

with the R142X mutation [29]. One explanation for these findings is alternative usage of methionine as a translation start site, which has been reported in OS patients with N-terminal RAG1 frameshift mutations [30,31]. A translation start prediction program NetStart 1.0 also indicated that methionines at codon 183 and 202, which were the first and second methionines found after the R142X mutations, could be alternative translation start sites with scores comparable to the conventional initiator codon 1 (<http://www.cbs.dtu.dk/services/NetStart/>) [32]. Therefore, it is possible that an N-terminal truncated and partially functional RAG1 protein generated by alternative usage of methionine led to the OS phenotype in our patient.

The clinical features of patient 4 were consistent with CID with $\gamma\delta$ /CMV. Despite decreased recombination activity, patient 4 exhibited normal immunoglobulin levels and a normal percentage of peripheral B cells. These findings were in contrast to SCID and OS, but were in agreement with previously described cases of this disease [4,5]. Moreover, our B cell analysis of patient 4 revealed normal maturation, normal production of IgE after stimulation with anti-CD40 and interleukin-4, and normal somatic hypermutation in CD27⁺ B cells. Taken together, our case provided additional data of the genetic and immunological features of this unique disease.

RAG mutations found in patients with typical T⁻B⁻ SCID have been usually shown to abrogate recombination activity almost completely [2,33]. The residual V(D)J recombination activity resulting from the E770K mutation in RAG1 was associated with the SCID phenotype in patient 5. Despite trends towards more severe mutations, such as nonsense and frameshift mutations in SCID patients, missense mutations can lead to the SCID phenotype [33]. It is also known that the same mutations may cause different clinical phenotypes, presenting as either T⁻B⁻ SCID or OS [18], and as either T⁻B⁻ SCID or CID with $\gamma\delta$ /CMV even within one family [34,35]. These findings suggest that that residual V(D)J recombination activity may not be solely responsible for the disease development. Further studies will be necessary to assess additional factors that influence the clinical phenotype of RAG deficiency.

In summary, our studies demonstrated the pathogenic significance of the 8 RAG mutations including 6 novel mutations from 5 patients with RAG deficiency. The characterization of the genetic defects and functional abnormalities in RAG-deficient patients will help define the role of RAG in V(D)J recombination and may lead to a better understanding of the variable phenotypic expression in RAG deficiency.

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