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CCL22 mutations in large granular lymphocytic leukemia

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Authorship

Contribution: 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.

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Data availability

Data are available on request from the corresponding author, Fumihiro Ishida (fumishi@shinshu-u.ac.jp).

To the Editor,

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 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 αβ (n=36), TCRγδ (n=7),

and $CD4^+$ TCR αβ (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 2 of the 52 patients (3.9%) with T-LGL leukemia (Table 1 and Figure 1a) using target sequencing (Ion Ampliseq Technology, Thermo Fischer Scientific), in addition to NK-LGL leukemia. Case 24 had $CD8^+$ TCR αβ type-LGL leukemia with a *STAT3* D661Y co-mutation of VAF at 2.3%. Case 25 had $CD4^+$TCR αβ type-LGL leukemia with *STAT5B* Y665F and *STAT3* K658R co-mutations. Notably, they shared the same *CCL22* mutation site, P46R, with VAFs 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 Supplemental 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 and Supplemental Table S1). The median variant allele frequency (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 three

presented with neutropenia. None of the six patients had anemia. *TET2* mutations were identified in 3 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 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 mRNAs in all the cell fractions. The mutated sequence was detected in DNA from peripheral blood mononuclear cells (Supplemental Figure S2a). The mutated sequence was also detected in mRNA from peripheral blood mononuclear cells by allele-specific PCR for L45R (Supplemental Figure S2b), whereas mutated mRNA was not detected by Sanger sequencing (Supplemental 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 $CD8^+$TCRαβ 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 $CD8^+$TCRαβ LGL leukemia was diagnosed.

In this study, *CCL22* mutations were detected in patients with both T-LGL and NK-LGLL. The VAFs in T-LGL leukemia cases tended to be lower than those in NK-LGLL cases (Table 1 and Supplemental 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 αβ type, while the other had the $CD8^+$TCR αβ type. Interestingly, *CCL22* and *STAT3* were co-mutated in 2 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 VAFs 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 not able 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*s. 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*s 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.

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Table 1. *CCL22*-mutated T-LGL leukemia.

	case 24	**case 25**
Age(years)/ sex	61/F	79/M
T-LGL leukemia type	$CD8^+$TCRαβ	$CD4^+$TCRαβ
CCL22 mutations, VAF%	P46R,1.6	P46R, 4.0
Complications	Pure red cell aplasia	Bronchial asthma
Neutrophils (×10□/L)	2.7	7.1
Lymphocytes (×10□/L)	1.3	5.4
Hb (g/dL)	6.5	15.5
Platelets (×10□/L)	234	245
Other mutated genes (VAF%)	*STAT3* D661Y (2.3%)	*STAT5B* Y665F (31%), *STAT3* K658R (2.7%)
Therapy	cyclophosphamide	none
Observation period(years)	16	3
Outcome	alive	alive

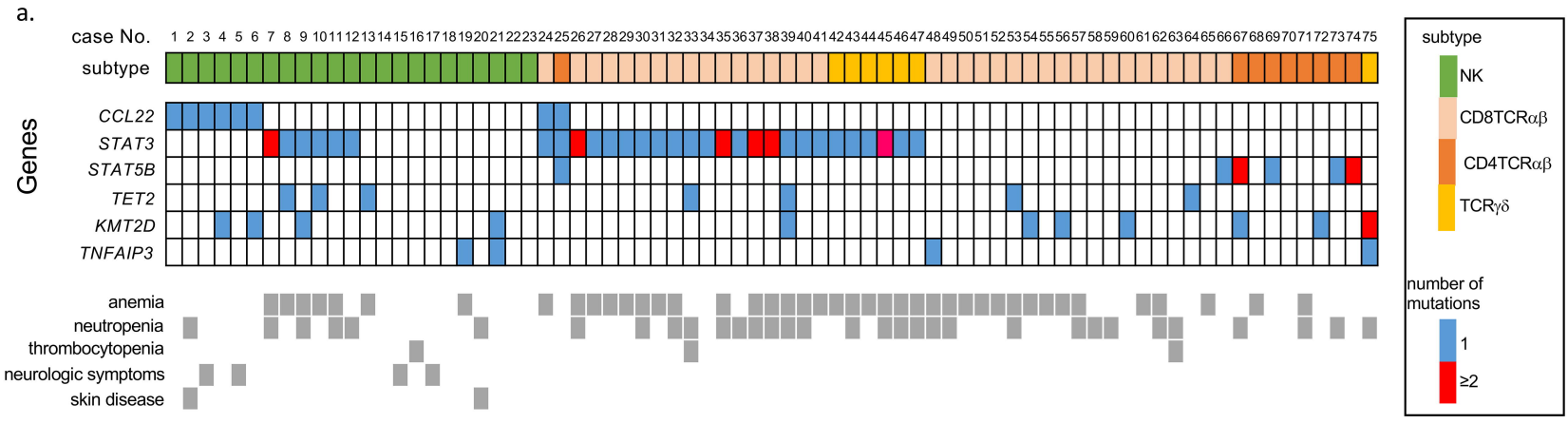
Figure legend

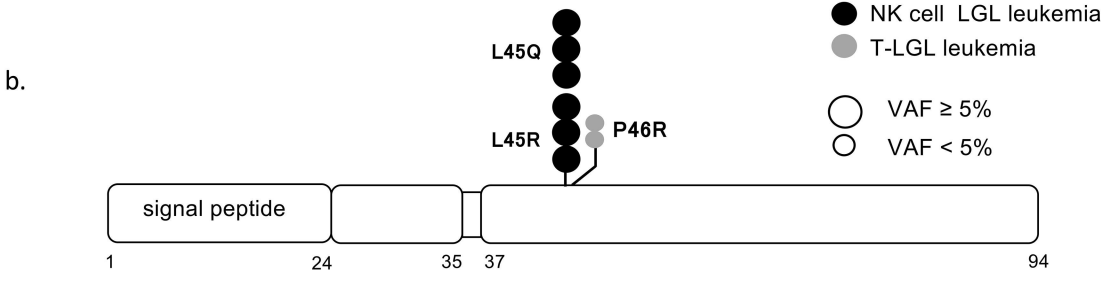
Figure 1. Mutational landscapes of large granular lymphocytic leukemia.

(A); Heatmap of recurrently mutated genes in NK cell granular lymphocytic leukemia and T cell large granular lymphocytic leukemia.

(B); Loliplot of *C-C motif chemokine ligand 22* mutation in large granular lymphocytic leukemia.

Figure 1.


a.
case No.
subtype
Genes
CCL22
STAT3
STAT5B
TET2
KMT2D
TNFAIP3
anemia
neutropenia
thrombocytopenia
neurologic symptoms
skin disease
subtype
NK
CD8TCRαβ
CD4TCRαβ
TCRγδ
number of mutations
1
≥2


b.
L45Q
L45R
P46R
NK cell LGL leukemia
T-LGL leukemia
VAF ≥ 5%
VAF < 5%
signal peptide
1
24
35
37
94

Supplemental Materials

Supplement to: Mizuno Y, et al. *CCL22* mutations in large granular lymphocytic leukemia

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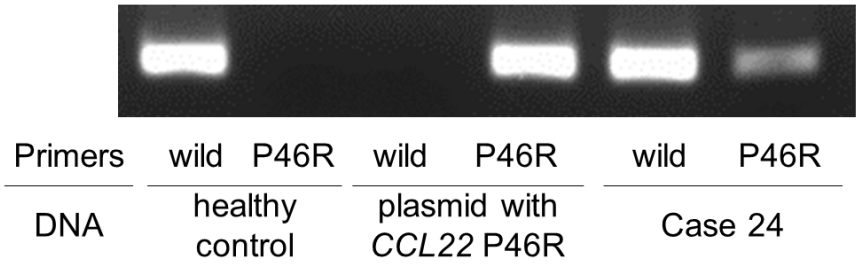
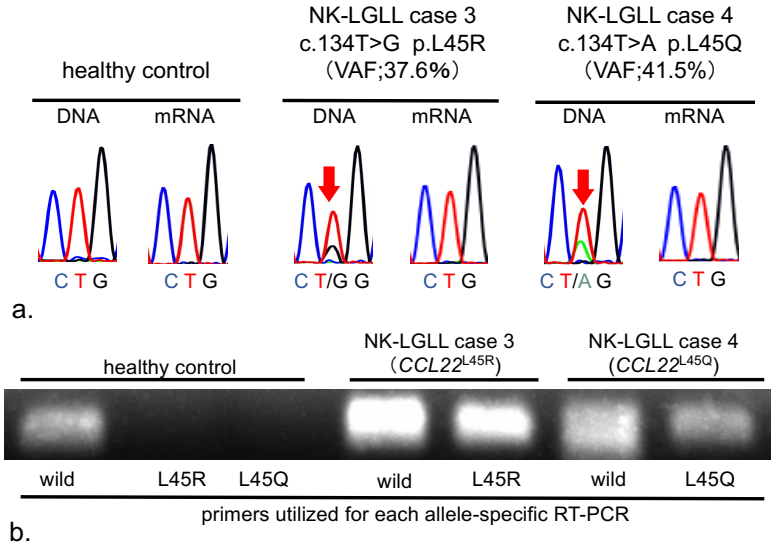
Supplemental Figure S1.



Allele-specific polymerase chain reaction (PCR) for *CCL22* P46R mutation.

Case 24 was a patient with $CD8^+$ TCRαβ type T cell large granular lymphocytic (T-LGL) leukemia. Allele-specific PCR was performed using primers for wild-type *CCL22* or *CCL22* P46R mutation, which validated a *CCL22* P46R mutation identified with the target sequencing. *CCL22* P46R mutation was also confirmed in case 25 with the same method. Primer sequences would be provided upon proper requests to the corresponding author.

Supplemental Figure S2.



The CCL22 mRNA expression in peripheral blood cells from patients with NK cell large granular lymphocytic leukemia.

a: Sanger sequencing analyses of a *CCL22* mutational site in NK-LGLL patients with *CCL22* mutations. Red arrow indicates a heterozygous C. 134T>G p.L45R or C. 134T>A. L45Q mutation in case 3 or 4, respectively, that was identified in amplified DNA from genome DNA derived from peripheral blood mononuclear cells (PBMC), while no mutational peak was recognized in cDNA reverse-transcribed from mRNA of PBMC.

b: Allele-specific RT-PCR analyses of *CCL22* mutational sites in NK-LGLL patients with *CCL22* L45 mutation.

Supplemental Table S1: NK-LGL leukemia patients with *CCL22* mutations.

case No.	Age (y.o.)	Sex	Neu (x10⁹/L)	Lym (x10⁹/L)	Hb (g/dL)	PLT (x10⁹/L)	*CCL22* mutations (VAF%)	complication	therapy	observation periods (years)	outcome
3	57	F	3.2	8.6	12.6	280	L45R(37.6)	neuropathy	Watch	3	alive
4	73	F	3.0	6.2	12.0	274	L45Q(41.5)	none	Watch	9	alive
5	82	M	3.9	7.3	15.9	286	L45R(31.8)	oculomotor palsy	Watch	2	alive
6	80	M	3.4	8.6	15.6	121	L45Q (9.9)	NAFLD	Watch	0.2	alive
7	56	M	2.5	6.4	14.3	231	L45Q(34.5)	none	Watch	1	alive
8	28	F	0.6	1.1	11.7	286	L45R(29.7)	SCPTCL	PSL	4	alive

NAFLD, non alcoholic fatty liver disease ; SCPTCL, subcutaneous panniculitis-like T cell lymphoma; PSL, prednisolone