and CD19^{+} B cells, with no evidence for somatic reversion mosaicism (Fig. 1a). The SNP array of the patient's genomic DNA revealed a karyotype of arr 11p13p12 (34,401,525–38, 380,493)×1 (Fig. 1b). The FISH analysis confirmed the non-mosaic heterozygous deletion of the 11p12 region, because one of the BAC clones, GS-44H16 (11p15.5, red dot), was represented twice and the other BAC clone, RP11-72A10 (11p12, blue dot), was represented only once (Fig. 1c). This deletion encompassed both *RAG1* and *RAG2*genes as confirmed by the SNP array (Fig. 1b). The patient's mother and brother were heterozygous for the same two missense mutations in *RAG1*. In contrast, the father's sequence was apparently normal, and his SNP array analysis revealed the same heterozygous deletion as the patient. In addition, the sister's sequence was apparently normal, although the SNP array was not analyzed (Fig. 1d, e).

In Vitro V(D)J Recombination Assay

An analysis of the recombination activity revealed an activity for the E455K, R764H and E455K+R764H recombinant proteins of 13.73, 22.42 and 2.65 %, respectively, compared with wild-type RAG1 (Fig. 2a, b). The effects of the patient's missense mutations were also evaluated using web-based analysis tools, including Mutation @ A glance, which predicted the mutations to be deleterious based on the results of the SIFT program [18].

Flow Cytometric Analysis of the BM

The relative frequency of pro B cells (95.1 %) in the patient's BM was higher, while that of pre-B1a cells (0.3 %) was lower, than that observed in normal BM. The number of later stage B

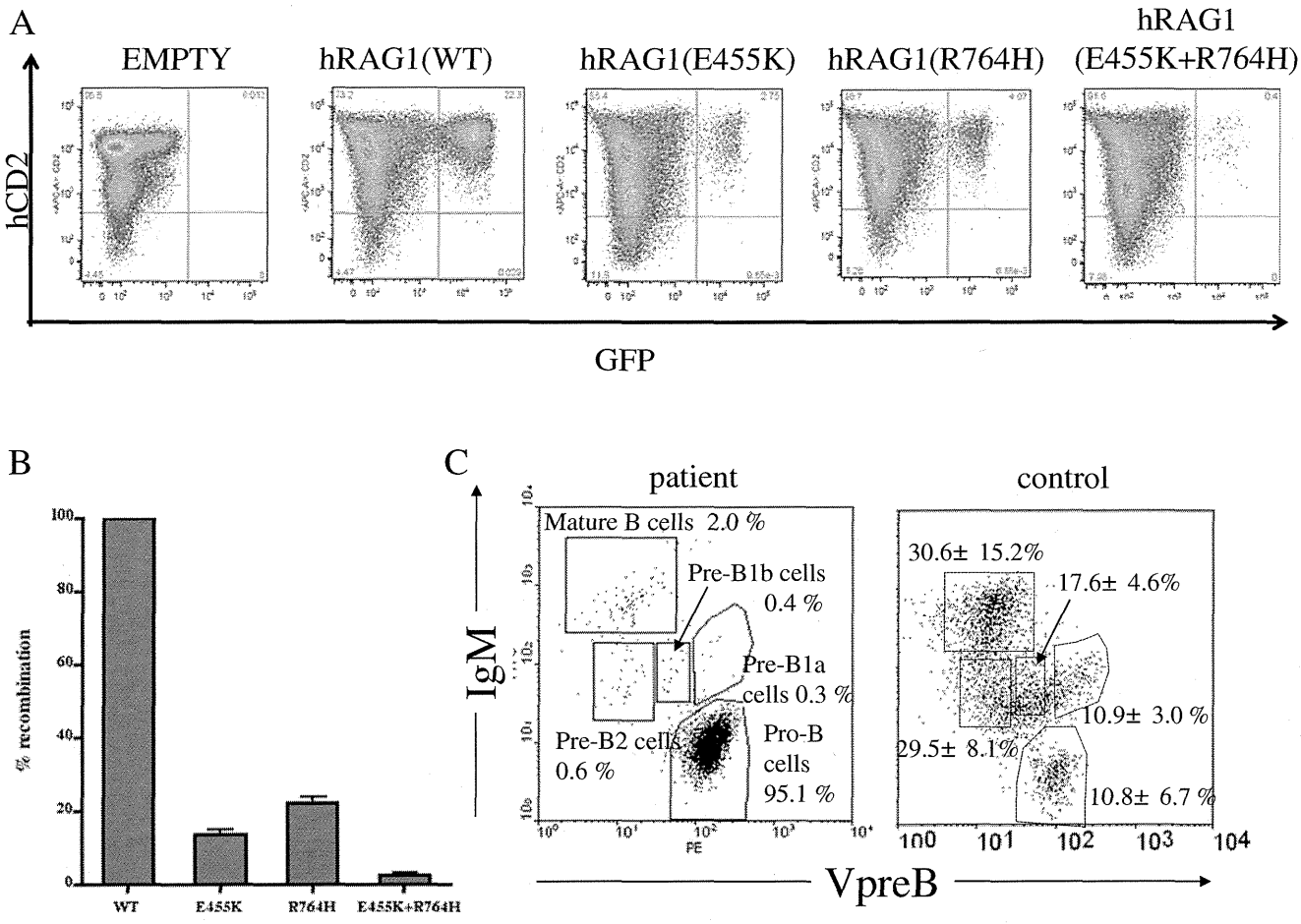

Fig. 2 V(D)J recombination activity. **a** GFP and hCD2 expression levels indicate the recombinase activity using an empty vector or vectors encoding for wild-type (WT) hRAG1 or hRAG1(E455K), hRAG1(R764H) or hRAG1(E455K+R764H), respectively. **b** The data are expressed as the percentage of activity compared with that of the wild-type protein and represent the mean±standard deviation of four independent experiments. The results revealed a recombination activity for the E455K, R764H and E455K+R764H recombinant proteins of 13.73, 22.42 and 2.65 %, respectively, compared with wild-type RAG1. **c** Flow cytometric analysis of the BM Flow cytometric analysis of the patient and a control using anti IgM and anti VpreB were shown. The relative frequency of pro B cells in the BM was significantly higher, while that of other cells was significantly lower, than that observed in normal BM (The reported percentage of B-lineage cells in BM samples of healthy control of children were indicated in the figure [16])

lineage cells, including pre-B1b (0.4 %), pre-B2 (0.6 %) and mature B cells (2.0 %), was markedly reduced, although a reduced but detectable proportion of mature B cells was also noted (Fig. 2c).

Clinical Course of the Patient

He was diagnosed as RAG deficient when 4 years old. Based on the in vitro analysis described above, we anticipated that his condition would become worse in the future. Thus he underwent bone marrow transplantation(BMT) from an HLA-matched sibling. The conditioning regimen consisted of fludarabine at a dose of 180 mg/m2 and busulfan at a dose of 16 mg/kg based on the proposed guideline of EBMT- inborn errors working party. The BMT course was uneventful with a full donor chimerism without acute and chronic graft-versus-host disease, and his TCR repertoire of $CD4^+$ and $CD8^+$ T cells was polyclonal after 1 month post-BMT(Fig. 3).

Discussion

With the detection and characterization of hypomorphic variants, it has become clear that SCID-causing mutations [19] may result in surprisingly mild phenotypes that do not match the definition of SCID [20, 21]. Modifier genes, epigenetic factors (e.g. systemic viral infection, such as that involving cytomegalovirus or varicella) and iatrogenic factors (immunoglobulin substitution and/or the use of anti-infective agents given therapeutically or prophylactically) may play a role in the onset of RAGD, although the precise mechanisms leading to certain phenotypes remain to be elucidated [6]. Recently, a family with RAGD associated with early-onset autoimmunity with preserved B lymphocytes was reported [22]. That case and the current report expand the clinical spectrum of RAGD.

The patient presented here was diagnosed with SIgAD without any evidence of opportunistic infections, rashes, hepatosplenomegaly, autoimmunity or granulomas. Surprisingly, however, he became infected with varicella, although

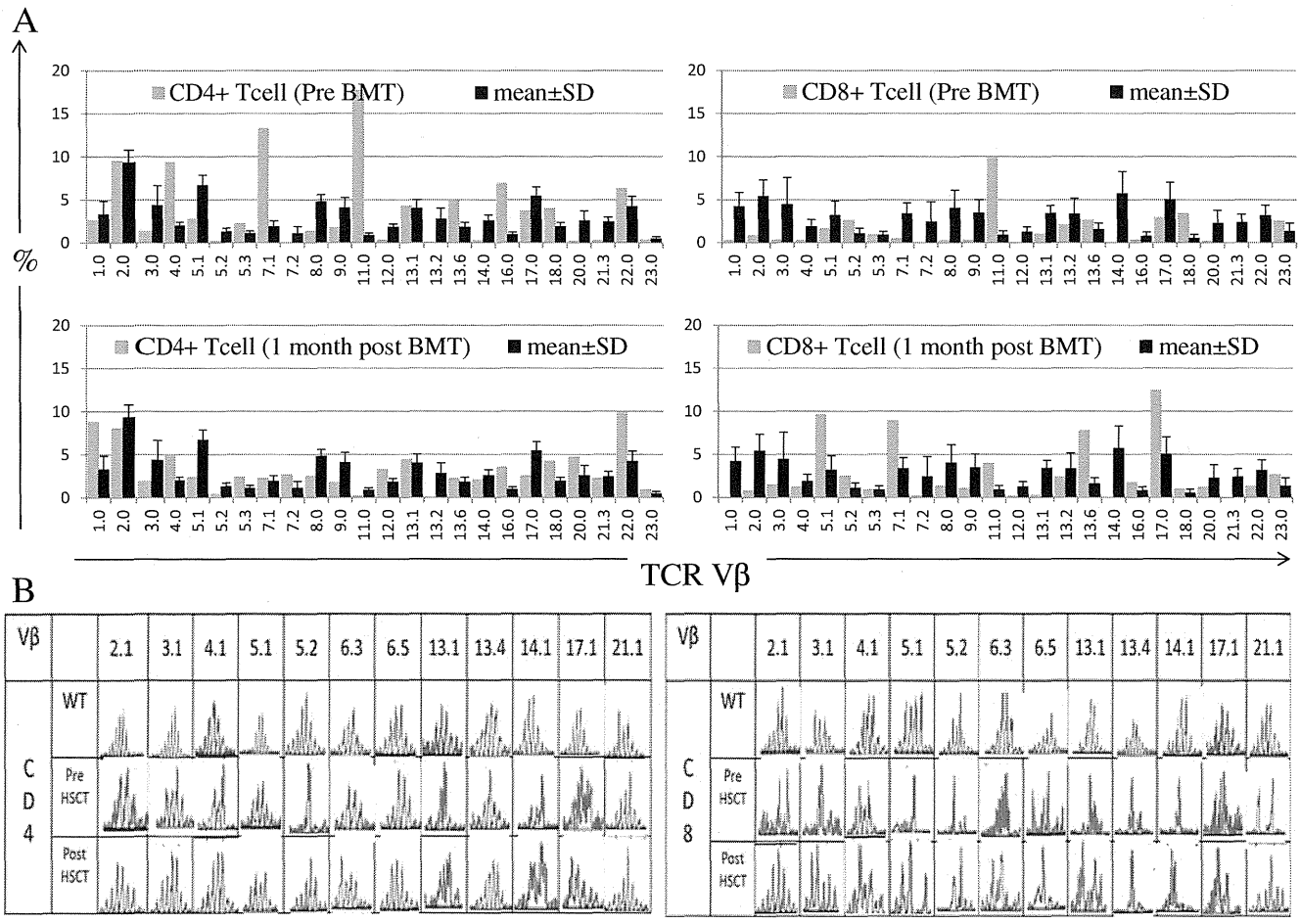

Fig. 3 TCR repertoire. **a** IO Test Beta Mark. Pre BMT, moderately oligoclonal for $CD4^+CD3^+$ and $CD8^+CD3^+$ cells. 1 month post BMT, both were polyclonal. **b** Spectratype analysis of the CDR3 region. Pre BMT, moderately oligoclonal for $CD4^+CD3^+$ and $CD8^+CD3^+$ cells. 1 month post BMT, both were polyclonal

his course was not serious. To the best of our knowledge, this case is the most moderate case associated with hypomorphic mutations reported to date. Nevertheless, the results of lymphocyte phenotyping and analyses of TRECs and KRECs prompted us to perform a sequence analysis of the *RAG* gene. Recently, we reported that TRECs and KRECs are useful markers for assessing the clinical severity and pathogenesis of CVID and distinguishing CID from CVID. Hence, developing a patient classification based on the levels of TRECs and KRECs would provide helpful information for determining an effective treatment plan for individual patients with CVID [14]. This case also indicates that lymphocyte phenotyping and TRECs and KRECs analyses are useful tools for detecting patients requiring treatment similar to that for SCID or CID.

A number of other types of primary immunodeficiency display autosomal recessive inheritance and, as such, require the presence of abnormalities in both alleles for the phenotypic expression. In the present case, the patient carried missense *RAG1* mutations on the maternal allele, and a deletion of both *RAG1* and *RAG2* loci on the paternal allele. This scenario has previously been reported for the gene encoding *Artemis* proteins, in which small deletions encompassing several exons result in a loss of function and SCID [23], and the gene encoding Coronin-1A, in which a 600-kb deletion encompassing *CORO1A* results in a loss of function and SCID [24]. The gross deletion of multiple contiguous genes (*PAX6*, *WT1*, *RAG1*, *RAG2*) at chromosome 11 compounded by point mutations leading to the development of Omenn syndrome and 11p13 syndrome has been reported [25]. Our findings and the results observed in other cases of autosomal recessive SCID in which deletions were found in one allele suggest that microarray analyses should be considered if sequencing reveals mutations in only one allele.

The present patient exhibited BM arrest at the transition between pro-B cells and pre-B1 cells, in line with the essential role of RAG proteins in initiating the V(D)J recombination process. In addition, minor leakiness was detected, which was clearly related to the low recombination activity [26, 27].

In summary, we herein reported a distinct hypomorphic RAGD phenotype who was initially given the diagnosis of SIgAD based on immunoglobulin serum levels. To the best of our knowledge, this case is the most moderate case associated with hypomorphic *RAG1* mutations reported to date. These findings suggest that TRECs and KRECs are useful markers for detecting hidden severe, but also not so severe cases.

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