Genomic and outcome analysis of adult T-cell lymphoblastic lymphoma

T-cell lymphoblastic lymphoma (T-LBL) is a rare and aggressive lymphoid neoplasm occurring predominantly in children and young adults.[1] T-LBL is characterized by a proliferation of T lymphoblasts arrested at an early stage of maturation and accounts for less than 2% of all the non-Hodgkin lymphomas. Currently, the molecular pathogenetic mechanisms of T-LBL are largely unknown. Previous studies have identified recurring genetic alterations in NOTCH, PI3K/AKT and RAS signaling pathways in T-LBL patients;[3-5] however, the genome-wide mutational landscape of T-LBL patients remains elusive. To address this question, we performed whole-exome sequencing in a cohort of 96 patients with T-LBL in the present study.

All patients (nine pediatric and 87 adult T-LBL patients) were reviewed and interpreted independently by three experienced pathologists. Diagnoses were made according to the current World Health Organization classification criteria. The clinical characteristics of the patient cohort are summarized in the *Online Supplementary Table S1*, and the experimental design is depicted in the *Online Supplementary Figure S1*. The study was conducted in accordance with the Declaration of Helsinki and with the approval of the Institutional Review Board of the First Affiliated Hospital of Zhengzhou University. Whole-exome sequencing was performed using DNA extracted from 96 T-LBL patient tumor samples and 41 paired normal tissue samples. Detailed descriptions of whole-exome sequencing and bioinformatics analysis are provided in the *Online Supplementary Materials and Methods*. The 41 patients with paired normal tissue were deemed a "discovery cohort," and the remaining patients were considered a "validation cohort."

In the discovery cohort, the mean sequencing depth was 217×, and a mean of 96.6% of the target sequence was covered to a depth of at least 50× after excluding the duplicates (*Online Supplementary Table S2*). A total of 1,599 nonsilent mutations (median 33, range 4-124) were identified (*Online Supplementary Figure S2A* and *Table S3*). We further evaluated the relationship between the somatic nonsilent mutation burden and the clinical features of T-LBL patients. The results showed that the somatic nonsilent mutation burden was associated with age (R^2=0.16, P=0.010; *Online Supplementary Figure S2B*) but not with other clinical features of these patients (sex, stage, or LDH). The predominant type of substitution was the C to T transition at NpCpG sites in T-LBL (*Online Supplementary Figure S3A*). Combined nonnegative matrix factorization clustering and correlation with the 30 curated mutational signatures defined by the Catalog of

Table 1. Characteristics of the 66 adult T-cell lymphoblastic lymphoma patients treated uniformly with Hyper-CVAD regimen according to their *PHF6* or *N/F* mutation status.

Characteristics	Total (n=66)	*PHF6* wild-type (n=47)	*PHF6* mutation (n=19)	*P*	*N/F* wild-type (n=39)	*N/F* mutation (n=27)	*P*
Age (years)				0.664			1.000
≤50	60	42	18		35	25	
>50	6	5	1		4	2	
Sex				0.304			1.000
Male	54	40	14		32	22	
Female	12	7	5		7	5	
Ann Arbor stage				0.203			0.078
I/II	16	9	7		6	10	
III/IV	50	38	12		33	17	
LDH				0.176			0.211
Normal	32	20	12		16	16	
Elevated	34	27	7		23	11	
CNS involvement				0.316			0.138
No	62	43	19		35	27	
Yes	4	4	0		4	0	
BM involvement				0.496			0.757
No	53	39	14		32	21	
Yes	13	8	5		7	6	
Mediastinal involvement				0.311			0.393
No	5	5	0		2	3	
Yes	61	42	19		37	24	
Effusion				0.273			0.203
No	27	17	10		13	14	
Yes	39	30	9		26	13	

T-LBL: T-cell lymphoblastic lymphoma; N/F: NOTCH1 and/or FBXW7; LDH: lactate dehydrogenase; CNS: central nervous system; BM: bone marrow, CVAD: cyclophosphamide, vincristine, doxorubicin in course A.

Somatic Mutations in Cancer (COSMIC) database[6] revealed three predominant signatures in T-LBL (*Online Supplementary Figure S3B*). The matched COSMIC signatures were signature 1 (signature A and C; cosine similarities, 0.94 and 0.93, respectively) and signature 26 (signature B; cosine similarities, 0.88) (*Online Supplementary Figure S3C-D*). Signature 1 is thought to result from the age-associated accumulation of 5-methylcytosine deamination events, while signature 26 is associated with defective DNA mismatch repair.[6]

The mutational significance detection tool MuSiC was used to identify genes that were mutated at significantly higher rates than the background mutation rates, and 32 genes were identified with a false discovery rate value less than 0.1 (*Online Supplementary Table S4*). The spectrum and frequency of mutations in T-LBL in our study were similar to those of a previously published series of T-ALL. We also found several novel mutations in MTRNR2L2, CDC27, TMEM200C and NOTCH3, which have not been previously reported in T-LBL. To validate the potential driver mutations in the discovery cohort and to better define the gene-mutation prevalence in T-LBL, we extended mutation detection to a validation cohort of 55 adults with T-LBL. In the validation cohort, all the common single-nucleotide polymorphisms (SNP) were filtered out except those identified as somatic mutations in our discovery cohort or in previous studies on T-cell acute lymphoblastic leukemia (T-ALL). The accuracy of the genetic variant identification by exome sequencing was verified by performing Sanger sequencing in 140 variants from the same cases (*Online Supplementary Table S5-6*). We found that the Sanger sequencing data agreed with the exome sequencing data in 91% of the variants, confirming that our methods for exome sequencing and computational approaches for identifying genetic variants gave accurate results.

In total, *NOTCH1* mutations were found in 35.6% (31 of 87) of adult patients with T-LBL (Figure 1 and *Online Supplementary Table S7*). These mutations were mainly clustered in the heterodimerization domain (HD) and/or

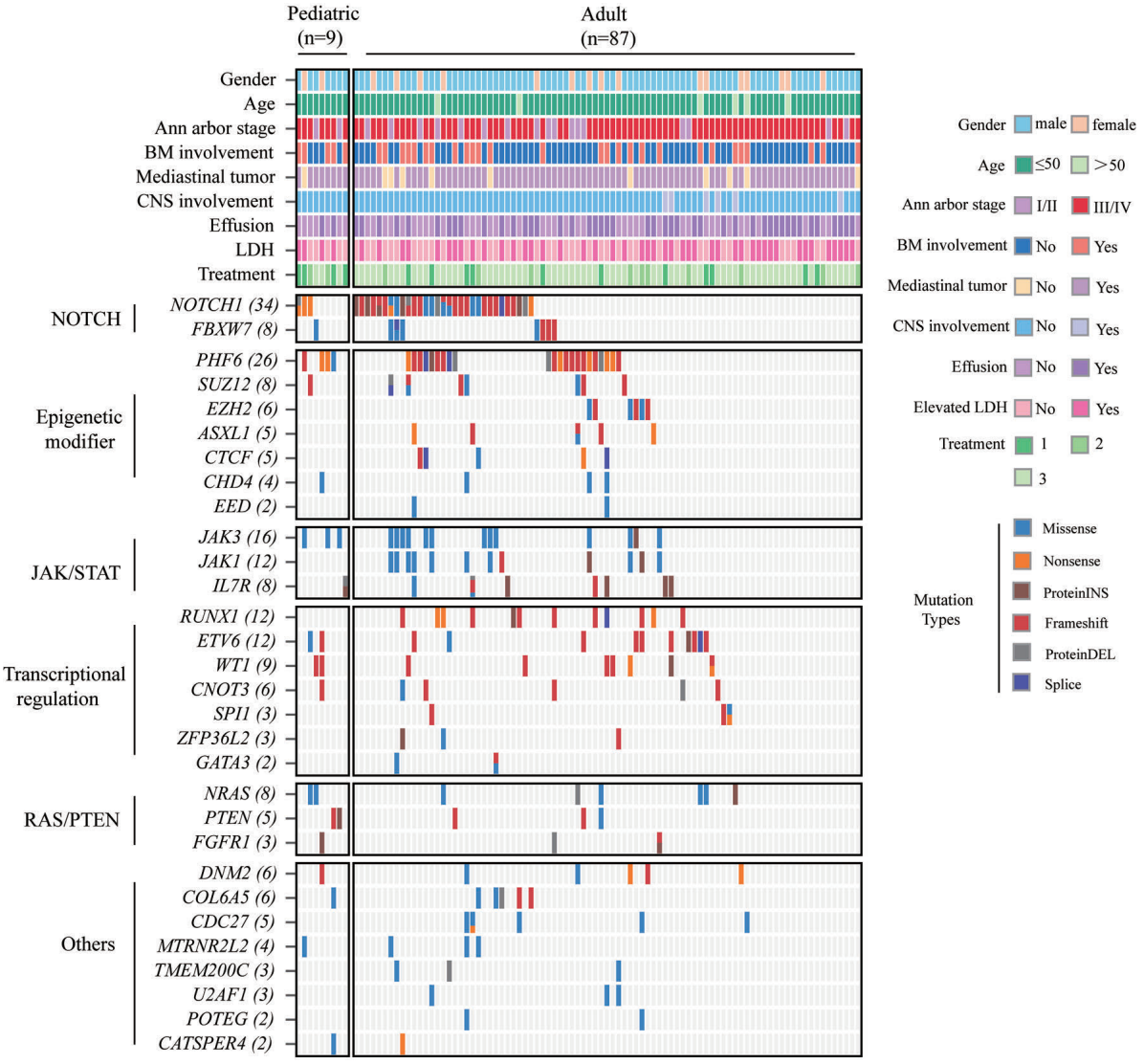

Figure 1. Mutational landscape of adult T-cell lymphoblastic lymphoma. The heat map shows the somatic mutational profile of the adult T-cell lymphoblastic lymphoma (T-LBL) cohort separated by gene functional groups. Rows represent individual genes, and columns represent individual tumors. Blocks are color-coded by the functional type of mutation. The top panel shows the clinical characteristics of the T-LBL patient cohort.

the proline, glutamine, serine and threonine (PEST) domain of *NOTCH1* (*Online Supplementary Figure S4*). The HD domain mutations occurred predominantly in highly conserved amino acid residues, whereas the PEST domain mutations were all frameshifting indels or nonsense mutations and were therefore predicted to truncate the normal protein sequence. Similar mutations have been reported in T-ALL and activate NOTCH signaling. Mutations in *FBXW7*, which functions as a negative regulator of NOTCH, were detected in 8.1% (7 of 87) of adult T-LBL cases. The majority of the mutations were identified in inactivating hotspots reported previously in T-ALL, including R465 (n=2), G423 and R505 (*Online Supplementary Figure 4*). In total, mutations in the NOTCH signaling pathway were identified in 40.2% (35 of 87) of adult patients with T-LBL.

PHF6 mutations were identified in 22 adult patients, representing the most frequently mutated epigenetic modifier in adult T-LBL (Figure 1). All mutations were truncating mutations (frameshift, nonsense or splice) and were expected to disrupt protein structure and function (*Online Supplementary Figure S4*). Other mutations were found in epigenetic modifiers, including *SUZ12* (n=7), *EZH2* (n=6) and *EED* (n=2), which encode the core component of Polycomb repressor complex 2 (PRC2) and mediate the repressive histone H3 lysine 27 trimethylation mark; *CTCF* (n=5), *ASXL1* (n=5) and *CHD4* (n=3), which are involved in chromatin organization, are also commonly found in adult patients with T-LBL (Figure 1). Altogether, we detected mutations in epigenetic modifiers in 36.8% (32 of 87) of the adult T-LBL cases.

We also found that recurrent mutations affected JAK/STAT signaling in 26.4% (23 of 87) of the cases, including *JAK3* (n=13), *JAK1* (n=12) and *IL7R* (n=7) (Figure 1). The majority of these mutations were detected in known activating hotspots or in close proximity, for example, *JAK3* (M511, n=7; A573, n=2; R657, n=2 and A572, n=1), *JAK1* (R724, n=3) and *IL7R* (IL241-242, n=4; VA253-254, n=2; and V78, n=2), which might result in constitutive activation of the JAK/STAT pathway (*Online Supplementary Figure S4*). Moreover, the mutation relation test revealed that *JAK3* was positively correlated with *JAK1* ($P < 0.01$, *Online Supplementary Figure S5*).

In addition, loss-of-function mutations in hematopoietic transcription factors, including *ETV6* (n=10), *RUNX1* (n=12), *WT1* (n=7) and *CNOT3* (n=5), and activating mutations in *NRAS* (G12 or G13, n=4), were also frequently observed in patients with adult T-LBL (Figure 1), which was consistent with previous studies in T-ALL .

T-LBL is commonly treated with T-ALL-derived proto-

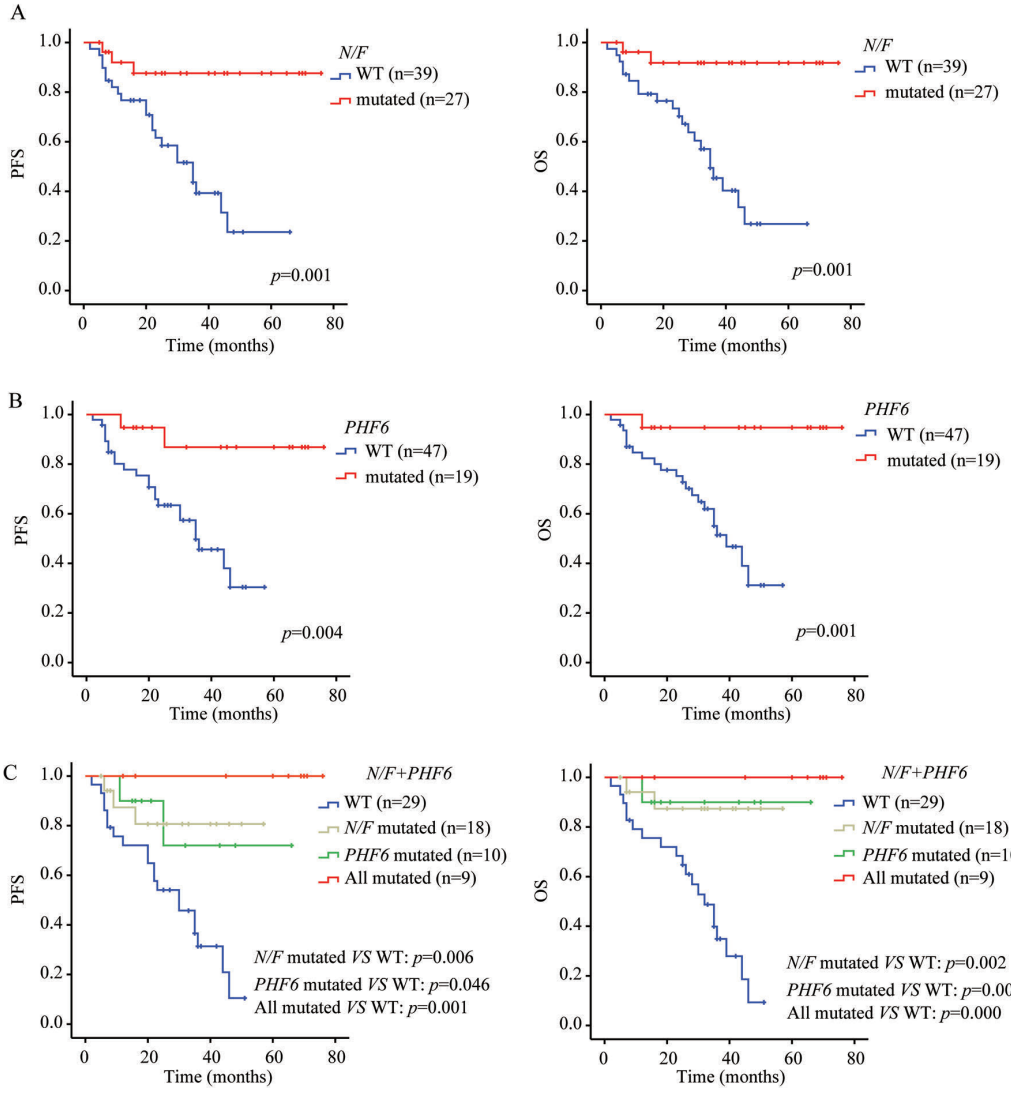

Figure 2. Mutational status and outcome analysis in adult T-cell lymphoblastic lymphoma patients who have been treated uniformly according to the hyper-CVAD regimen (n=66). (A) PFS and OS in adult T-cell lymphoblastic lymphoma (T-LBL) patients according to N/F mutational status. (B) PFS and OS in adult T-LBL patients according to *PHF6* mutational status. (C) PFS and OS in adult T-LBL patients according to the combined genotype of *N/F* and *PHF6*. Survival curves were estimated using the Kaplan–Meier method and compared using a two-sided log-rank test. PFS: progression-free survival; OS: overall survival.

cols.[9] Therapeutic stratification based on prednisone response and minimal residual disease assessment is well established in T-ALL but not easy to extrapolate to T-LBL. Molecular genetic markers are promising candidates for risk stratification because they represent underlying biological properties of the subgroups.[10] Currently, molecular genetic markers such as *NOTCH1* and/or *FBXW7* (N/F) mutations, RAS/PTEN alterations, FLASH deletion, TCR status, and loss of heterozygosity at chromosome 6q, have been identified for risk stratification in pediatric T-LBL patients. To identify additional prognostic molecular markers in adult T-LBL, we further evaluated the prognostic value of the genetic mutations detected in our study. To avoid the potential bias caused by different therapeutic regimens, we analyzed 66 adult T-LBL patients who were treated uniformly according to the hyper-in course A: cyclophosphamide, vincristine, doxorubicin (CVAD) regimen14 (Table 1).

The univariate analysis showed that adult T-LBL patients with N/F mutations had improved overall survival (OS, *P*=0.001) and progression-free survival (PFS, *P*=0.001) compared to those without the mutations (Figure 2A and *Online Supplementary Table S8*), which is consistent with previous reports. Of note, *PHF6* mutations were significantly correlated with good prognosis in adult T-LBL patients. The estimated 3-year OS rates of patients with *PHF6* mutations and those without *PHF6* mutations were 94.7±5.1% and 51.4±8.4%, respectively (*P*=0.001; Figure 2B). The estimated 3-year PFS rates of the two groups of patients were 86.8±8.9% and 45.6±8.7%, respectively (*P*=0.004; Figure 2B).

Moreover, Cox multivariate regression analysis demonstrated that *N/F* and *PHF6* mutation status were independent favorable prognostic markers in adult T-LBL after adjusting for multiple potential confounding clinical factors (age, Ann Arbor stage, bone marrow involvement, central nervous system involvement and LDH level) (*Online Supplementary Table S8*). Importantly, we found that adult T-LBL patients without *N/F* or *PHF6* mutations had a much worse prognosis than individuals with mutations in the three genes, as reflected by the OS time and the PFS time (Figure 2C). Therefore, the *N/F* and *PHF6* mutational status in adult T-LBL might provide an alternative for therapeutic stratification. However, these findings need to be further verified in large independent cohorts.

In summary, we provided the first comprehensive portrait of the mutational landscape of adult T-LBL. *PHF6* mutational status may provide a novel marker of good prognosis in adult T-LBL.

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