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ASTUTE CLINICIAN REPORT

Exome Sequencing Reveals *RAG1* Mutations in a Child with Autoimmunity and Sterile Chronic Multifocal Osteomyelitis Evolving into Disseminated Granulomatous Disease

Andreas Reiff · Alexander G. Bassuk · Joseph A. Church · Elizabeth Campbell · Xinyu Bing · Polly J. Ferguson

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Abstract We describe a boy who developed autoinflammatory (chronic sterile multifocal osteomyelitis) and autoimmune (autoimmune cytopenias; vitiligo) phenotypes who subsequently developed disseminated granulomatous disease. Whole exome sequencing revealed homozygous *RAG1* mutations thus expanding the spectrum of combined immunodeficiency with autoimmunity and granuloma that can occur with RAG deficiency.

Keywords Granulomatous disease · chronic recurrent multifocal osteomyelitis (CRMO) · immune deficiency · recombinase activating gene (RAG) · autoimmune hemolytic anemia

Mutations in the recombinase-activating gene (RAG) 1 or 2 can lead to a range of phenotypes including classic severe combined immune deficiency (SCID; OMIM # 601457) [1], Omenn syndrome (OMIM# 603554) [2, 3] as well as milder immune deficiency. Patients with hypomorphic *RAG1* mutations may present with a combination of increased susceptibility to infections and autoimmunity [4–7] or granulomatous disease [7–9]. These hypomorphic mutations result in decreased RAG1 activity, the production of low-affinity self-reactive antibodies, increased autoantibody production and moderate immune deficiency [6]. Autoimmune hemolytic anemia (AIHA), autoimmune neutropenia, idiopathic thrombocytopenic purpura, vitiligo, psoriasis, Guillian-Barre syndrome and granulomatous dermatitis have been reported [7].

Chronic recurrent multifocal osteomyelitis (CRMO) is an inflammatory bone disorder that presents with sterile osteomyelitis, with or without granulomas and is frequently associated with psoriasis and Crohn disease [10–13]. Anti-inflammatories provide clinical improvement while antimicrobials are ineffective. There is no known relationship to immune deficiency and the pathogenesis remains unknown, but there are two autosomal recessive forms of CRMO that are due to dysregulation of the IL-1 pathway [14–17].

Our patient was a Lebanese boy born to first cousins who presented at age 10 months with otitis media, fever, ankle swelling and rash. He took aspirin for 1 month and did well until 17 months of age when he was admitted with otitis media, pallor and splenomegaly. His hemoglobin was 5.9, platelets 62 K, WBC was 5.6 K (ANC of 1680, ALC 2184, AEC 728) and ESR was 122 mm/hr. He had a positive Coombs' test, positive ANA and an equivocal anti-dsDNA. Despite treatment with IVIG 2 g/kg/month he required up to 2 mg/kg/day of prednisone to control his AIHA. At age 5, he developed vitiligo. From 3–5 years of age, while on daily steroids, he developed uncomplicated varicella, and 2 pneumonias. Serum immunoglobulins were normal.

At age 6, he sustained pathologic fractures of the ulna and olecranon. A bone scan revealed uptake in the distal humerus,

A. Reiff · J. A. Church
The Department of Pediatrics, Children's Hospital Los Angeles and the Keck School of Medicine, University of Southern California, Los Angeles, CA, USA

A. G. Bassuk · E. Campbell · X. Bing · P. J. Ferguson
The Department of Pediatrics, University of Iowa Carver College of Medicine, Iowa City, IA, USA

P. J. Ferguson (✉)
Department of Pediatrics, Division of Rheumatology, University of Iowa Carver College of Medicine, 200 Hawkins Drive, 4038 Boyd Tower, Iowa City, IA 52240, USA
e-mail: polly-ferguson@uiowa.edu

proximal ulna, proximal tibia and calcaneus. Olecranon biopsy revealed necrotizing granulomatous inflammation and bone necrosis, fibrosis and chronic inflammation; calcaneal biopsy revealed marrow fibrosis, chronic inflammation but no granulomas. Cultures and stains for bacteria, mycobacteria and fungi were negative. He was treated empirically for mycobacteriosis with isoniazide, ethambutol, pyrazinamide and rifampicin, which was stopped 4 months later due to a lack of efficacy. He improved on steroids.

At 9 years of age, he presented with an erythematous wrist mass and worsening multifocal bone lesions; biopsy revealed sterile necrotizing granulomatous inflammation of the subcutaneous tissue. Chest imaging was concerning for early interstitial lung disease. Plain radiographs and MRI of the right ankle revealed lytic bone lesions (Fig. 1). Biopsy of the fibula revealed sterile necrotizing granulomatous inflammation. Serologic and antigen testing for Toxoplasma, Bartonella, Brucella, Coccidioides, Cryptococcus, Histoplasma, Aspergillus, Syphilis, CMV, EBV, Pneumocystis, Legionella, Mycoplasma and Chlamydia were negative. Treatment for mycobacteriosis was reinitiated with clarlithromycin, ethambutol and moxifloxicin without improvement. Immunologic assessment revealed elevated serum IgG [2480 mg/dL (423-1187)] and IgA [381 mg/dL (22-157)] with a normal IgM and IgE. He made protective antibody titers to tetanus, varicella and polio virus but poor responses to pneumococcal antigens. Reassessment of response to immunization with 23-valent pneumococcal vaccination was not performed due to continued immune suppressive drug regimens. When tested at ages 9 and 10 years, he made normal T cell lymphoproliferative responses to PWM, ConA, VZV, tetanus; equivocal responses to candida and CMV and negative responses to Herpes simplex virus and Adenovirus. Lymphocyte populations revealed T and B cell lymphopenia with low absolute and relative numbers of CD3 [250; 29 %], CD3+ CD4+ [109; 18 %], CD3+CD8+ [26; 5 %], CD19+[96; 11 %], CD20+ [71; 9 %] cells with an absolute and relative increase in CD3-CD16+CD56+ NK cells [369; 62 %] collected while on corticosteroids. No studies on T cell receptor repertoire diversity were performed. His Nitroblue tetrazolium assay was normal.

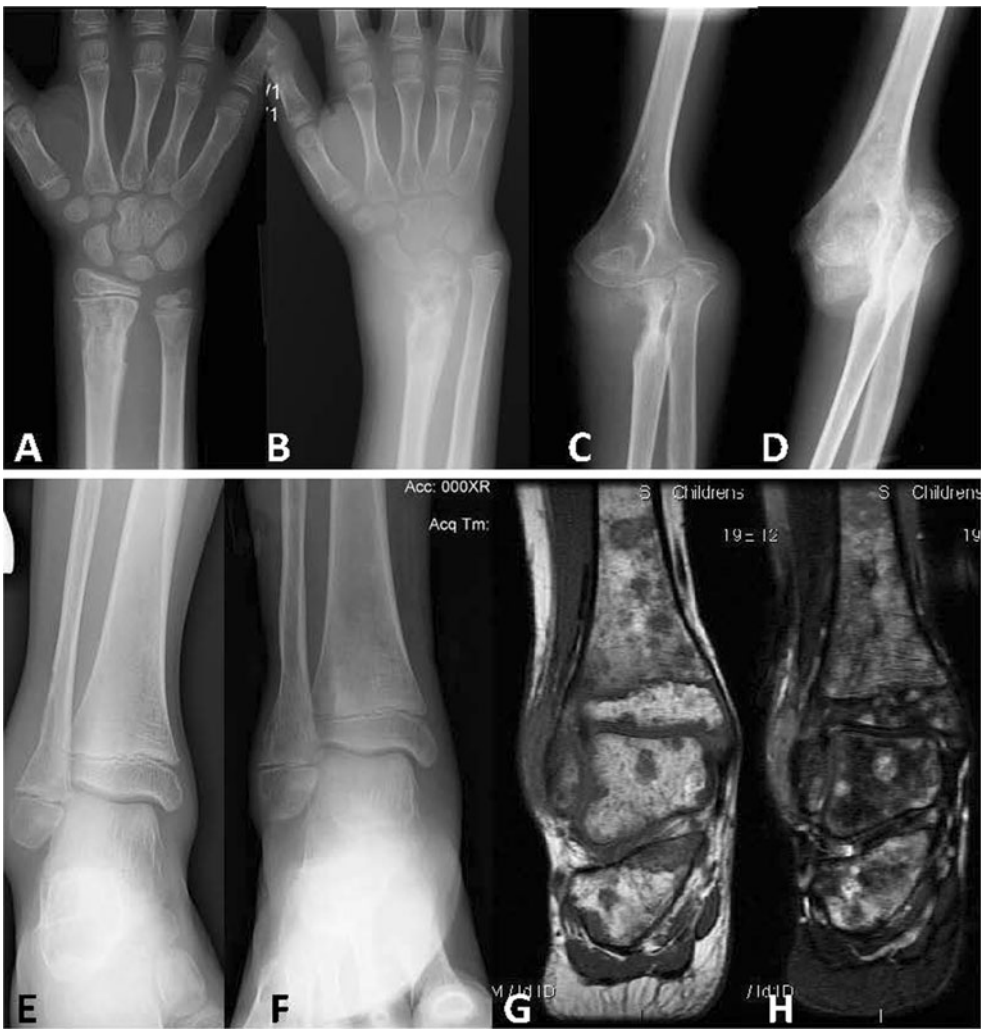

Fig. 1 Multifocal Osteomyelitis. Plain radiograph demonstrate progressive osteolytic lesions in the right distal radius and ulna over 4 months (**a**,**b**) at age 9. Progressive destruction of the olecranon (**c**,**d**) and right distal tibia/fibula (**e**,**f**) from age 9–11 years which are accompanied by diffuse osteolytic T1 hypointense (**g**), T2 hyperintense (**h**) lesions on MRI

Between 10– 12 years of age, he developed recurrent AIHA, non-granulomatous anterior uveitis and diffuse sterile non-caseating granulomatous disease of his lungs, bone marrow, testis, liver and pancreas. Immunologic studies showed worsening hypergammaglobulinemia [IgG of 3340 mg/dL], with improved T cell numbers but continued B cell lymphopenia: CD3 [943; 50 %], CD3+CD4+ [850; 45 %], CD3+CD8+ [89; 5 %], CD19+[90; 5 %], CD20+ [4 %] cells with a continued absolute and relative increase in CD3-CD16+CD56+ NK cells [917; 44 %]. Treatment with rituximab resulted in no improvement in the diffuse granulomatous disease, but his AIHA did improve. Treatment with infliximab at 10 mg/kg/dose was associated with significant improvement in his lung disease with partial improvement in his bone lesions. At the age of 12, he developed a diffuse erythematous rash. Histology showed dense lymphohistiocytic dermal infiltrate with a thin layer of parakeratosis, multifocal spongiosis without acanthosis, eosinophils or granulomas in the epidermis. At age 11 he succumbed to rapidly progressive pneumonia due to H1N1 infection with ARDS and subsequent multi-organ system failure. It was unclear if infection or uncontrolled innate immune system activation was the primary factor in his death.

Whole exome sequencing was performed on DNA from the child and both parents and was analyzed for variations that were homozygous in the child, heterozygous in both parents and not in dbSNP, 1000 genomes or the Exome Variant Server [http://evs.gs.washington.edu/EVS/] [18]. This led to the identification of non-synonymous variations in 12 genes (*RBM15, C1orf111, OBSCN, PDS5A, PCDHA2, ANKRD26, RAG1, HEPHL1, DNAH3, KLKBL4, ZNF208*). Only *RAG1* mutations have been associated with autoimmune cytopenia, vitiligo and sterile granulomatous disease [7]. The c.2095C>T *RAG1* variation was verified by Sanger resequencing as homozygous in the affected child; heterozygous in both parents, heterozygous in one sibling and absent in the other sibling. PolyPhen-2 v2.2.2r398 predicts the p.Arg699Trp variant protein to be probably damaging [http://genetics.bwh.harvard.edu/ggi/pph2].This mutation is in the heptamer binding region of *RAG1* which has been previously reported in a compound heterozygous state in a patient with Omenn syndrome and a child with autoimmune phenomena [7, 19]. There is no evidence of maternal engraftment as the X chromosome showed uniform homozygosity in the whole exome analysis.

Complete RAG deficiency results in the inability to functionally rearrange T and B cell receptors which results in B and T cell deficiency and hypogammaglobuliemia [20]. Our patient had low but present B and T cells, produced excess levels of IgG and retained the ability to make functional antibody to protein antigens demonstrating that there was residual RAG activity in his lymphocytes. His ability to make NK cells is consistent with RAG deficiency and may be due to a compensatory expansion in this setting of immune dysregulation [20, 21]. Genetic studies on this boy demonstrated uniform homozygosity on the X chromosome which argues against maternal engraftment as an explanation for the residual RAG function. This supports the conclusion that the p.Arg699Trp mutation only partially disrupts RAG function.

This case is the first report of chronic multifocal osteomyelitis associated with hypomorphic RAG1 mutations and provides further evidence that primarily rheumatologic symptoms may occur in children with hypomorphic RAG1 mutations. Corticosteroids and treatment with TNF-alpha antagonists (but not rituximab) improved his lung and bone inflammation, which suggests a role for activated T cells in disease pathogenesis [22–24]. The rash that occurred in this patient shared features with erythroderma seen in Omenn syndrome but lacked acanthosis [25]. This case should alert clinicians to include atypical immune deficiency in the differential diagnosis when recurrent infections and granulomatous inflammation occur in patients with rheumatic disease. Early diagnosis and bone marrow transplant is essential to prevent severe complications of autoimmunity and infections and can be lifesaving [9].

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