyclophosphamide and pharmacokinetically-dosed busulfan) and matched unrelated donor T-cell replete allogeneic bone marrow transplantation (BMT) as a therapy known to be curative for his immunodeficiency and hoped to halt his progressive autoimmune myopathy. Graft versus host disease (GVHD) prophylaxis was provided with high-dose post-transplantation cyclophosphamide, mycophenolate mofetil and sirolimus. He engrafted with donor cells across all lineages, with chimerism at 1-year post BMT showing 97% donor CD8 T cells, 94% donor CD4 T cells, 91% donor myeloid cells, 100% donor NK cells, and 100% donor B cells and appropriate immune reconstitution, with T cell counts in the expected range (Supp. Table 1). TCRVb spectratyping post-BMT was also essentially normal (Supp. Table 2B). His BMT course was complicated by grade 2 acute GVHD of the gut that resolved after a 10-day course of steroids. His GVHD prophylaxis was stopped, per schedule, at day +180; he is now over 1 year post-BMT and remains off all immunosuppression. His myopathy has notably stabilized after BMT, now over 6 months without any need for immunosuppressive therapy. He has increased exercise tolerance, has returned to work full-time at a physically demanding occupation and has even shown improvement such as being able to stand on his toes for the first time in years.

The presence of invading T cells on pathology and anti-myosin antibodies are consistent with an autoimmune dysregulatory basis for his myopathy. While there have been many reports of autoimmunity with *RAG* mutation (including myasthenia gravis[11]), and reports of muscle disease in CVID [12,13] and Good syndrome[14] and very early onset myopathy in *RAG1* mutation [4], this is to our knowledge, the first reported case of myopathy and immunodeficiency in a patient with *RAG2* mutation. Further studies are needed to investigate the frequency and pathogenesis of muscle disease in the context of *RAG* mutation. This case demonstrates the importance of actively assessing all patients diagnosed with CVID patients for underlying diagnoses through WES, as monogenic causes of CVID, as well as CID and leaky SCID (as in our patient) and associated complications may be amenable to targeted therapy or allogeneic hematopoietic cell transplantation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig 1. Muscle biopsy in RAG2 hypomorphic SCID patient.

Muscle biopsy with CD3 T cell infiltrate. Top: Hematoxylin and eosin stained section demonstrates perivascular lymphocytic inflammation, ectatic capillaries, fibrosis, atrophic fibers and myophagocytosis. Bottom: CD3 immunohistochemistry with hematoxylin counterstain demonstrates perivascular and interstitial T lymphocytes. Scale bars indicate 200 microns.