CCL22 mutations in large granular lymphocytic leukemia

Large granular lymphocytic (LGL) leukemia is an indolent, mature cytotoxic T-cell or NK-cell proliferative disorder that is often associated with immune-mediated complications, including cytopenia and autoimmune diseases.[1,2] The genetic landscape of LGL leukemia has been elucidated, including frequent somatic mutations in *STAT3*, *STAT5B* and *TET2* genes.[3-9] Recently, somatic mutations in C-C motif chemokine ligand 22 (*CCL22*) were identified in patients with NK-cell LGL leukemia (NK-LGLL),[10] suggesting a novel mechanism of LGL proliferation in mutated chemokine-induced microenvironmental alterations. However, information on the mutational profile of *CCL22* and its clinical significance in LGL leukemia remains limited, and its genetic-phenotypic associations remain to be clarified. We investigated mutational profiles, including *CCL22* gene mutations, in patients with LGL leukemia and identified *CCL22* mutations in T-cell LGL (T-LGL) leukemia and NK-LGLL, and found that their associations with clinical features varied among the subtypes of LGL leukemia.

The study cohort included 23 patients with NK-LGLL and 52 with T-LGL leukemia. Immunophenotypes of T-LGL leukemia included CD8$^+$TCR$\alpha\beta$ (N=36), TCR$\gamma\delta$ (N=7), and CD4$^+$TCR$\alpha\beta$ (N=9). This study was conducted in line with the Declaration of Helsinki and written informed consent was obtained from all patients. The study protocol was approved by the institutional review board of each participating institution.

We identified *CCL22* mutations in two of the 52 patients (3.9%) with T-LGL leukemia (Table 1; Figure 1A) using target sequencing (Ion Ampliseq Technology, Thermo Fischer Scientific), in addition to NK-LGL leukemia. Case 24 had CD8$^+$ TCR $\alpha\beta$ type-LGL leukemia with a *STAT3* D661Y co-mutation of variant alle frequency (VAF) at 2.3%. Case 25 had CD4$^+$TCR $\alpha\beta$ type-LGL leukemia with *STAT5B* Y665F and *STAT3* K658R co-mutations. Notably, they shared the same *CCL22* mutation site, P46R, with VAF of 4.0% and 1.6%, respectively (Figure 1B). The *CCL22* mutation at P46R was confirmed by allele-specific polymerase chain reaction (PCR) in both patients (representative data of case 24 are shown in *Online Supplementary Figure S1*). Sorted CD8$^+$ T cells in case 24 were negative for *CCL22* mutation but positive for *STAT3* D661Y mutation. No cutaneous or neurological manifestations were observed in either of the patients.

Recurrent *CCL22* mutations in six of the 23 patients (26%) with NK-LGLL were at L45R (N=3) and L45Q (N=3) (Figure 1; *Online Supplementary Table S1*). The median VAF of *CCL22* mutations was 33.2% (range, 9.9-41.5%). Among the six patients with NK-LGLL, two presented with neurological symptoms and one presented with neutropenia. None of the six patients had anemia. *TET2* mutations were identified in three of the 23 NK-LGLL cases, with a median VAF of 20.9%. All three patients with *TET2* mutations had cytopenia and two had *STAT3* co-mutations. Among NK-LGLL cases, *CCL22* mutations were mutually exclusive of *STAT3* and *TET2* mutations. The immunophenotypes of NK-LGLL cells were CD16$^+$CD56^{bright} in *CCL22*-mutated patients and CD16$^+$CD56$^{-/dim}$ in *STAT3*- and/or *TET2*-mutated patients (P=0.00072).

We performed real-time PCR (RT-PCR) to search for CCL22 mRNA in the peripheral blood of patients with *CCL22* L45R mutation (case 3) or L45Q mutation (case 4). RT-PCR identified *CCL22* mRNA in all the cell fractions. The mutated sequence was detected in DNA from peripheral blood mononuclear cells (*Online Supplementary Figure S2A*). The mutated sequence was also detected in mRNA from peripheral blood mononuclear cells by allele-specific

Table 1. *CCL22*-mutated T-cell large granular lymphocytic leukemia.

Characteristics	Case 24	Case 25
Age in years/sex	61/F	79/M
T-LGL leukemia type	CD8$^+$TCR$\alpha\beta$	CD4$^+$TCR$\alpha\beta$
CCL22 mutations, VAF%	P46R, 1.6	P46R, 4.0
Complications	pure red cell aplasia	bronchial asthma
Neutrophils $\times10^9$/L	2.7	7.1
Lymphocytes $\times10^9$/L	1.3	5.4
Hb g/dL	6.5	15.5
Platelets $\times10^9$/L	234	245
Other mutated genes, VAF%	*STAT3* D661Y, 2.3	*STAT5B* Y665F, 31 *STAT3* K658R, 2.7
Therapy	cyclophosphamide	none
Observation period in years	16	3
Outcome	alive	alive

F: female; M: male; T-LGL: T-cell large granular lymphocytic leukemia; VAF: variant allele frequency; Hb: hemoglobin.

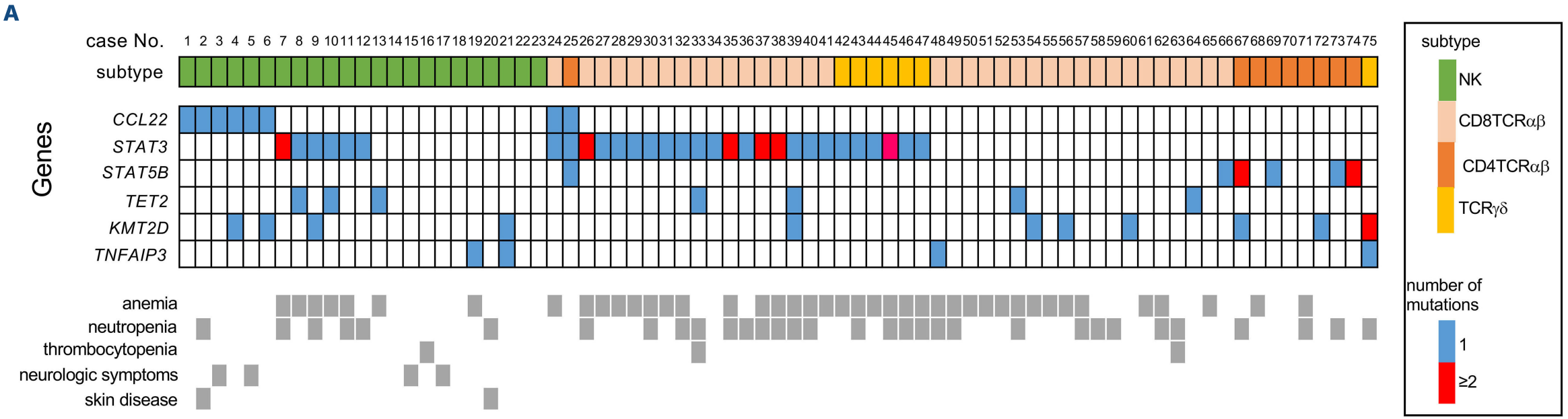

Figure 1. Mutational landscapes of large granular lymphocytic leukemia. (A) Heatmap of recurrently mutated genes in natural killer (NK) cell granular lymphocytic leukemia and T-cell large granular lymphocytic leukemia (T-LGLL). (B) Loliplot of C-C motif chemokine ligand 22 (*CCL22*) mutation in LGLL.

PCR for L45R (*Online Supplementary Figure S2B*), whereas mutated mRNA was not detected by Sanger sequencing (*Online Supplementary Figure S2A*). The sorted NK cells were positive for DNA mutations.

Two of the eight patients with *CCL22* mutations required medical intervention for LGL leukemia and/or its complications; one had NK-LGLL complicated by subcutaneous panniculitis-like T-cell lymphoma at the time of diagnosis (case 2). Oral prednisolone therapy improved the cutaneous lesions, and a significant decrease was observed in NK cells in the peripheral blood. The other patient had CD8$^+$TCR$\alpha\beta$ LGL leukemia (case 24), the diagnosis of which was preceded by a history of idiopathic pure red cell aplasia (PRCA) that had been in complete remission with cyclosporine and prednisolone for >10 years. Her anemia eventually relapsed, and CD8$^+$TCR$\alpha\beta$ LGL leukemia was diagnosed.

In this study, *CCL22* mutations were detected in patients with both T-LGL and NK-LGLL. The VAF in T-LGL leukemia cases tended to be lower than those in NK-LGLL cases (Table 1; O*nline Supplementary Table S1*). In a previous report describing the results of an extensive *in silico* analysis of 2,837 cases of various T/NK neoplasms, including T-LGL leukemia, *CCL22* mutations were not identified.[10] The immunophenotypes of *CCL22*-mutated T-LGL leukemia seemed variable; one patient had the CD4$^+$TCR$\alpha\beta$ type, while the other had the CD8$^+$TCR$\alpha\beta$ type. Interestingly, *CCL22* and *STAT3* were co-mutated in two cases of T-LGL leukemia in our cohort. In contrast, *CCL22* and *STAT3* were mutually exclusive in patients with NK-LGLL, which is consistent with a previous study.[10] Collectively, our results imply that *CCL22* mutations may not be limited to NK-LGLL and may also be prevalent in other T/NK neoplasms with a unique set of molecular and clinical features, although the frequency of *CCL22* mutations differed significantly between NK-LGLL and T-LGL leukemia (26% *vs*. 3.8%; *P*=0.007), and VAF were lower in T-LGL leukemia, suggesting a different impact of *CCL22* mutations on T-LGL leukemia.

CCL22 mutations in NK-LGLL resulted in a high VAF, implying that mutated NK cells represent a major clone. In contrast, the low burden of *CCL22* mutations in T-LGL leukemia implies subclones of T-LGL leukemic cells or small clonal cells in non-T-LGL cells, perhaps NK cells. The *CCL22*-non-mutated T-LGL leukemic cells in case 24 support the latter possibility, although we were unable to confirm the *CCL22*-mutated cell lineage in the patient due to insufficient material. The frequencies of the reported *CCL22* mutational sites in LGL leukemia were quite low (<0.00001) in the databases, including Togovar and gnomAD. Mutational sites in LGL leukemia are unique, although various *CCL22* mutations have been reported in solid tumors[11] (COSMIC, accessed February 2024).

CCL22 mRNA expression was weak in both wild-type and *CCL22*-mutated NK cells, which implies that a low level of mutated CCL22 may be sufficient to play a pathophysiological role in NK-LGLL with *CCL22* mutations. It is also possible that mutant *CCL22* expression is enhanced in specific environments (e.g., bone marrow).

The genetic and phenotypic characteristics of *CCL22*-mutant NK-LGLL cases include infrequent cytopenia, predominant immunophenotype of CD16$^+$CD56^{bright} NK cells, and absence of *STAT3* or *TET2* mutations.[10] However, *CCL22*-mutated T-LGL leukemia presented unique characteristics with lower VAF levels and co-mutational status in *STAT*. In T-LGL leukemia with *CCL22* mutations, the mutated *CCL22* - originating from either non-leukemic cells or LGL cells bearing these mutations - may, in conjunction with activating mutations in *STAT* within LGL cells, contribute to LGL proliferation and the emergence of associated complications. The prevalence and pathophysiological implications of *CCL22* mutations in individuals with LGL leukemia, other disorders, and within healthy populations, necessitate further investigation.

Authors

Yuga Mizuno,[1*] Toru Kawakami,[2*] Daigo Higano,[1] Shotaro Miyairi,[1] Ami Asakura,[1] Fumihiro Kawakami,[2] Keijiro Sato,[3] Shuji Matsuzawa,[2] Sayaka Nishina,[2] Hitoshi Sakai,[2] Yumiko Higuchi,[1,4] Kazuyuki Matsuda,[1,5] Hideyuki Nakazawa[2] and Fumihiro Ishida[1,2,4]

[1]Department of Clinical Laboratory Investigation, Graduate School of Medicine, Shinshu University, Matsumoto; [2]Department of Hematology, Shinshu University School of Medicine, Matsumoto; [3]Department of Hematology, Nagano Red Cross Hospital, Nagano; [4]Department of Biomedical Laboratory Sciences, Shinshu University School of Medicine, Matsumoto and [5]Department of Health and Medical Sciences, Graduate School of Medicine, Shinshu University, Matsumoto, Japan

**YM and TK contributed equally as first authors.*

Correspondence:
F. ISHIDA - fumishi@shinshu-u.ac.jp

https://doi.org/10.3324/haematol.2024.285404

Received: March 14, 2024.
Accepted: May 17, 2024.
Early view: May 30, 2024.

Disclosures

No conflicts of interest to disclose.

Contributions

YM, TK, and FI designed the study, performed the experiments, and analyzed the data. DH, SM, AA, YH, and KM performed the experiments

and analyzed the data. FK, KS, SM, SN, HS, HN, and FI collected the samples and clinical data. YM, TK, NH and FI wrote the manuscript.

Acknowledgments

The authors would like to thank Dr. Keiki Nagaharu of Kuwana City Medical Center, Dr. Takeki Mitsui of Gunma University, Dr. Yukio Hirabayashi of Matsumoto Medical Center, Dr. Toshiharu Yujiri of Yamaguchi University, and Dr. Hiroyuki Takamatsu of Kanazawa University for providing the samples and clinical data.

Funding

This work was supported in part by Kaken20K080709 and 21K16302 from a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan..

Data-sharing statement

Sequencing data were deposited in the Japanese Genotype-Phenotype Archive under accession code JGAS000709.

References

1. Lamy T, Moignet A, Loughran TP, Jr. LGL leukemia: from pathogenesis to treatment. Blood. 2017;129(9):1082-1094.
2. Semenzato G, Calabretto G, Barila G, Gasparini VR, Teramo A, Zambello R. Not all LGL leukemias are created equal. Blood Rev. 2023;60:101058
3. Cheon H, Xing JC, Moosic KB, et al. Genomic landscape of TCRalphabeta and TCRgammadelta T-large granular lymphocyte leukemia. Blood. 2022;139(20):3058-3072.
4. Olson TL, Cheon H, Xing JC, et al. Frequent somatic TET2 mutations in chronic NK-LGL leukemia with distinct patterns of cytopenias. Blood. 2021;138(8):662-673.
5. Kawakami T, Sekiguchi N, Kobayashi J, et al. STAT3 mutations in natural killer cells are associated with cytopenia in patients with chronic lymphoproliferative disorder of natural killer cells. Int J Hematol. 2019;109(5):563-571.
6. Andersson EI, Tanahashi T, Sekiguchi N, et al. High incidence of activating STAT5B mutations in CD4-positive T-cell large granular lymphocyte leukemia. Blood. 2016;128(20):2465-2468.
7. Pastoret C, Desmots F, Drillet G, et al. Linking the KIR phenotype with STAT3 and TET2 mutations to identify chronic lymphoproliferative disorders of NK cells. Blood. 2021;137(23):3237-3250.
8. Ishida F, Matsuda K, Sekiguchi N, et al. STAT3 gene mutations and their association with pure red cell aplasia in large granular lymphocyte leukemia. Cancer Sci. 2014;105(3):342-346.
9. Koskela HL, Eldfors S, Ellonen P, et al. Somatic STAT3 mutations in large granular lymphocytic leukemia. N Engl J Med. 2012;366(20):1905-1913.
10. Baer C, Kimura S, Rana MS, et al. CCL22 mutations drive natural killer cell lymphoproliferative disease by deregulating microenvironmental crosstalk. Nat Genet. 2022;54(5):637-648.
11. Liu J, Lee W, Jiang Z, et al. Genome and transcriptome sequencing of lung cancers reveal diverse mutational and splicing events. Genome Res. 2012;22(12):2315-2327.