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STAT3 and STAT5B mutations have unique distribution in T-cell large granular lymphocyte proliferations and advanced myeloid neoplasms

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Author contributions

Z.J.X. designed the research, was the principal investigator, and took primary responsibility for the paper; S.Q.Q., Y.J.J., H.J.W., X.F.A., and Z.J.X. acquired the data, analyzed and interpreted the data, performed statistical analysis and drafted the article; S.Q.Q., Z.F.X., T.J.Q., L.J.P., B.L., and Z.J.X. recruited the patients; Z.J.X., G.H. and R.P.G. prepared the typescript.

Supplemental data for this article can be accessed [here](#).

Disclosure statement

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T-cell large granular lymphocyte leukemias (T-LGLL) is a heterogeneous disease with special histology, immune phenotype and genetic abnormalities. The 2017 World Health Organization (WHO) Classification recommends $2-20 \times 10^9/L$ for LGL population in peripheral blood [1]. In practice, persons with leukopenia fails to meet this cutoff value, but they have other clinical features of T-LGLL, such as anemia, high proportion of clonal LGL population, and *STAT3* mutations. We usually considered these oligolymphocytic large granular lymphocyte proliferations (LGLP) as T-LGLL.

Some recurrent mutations such as *STAT3*, *STAT5B* and *TNFAIP3* are found in T-LGLL [2–4]. *STAT3* mutations are reported in 30–75% of persons with T-LGLL resulting in *STAT3* activation [2,5–8]. However, *STAT3* activation is also reported in persons without a *STAT3* mutation suggesting *STAT3* is important in the pathogenesis of T-LGLL [9]. But *STAT3* mutations are not included in the current diagnostic criteria of T-LGLL and T-LGLLs with and without *STAT3* mutations are not distinguished in present classifications [1]. *STAT5B* activating mutations are reported previously in only 1 percent of persons with T-LGLL [3]. *STAT5B* mutations occurred mainly in CD4 positive T-LGLL [10]. Only one study detected *TNFAIP3* mutations in 8 percent of persons with T-LGLL, and this finding needs to be confirmed by more studies [4].

Some studies report LGLP can be found in solid cancers and myeloid neoplasms (MN), especially in myelodysplastic syndromes (MDS) [11–14]. One hypothesis is that cancer-related neoantigens trigger an immune response activating cytotoxic T-cells. These activated polyclonal cells transform into monoclonal T-cell population under selection pressures [1,8]. Here, we compare the clinical and molecular features of T-LGLPs with or without MN. In addition, we also explored the distribution of LGLL related gene mutations.

1622 subjects including 983 with MDS, 195 with myeloproliferative neoplasm (MPN), 102 with MDS/MPN, 89 with acute myeloid leukemia (AML) and 253 cytopenias caused by other reasons were enrolled in the study From June 2018 to May 2020. Subjects provided informed consent in compliance with the Declaration of Helsinki. Flow cytometric immune phenotypic analyses were done on blood samples by usual multi-parameter flow cytometry. Antigens analyzed included CD2, CD3, CD4, CD5, CD7, CD8, CD16, CD56, CD57, TCR α/β and TCR γ/δ . T-cell receptor (TCR) rearrangement was assessed by multiplex polymerase chain reaction (PCR). Next generation sequencing (NGS) was done for coding sequences of 137 genes including *STAT3*, *STAT5b* and *TNFAIP3* associated with hematologic neoplasms as described (Supplementary Table 1).

Diagnosis of T-LGLP requires these criteria: (1) expression of abnormal T-LGL immune phenotype such as CD3 +, CD8 +, CD5 dim+/- CD7 dim+/- and CD57 +; (2) clonal TCR gene rearrangement by PCR; (3) blood LGL concentration $0.5 \times 10^9/L$ or $<0.5 \times 10^9/L$ if there was an unexplained cytopenia. When the 1st 2 criteria were met in someone with a WHO-defined myeloid neoplasm the assigned diagnosis was *myeloid neoplasms with*

coexisting LGLP (MN-LGLP). Using these criteria 23 subjects were diagnosed as simple T-LGLP and 16 as MN-LGLP including 10 with MDS (Supplementary Table 2).

STAT3 mutations were detected in 19 subjects with T-LGLP 80 percent of which were in the SH2 domain. p.Y640F was the most common variant (Figure 1(A)). *STAT3* mutations were not detected in MN-LGLP cohort but *STAT5B* mutations were detected in 3 higher-risk MDS subjects with CD8 positive T-LGLP including 2 with MDS with excess blasts (MDS-EB) and 1 with MDS with ring sideroblasts (MDS-RS). The subjects with MDS-RS had a *TP53* mutation and a complex karyotype. An abnormal karyotype (-Y) was detected in only 1 subject in the T-LGLP cohort, but -Y is common in older males and may not really be an abnormality. In contrast, seven of 12 subjects in the MN-LGLP cohort had an abnormal karyotype.

Subjects in the T-LGLP cohort had higher percent blood LGLs and a higher blood concentration, more severe anemia and lower percent reticulocytes compared with MN-LGLP cohort (Table 1). Eight subjects had pure red cell aplasia (PRCA). Average number of mutations was 1.48 and the average variant allele fraction (VAF) < 10 percent in the T-LGLP cohort except for *STAT3* mutation. Subjects in the MN-LGLP cohort had more mutations and higher VAFs (Table 1, Figure 1(B)).

We screened the sequencing data of another 1583 subjects without LGLP detecting *STAT3* mutations ($n = 6$), *STAT5B* mutations ($n = 10$) and *TNFAIP3* mutations ($n = 2$) in 18 subjects (Supplementary Table 3). Clonal TCR gene rearrangement was detected in 3 subjects. Diagnoses of the 6 subjects with *STAT3* mutations included idiopathic PRCA ($n = 2$), idiopathic cytopenia of undetermined significance (ICUS; $n = 3$) and CD5-/CD10- small B-cell lymphoma, unclassifiable ($n = 1$; Figure 1(B)). Average VAF of *STAT3* mutations was 2.53% (range, 1.17–3.89%) which was significantly lower than 15.66% (range, 6.9–24.42%) of VAFs of the T-LGLL cohort ($p = .001$). Diagnoses of the 10 subjects with *STAT5B* mutations included MDS-EB ($n = 4$), primary myelofibrosis, accelerated phase (PMF; $n = 2$), PMF, chronic phase ($n = 1$), polycythemia vera (PV; $n = 1$), chronic myelomonocytic leukemia-0 (CMML; $n = 1$) and MDS/MPN with ring sideroblasts and thrombocytosis (RS-T, $n = 1$). The most common variants were in the SH2 domain with p.N642H ($n = 3$) and p.T628S ($n = 2$; Figure 1(A)). 5 subjects with *STAT5B* mutations had co-mutations of other JAK-STAT signal pathway genes including *JAK2*^{N617F} ($n = 3$), *CBL*^{C404Y} ($n = 1$) and *MPL*^{Y591N} ($n = 1$; Figure 1(B)). The 2 subjects with a *TNFAIP3* mutation had MDS-EB1 and splenic marginal zone lymphoma (SMZL). A *SH2B3* mutation was found in 1 subject. These results clearly indicate that *STAT3*, *STAT5B* and *TNFAIP3* mutations are not specific for LGLL.

Our results have several interesting implications. 1st, there are different clinical and molecular features between MN-LGLP and simple T-LGLP. In particular, *STAT3* mutations were detected in most subjects with T-LGLP but not in subjects with MN-LGLP. These data indicate that the pathogenesis of LGLP in two cohorts is different. Although the definition of LGL leukemia subsets according to associated diseases does not improve the diagnosis and treatment of this rare disease, the presence of a driver gene contributes to distinguish whether LGLP is a real leukemia or a transient response to neoantigens stimulation.

2nd, the median number of LGL in T-LGLP cohort was $1.12 \times 10E + 9/L$ with only 4 subjects greater than $2 \times 10E + 9/L$, the frequency of *STAT3* mutations was as high as 83%. It suggests that these oligolymphocytic T-LGLP have the same pathogenesis as other typical T-LGLL with *STAT3* mutation. We suggest that T-LGLL with *STAT3* mutations should be classified into one subtype of T-LGLLs, instead of being defined according to the arbitrarily LGL threshold. It is reasonable to stratify this heterogeneous disease according to molecular profiles, which can be relevant for targeted treatment options.

3nd, subjects with a low *STAT3* mutation VAF typically present with unexplained cytopenia(s). Although no abnormal T-LGL phenotype was detected it is impossible to exclude a *STAT3* mutation as the cause of the cytopenia(s). These persons may eventually develop T-LGLP. Consequently, we recommend that persons with an unexplained cytopenia(s) should be screened with NGS or sensitive techniques to sequence *STAT3*.

Lastly, eight of 13 subjects with *STAT5B* mutations had an advanced myeloid neoplasm including MDS-EB ($n = 6$) and PMF, accelerated phase ($n = 2$). A previous study has shown that *STAT5BN642H* mutation is associated with myeloid neoplasms with eosinophilia [15]. To our knowledge, this is the first report that *STAT5B* mutations are associated with advanced myeloid neoplasms. *STAT5B* is often co-mutated with other JAK-STAT signaling pathway genes. We speculate continuous *STAT5B* activation may induce mutation. Due to the small number of subjects, these findings need to be confirmed by more studies.

Our research has several limitations. 1st, as a national tertiary blood disease hospital our subjects may reflect selection biases. 2nd, most subjects are lost to long-term follow-up because they return to the referral hospital.

In summary, our data indicate *STAT3* mutations occur predominately in persons with simple T-LGLP and unexplained cytopenia(s) whereas *STAT5B* mutations occur predominately in persons with advanced myeloid neoplasms and who often have co-mutations in other JAK-STAT signaling pathway genes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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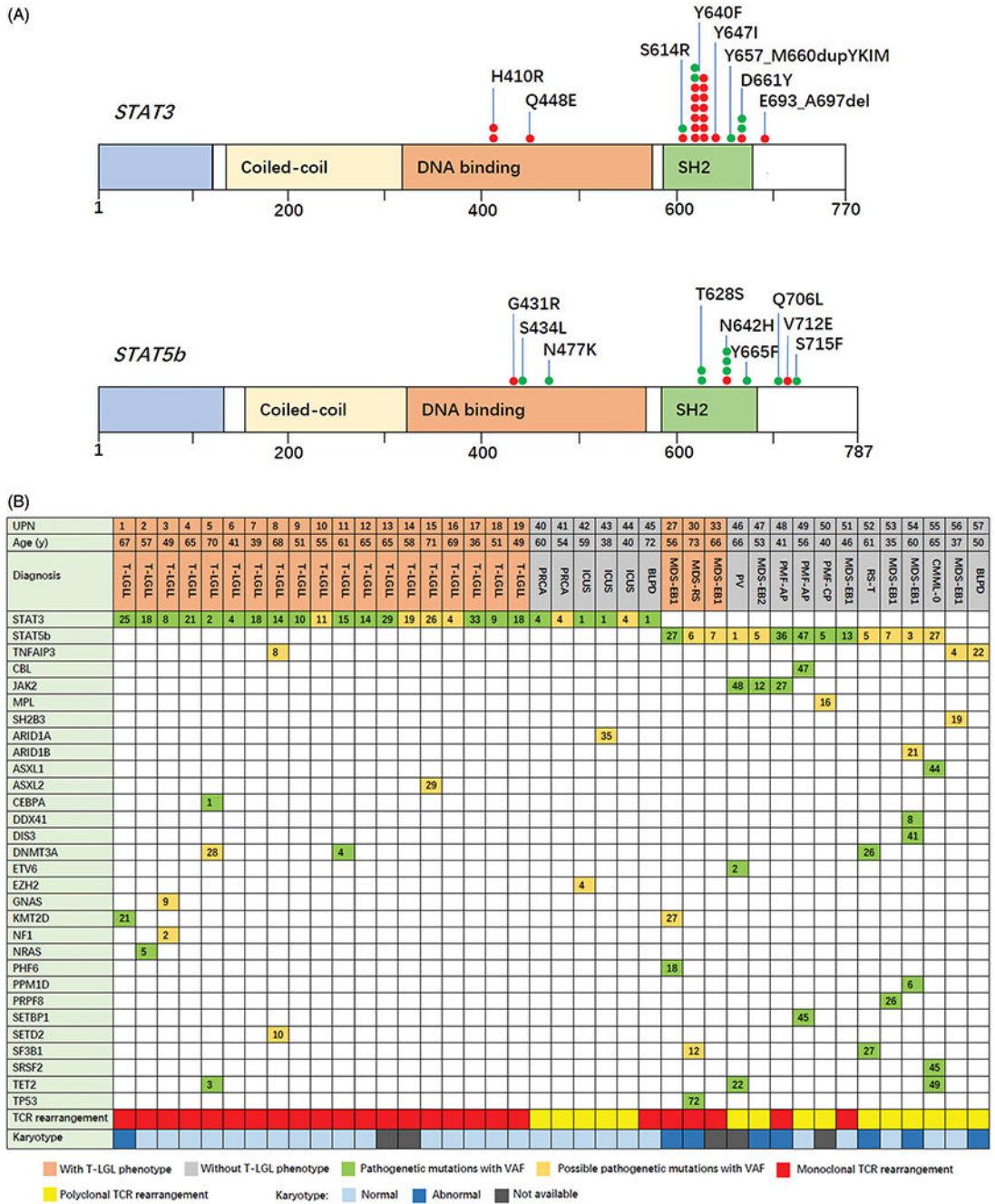


Figure 1. *STAT3* and *STAT5B* gene variant sites and concomitant mutations. (A) The upper gene structure map represents the *STAT3* variant site and the lower map, the *STAT5B* variant site. Red circles represent presence of large granular lymphocyte proliferation and the green circles, absence of large granular lymphocyte proliferation; (B) The Oncoplot represents the diagnosis and concomitant mutations of each subject with *STAT3*, *STAT5B* or *TNFAIP3* variants. Number in the box represents the mutant gene variant allele fraction (%). AP: accelerated phase; BLPD: B-cell lympho-proliferative diseases; CMML: chronic

myelomonocytic leukemia; CP: chronic phase; PMF: primary myelofibrosis; RS-T: ring sideroblasts and thrombocytosis; UPN: unique patient number.

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Table 1.

Comparison of clinical features between T-LGLP and MN-LGLP.

Co-variate	T-LGLP (n = 23)	MN ^a -LGLP (n = 16)	p-value
Male	15	11	.818
Age, years (median, range)	58 (33–71)	62 (33–75)	.558
WBC × 10E+9/L (median, range)	3.96 (0.65–27.7)	2.7 (1.11–28)	.475
ANC × 10E+9/L (median, range)	1.38 (0.09–21.86)	1.05 (0.43–18)	.711
ALC × 10E+9/L (median, range)	1.85 (0.53–5.45)	1.29 (0.47–4.58)	.061
LGL %	36 (6–66)	17.3 (4–24.5)	.001
ALGLC × 10E+9/L (median, range)	1.12 (0.3–4.15)	0.49 (0.15–1.78)	.005
ALGLC 0.5 × 10E+9/L	21	8	.011
Hemoglobin g/L (median, range)	63 (41–139)	103 (54–140)	.001
MCV, fL (median, range)	99.3 (78.7–118)	105.9 (78.3–124.8)	.166
Reticulocytes % (median, range)	0.7 (0.14–4.4)	2.25 (0.81–6.09)	.000
PLT, × 10 ⁹ /L (median, range)	232 (4–287)	74.5 (22–935)	.07
PRCA phenotype	8	0	.004
Abnormal karyotype	1/21	7/12	.002
<i>STAT3</i> mutation	19	0	.000
<i>STAT5B</i> mutation	0	3	.061
<i>TNFAIP3</i> mutation	1	0	.335
N mutations (mean ± SD)	1.48 ± 1.04	3 ± 1.79	.002
VAF other than <i>STAT3/5B</i> , % (mean ± SD)	9.79 ± 9.96	24.85 ± 16.99	.000

ALC: Absolute lymphocyte concentration; ALGLC: Absolute large granular lymphocyte concentration; ANC: Absolute neutrophil concentration; MCV: mean corpuscular volume; WBC: white blood cell concentration.

^aMyeloid neoplasms include MDS with single lineage dysplasia (n = 1), MDS with multi-lineage dysplasia (n = 3), MDS with excess blasts (n = 5), MDS with ring sideroblasts (n = 1), primary myelofibrosis (n = 3), essential thrombocythemia (n = 1), atypical chronic myeloid leukemia (n = 1), and systemic mastocytosis (n = 1).