

Letter to the Editor

Common variable immunodeficiency classification by quantifying T-cell receptor and immunoglobulin κ -deleting recombination excision circles

To the Editor:

Common variable immunodeficiency (CVID) is the most frequent primary immunodeficiency associated with hypogammaglobulinemia and other various clinical manifestations. CVID was originally reported to be a disease primarily caused by defective B-cell function, with defective terminal B-cell differentiation rendering B cells unable to produce immunoglobulin. However, combined immunodeficiency (CID) involving both defective B and T cells is often misdiagnosed as CVID.¹ Indeed, one study reported that CD4⁺ T-cell numbers were decreased in 29% of 473 patients with CVID²; similarly, another study found that naive T-cell numbers were markedly reduced in 44% (11/25) of patients with CVID.³ These observations indicated that a subgroup of patients with clinically diagnosed CVID is T-cell deficient. Consistently, some patients with CVID have complications that might be related to T-cell deficiency, including opportunistic infections, autoimmune diseases, and malignancies, which is similar to that observed in patients with CID.^{1,4} Therefore identifying novel markers to better classify CVID and distinguish CID from CVID will be required to best manage medical treatment for CVID.

We recently performed real-time PCR-based quantification of T-cell receptor excision circles (TREC) and signal joint immunoglobulin κ -deleting recombination excision circles (KREC) for mass screening of severe combined immunodeficiency (SCID)⁵ and B-lymphocyte deficiency⁶ in neonates. TREC and KREC are associated with T-cell and B-cell neogenesis, respectively.⁷ Here we retrospectively report that TREC and KREC are useful for classifying patients with clinically diagnosed CVID.

Hypogammaglobulinemic patients (n = 113) were referred to our hospital for immunodeficiency from 2005-2011, and the following patients were excluded from the CVID pool by estimating their SCID genes based on clinical manifestations and lymphocyte subset analysis: 18 patients with SCID diagnoses; 14 patients less than 2 years of age (transient infantile hypogammaglobulinemia); 10 patients with IgM levels of greater than 100 mg/dL (hyper-IgM syndrome); 26 patients with diseases other than CVID caused by known gene alterations (10 with X-linked agammaglobulinemia and 11 with hyper-IgM syndrome [CD40L or AICDA mutated]), (2 with DiGeorge syndrome, and 3 with FOXP3, IKBKG, or 6p deletions); and 5 patients with drug-induced hypogammaglobulinemia. The remaining 40 patients with decreased IgG (≥ 2 SDs below the mean for age), IgM, and/or IgA levels, as well as absent isohemagglutinins, poor response to vaccines, or both were included in this study as patients with CVID and analyzed for TREC/KREC levels, retrospectively.

Ages of patients with CVID ranged from 2 to 52 years (median age, 15.5 years). The sex ratio of the patients was 21 male/19 female patients. Serum IgG, IgA, and IgM levels were 370 ± 33 mg/dL (0-716 mg/dL), 30 ± 7 mg/dL (1-196 mg/dL), and 40 ± 6 mg/dL (2-213 mg/dL), respectively. TREC and KREC quantification was performed by using DNA samples extracted from peripheral blood, as reported previously.^{5,6} Clinical symptoms were then assessed

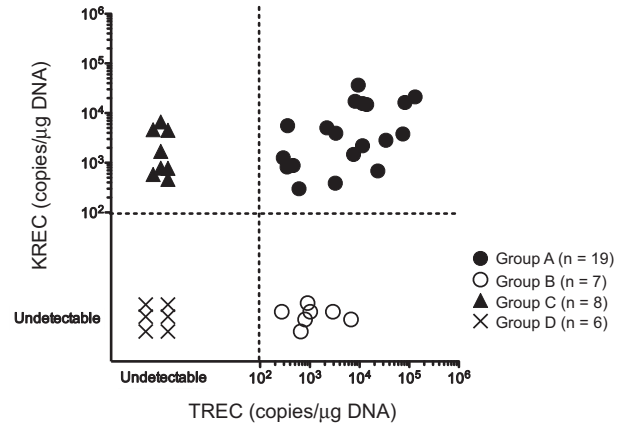


FIG 1. Quantifying TREC and KREC classifies patients with CVID into 4 groups. Patients with CVID were classified as follows: TREC(+)/KREC(+), group A (19 patients); TREC(+)/KREC(-), group B (7 patients); TREC(-)/KREC(+), group C (8 patients); and TREC(-)/KREC(-), group D (6 patients). Undetectable, Less than 100 copies/ μ g DNA.

retrospectively. The study protocol was approved by the National Defense Medical College Institutional Review Board, and written informed consent was obtained from adult patients or parents of minor patients in accordance with the Declaration of Helsinki.

Based on TREC and KREC copy numbers, the 40 patients with CVID were classified into 4 groups (groups A, B, C, and D; Fig 1). Comparing lymphocyte subsets, CD3⁺ T-cell numbers were similar among groups A, B, and D but were significantly lower in group C ($P < .05$; group A, 1806 ± 204 cells/ μ L; group B, 1665 ± 430 cells/ μ L; group C, 517 ± 124 cells/ μ L; and group D, 1425 ± 724 cells/ μ L; $P = .0019$, Tukey multiple comparison test based on 1-way ANOVA). CD3⁺CD4⁺CD45RO⁺ memory T-lymphocyte percentages in groups B, C, and D were significantly higher than those in group A ($P < .0001$; group A, $37\% \pm 16\%$; group B, $67\% \pm 13\%$ [$P = .0006$]; group C, $92\% \pm 8.2\%$ [$P < .0001$]; and group D: $83\% \pm 14\%$ [$P < .0001$]; see Fig E1 in this article's Online Repository at www.jacionline.org); additionally, the percentages of these cells in groups C and D were higher than in group B ($P = .0115$). These results indicate that group C and D patients have markedly decreased CD4⁺CD45RA⁺ naive T-cell counts than group A patients and that counts in group B are also significantly decreased, although less so than in groups C or D, which is consistent with a report showing lower TREC copy numbers in CD4⁺CD45RO⁺ cells. Some patients in groups B, C, and D exhibited normal CD4⁺CD45RO⁺ percentages, although TREC levels, KREC levels, or both decreased. This discrepancy indicates that TREC/KREC levels could be independent markers to determine the patient's immunologic status in addition to CD4⁺CD45RA⁺; the reasons underlying the discrepancy between CD4⁺CD45RA⁺ and TREC/KREC levels remain unsolved.

CD19⁺ B-cell numbers in group A were significantly higher ($P < .05$) than those in groups B and D (group A, 269 ± 65 cells/ μ L; group B, 35 ± 16 cells/ μ L; group C, 60 ± 11 cells/ μ L; and group D, 29 ± 16 cells/ μ L; $P = .0001$). However, B-cell subpopulations, including CD27⁻, IgD⁺CD27⁺, and

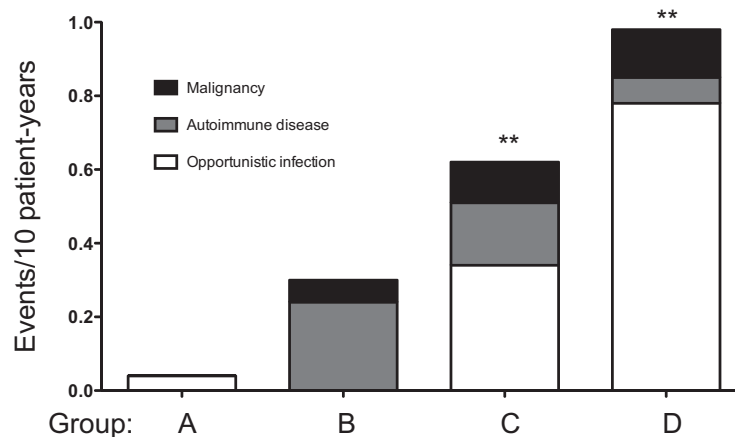


FIG 2. Cumulative incidence of complication events per 10 patient-years differs among groups. Opportunistic infections, autoimmune diseases, and malignancies were evaluated for each patient group. Complication incidences in group D (0.98 events/10 patient-years), group C (0.63 events/10 patient-years), and group B (0.30 events/10 patient-years) were higher than in group A (0.04 events/10 patient-years). Group A versus group D: $**P = .0022$; group A versus group C: $**P = .0092$; group A versus group B: $P = .0692$.

IgD⁺CD27⁺ cells, were not significantly different among the groups. Standardizing KREC copy numbers for each patient by dividing their CD19⁺ by their CD27⁺ percentages revealed the same patient classification as that shown in Fig 1 (data not shown), indicating that the original classification was independent of CD19⁺ B-cell or CD27⁺ memory B-cell percentages.

Because TREC and KREC levels decrease with age (see Fig E2 in this article's Online Repository at www.jacionline.org)^{5,6} and age distribution was wide in this study, we compared patients' ages among groups at the time of analysis to determine whether classification was associated with age. TREC/KREC-based classification was independent of both age and sex because age distribution was not significantly different among groups ($P > .05$; group A, 12.7 ± 2.3 years [2-30 years]; group B, 23.4 ± 4.2 years [6-39 years]; group C, 21.5 ± 6.1 years [4-52 years]; and group D, 25.5 ± 4.4 years [15-46 years]; data not shown) nor was male/female sex ratio (overall, 21/19; group A, 10/9; group B, 2/5; group C, 5/3; and group D, 4/2; $P = .4916$, χ^2 test; data not shown).

We next evaluated whether any correlation existed between TREC/KREC-based classification and clinical symptoms in each patient group. All patients in the study had been treated with intravenous immunoglobulin (IVIG) substitution at the time of analysis. We found that the cumulative events of complications (opportunistic infections, autoimmune diseases, and malignancies) per 10 patient-years were highest in group D (0.98 events/10 patient-years), followed by group C (0.63 events/10 patient-years), group B (0.30 events/10 patient-years), and group A (0.04 events/10 patient-years), where events in groups D and C were significantly higher than group A (group A vs group D, $P = .0022$; group A vs group C, $P = .0092$; group A vs group B, $P = .0692$; Fig 2). Furthermore, we found similar results when evaluating only patients 19 years old or older for group D (1.01 events/10 patient-years), group C (0.56 events/10 patient-years), group B (0.32 events/10 patient-years), and group A (0.06 events/10 patient-years; group A vs group D, $P = .0074$; group A vs group C, $P = .0407$; group A vs group B, $P = .1492$; data not shown). Categorizing patients by using several different previously reported CVID classifications (focused primarily on separating patients based on levels of circulating B-cell subsets), we found

that no classification scheme showed any significant event increases in any particular group (see Fig E3 in this article's Online Repository at www.jacionline.org). Assessing longitudinal cumulative opportunistic infection incidence among the groups, group D and C values were significantly higher than in group A (see Fig E4, A, in this article's Online Repository at www.jacionline.org; $P = .0059$). Autoimmune and malignant diseases ($P = .5168$ and $P = .6900$, respectively) were observed in groups B and D but not in group A (see Fig E4, B and C). Cumulative events were significantly different between groups ($P = .0313$, log-rank test; group A, 5.3% and 5.3%; group B, 14.3% and 57.1%; group C, 27.1% and 63.5%; and group D, 33.3% and 83.3% at 10 and 30 years of age, respectively; see Fig E4, D). One patient in group D died of *Pneumocystis jirovecii* pneumonia, and 2 other patients in the same group received hematopoietic stem cell transplantation after complications caused by EBV-related lymphoproliferative disorder.

Assessing these data, TREC/KREC-based classification matches clinical outcomes. Because group D patients exhibited the most frequent complications (opportunistic infections, autoimmune diseases, and malignancies), they could receive a diagnosis of CID based on these symptoms. If they are indeed determined to have CID, then TREC/KREC analysis is helpful to distinguish between CID and CVID. Their TREC(−)/KREC(−) phenotype might relate to defective V(D)J recombination in T- and B-cell development⁸ because patients with B-negative SCID (*RAG1*, *RAG2*, *Artemis*, and *LIG4*), as well as patients with ataxia-telangiectasia (AT) and Nijmegen breakage syndrome (NBS; see Fig E5 in this article's Online Repository at www.jacionline.org)^{5,6} were also negative for both TREC and KREC; it is intriguing to speculate that an unknown V(D)J recombination gene or genes is responsible. As for treatment, hematopoietic stem cell transplantation should be considered the preferred treatment to "cure" group D patients, as reported in patients with severe CVID/CID, because event-free survival is poor.⁹

In contrast to group D patients, TREC(+)/KREC(+) group A patients treated with IVIG substitution therapy remained healthy. One possible explanation is that these patients harbor

defects only in terminal B-cell differentiation, but not in T cells, and represent typical patients with CVID, as originally reported.

Group C patients had a high frequency of both opportunistic infections and malignancies, suggesting that these TREC(−) patients have T-cell defects. Although group C patients had a similar TREC/KREC pattern to patients with SCID with B cells (*IL2RG* and *JAK3*; see Fig E5, A), they do not fulfill the European Society for Immunodeficiencies criteria for SCID, and no mutation was identified in the SCID genes estimated from clinical manifestation and lymphocyte subset analysis. However, from our data, they would likely benefit from undergoing similar treatment to patients with SCID or CID to prevent these complications.

Although opportunistic infections were rare in group B patients, autoimmune diseases were often observed. This is consistent with this group being TREC(+)/KREC(−) and the idea that balance between T and B cells is important to prevent autoimmune diseases in patients with CVID.¹ Intriguingly, a group of patients with AT and NBS were also TREC(+)/KREC(−) (see Fig E4, B), which is similar to group B patients. Additionally, CD45RA⁺CD4⁺ naive T-cell numbers were reduced in most group B patients, which is similar to the phenotype exhibited by patients with AT and NBS. This finding raises the possibility that although some group B patients are also T-cell deficient, as well as B-cell deficient, and should be treated similarly to patients with CID, other patients have only B-cell deficiency and are effectively treated with IVIG substitution therapy.

By analyzing a large CVID patient cohort, the overall survival rate of patients with more than 1 complication was worse than that for patients without other complications.⁴ Our findings indicate that low TREC levels, KREC levels, or both are useful markers that correlate well with the overall survival rate in patients with CVID. Therefore we conclude that TREC and KREC are useful markers to assess the clinical severity and pathogenesis of each patient with CVID and to distinguish CID from CVID. Thus patient classification based on TREC/KREC levels would provide a helpful tool for deciding on an effective treatment plan for each patient with CVID.

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REFERENCES

1. Yong PFK, Thaventhiran JED, Grimbacher B. "A rose is a rose is a rose," but CVID is not CVID. common variable immune deficiency (CVID), what do we know in 2011? *Adv Immunol* 2011;111:47-107.
2. Resnick ES, Moshier EL, Godbold JH, Cunningham-Rundles C. Morbidity and mortality in common variable immune deficiency over 4 decades. *Blood* 2012;119:1650-7.
3. Moratto D, Gulino AV, Fontana S, Mori L, Pirovano S, Soresina A, et al. Combined decrease of defined B and T cell subsets in a group of common variable immunodeficiency patients. *Clin Immunol* 2006;121:203-14.
4. Chapel H, Lucas M, Lee M, Bjorkander J, Webster D, Grimbacher B, et al. Common variable immunodeficiency disorders: division into distinct clinical phenotypes. *Blood* 2008;112:277-86.
5. Morinishi Y, Imai K, Nakagawa N, Sato H, Horiuchi K, Ohtsuka Y, et al. Identification of severe combined immunodeficiency by T-cell receptor excision circles quantification using neonatal Guthrie cards. *J Pediatr* 2009;155:829-33.
6. Nakagawa N, Imai K, Kanegane H, Sato H, Yamada M, Kondoh K, et al. Quantification of κ-deleting recombination excision circles in Guthrie cards for the identification of early B-cell maturation defects. *J Allergy Clin Immunol* 2011;128:223-5.e2.
7. van Zelm MC, Szczepanski T, Van Der Burg M, Van Dongen JJM. Replication history of B lymphocytes reveals homeostatic proliferation and extensive antigen-induced B cell expansion. *J Exp Med* 2007;204:645-55.
8. Verbsky JW, Baker MW, Grossman WJ, Hintermeyer M, Dasu T, Bonacci B, et al. Newborn screening for severe combined immunodeficiency: the Wisconsin experience (2008-2011). *J Clin Immunol* 2012;32:82-8.
9. Rizzi M, Neumann C, Fielding AK, Marks R, Goldacker S, Thaventhiran J, et al. Outcome of allogeneic stem cell transplantation in adults with common variable immunodeficiency. *J Allergy Clin Immunol* 2011;128:1371-2.

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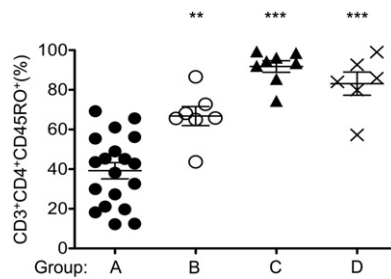


FIG E1. CD45RO⁺CD3⁺CD4⁺ T-cell frequency within CD4⁺CD3⁺ lymphocytes was analyzed among groups. CD45RO⁺CD3⁺CD4⁺ lymphocyte counts were significantly higher in groups B, C, and D compared with those in group A ($P < .0001$). Group A: 37% ± 16%; group B: 67% ± 13% (** $P < .01$); group C: 92% ± 8.2% (*** $P < .001$); and group D: 83% ± 14% (*** $P < .001$).

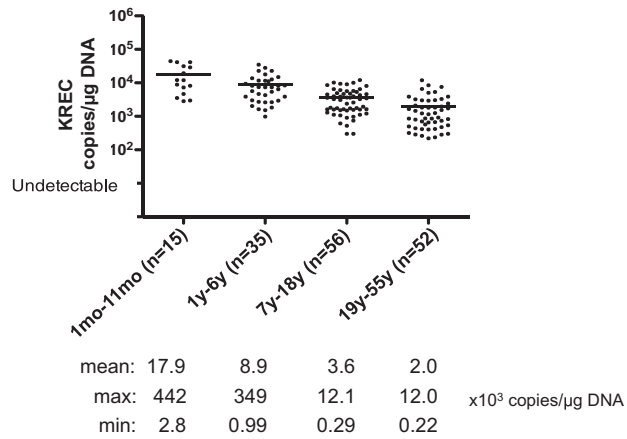


FIG E2. KREC levels were analyzed in genomic DNA samples extracted from peripheral blood of control subjects at different age groups ($n = 158$; age range, 1 month to 55 years). KREC levels were significantly higher in infants ($17.9 \pm 3.9 \times 10^3$ copies/ μ g DNA) compared with other children's age groups ($8.9 \pm 1.3 \times 10^3$ copies/ μ g DNA in the 1- to 6-year-old group and $3.6 \pm 3.8 \times 10^3$ copies/ μ g DNA in the 7- to 18-year-old group) and adults ($2.0 \pm 3.3 \times 10^3$ copies/ μ g DNA; $P < .0001$).

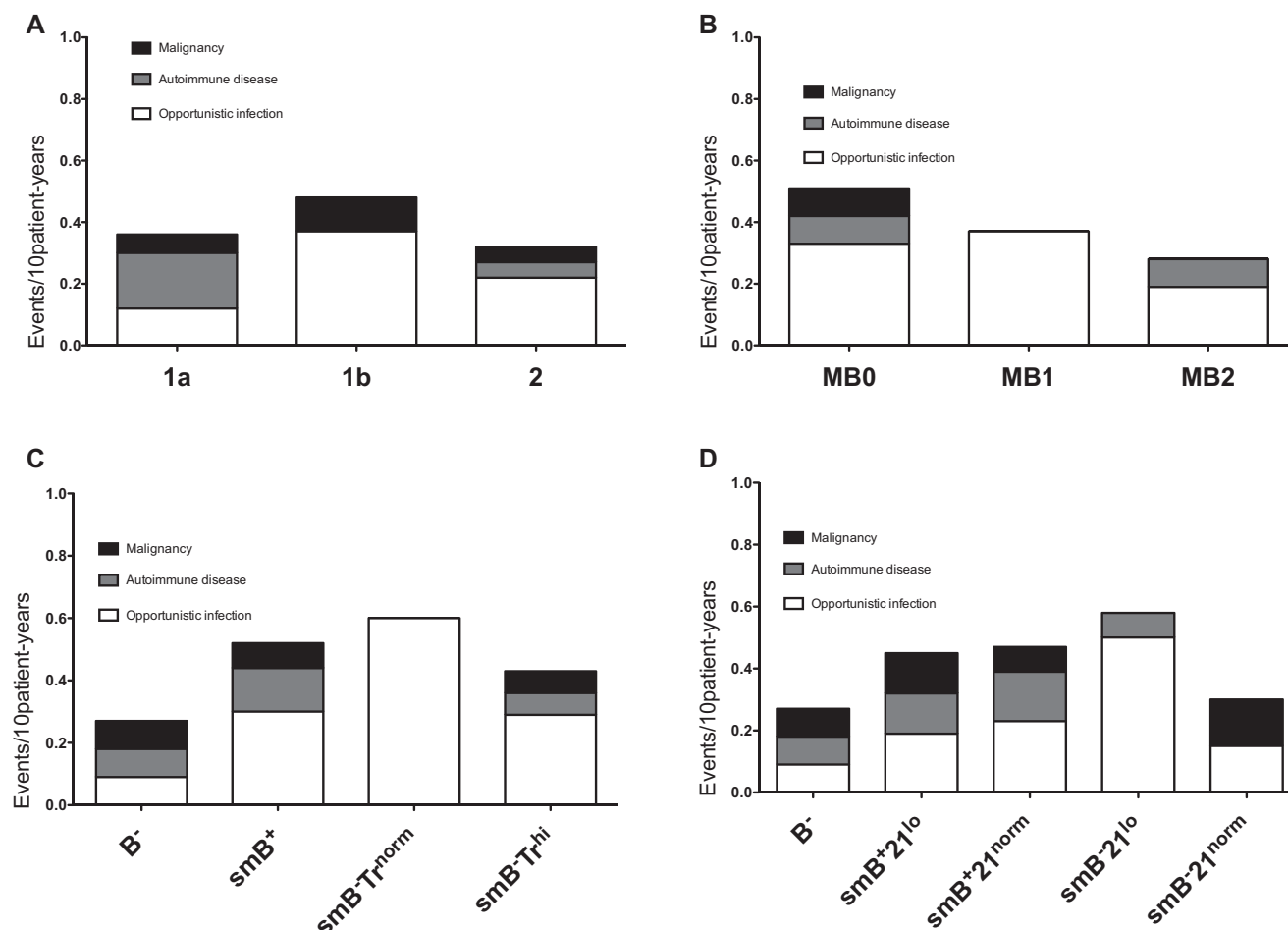


FIG E3. Patients were classified in the following way and analyzed for cumulative incidence of complications: **A**, Freiburg; **B**, Paris; and **C**, EUROclass classifications, according to CD38^{hi}IgM^{hi} transitional B cells (Fig E3, A-C) or CD21^{lo} B cells (**D**). Five patients were excluded from the Freiburg and Paris classifications because of decreased B-cell numbers (<1%). Additionally, we excluded 4 patients in the Freiburg classification, 1 patient in the Paris classification, and 4 patients in the EUROclass classification for transitional B cells and 8 in the EUROclass classification for CD21^{lo} B cells because of lack of data. The following cumulative events/10 patient-years were found. Freiburg classification: 1a, 0.36; 1b, 0.48; 2, 0.32. Paris classification: MB0, 0.50; MB1, 0.37; MB2, 0.28. EUROclass classification according to transitional B cells: B⁻, 0.27; smB⁺, 0.52; smB⁻Tr^{norm}, 0.60; smB⁻Tr^{hi}, 0.43. EUROclass classification according to CD21^{lo} B cells: B⁻, 0.27; smB⁺21^{lo}, 0.45; smB⁺21^{norm}, 0.47; smB⁻21^{lo}, 0.58; smB⁻21^{norm}, 0.30. No classification showed any significantly increased events in any particular group according to calculated *P* values, as follows—Freiburg classification: 1a vs 2 = .898, 1b vs 2 = .479, 1a vs 1b = .838; Paris classification: MB0 vs MB1 = .179, MB1 vs MB2 = .654, MB0 vs MB2 = .764; EUROclass classification according to transitional B cells: B⁻ vs smB⁺ = .298, smB⁻Tr^{norm} vs smB⁺ = .809, smB⁻Tr^{hi} vs smB⁺ = .702, smB⁻Tr^{hi} vs smB⁻Tr^{norm} = .641, smB⁻Tr^{norm} vs B⁻ = .329, smB⁻Tr^{hi} vs B⁻ = .508; EUROclass classification according to CD21^{lo} B cells: B⁻ vs smB⁺21^{norm} = .443, smB⁺21^{lo} vs smB⁺21^{norm} = .930, smB⁻21^{lo} vs smB⁺21^{norm} = .695, smB⁻21^{norm} vs smB⁺21^{norm} = .575, B⁻ vs smB⁻21^{norm} = .926, smB⁺21^{lo} vs smB⁻21^{norm} = .609, smB⁻21^{lo} vs smB⁻21^{norm} = .399, B⁻ vs smB⁺21^{lo} = 0.474, B⁻ vs smB⁻21^{lo} = 0.270, smB⁺21^{lo} vs smB⁻21^{lo} = 0.618.

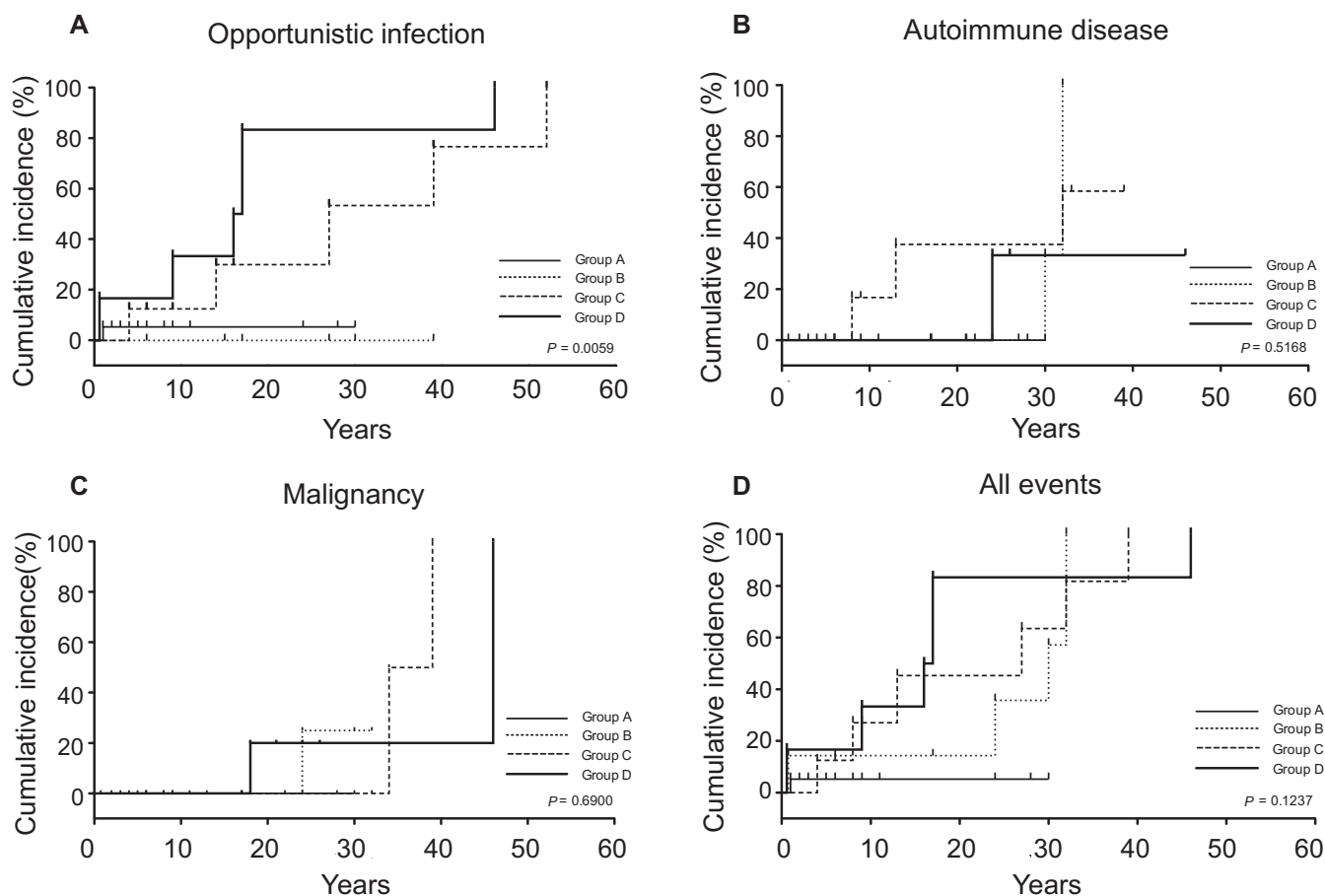


FIG E4. Comparing longitudinal cumulative incidence of complication events among groups. Cumulative incidence was estimated separately and longitudinally by using the Kaplan-Meier method and statistically compared between groups by using the log-rank test. The cumulative incidence of opportunistic infections (A), autoimmune diseases (B), malignancies (C), and all events (D) is shown.

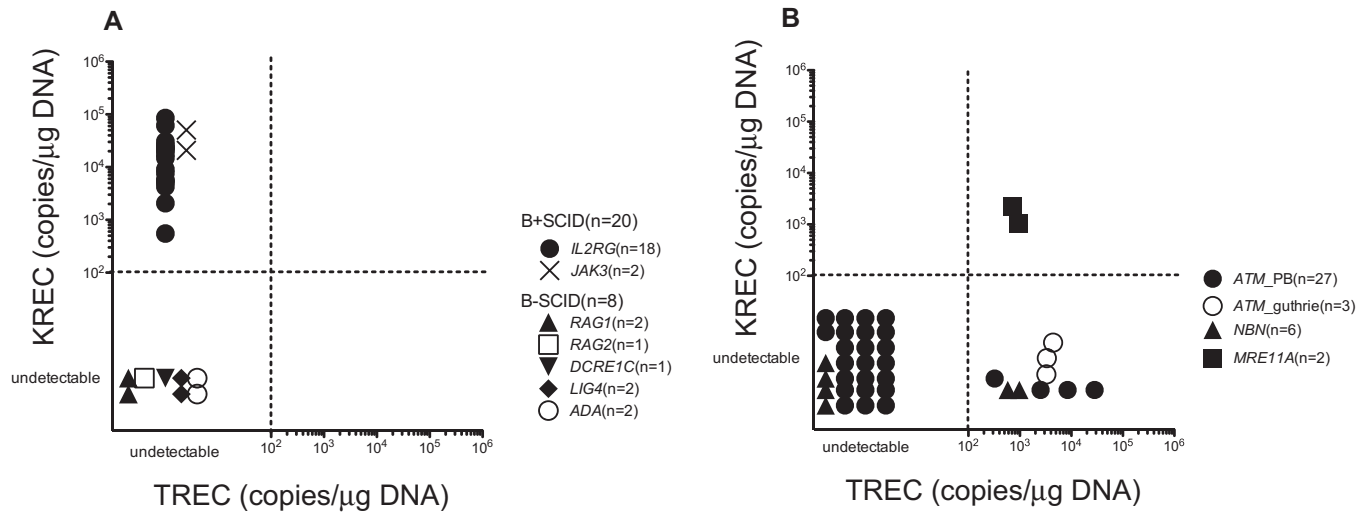


FIG E5. TREC and KREC quantification classifies patients with SCID, AT, NBS, or ataxia-telangiectasia-like disease (ATLD) into 4 groups. **A**, Patients with B⁺SCID (n = 20) were classified as group C, and patients with B⁻SCID (n = 8) were classified as group D; these patients were included in the previous studies.^{5,6} **B**, Although most patients with AT (n = 23) and patients with NBS (n = 4) were classified as group D, TRECs were detected in peripheral blood samples (n = 4 in patients with AT and n = 2 in patients with NBS) and neonatal Guthrie cards (n = 3) of some patients with AT, who were classified as group B. Patients with ATLD with *MRE11A* mutations were classified as group A.