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Estimated disease incidence of *RAG1/2* mutations: a case report and querying the Exome Aggregation Consortium

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Summary

RAG deficiency is emerging as one of the leading causes of SCID and leaky SCID with an estimated incidence of 1:336,000. Hypomorphic mutations in the *RAG* genes can also lead to highly variable delayed-onset combined immunodeficiency diseases. We estimate the population genetic frequency of these hypomorphic diseases as up to 1:181,000, suggesting that *RAG1/2* mutations are likely to contribute to undiagnosed cases of combined immunodeficiencies.

Keywords

Recombination activating gene 1/2 (RAG1/2); combined immunodeficiency; population frequency; pathogenic mutation

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To the Editor

There is a great diversity of RAG1 and RAG2-dependent primary immunodeficiencies (PID) ranging from severe combined immunodeficiency (SCID) to various late-onset combined immunodeficiencies, as complete lack of RAG activity leads to absence of mature B and T cells (T- B- SCID), whereas hypomorphic *RAG* mutations allow limited generation of T and or B cells, resulting in various clinical phenotypes distinct from SCID.[1, 2]

Currently, there are no peripheral blood-based functional assays for the evaluation of RAG activity. Therefore, the pathogenicity of *RAG* variants is determined utilizing prediction programs and *in vitro* V(D)J recombination assays.[3] Critical to this evaluation is the population frequency of the variants. Querying the 1000 Genomes Project (http://www.1000genomes.org) with *RAG1/2* mutations listed in Human Gene Mutation Database (HGMD), we predicted the population frequency of hypomorphic RAG1/2 deficiency to be approximately 1:5700 individuals of European descent.[4] With the availability of larger databases, this observation can now be revisited. In this study we present a clinical case of SCID in which three *RAG1* variants previously described as pathogenic were detected. To evaluate the pathogenicity of these variants, we estimated the carrier frequency of *RAG1* mutations by intersecting large population databases with functional datasets.

Case report

A female patient from Hungary presented with hand-foot-mouth disease at 3 months of age. At 5 months, she developed left-sided lymphadenopathy after Bacille Calmette-Guerin (BCG) immunization, which required antibiotic therapy and resection. At 17 months of age, she was hospitalized for prolonged febrile episodes complicated by bronchitis and pyuria. A second hospitalization occured at 20 months of age for fever, severe diarrhea with dehydration, apthous oral ulcers and extensive maculopapular dermatitis. Laboratory evaluation (Supplementary Table 1.) was notable for decreased T and B cells with a preserved NK cell compartment, and the clinical diagnosis of T-/B-/NK+ SCID was made. T cell count of less than 300 T cells/uL is indeed part of the criteria for SCID now internationally adopted.[5] Although absent or markedly reduced (<10% of lower limit of normal) proliferative response to PHA is also required to definitely make a diagnosis of SCID, this test was not performed in this case, and therefore the diagnosis of SCID remains presumptive. At 21 months of age, the patient received a successful HLA-matched (10/10) allogeneic cord blood transplant.

Genetic testing

Genetic testing (Suppl Material) revealed three reportedly pathogenic variants in the *RAG1* gene. **p.R778Q (c.2333G>A)** was first reported in a compound heterozygous (p.R778Q/p.R975W) patient with infections and granulomatous disease.[1] We measured that p.R778Q has 8.6 (+/− 1.0)% residual RAG1 enzymatic activity in the Abelson pro-B cell line system[3], and Schuetz at al. reported that p.R778Q has 3.7% residual RAG1 enzymatic activity in a recombination assay utilizing fibroblast lines.[1] **p.R410Q (c.1229G>A)** was first described in a *RAG1* compound heterozygous patient (p.R410Q/p.R841W) diagnosed with atypical

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SCID/Omenn syndrome.[6] We have shown that p.R410Q is a complete loss-of-function variant.[3] **p.R449K (c.1346G>A)** was reported as a homozygous mutation in an Omenn syndrome patient.[7] This variant has 92.1(+/− 3.6)% residual RAG1 activity.[3] To determine the population frequency of p.R449K, we interrogated the Exome Aggregation Consortium (ExAC) database (Suppl. Material), and identified 1266 heterozygous alleles and 13 homozygous **p.R449K** (c.1346G>A) individuals out of 121,268 chromosomes tested, leading to an allele frequency of 1.044%. On the contrary, p.R410Q and p.R778Q are not found in the ExAC database.

Sequencing (Suppl. Material) demonstrated that both p.R410Q and p.R449K variants are on the same chromosome (Supplementary Figure 1A) and of maternal origin, whereas p.R778Q was inherited from the father (Supplementary Figure 1B). In addition, protein crystallography of RAG1[8,9] has previously demonstrated that R410 makes critical contacts with the DNA nonamer of the RAG recombination signal sequence (Supplementary Figure 1C) and R778 is important for the structural integrity of the RAG1/2 binding interface (Supplementary Figure 1D). This is in contrast to R449 (Supplementary Figure 1C), which does not appear to be in a position important for protein fold integrity, homodimer interaction, RAG2 complex formation, or DNA binding.

In summary, the **p.R449K (c.1346G>A)** variant is common (>1 % allele frequency; rs4151031), and is present in homozygous form in the ExAC database, which excludes severe childhood diseases, suggesting that it does not lead to severe congenital disease such as Omenn syndrome or SCID. Our *in vitro* functional studies have demonstrated that it is a neutral variant with full functional activity (92%). This case report demonstrates that R449K can be found in *cis* with a complete loss-of-function variant (p.R410Q). In addition, insight from RAG1 crystal structures does not reveal an obvious effect on DNA binding with the p.R449K substitution. Based on these findings, we conclude that p.R449K is unlikely to be a pathogenic variant. Since the two variants on the same chromosome have very different population frequencies (p.R410Q is not found in ExAC, whereas p.R449K is common with >1 % population frequency), we find linkage disequilibrium unlikely.

Carrier frequency of RAG deficiency

The carrier frequency of presumed pathogenic *RAG* mutations was previously determined utilizing the frequency of the **p.R449K (c.1346G>A)** variant in the 1000 Genomes Project Database and HGMD.[4] Here we used the ExAC dataset to evaluate the frequency of pathogenic variants in *RAG1/2* genes. Of the approximately 121,000 alleles in ExAC, 47 and 13 are predicted as complete loss-of-function *RAG1* and *RAG2* mutations, respectively, based on being frameshift, gain-of-stop, or canonical splice-site alleles. Evaluation of missense variants is more challenging, as computational predictions are fraught with error. Instead of running various prediction programs or using HGMD variants (which annotates p.R449K as pathogenic), we counted the experimentally evaluated loss- or decreased-function variants in this database, which resulted in the calculated population frequency (Suppl. Material) as high as about 1:181,000 individuals who are homozygous or compound heterozygous for pathogenic *RAG1/2* mutations.

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Discussion

Newborn screening in the United States showed that *RAG1/2* mutations were the cause of about 12% of typical SCID, and 40% leaky SCID/Omenn syndrome cases for a combined incidence of 1:336,000 together[10]). Hypomorphic mutations in the *RAG* genes can also lead to highly variable delayed-onset combined immunodeficiency diseases[11,12], but the frequency of these hypomorphic *RAG* mutations is unknown. Recent advances in high-throughput sequencing permit the generation of gene variant databases on population scales. The first of these, the 1000 Genomes Project, allowed the estimation of common variants (1% or more). The ExAC database includes exome-sequencing data from over 60,000 unrelated individuals. Querying this dataset using variants with experimentally evaluated function, we estimate that there are about twice as many RAG1/2 hypomorphic patients (1:181,000) than RAG1/2-deficient SCID, leaky SCID, and Omenn syndrome patients. Our population genetic estimate suggests that *RAG1/2* mutations are likely to contribute to undiagnosed cases of combined immunodeficiencies. This estimate, however, is significantly lower than the previous estimate based on the much smaller 1000 Genomes Project database (~1 in 5,700)[4].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Schuetz C, Huck K, Gudowius S, Megahed M, Feyen O, Hubner B, et al. An immunodeficiency disease with RAG mutations and granulomas. N Engl J Med. 2008; 358:2030–8. [PubMed: 18463379]
2. Schwarz K, Gauss GH, Ludwig L, Pannicke U, Li Z, Lindner D, et al. RAG mutations in human B cell-negative SCID. Science. 1996; 274:97–9. [PubMed: 8810255]
3. Lee YN, Frugoni F, Dobbs K, Walter JE, Giliani S, Gennery AR, et al. A systematic analysis of recombination activity and genotype-phenotype correlation in human recombination-activating gene 1 deficiency. J Allergy Clin Immunol. 2014; 133:1099–108. [PubMed: 24290284]
4. Chen K, Wu W, Mathew D, Zhang Y, Browne SK, Rosen LB, et al. Autoimmunity due to RAG deficiency and estimated disease incidence in RAG1/2 mutations. J Allergy Clin Immunol. 2014; 133:880–2 e10. [PubMed: 24472623]
5. Shearer WT, Dunn E, Notarangelo LD, Dvorak CC, Puck JM, Logan BR, et al. Establishing diagnostic criteria for severe combined immunodeficiency disease (SCID), leaky SCID, and Omenn syndrome: the Primary Immune Deficiency Treatment Consortium experience. J Allergy Clin Immunol. 2014; 133:1092–8. [PubMed: 24290292]
6. Villa A, Sobacchi C, Notarangelo LD, Bozzi F, Abinun M, Abrahamsen TG, et al. V(D)J recombination defects in lymphocytes due to RAG mutations: severe immunodeficiency with a spectrum of clinical presentations. Blood. 2001; 97:81–8. [PubMed: 11133745]

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7. Sobacchi C, Marrella V, Rucci F, Vezzoni P, Villa A. RAG-dependent primary immunodeficiencies. Hum Mutat. 2006; 27:1174–84. [PubMed: 16960852]
8. Kim MS, Lapkouski M, Yang W, Gellert M. Crystal structure of the V(D)J recombinase RAG1-RAG2. Nature. 2015; 518:507–11. [PubMed: 25707801]
9. Yin FF, Bailey S, Innis CA, Ciubotaru M, Kamtekar S, Steitz TA, et al. Structure of the RAG1 nonamer binding domain with DNA reveals a dimer that mediates DNA synapsis. Nat Struct Mol Biol. 2009; 16:499–508. [PubMed: 19396172]
10. Kwan A, Abraham RS, Currier R, Brower A, Andruszewski K, Abbott JK, et al. Newborn screening for severe combined immunodeficiency in 11 screening programs in the United States. JAMA. 2014; 312:729–38. [PubMed: 25138334]
11. Abolhassani H, Wang N, Aghamohammadi A, Rezaei N, Lee YN, Frugoni F, et al. A hypomorphic recombination-activating gene 1 (RAG1) mutation resulting in a phenotype resembling common variable immunodeficiency. J Allergy Clin Immunol. 2014; 134:1375–80. [PubMed: 24996264]
12. Buchbinder D, Baker R, Lee YN, Ravell J, Zhang Y, McElwee J, et al. Identification of Patients with RAG Mutations Previously Diagnosed with Common Variable Immunodeficiency Disorders. J Clin Immunol. 2015; 35:119–24. [PubMed: 25516070]
13. Al-Herz W, Al-Mousa H. Combined immunodeficiency: the Middle East experience. J Allergy Clin Immunol. 2013; 131:658–60. [PubMed: 23321211]

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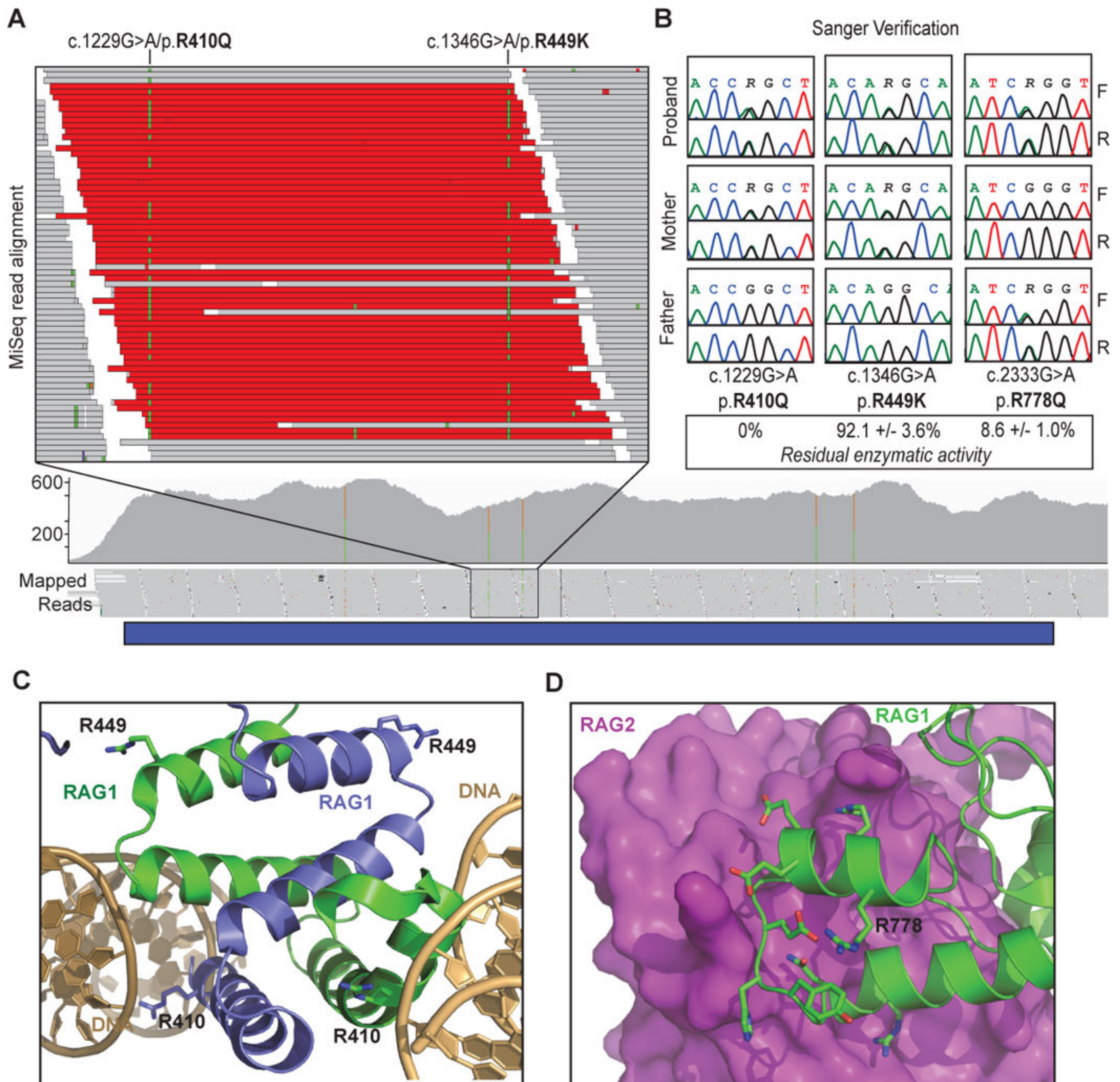

Figure 1.

(A) Sixty-eight sequence reads (shown in red) span both R410 and R449 and show that R410Q and R449K occur in *cis*. **(B)** Sanger sequencing confirms the presence of both R410Q and R449K in the healthy mother. **(C–D)** Modeling of superimposed x-ray crystal structures of a RAG1 homodimer (two identical chains shown in green and blue) complexed with DNA (PDB ID 3GNA[8] and a RAG1/2 complex (PDB ID 4WWX[7]) reveal a role for R410 in DNA binding, R778 for the RAG2 interaction, but finds no intra- or inter-molecular contacts for R449. In addition, the amino acid change of arginine (R) to lysin (K) observed in the R449K mutation corresponds to a mild change of the side chain (both basic side

chains). However, the arginine to glutamine (Q) change in both R410Q and R778Q results in significant alteration of the amino acid side chain polarity; from non-polar to polar side chain.

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