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Genetic profile of adult T-cell leukemia/lymphoma in Okinawa: Association with prognosis, ethnicity, and HTLV-1 strains

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Abstract

Genetic alterations in adult T-cell leukemia/lymphoma (ATLL), a T-cell malignancy associated with HTLV-1, and their clinical impacts, especially from the perspective of viral strains, are not fully elucidated. We employed targeted next-generation sequencing and single nucleotide polymorphism array for 89 patients with ATLL in Okinawa, the southernmost islands in Japan, where the frequency of HTLV-1 *tax* subgroup-A (HTLV-1-*tax*A) is notably higher than that in mainland Japan, where most ATLL cases have HTLV-1-*tax*B, and compared the results with previously reported genomic landscapes of ATLL in mainland Japan and the USA. Okinawan patients exhibited similar mutation profiles to mainland Japanese patients, with frequent alterations in TCR/NF-κB (eg, *PRKCB*, *PLCG1*, and *CARD11*) and T-cell trafficking pathways (*CCR4* and *CCR7*), in contrast with North American patients who exhibited a predominance of epigenome-associated gene mutations. Some mutations, especially *GATA3*

and *RHOA*, were detected more frequently in Okinawan patients than in mainland Japanese patients. Compared to HTLV-1-*tax*B, HTLV-1-*tax*A was significantly dominant in Okinawan patients with these mutations (*GATA3*, 34.1% vs 14.6%, *P* = .044; *RHOA*, 24.4% vs 6.3%, *P* = .032), suggesting the contribution of viral strains to these mutation frequencies. From a clinical viewpoint, we identified a significant negative impact of biallelic inactivation of *PRDM1* (*P* = .027) in addition to the previously reported *PRKCB* mutations, indicating the importance of integrated genetic analysis. This study suggests that heterogeneous genetic abnormalities in ATLL depend on the viral strain as well as on the ethnic background. This warrants the need to develop therapeutic interventions considering regional characteristics.

KEYWORDS

adult T-cell leukemia/lymphoma, geographical mutation heterogeneity, human T-cell leukemia virus type I, integrated clinico-genetic analysis, *tax* subgroup

1 | INTRODUCTION

Human T-cell leukemia virus type I (HTLV-1) is a retrovirus associated with adult T-cell leukemia/lymphoma (ATLL),[1-4] a distinct peripheral T-cell malignancy. ATLL is classified into four clinical subtypes, namely acute, lymphoma, chronic, and smoldering.[5] Patients with the former two subtypes, which are referred to as aggressive ATLLs, show very poor prognosis, with a median survival time (MST) of approximately 1 year, even if they receive combined chemotherapy.[6] On the contrary, patients with the latter two types, referred to as indolent ATLLs, show relatively long-term survival without treatment.[7]

HTLV-1 carriers are endemically distributed worldwide, mainly in Central Africa, South America, the Caribbean coast, Melanesia, and Japan.[8] Phylogenetic variations in HTLV-1 are related to geography and/or ethnicity.[8] The polymorphism of the HTLV-1 *tax* gene, encoding the viral oncoprotein, Tax,[9] is closely associated with subgrouping. HTLV-1 *tax* subgroup-A (HTLV-1-*tax*A) and *tax* subgroup-B (HTLV-1-*tax*B) are prevalent in Japan.[10] These two viral strains differ in two amino acids in Tax and one amino acid in HTLV-1 bZIP factor (HBZ),[10] another viral oncoprotein on the antisense strand.[11] Infection with HTLV-1-*tax*A increases the risk of HTLV-1-associated myelopathy (HAM), an inflammatory disease caused by HTLV-1, compared with HTLV-1-*tax*B infection.[10] These differences in viral strains have been described as affecting the biological characteristics of infected T-cells, such as different expression levels of several genes, including *HBZ* and *FOXP3*.[12,13] However, the effect of variations in the virus on the biological characteristics of ATLL tumor cells remains to be elucidated.

The Okinawa islands, located in the southernmost area off mainland Japan, are one of the largest endemic areas for HTLV-1. The germline genomic background of Okinawa residents has been reported to differ from that of the mainland Japanese population.[14,15] We previously reported that HTLV-1-*tax*A and HTLV-1-*tax*B had similar frequencies in Okinawa (*tax*A = 44%, *tax*B = 56%)[16] in contrast to the extremely higher frequency of HTLV-1-*tax*B in mainland Japan (*tax*A = 11%, *tax*B = 89%).[10]

Although previous studies have identified numerous genetic aberrations in ATLL,[17-21] there have been few studies that analyzed their clinical significance.[20,22] Recent studies conducted in North America and Japan suggest regional or racial heterogeneity in the genetic profiles of patients with ATLL and thereby imply that the outcome of a therapeutic intervention may vary among different regions or races.[19,20] Based on these reports, we hypothesized that phylogenetic variations in HTLV-1 contribute to the geographically heterogeneous genetic profiles of ATLL and their clinical impacts. In this study, we employed targeted next-generation sequencing and a single nucleotide polymorphism (SNP) array to unravel the genetic alteration profile of aggressive ATLL in Okinawa, which was not included in prior studies,[17-22] and to determine how it is associated with the *tax* subgroup and clinical outcome. In addition, we compared the frequency of genetic alterations among patients in our cohort with those in prior studies conducted in mainland Japan and North America to identify geographical variations of the genetic landscape of ATLL. The results of this study indicate that viral strains might be associated with the heterogeneity of genetic alterations in ATLL.

2 | MATERIALS AND METHODS

2.1 | Materials and patients

We used genomic DNA samples from 89 patients with aggressive ATLL (64 acute type, 12 lymphoma type, 2 chronic type with poor prognosis, and 11 indolent form with disease progression) in Okinawa, diagnosed between 2013 and 2017 (Table S1). Monoclonal integrations of HTLV-1 provirus were observed in all DNA samples analyzed in this study. ATLL subtypes were assessed based on the Shimoyama classification.[5] Genomic DNA

was extracted from 76 peripheral blood mononuclear cell samples, 11 lymph nodes, and 2 tissue samples from the mammary glands and subcutaneous tissue using the QIAamp Blood/DNA Mini Kit (QIAGEN, Hilden, Germany). We also analyzed the *tax* subgroups as described in our prior research.[16] Viral genotyping identified HTLV-1-*tax*A and HTLV-1-*tax*B in 41 and 48 cases, respectively (Table S1).

This study was approved by the ethics review board of the University of the Ryukyus and each participating institution, adhering to the Helsinki Declaration revised in 2008 or 2013. Before sampling, all the patients provided their physicians at the hospitals with informed consent for participation in the study.

2.2 | Targeted sequencing

We performed targeted next-generation sequencing for 89 genomic DNA samples using the HaloPlex HS target enrichment system (Agilent Technology) and the Miseq platform (Illumina). The SureCall software (Agilent Technologies) was used for identifying the variants. We designed probes for 51 cancer-related genes, especially focusing on the genes frequently mutated in ATLL patients (Table S2).[19,20]

Variants were filtered out under the following exclusion criteria: (a) synonymous mutations and variants in introns except for splice sites and a promoter region (*TERT*) or (b) variants with ≥1% allele frequency in online SNP databases (details in Document S1). Subsequently, putative driver mutations were selected when they satisfied at least one of the following conditions: (a) frameshift, in-frame, or splice-site mutations, (b) variants considered to be recurrent mutations in cancers by the Catalogue of Somatic Mutations in Cancer (https://cancer.sanger.ac.uk/cosmic) or a previous report,[19] or (c) nonsynonymous mutations with a SIFT score < 0.05.[23]

2.3 | SNP array

The OncoScan® CNV FFPE Assay Kit (Thermo Fisher Scientific) was used to analyze copy number alterations (CNAs) in the DNA samples from 87 cases. Preparation of the PCR amplicon library, array hybridization, and array scanning were performed in accordance with the protocol recommended by Affymetrix. The Chromosome Analysis Suite v4.0 software (Thermo Fisher Scientific) was used for detecting CNAs. The probes on chromosomes X and Y were excluded from the analysis because each experiment was not sex matched.

2.4 | Statistical analysis

Fisher's exact test was used for assessing the significance of coexistence or mutual exclusiveness of mutations, alteration frequency, and/or clinical characteristics between *tax* subgroups or clinical subtypes, and mutation frequencies between patients in Okinawa and those in other regions.[19,20] Altered genes selected for statistical analysis are shown in Table S3. Overall survival (OS) was calculated from the day of diagnosis or disease progression to the last follow-up day or death by any cause. Univariate analysis was performed using the Kaplan-Meier method and log-rank test. We adjusted the effect of variables with statistical significance by multivariate analysis with the Cox proportional hazard model. ATLL-Prognostic Index (high/intermediate risk and low risk groups)[24] and corrected calcium,[25] a previously reported prognostic predictor, were selected as clinical covariates. $P < .05$ were considered significant in all statistical analyses. All analyses were performed using the Stata software version 14 (Stata Corporation).

Detailed methods for each section are provided in the online Supporting Information.

3 | RESULTS

3.1 | Genetic alteration profile of aggressive ATLL in Okinawa prefecture

Eighty-eight of the 89 (98.9%) patients carried two or more mutations. A total of 470 mutations were identified in 44 out of 51 genes (Figure 1A and Table S4). The most frequently mutated gene was *PRKCB* (39/89, 43.8%), followed by *PLCG1* (36/89, 40.4%), *TP53* (31/89, 34.8%), *TBL1XR1* (22/89, 24.7%), *GATA3* (21/89, 23.6%), *CCR4* (21/89, 23.6%), *IRF4* (18/89, 20.2%), and *CARD11* (18/89, 20.2%). The mutations included components of TCR/NF-κB signaling (*PRKCB*, *PLCG1*, *IRF4*, *CARD11*, *RHOA*, *VAV1*, *FYN*, and *CD28*), G protein-coupled receptors (*CCR4* and *CCR7*), and other T-cell related genes (*TBL1XR1*, *GATA3*, *NOTCH1*, and *STAT3*), in agreement with those identified in a prior study.[19] We analyzed the coexistence and mutual exclusiveness of the mutated genes (Table 1). In particular, strong coexistence-association was observed in the combinations of *PRKCB/CARD11*, *EP300/CARD11*, *CD28/VAV1*, and *PRKCB/IRF4* ($P < .01$).

The CNAs in 87 cases are shown in Figure 1B and Table S5. Several reported alterations in aggressive ATLL[17-19] were also frequently observed (>20%) in our cohort: gains on chromosome 3, the long-arm of chromosomes 1, 7, 18, and 21, 1p36.33 (*SKI*), 2q33.2 (*CD28*, *CTLA4*, and *ICOS*), 6p25.3 (*IRF4*), 9q34.3 (*NOTCH1*), and 14q31.1-32.33, and loss of 6q14.3 (*SYNCRIP*), 7q31.1 (*IMMP2L*), 7q34 (*TRB*), 9p21.3 (*CDKN2A*), 13q32.2-34, 14q11.2 (*TRA*), and 17p13.1 (*TP53*). We investigated the frequency of biallelic alterations, namely, homozygous deletions or co-occurrences of heterozygous deletions and mutations. Co-occurrences of deletions and mutations were significantly frequent in *TBL1XR1*, *PRDM1*, *ITGB1*, and *TP53* (Figure 1C). Homozygous deletions were frequently identified in *CDKN2A* (21/87, 24.1%), whereas no homozygous deletions of the other analyzed genes were identified in more than 5% of the patients. In particular, more than 50% of the mutations in *CD58*, *PRDM1*, and *TP53* were truncating mutations, probably leading to loss of function (Figure 1A). This suggests that biallelic inactivation
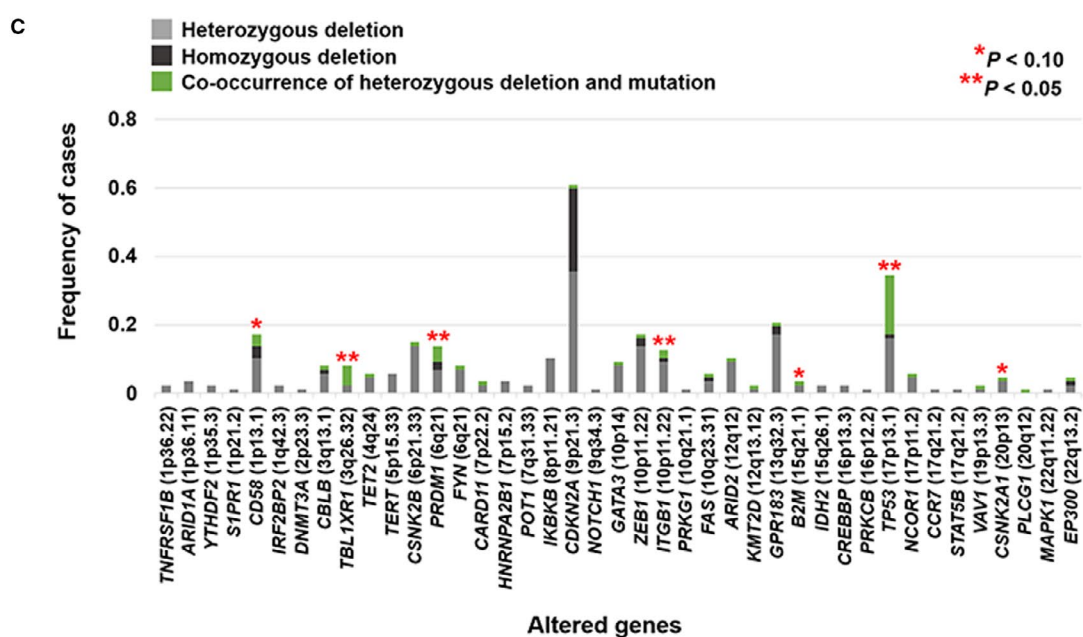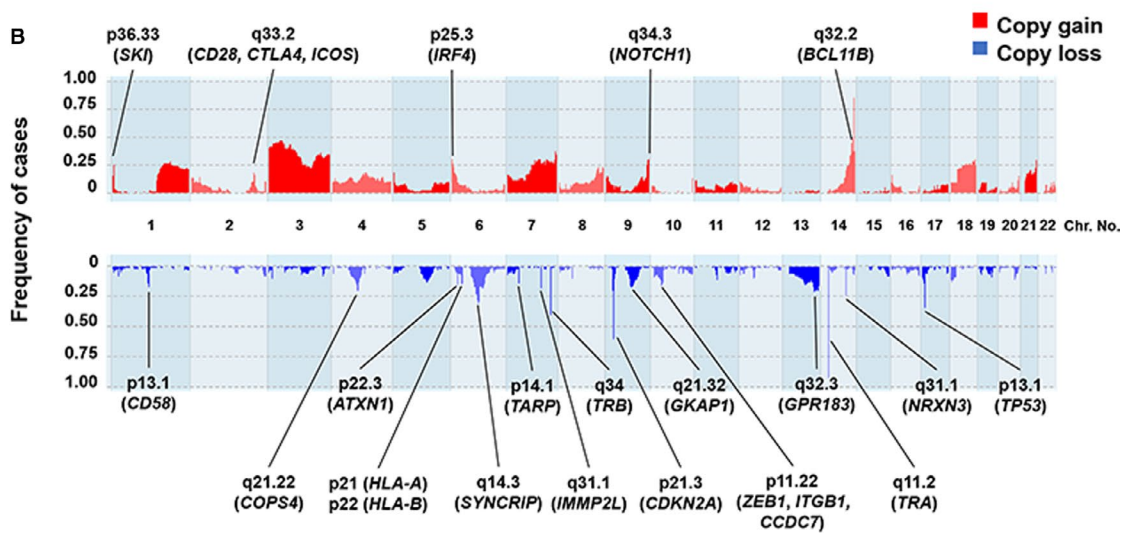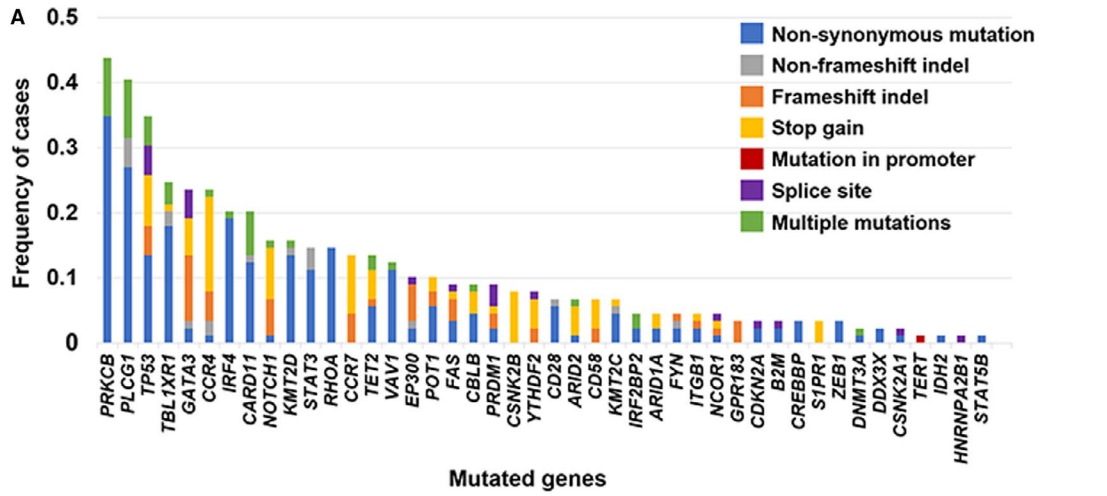
FIGURE 1 (A) The mutational profile of 44 mutated genes out of 51 genes in 89 patients with ATLL, analyzed by targeted next-generation sequencing. (B) The frequency histograms of copy number alterations in 87 patients. Genes frequently altered (>10%) and previously reported in ATLL are annotated. (C) Analysis of genetic status via combinations of mutations and copy number losses in 87 patients. The association between mutations and deletions was assessed by the two-sided Fisher's exact test

TABLE 1 Mutual association of mutated genes

Coexistence	Number of cases with mutations in both genes, n (%)		
	Left gene	Right gene	P
PRKCB/CARD11	15/39 (39)	15/18 (83)	<.001
EP300/CARD11	6/9 (67)	6/18 (33)	.002
CD28/VAV1	4/6 (67)	4/11 (36)	.002
PRKCB/IRF4	13/39 (33)	13/18 (72)	.008
CSNK2B/PLCG1	6/7 (86)	6/36 (17)	.016
NOTCH1/FAS	4/14 (29)	4/8 (50)	.019
CBLB/TBL1XR1	5/8 (63)	5/22 (23)	.020
PLCG1/ITGB1	4/36 (11)	4/4 (100)	.024
PRKCB/NOTCH1	10/39 (26)	10/14 (71)	.038
CCR7/CARD11	5/11 (45)	5/18 (28)	.041
POT1/CBLB	3/10 (30)	3/8 (38)	.044
CCR7/PLCG1	8/11 (73)	8/36 (22)	.045
FYN/TBL1XR1	3/4 (75)	3/22 (14)	.045
Mutual exclusiveness			
TP53/PLCG1	7/31 (23)	7/36 (19)	.014
CARD11/TP53	2/18 (11)	2/31 (6)	.025
CCR4/STAT3	0/21 (0)	0/13 (0)	.033
CCR4/RHOA	0/21 (0)	0/13 (0)	.033

is induced by co-occurrences of deletions and mutations of these genes.

3.2 | Association of the HTLV-1 *tax* subgroup with genetic alterations

We examined the association between each *tax* subgroup (HTLV-1-*tax*A, 41 cases; HTLV-1-*tax*B, 48 cases) and genetic alterations. Regarding mutations (Figure 2A and Table S6), *GATA3* mutations were detected among 34.1% (14/41) and 14.6% (7/48) in patients with HTLV-1-*tax*A and HTLV-1-*tax*B, respectively (P = .044). *RHOA* mutations were also more frequent among patients with HTLV-1-*tax*A (10/41, 24.4%) compared with those with HTLV-1-*tax*B (3/48, 6.3%) (P = .032). Patients with HTLV-1-*tax*B had more frequently mutated *EP300* (8/48, 16.7%) than those with HTLV-1-*tax*A (1/41, 2.4%) (P = .035). Copy number gains of *RHOA* (3p21.31) were more frequently observed in patients with HTLV-1-*tax*A (22/41, 53.7%) than in patients with HTLV-1-*tax*B (13/46, 28.3%), showing the same tendency for this *RHOA* mutation (P = .018) (Figure 2B and Table S7). The association between types of *RHOA* mutations, copy number gains, and viral strains was further analyzed. However, no significant relationship was identified among them.

3.3 | Comparison of genetic alteration profiles between patents in Okinawa, mainland Japan, and North America

We compared the frequency of each mutation between patients included in studies in Okinawa, mainland Japan,[19] and North America.[20] *STAT3* mutations were significantly more frequent among patients in Okinawa (13/89, 14.6%) than in patients in North America (0/30, 0%) (Figure 3A). *CARD11* and *GATA3* mutations tended to be more frequently detected among patients in Okinawa (*CARD11*, 18/89, 20.2%; *GATA3*, 21/89, 23.6%) than among patients in North America (*CARD11*, 2/30, 6.7%; *GATA3*, 2/30, 6.7%). Although *EP300* mutations were reported to be significantly more frequent in North American patients with ATLL than in the Japanese counterparts,[20] no significant difference was identified in the frequency between patients in Okinawa (9/89, 10.1%) and North America (6/30, 20.0%) in the present study.

TP53 and *YTHDF2* showed significantly higher mutation rates among patients in Okinawa than among patients in mainland Japan (Figure 3B). Patients with mutated *PRKCB*, *GATA3*, *RHOA*, and *CD28* were also more frequently observed in Okinawa, but with marginal significance (P values 0.051-0.063). Although the number of patients was small, *CSNK1A1* and *HNRNPA2B1* mutations, which were reported in 5-6% of mainland Japanese patients with ATLL,[19] were rarely detected among patients in Okinawa (*CSNK1A1*, P = .031; *HNRNPA2B1*, P = .095).

3.4 | Association between genetic alteration and prognosis in patients with aggressive ATLL in Okinawa

Detailed clinical findings for 87 cases with available follow-up data were analyzed as described in Tables 2 and S8. The MST of patients with the acute and lymphoma types in this cohort was 7.5 and 10.8 months, respectively (Figure S1A). These were equivalent to 8.3 months for the acute type and 10.6 months for the lymphoma type in the largest retrospective study of ATLL.[7] Mutated *PRKCB*, reported as an independent prognostic predictor in aggressive ATLL,[22] and loss of *CD58* (1p13.1), a characteristic genetic alteration in the aggressive type,[18] were significantly associated with poor OS (Figure 4A,B, and Tables S9 and S10). We further analyzed prognosis by focusing on frequent biallelic alterations: *TBL1XR1* (3q26.32), *PRDM1* (6q21), *CDKN2A* (9p21.3), *ITGB1* (10p11.22), and *TP53* (17p13.1). Among them, only the biallelic alteration of *PRDM1* (6q21) was significantly associated with inferior OS, whereas heterozygous deletion or mutation of *PRDM1* did not affect the prognosis (Figure 4C,D and Table S11). We subsequently performed multivariate analysis for 73 patients with complete clinical data to adjust the

FIGURE 2 Association between genetic alterations and *tax* subgroups as assessed by the two-sided Fisher's exact test. (A) The association between each mutated gene and *tax* subgroup. Genes mutated in more than 5% of patients were analyzed. (B) The frequency histograms of copy number alterations by the *tax* subgroup. Altered genes found in more than 10% of patients, including genes reported as strongly significant in ATLL,[22] were analyzed. Arrows indicate altered regions more frequently detected at a statistically different level in HTLV-1-*tax*A or HTLV-1-*tax*B
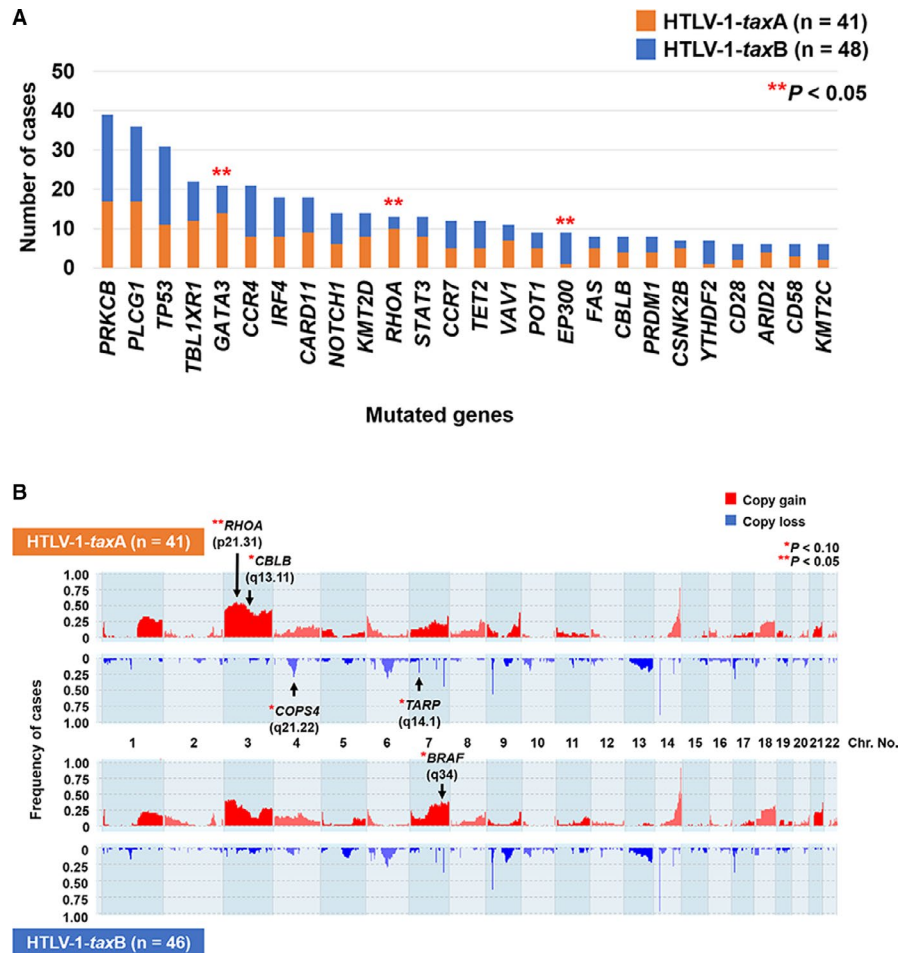
prognostic impact of *PRKCB* mutations (31 cases), *CD58* (1p13.1) losses (11 cases), and biallelic alterations of *PRDM1* (6q21) (6 cases). Multivariate analysis showed independent negative impacts of all these three alterations on OS (Table 3).

We did not find statistically significant differences in clinical characteristics and prognosis between HTLV-1-*tax*A and HTLV-1-*tax*B patients (Table 2 and Figure S1B). A recently proposed prognostic model for ATLL based on *PRKCB* mutations, 9p24.1 (*PD-L1*) amplifications, and older age (≥70) significantly distinguished cases with poorer prognosis among aggressive ATLL patients.[22] We applied this clinico-genetic prognostic index to our cohort. The worst prognoses with statistical significance were observed in patients with more risk factors (Figure S1C).

4 | DISCUSSION

In this study, we confirmed the importance of genetic alterations involved in several molecular pathways, such as TCR/NF-κB signaling, G protein-coupled receptors, and other T-cell related genes, in ATLL cases in Okinawa as well as in mainland Japan through comprehensive genetic analysis.[19] On the contrary, genetic alterations in *RHOA* and *GATA3*, which were frequent in patients with HTLV-1-*tax*A, were significantly more frequent among patients in Okinawa compared

with cases in other areas.[19,20] These results suggest that the different *tax* subgroups contribute to the geographical heterogeneity of genetic alterations in ATLL. Furthermore, we identified the negative prognostic impact of biallelic alterations in *PRDM1* by examining combinations of mutations and copy number alterations.

Genetic alterations of *GATA3* and *RHOA*, characterized by ATLL associated with HTLV-1-*tax*A, are related to Tax protein as with *PRKCB*, *CARD11*, *CD28*, *TP53*, and *CDKN2A*,[19,26] although Tax is downregulated in ATLL cells to escape from immune surveillance.[19,27] These alterations seem to replace the functions of Tax to promote proliferation and survival of ATLL cells. The promoter of *GATA3*, a transcription factor associated with Th2 cell differentiation, is regulated by Tax via the repressor ZEB protein, resulting in reduction of gene expression activity.[26] The sites of mutations inducing protein truncation were widely distributed in the entire coding region of *GATA3* in the present and prior studies,[19] suggesting the essential role of the loss of function in this gene to ATLL pathogenesis. These interactions support the possibility that Tax variants affect the context of genetic alterations in ATLL cells. Moreover, RHOA, one of the Tax-binding proteins,[28] belongs to the small GTPase Rho family, which is involved in cell cycle progression, cell survival, and cytoskeletal rearrangement.[29] Interestingly, not only mutations but also copy number gains of *RHOA* were more frequently identified in the HTLV-1-*tax*A subgroup (Figure 2A,B). However, no significant
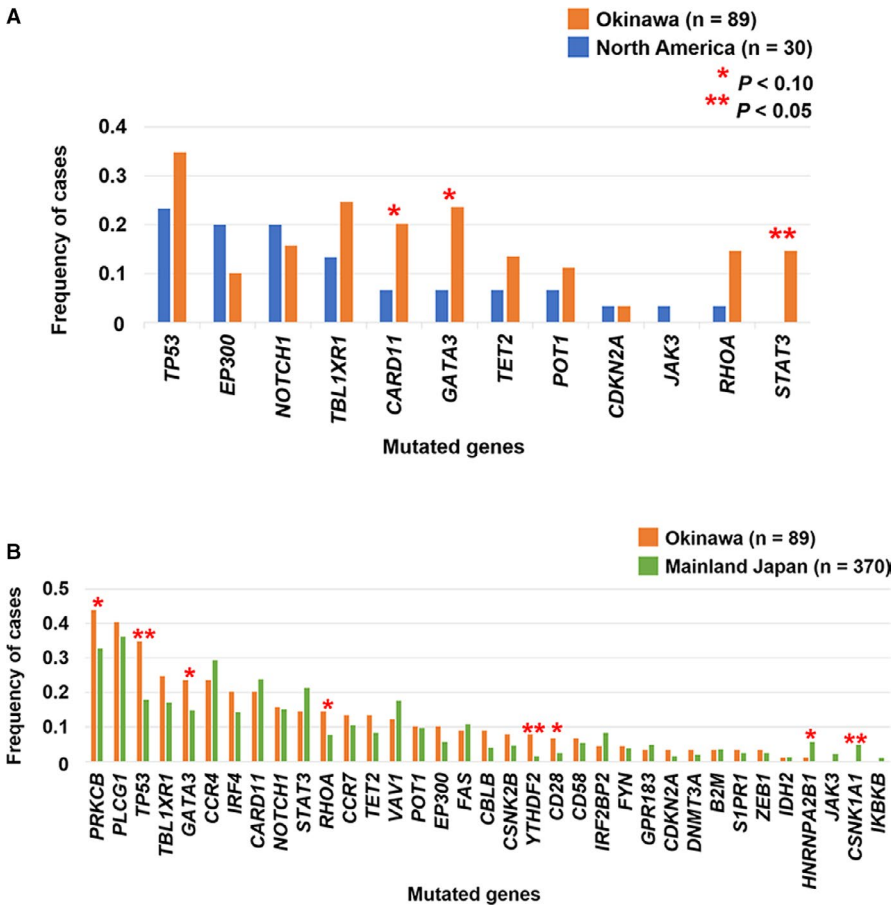
A

B

association was identified among each type of mutation, copy number status, and viral strain in the present cohort. Expanded studies in other HTLV-1 endemic areas, especially where different HTLV-1 subgroups are prevalent, will clarify the relationship between the viral strain and tumor biology of ATLL.

Differences in HTLV-1 strains do not completely explain the geographical heterogeneity of mutation rates. A recent genomic study reported a higher frequency of mutations in genes associated with the epigenome and a lower frequency of mutations in the components of TCR/NF-κB and JAK/STAT signaling in North American patients (Caribbean descent) than in Japanese patients.[19,20] In this study, *STAT3* and *CARD11* mutations were more frequent in Okinawan patients than in North American patients. However, there was no statistically significant difference in these mutations between ATLL cases in Okinawa and those in mainland Japan. These results suggest that TCR/NF-κB and JAK/STAT3 signaling are important pathways for ATLL tumor formation in Japanese patients, unlike in North American patients. Moreover, mutation rates in *PRKCB*, *TP53*, *YTHDF2*, *CD28*, *HNRNPA2B1*, and *CSNK1A1* differed among patients in mainland Japan and Okinawa. It was previously reported that mutation rates of *PRKCB* and *TP53* were significantly higher in aggressive ATLL compared with indolent ATLL.[22] Higher rates of *PRKCB* and *TP53* mutations among patients in Okinawa might be ascribed to the fact that all patients in this cohort had aggressive ATLL. Regarding other genes showing geographical variations in mutation frequency, it is possible that the genetic background contributes to

the incidence of somatic mutations as people from Okinawa showed different genetic polymorphism compared with those from mainland Japan in studies using SNP genotyping.[14,15] Several cancers show heterogeneous mutation profiles in patients between different geographical regions. For example, trans-ethnic analysis of genetic alterations identified race-dependent differences in the frequency of *BRAF/KRAS* mutations in colon cancer[30] and ancestry-dependent diversity of mutational signatures in hepatocellular carcinoma.[31] Some genetic polymorphisms in HLA class II locus were reported to influence the susceptibility to *EGFR* mutation-positive lung adenocarcinoma in Japanese.[32] These reports suggest that some germline genetic variations might contribute to the different incidence of some somatic mutations in tumor cells among patients in Okinawa and in mainland Japan.[14,15]

PRKCB mutations, *CD58* (1p13.1) losses, and biallelic alterations in *PRDM1* had a negative impact on survival. In a prior large cohort, *PRKCB* mutation was identified as an independent factor of poor prognosis.[22] The loss of *PRDM1* (6q21) was also reported to be significantly associated with an unfavorable OS in activated B-cell-like DLBCL.[33] In DLBCL, homozygous deletions, not heterozygous deletions, were significantly associated with poor prognosis and induced upregulation of several transcription factors compared to the cases without homozygous deletions. In the prior study,[22] although multivariate analysis did not show statistical significance, the loss of *PRDM1* (6q21) was a candidate for poor prognosis predictor in patients with ATLL in univariate analysis.

TABLE 2 Clinical characteristics of patients

| Clinical characteristics | Number of patients | | | |
	Total (n = 87)	*tax*A (n = 40)	*tax*B (n = 47)	P
Sex				
M	38	19	19	.328
F	49	21	28	
Age				
<70	46	21	25	.560
≥70	41	19	22	
Median (range)	69 (35-90)	69 (53-90)	69 (35-90)	
Clinical subtype				
Acute	62	27	35	.477
Lymphoma	12	7	5	
Unfavorable chronic	2	0	2	
Disease progression from indolent form	11	6	5	
PS				
0-1	40	17	23	.667
2-4	47	23	24	
Ann Arbor stage				
I-II	5	1	4	.227
III-IV	81	39	42	
Unevaluable	1	0	1	
LDH (U/L)				
<230	6	2	4	.683
≥230	81	38	43	
Serum albumin (g/dL)				
<3.5	38	18	20	.832
≥3.5	49	22	27	
Corrected Ca (mmol/L)				
<2.75	58	26	32	.469
≥2.75	29	14	15	
sIL-2R (U/mL)				
≤20 000	29	13	16	.536
>20 000	56	26	30	
Not tested	2	1	1	
Regimen of chemotherapy				
VCAP-AMP-VECP	17	5	12	.223
CHOP/CHOP-like	42	20	22	
Etoposide	11	5	6	
Others	9	7	2	
Untreated	8	3	5	

(Continues)

TABLE 2 (Continued)

| Clinical characteristics | Number of patients | | | |
	Total (n = 87)	*tax*A (n = 40)	*tax*B (n = 47)	P
Mogamulizumab				
−	24	10	14	.400
+	63	30	33	
Stem cell transplantation				
−	79	38	41	.192
+	8	2	6	
ATLL-PI				
Low	11	4	7	.934
Intermediate	37	17	20	
High	27	13	14	
Unevaluable	12	6	6	
JCOG-PI				
Moderate	30	12	18	.280
High	57	28	29	
Follow-up period (month)				
Median (range)	7.7 (0.2-64)	7.6 (0.2-50.7)	10.0 (0.2-64.0)	

Notes: P values are calculated by two-sided Fisher's exact test.
Abbreviations: ATLL-PI, ATLL Prognostic Index; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; JCOG-PI, Japan Clinical Oncology Group-Prognostic Index; LDH, lactate dehydrogenase; PS, performance status; sIL-2R, soluble interleukin 2 receptor; VCAP-AMP-VECP, vincristine, cyclophosphamide, doxorubicin, ranimustine, vindesine, carboplatin, etoposide, and prednisone; WBC, white blood cell.

Our observations suggest that the biallelic inactivation of *PRDM1* affects the malignant potential of ATLL tumor cells more than the heterozygous alteration.

We found a high frequency of coexistence between *PRKCB* and *CARD11* mutations, which is consistent with a previous report.[19] These two genes were some of the most frequently mutated genes in aggressive ATLL. In vitro experiments in a prior study demonstrated that co-expression of the p.D427N PKCβ and the p.E626K CARD11 mutants enhanced NF-κB activation more than either of the mutants alone.[19] Reproducible results in the two independent cohorts in Okinawa and mainland Japan indicated the crucial cooperative roles of *PRKCB* and *CARD11* mutations in the pathogenesis of ATLL. Previous studies also described the importance of intragenic focal deletions in the inhibitory domain of *CARD11*, which cause CARD11 to be constitutively active.[19,34] However, these small deletions were not detected in the present study owing to the resolution of the SNP array, which is one of the limitations in this study. Detailed examination of the intragenic focal deletions in *CARD11* will accurately determine the significance of the cooperation between *CARD11* and *PRKCB* alterations.

Another limitation of this study is that mutational signature has not been understood because we performed targeted sequencing
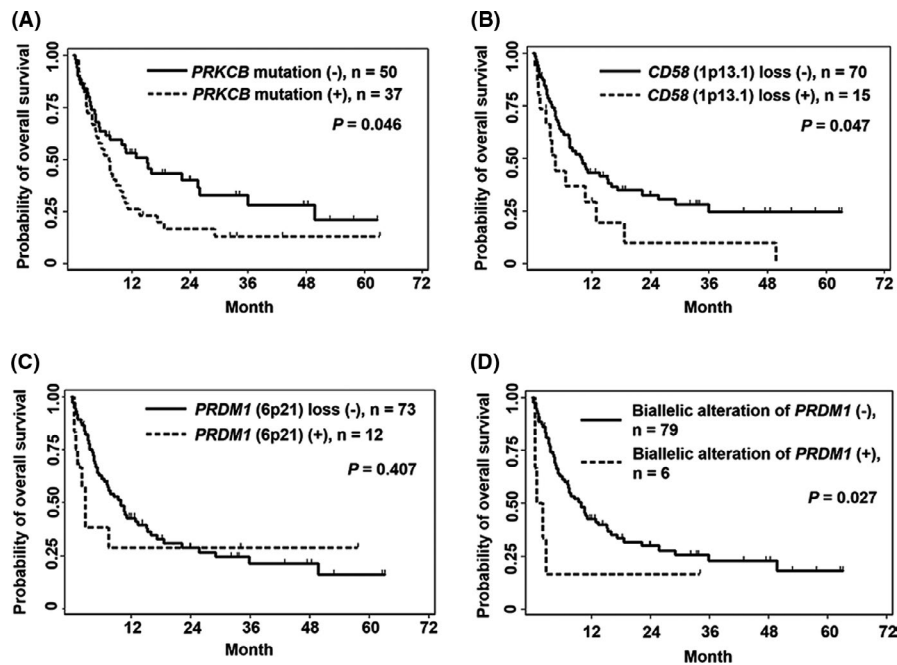
FIGURE 4 Survival analysis by the Kaplan-Meier method for *PRKCB* mutation (A), *CD58* (1p13.1) loss (B), *PRDM1* (6q21) loss (C), and biallelic alteration of *PRDM1* (D) are shown. Survival curves were evaluated by the log-rank test

TABLE 3 Results of multivariate analysis

Variable	Hazard ratio	95% confidence interval	P
ATLL-prognostic index			
High/intermediate	3.31	1.22-8.98	0.019
Low	1.00		
Corrected Ca (mmol/L)			
≥2.75	0.72	0.38-1.36	0.306
<2.75	1.00		
PRKCB mutation			
Positive	1.87	1.01-3.46	0.047
Negative	1.00		
CD58 copy number loss			
Positive	3.62	1.68-7.82	0.001
Negative	1.00		
PRDM1 biallelic alteration			
Positive	2.86	1.07-7.64	0.036
Negative	1.00		

Notes: Seventy-three patients with evaluable ATLL-Prognostic Index scores, data of mutation, and copy number alteration were analyzed using Cox's proportional hazard model.

and not whole genome sequencing. Furthermore, the driver mutations were defined based on several databases as described in the Materials and Methods section, and possibly somatic passenger mutations as well as germline polymorphisms were excluded. Therefore, it was difficult to completely distinguish somatic mutations, including driver and passenger ones from germline polymorphisms due to the lack of normal control samples.

In summary, this study uncovers genetic alterations in aggressive ATLL in Okinawa and suggests an association between HTLV-1 strains and the regional heterogeneity of genetic alterations. In addition, we discovered adverse outcomes in patients with *CD58* (1p13.1) losses and *PRDM1* biallelic alterations and confirmed the clinical impact of *PRKCB* mutations and the importance of several molecular pathways, such as TCR/NF-κB signaling. Our results will contribute to the identification of optimal therapeutic targets for different geographical regions. Our integrated clinico-genetic data, along with those of prior genomic studies,[19,20,22] form the foundation for future studies on ATLL from biological, clinical, virologic, and epidemiological viewpoints.

DISCLOSURE

The authors have no conflict of interest to declare.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.