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Original Article

# Prognostic analysis of smoldering ATLL with skin eruptions based on genomic aberrations

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ABSTRACT

*Background:* Patients with smoldering ATLL often present with a skin eruption due to skin infiltration of ATLL cells. Although skin eruption type is known to be associated with prognosis based on its pattern, it is unknown why different types of skin eruptions are associated with different prognoses.
*Objective:* Genomic analysis of patients with skin eruptions of smoldering ATLL will be performed to determine the mechanism of ATLL development and its association with prognosis.
*Methods:* DNA from skin biopsy specimens was used for targeted sequencing of 280 genes to examine the association between genomic variation and prognosis.
*Results:* Due to the small number of smoldering ATLL patients (27 cases), we could not find a clear relationship between skin eruption and prognosis in this study. Genomic analysis identified 247 genomic variants (108 genes), with an average of 9.2 variants and 3.2 variants as driver genes. Pathway analysis of the driver genes showed activation of the pathway associated with HTLV-1 infection, as well as activation of the signaling pathway observed throughout ATLL. Furthermore, multivariate analysis identified age > 70 years and STAT3 mutation as prognostic risk factors and TBL1XR1 mutation as a risk factor for progression-free survival.
*Conclusion:* Although the small number of patient samples did not allow us to determine a prognostic association with skin eruption, STAT3 mutation was identified as a prognostic risk factor for smoldering ATLL with skin eruption. Further studies are needed to increase the number of patients with this disease.


## 1. Introduction

Adult T-cell leukemia/lymphoma (ATLL) is a refractory leukemia of mature T cells, caused by infection with human T-cell leukemia virus type 1 (HTLV-1) [1]. HTLV-1 infection is endemic in southwestern Japan, mainly in Kyushu and the Caribbean islands, Papua New Guinea, South America, and Africa. In recent years, the discovery of a cluster of HTLV-1 carriers with over 40 % of the population among the Aborigines of Australia has resulted in a large splash [2]. The lifetime risk of developing ATLL among HTLV-1 carriers is estimated to be approximately 6.6 % in men and 2.1 % in women [3].

*Abbreviations:* ACAN, aggrecan; CCR4, C-C motif chemokine receptor 4; CSNK2A1, casein kinase 2 alpha 1; DMD, Duchenne muscular dystrophy; FLG, filament-aggregating protein gene; HSPA6, heat shock protein family A (Hsp70) member 6; IRF4, interferon regulatory factor 4; LGL, T-cell large granular leukemia; LRP1B, LDL receptor RELA proto-oncogene; NF-κB, nuclear factor-kappa B; NK-CLL, natural killer cells in chronic lymphocytic leukemia; PD-L1, programmed cell death ligand 1; PRKCB, protein kinase C beta; RPL10, ribosomal protein L10; STAT3, signal transducer and activator of transcription 3; TBL1XR1, transducin (beta)-like 1X/Y related 1; TP53, tumor protein p53; ATLL, adult T-cell leukemia lymphoma; HTLV-1, human T-cell leukemia virus 1; CGH, comparative genomic hybridization; iATL-PI, indolent ATL prognostic index; sIL-2R, soluble interleukin-2 receptor; LDH, lactate dehydrogenase; PFS, progression-free survival; OS, overall survival; SNV, single nucleotide variant

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**Table 1**
Clinical and genomic characteristics of patients with smoldering ATLL with skin eruption.

| Factor | All patients | poor group | better group | p value |
|---|---|---|---|---|
| **Total No. of evaluated cases** | 27 | | | |
| **Clinical subtype** | | | | |
| Acute, lymphoma, Chronic type | 0 | 0 | 0 | |
| Smoldering type | 27 | 23 | 4 | |
| **Patient-related factors** | | | | |
| **Sex** | | | | |
| Male | 14 | 12 | 2 | 1 |
| Female | 13 | 11 | 2 | |
| **Age, y** | | | | |
| Average (range) | 67 (47-90) | 64.8 (53-90) | | 1 |
| ≦70 | 17 | 14 | 3 | |
| > 70 | 10 | 9 | 1 | |
| **Complications at diagnosis** | | | | |
| Absent | 17 | 13 | 4 | 0.264 |
| Present | 10 | 10 | 0 | |
| Diabetes mellitus | 5 | 5 | 0 | 0.561 |
| Hypertension | 7 | 7 | 0 | 0.545 |
| Opportunistic infections | 1 | 1 | 0 | 1 |
| Stroke | 1 | 1 | 0 | 1 |
| Nephropathy | 2 | 2 | 0 | 1 |
| **Complication of skin diseases at diagnosis** | 4 | 3 | 1 | 0.495 |
| Atopic dermatitis | 3 | 2 | 1 | 0.395 |
| Seborrheic dermatitis | 1 | 1 | 0 | 1 |
| **Hematologic factors** | | | | |
| WBC count, /μL | 6700 | 6900 | 6350 | 0.608 |
| median (range) | (4200-13000) | (4200-13000) | (4500-8300) | |
| Total lymphocyte count, /ml median (range) | 1644 (73-3321) | 1644 (73-3321) | 1691.5 (960-2258) | 0.818 |
| **Laboratory factors** | | | | |
| LDH IU/L | 214 | 217 | 120.5 | 1 |
| median (range) | (147-353) | (147-353) | (207-213) | |
| Calcium mg/dL | 9.7 | 9.65 | 9.7 | 0.862 |
| median (range) | (8.7-10.1) | (8.7-10.1) | (9.1-9.8) | |
| serum sIL-2R U/mL | 1150 | 1020 | 1430 | 0.539 |
| median(range) | (292-14100) | (292-14100) | (808-1860) | |
| Low albumin (< LLN) | | | | 1 |
| (-) | 21 | 18 | 3 | |
| (+) | 6 | 5 | 1 | |
| High BUN (> ULN) | | | | 1 |
| (-) | 26 | 22 | 4 | |
| (+) | 1 | 1 | 0 | |
| High LDH (> ULN) | | | | 1 |
| (-) | 15 | 13 | 2 | |
| (+) | 12 | 10 | 2 | |
| Unfavorable factor | | | | |
| (-) | 14 | 12 | 2 | |
| (+) | 13 | 11 | 2 | |
| iATL-PI 1.51 × log10 (serum sIL-2R level, U/mL) | | | | 0.715 |
| low-risk 1 (< 4.62) | 11 | 10 | 1 | |
| Intermediate-risk 2 (4.62 < ≤5.79) | 14 | 11 | 3 | |
| high-risk 3 (> 5.79) | 2 | 2 | 0 | |
| **Skin lesions** | | | | 0.000057* |
| Patch type | 4 | 0 | 4 | |
| Plaque type | 11 | 11 | 0 | |
| Multipapular types | 1 | 1 | 0 | |
| Nodulotumoral type | 10 | 10 | 0 | |
| Erythrodermic type | 1 | 1 | 0 | |
| Purpuric type | 0 | 0 | 0 | |
| **Mutation** | | | | |
| All of the genes: average (+/- SD) | 9.2 ( ± 7.6) | 9.6 ( ± 8.0) | 6.5 ( ± 5.5) | 0.472 |
| Driver genes: average (+/- SD) | 3.2 ( ± 2.5) | 3.3 ( ± 2.6) | 2.5 ( ± 2.5) | 0.781 |
| **Mutation** | | | | |
| All of the genes: median (range) | 6 (0-28) | 8 (0-28) | 7 (1-11) | |
| Driver genes: median (range) | 2 (0-9) | 3 (0-9) | 2 (0-6) | |
| **Acute crisis** | | | | |
| no | 9 | 8 | 1 | 1 |
| yes | 18 | 15 | 3 | |

Star (*) indicates significant correlations in the significance test ($p < 0.001$)

ATLL is classified into 4 clinical categories: smoldering, chronic, acute, and lymphoma. Chronic and smoldering types are called indolent types and have a relatively good prognosis, with a median survival of 31.5 and 55.0 months, respectively. The acute and lymphoma types, called aggressive types, have a very poor prognosis, with a median survival of 8.3 and 10.6 months, respectively. Among patients with chronic ATLL, a factor that satisfies at least one of the three components of low serum albumin level, high LDH, or

high blood urea nitrogen is defined as an unfavorable progressive type [4,5]. The prognosis of patients with unfavorable chronic ATLL is as poor as that of patients with acute lymphoma-type ATLL [6]. Therefore, unfavorable chronic-type ATLL is included in the aggressive-type ATLL and has an adverse clinical course.

To summarize, in the aggressive type, the treatment of leukemia is mainly chemotherapy, bone marrow transplantation, or other molecular targeted therapies, such as the CCR4 antibody. The smoldering type progresses slowly and is carefully monitored by watchful waiting, but patients occasionally present with various clinical courses and survival rates [5]. To provide appropriate treatment based on a risk-adapted strategy, it is important to determine the prognostic factors for smoldering ATLL. A prognostic index for indolent ATLL (iATL-PI) has been previously reported as a prognostic parameter for chronic and smoldering ATLL, with soluble interleukin-2 receptor (sIL-2R) levels as independent prognostic factor [7,8]. In addition, genetic alterations such as IRF4, PD-L1 amplification, and CDKN2A deletion were associated with poor prognosis in patients with indolent-type ATLL [9].

In contrast, skin lesions are found in approximately 50 % of ATLL patients. Therefore, it is important to evaluate the relevance of skin lesions to disease severity and prognosis. In patients with ATLL, except for the lymphoma type, the presence of a skin eruption is considered to be a poor prognostic factor [10,11]. The type of eruption is an independent prognostic factor for ATLL, and erythrodermic, nodulotumoral, multipapular/purpuric, plaque, and patch eruptiones are reported to have a poor prognosis in that order [11]. In the smoldering type with skin lesions, phosphorylated STAT3 expression by immunohistochemistry was significantly associated with overall survival and progression-free survival, but STAT3 mutation in skin lesions was not associated with prognosis [12]. Genomic profiling of skin lesions using array comparative genomic hybridization (array-CGH) showed that a gain of 1p36.33-32 or a loss of 13q33.1-3 was associated with poor prognosis [13]. However, few studies have reported the prognostic correlates of the characteristic genomic profiles of skin lesions in smoldering ATLL cases with skin lesions. Similarly, few reports have identified the prognostic factors associated with skin lesions in smoldering ATLL.

In this study, we aimed to identify the genomic profiles characteristic of smoldering ATLL with skin lesions, clarify the relationship between genomic abnormalities and clinical features of skin lesions, and identify prognostic factors.

## 2. Materials and methods

### 2.1. Patients and tissue samples

We retrospectively collected data from 17 patients and prospectively collected data from 10 patients diagnosed with smoldering-type ATLL with skin lesions between January 2000 and December 2020 at the Department of the University Hospital of Miyazaki, Japan. The patients in this retrospective study had to have provided consent using the opt-out method, as the prospective study included those who had provided their consent in the consent explanation form, consent withdrawal form, and consent form.

ATLL was diagnosed on the basis of the criteria proposed by Shimoyama et al. [14]. In this study, smoldering-type ATLL with skin lesions was defined as (i) positive serum anti-HTLV-1 antibody, (ii) abnormal lymphocytes with convoluted or lobulated nuclei from skin biopsy, and (iii) tumor cells manifesting the mature T-cell phenotype. (iv) monoclonal integration of HTLV-1 proviral DNA into the blood and/or skin tumor cells, (v) a normal lymphocyte level ($< 4\times10^9$/L) (vi) no hypercalcemia (corrected calcium level, < 2.74 mmol/L); (vii) lactate dehydrogenase (LDH) values less than 1.5 times the normal upper limit; (viii) no lymphadenopathy; and (ix) no involvement of extranodal organs including the liver, spleen, central nervous system, bone, and gastrointestinal tract, and neither ascites nor pleural effusion.

This study was approved by the institutional review board of the Faculty of Medicine, University of Miyazaki, in accordance with the Declaration of Helsinki. (No. 2015-181 (O)).

Subsequent methods will be provided as supplementary methods.

## 3. Results

### 3.1. Prognostic analysis of smoldering ATLL dependent on skin eruptions

To investigate the relationship between skin eruptions associated with smoldering ATLL and patient prognosis, we collected 27 patients with smoldering ATLL and skin eruptions (Table 1). All 27 patients met the criteria for smoldering ATLL of the Shimoyama classification. The median age of the 27 ATLL patients collected was 67 years (47-90 years), including 14 males and 13 females. According to the previously reported classification of eruption types associated with ATLL, four patients were patch, 11 were plaque types, one was multipapular or erythrodermic, and ten were nodulotumoral types. The median survival time (MST) could not be calculated for patch, multipapular, and erythrodermic lesions because of the small number of patients; however, it was 60 months for the nodulotumoral type and 29 months for the plaque type (Supplementary Fig. 1**A**). As patch-type ATLL patients showed good survival rates in all types of ATLL [5], we analyzed the patch type as the better (prognosis) group and the other plaque, nodulotumoral, multipapular, and erythordermic types as the poor (prognosis) group (Table 1**,** Supplementary Fig **.1B**). Therefore, four ATLL patients with skin eruptions in the better group and 23 patients in the poor group were included in the study to compare their laboratory data, clinical images, and prognoses. We compared patient-related factors, associated diseases at diagnosis, skin diseases at diagnosis, hematological factors, and laboratory factors between the better and poor groups (Table 1). The results showed no differences in any of the factors between the two groups or in the nodulotumoral type alone, although this factor group was compared to the other groups. The results were not associated with prognosis depending on the type of skin eruption seen in smoldering ATLL patients and could not be identified as a poor or good prognostic factor in this study with a small number of patients.

### 3.2. Deep DNA sequencing of skin specimen from smoldering ATLL patients using panel-based capture sequencing

To clarify the relationship between skin lesions and prognosis in 27 smoldering ATLL patients, we performed targeted sequencing of 280 genes that are repeatedly mutated in hematologic cancers and other types of malignancies [15] using DNA purified from biopsy specimens of the skin lesions. A total of 247 mutations were identified in 108 different genes, and the average number of mutations in ATLL patients was 9.2, and the driver genes identified by Kataoka et al. [16] had an average of 3.2 mutations. Filaggin (filament aggregating protein, FLG) was the most frequently mutated gene, found in seven of 27 cases (26 %), followed by ACAN, CCR4, HSPA6, LRP1B, RPL10, STAT3, TP53, and TBL1XR1 (Fig. 1**,** Supplementary Fig 2**,** Supplementary Table 1). Interestingly, filaggrin gene (*FLG*) expression, particularly in the skin, has been linked to the development of skin barrier and is associated with eczema risk [17]. FLG is a filament-associated protein that binds to keratin fibers in epithelial cells, and the truncation mutation is strongly predisposed to a severe form of dry skin, ichthyosis vulgaris, atopic dermatitis, and eczema [17]. However, mutations in FLG have not been reported in ATLL or lymphoid tumors. Since genomic mutations in the FLG gene in this study were not truncation mutations but amino acid substitutions (Supplementary Fig. 2), even if the FLG gene abnormality is derived from cutaneous origin, the functional abnormality of the FLG protein

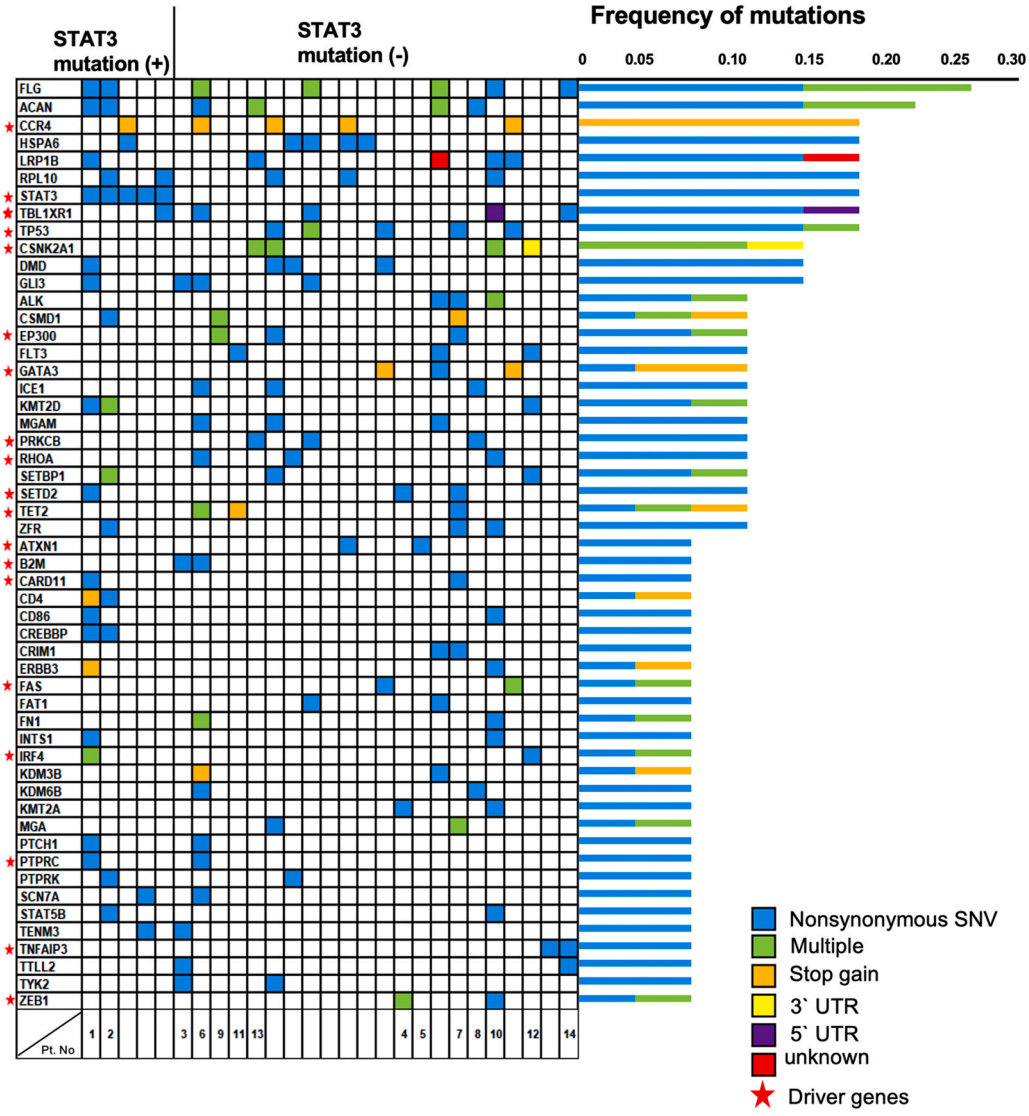


**Fig. 1.** Higher frequencies of somatic mutations in smoldering ATLL with skin eruption.This figure was ordered according to the frequency of genomic variation, starting with the most frequently mutated genes. The listed genes were divided into two groups, one with STAT3 mutation (left side) and the other without STAT3 mutation (right side). The genetic abnormalities found in each case were color-coded to indicate the type of mutation. Asterisks next to these genes indicate driver genes, as reported by Kataoka et al. [9].

based on this genomic abnormality is unknown. STAT3 mutations are more frequent in indolent ATLL than in aggressive ATLL. These mutations were found in almost the same SH2 domain and contained the same type of mutations (G618R and Y640F) at high frequencies [9] (Supplementary Fig. 2). TBL1XR1 mutations have been found to be driver mutations in ATLL and are known to cause extranodal immunoblastic lymphoma as a transcriptional corepressor [18]. Most TBL1XR1 mutations were concentrated within the numerous WD40 domains (Supplementary Fig. 2), suggesting aberrant function due to the inhibition of protein binding, which acts jointly as a corepressor. Protein kinase C beta (PRKCB), a driver mutation in aggressive ATLL, is an activating kinase in the NF-κB signaling pathway, and most mutations are active mutations. Since D630N has been found in many cases of aggressive ATLL [16], D630Y, which is likely to be an active mutation, was also identified in this report.

Of the 88 driver genes identified in ATLL, 52 were included in the 280-gene panel. As a result, 28 genes were identified as candidate driver genes in specimens with smoldering ATLL and skin eruptions. Eleven of the 28 genes belong to the NF-κB/TCR signaling pathway, which is the most important pathway in ATLL. In addition, pathway analysis was performed using the identified driver genes with point mutations in smoldering ATLL patients with skin eruptions (Supplementary Table 2). Among the 23 pathways reported in ATLL [16], nine pathways were identical, including activating signaling in T cells, such as the T cell receptor/NF-κB, Wnt signaling, and chemokine signaling pathways (Supplementary Table 2). Based on these results, the basic activation mechanism of the intracellular signaling pathway is almost identical to that of the entire type of ATLL. In addition, the JAK/STAT, HIF1α, and cAMP signaling pathways, which have been reported to be activated in T-lymphoblastic tumors were included [19–21]. Moreover, several genes are activated by viral infections, including HTLV-1, measles, and hepatitis B (yellow column in Supplementary Table 2), which are not found in all types of ATLL cells [16]. This result suggests that the leukemic cells of smoldering ATLL are more characteristic of HTLV-1-infected T lymphocytes.

### *3.3. Analysis of risk factors for acute crisis of smoldering ATLL with skin eruptions*

Since the association between prognosis dependent on the types of skin lesions and genomic abnormalities is not clear (Supplementary Fig. 1), we examined the association between acute

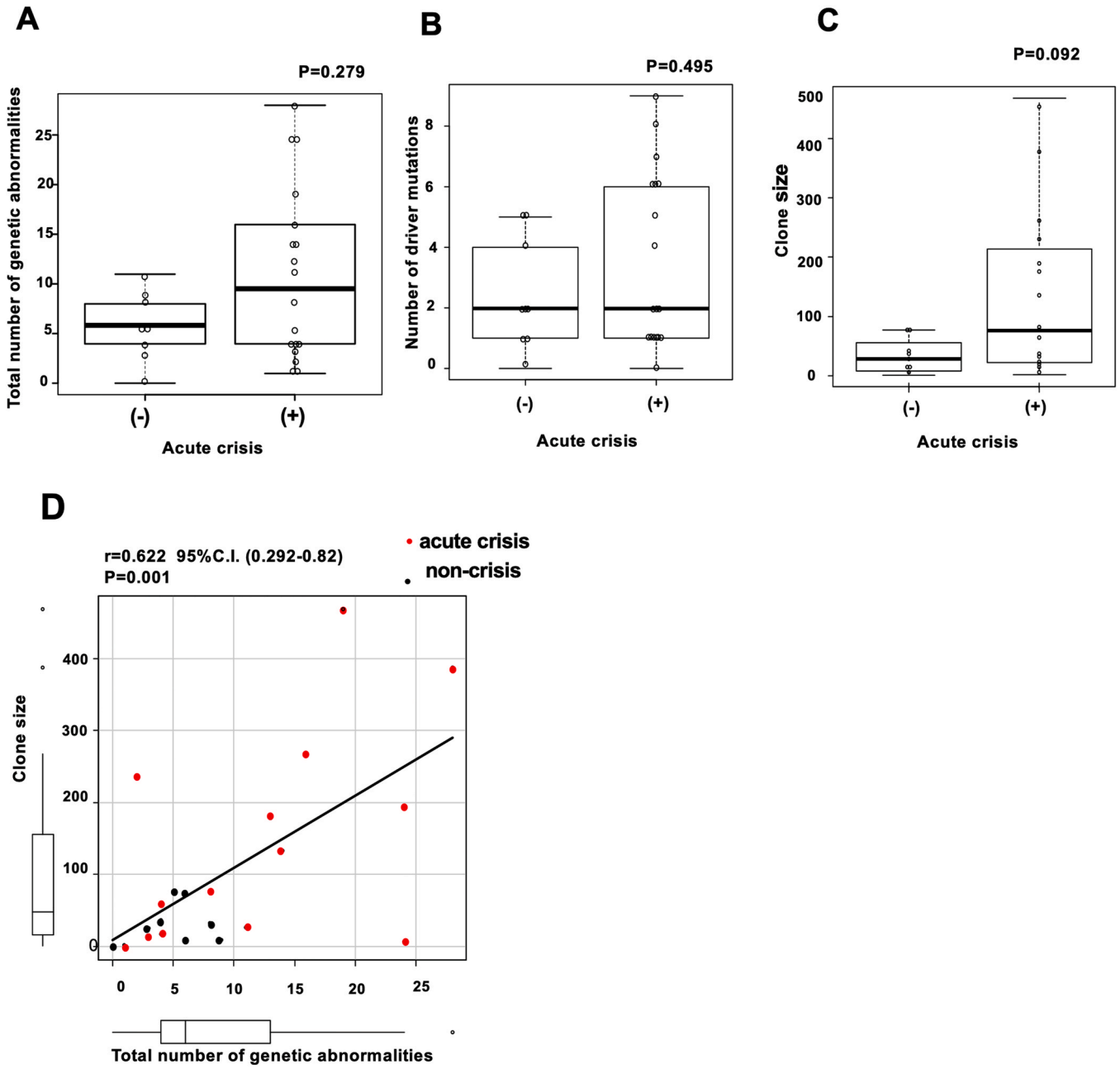


**Fig. 2.** Association of Genomic Abnormalities in Two Different Prognostic Skin Lesion Groups. A–C. The number of genomic mutations (**A**), number of mutations in the driver gene group (**B**), and clone size (**C**) were compared between patient group with acute crisis and without acute crisis. The number of cases with acute transformation is circled in red and those without acute transformation are circled in white. **D.** The correlation between genomic abnormalities and clone size was examined separately for the patients with acute crisis (red) and without acute crisis (black).

crisis and genomic abnormalities in all smoldering ATLL patients with skin eruptions. Comparisons were made for the total number of genomic mutations, number of driver mutations, and clone size with and without acute transformation. For these three items, the predominant difference with acute conversion could not be considered statistically significant (Fig. 2**A–2C**). However, patients with elevated total genomic mutations, driver mutations, and clone size were more likely to be in the acute conversion group. The relationship between clonal size and number of genomic mutations was compared between the acute and non-acute transformation groups. Clone size was strongly correlated with the number of genomic variants (r=0.622). In other words, patients with a larger viral clone size and a greater number of genomic mutations were more likely to develop acute transformation. (Fig. 2**D**).

### *3.4. Univariate and multivariate risk stratification for overall survival (OS) of smoldering ATLL with skin eruptions according to genetic alterations*

Next, we investigated the association between genomic abnormalities and prognosis in smoldering ATLL with all types of skin eruptions. First, among the gene groups in which mutations were found in ATLL with skin lesions, we selected 27 genes with mutation rates of 10 % (three cases) or more. Overall survival (OS) analysis was performed for the 27 genes using univariate analysis (log-rank test). Among the 27 genes, STAT3 and PRKCB showed significant differences ($p < 0.05$) with respect to OS in the present study, and STAT3 was found to be a poor prognostic factor (Fig. 3**A and B**, Supplementary Table 4) however, STAT3 mutation or

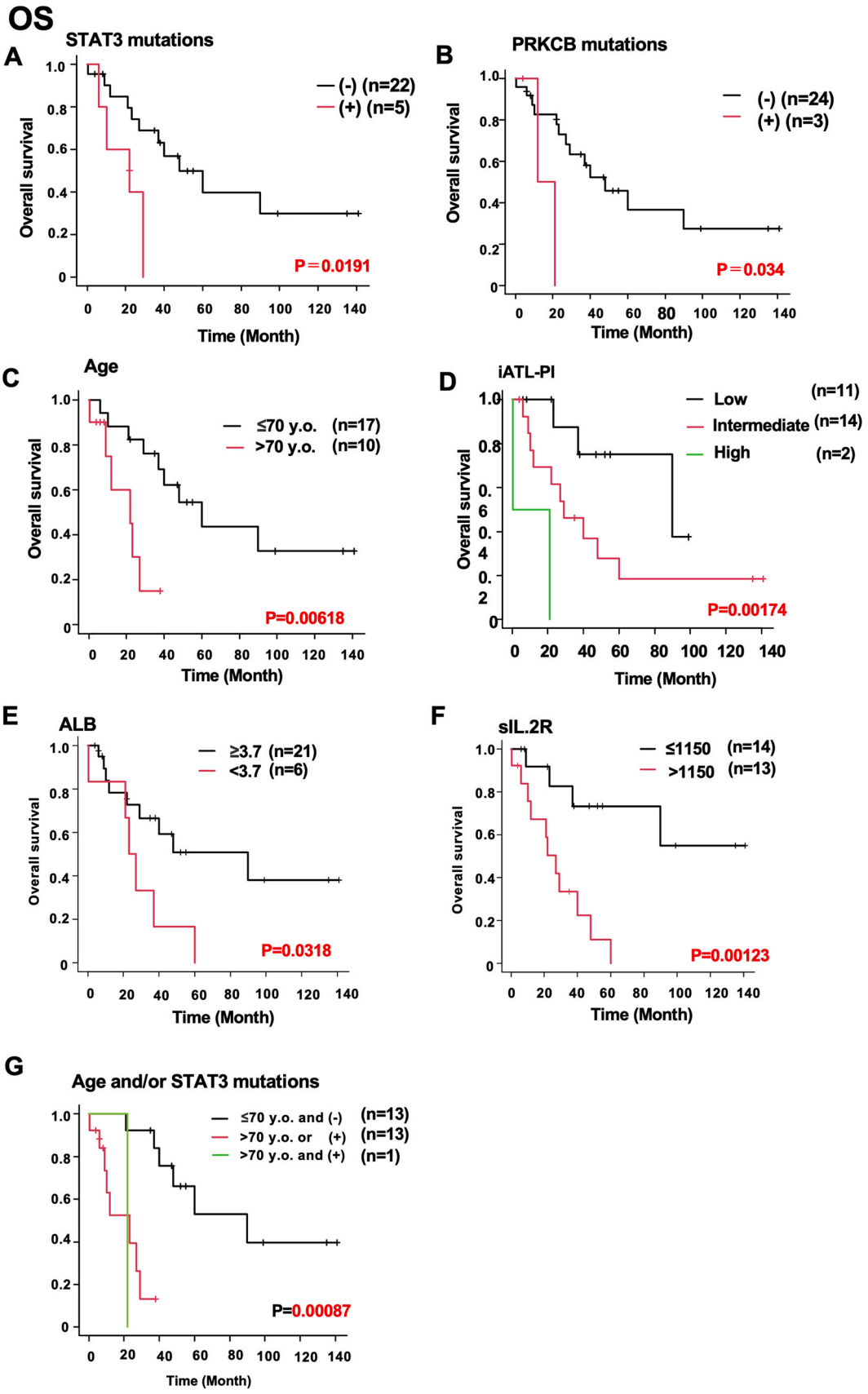


**Fig. 3.** Prognostic impact of the genetic alterations in ATLL patients with skin eruptions by univariate or multivariate analysis. A–F. Univariate analysis showing the prognostic curves for each factor that yielded significant differences in the correlation between genomic variation (A, B) and prognosis as well as in the correlation between prognosis using clinical indicators (C–F). **G.** Prognostic curves are shown for combinations of age > 70 years and/or STAT3 mutations identified using multivariate analysis in patients with ATLL and skin eruptions.

**Table 2**
Multivariate analysis of the risk factors for overall survival (OS) and progression-free survival (PFS) in smoldering type ATLL with skin eruption.

| **Multivariate analysis of the risk factors for OS** | | |
|---|---|---|
| **Factor** | **Hazard ratio** | **p. value** |
| Age > 70 | 6.04 (1.41-25.91) | 0.015* |
| iATL- PI | 2.21 (0.40-12.37) | 0.37 |
| ALB < 3.7 LLN | 3.48 (0.72-16.74) | 0.12 |
| sIL-2R > 1150 | 1.43 (0.19-10.49) | 0.73 |
| PRKCB mutation (+) | 4.91 (0.48-50.61) | 0.18 |
| STAT3 mutation (+) | 9.77 (1.17-81.35) | 0.035** |
| Star (**) in p-values indicate significant correlations (p < 0.05) | | |
| **Multivariate analysis of the risk factors for PFS** | | |
| **Factor** | **Hazard ratio** | **p. value** |
| Age > 70 | 2.72 (0.97-7.64) | 0.057 |
| TBL1XR1 mutation (+) | 3.17 (1.06-9.46) | 0.038** |
| Star (**) in p-values indicate significant correlations (p < 0.05) | | |

phosphorylated STAT3 expression were considered a good prognostic indicator for indolent ATLL [9,12] In contrast, PRKCB mutations were associated with shorter OS in aggressive subtypes but not in indolent types of ATLL in previous reports [9]. Therefore, further multivariate analysis is required to determine whether mutations in PRKCB and STAT3 play a prognostic role in indolent ATLL with skin eruptions.

Finally, a combination of clinical indicators and genomic abnormalities were used to determine patient prognosis. First, we examined the prognostic correlates of each patient's clinical indicators using a univariate analysis (Supplementary Table 5). In addition to PRKCB and STAT3 mutations, we identified the following as poor prognostic factors for overall survival (OS): age > 70 years, high sIL-2R levels, low serum albumin levels, high iATL-PI levels (Fig. 3**C to F,** Supplementary Table 5). Therefore, we performed multivariate analysis using these risk factors, including genomic abnormalities. Age > 70 years and STAT3 mutations were identified as poor prognostic factors with significant differences in OS (Table 2-1). Prognostic analysis was performed by combining age factors and STAT3 mutations:13 patients were under 70 years of age and had no STAT3 mutations, 13 patients were over 70 years of age or had STAT3 mutations, and only one patient had both (Fig. 3**G**). On the other hand, in patients with and without STAT3 mutations, no correlation was found between the mutations and clinical factors, including age (Supplementary Table 6). Moreover, the total numbers of genomic mutations, the numbers of driver mutations, and clonal size were not correlated (Supplementary Fig. 3**A to C**). The association of other genomic variants with STAT3 mutations in patients with STAT3 mutations revealed that CD4 and CREBBP were significantly associated with STAT3 mutations (Supplementary Fig. 3D). CD4 gene is involved in immune responses of helper T cells, and CREBBP (CREB binding protein) is a transcriptional cofactor with histone acetyltransferase activity and the CREBBP are considered as tumor suppressor genes, which somatic mutations are widely observed not only in solid tumors but also in hematological cancers [22]. Accordingly, the STAT3 mutations independently worsen prognosis regardless of age.

### *3.5. Association of phosphorylated STAT3 expression and genomic abnormalities in skin lesions*

It has been reported that the expression of phosphorylated STAT3 in skin lesions associated with leukemic cell infiltration in ATLL patients has a good prognosis. Therefore, we examined the expression of phosphorylated STAT3 by histopathological staining using specimens from smoldering ATLL patients with skin lesions in the present study. We collected remaining formalin-fixed skin tissues, of which 14 cases were histopathologically stainable. Among them, two cases had STAT3 mutations, H147Y mutation and I659L (Supplementary Table 7). The ATLL cells were immunopositive for CD3 in all 14 cases (Supplementary Fig 4**,** Supplementary Table 7). The positive control, HUT102/HTLV-1-infected T cell line, as well as vascular endothelial cells in skin tissue were immunostained for phosphorylated STAT3, but pSTAT3 was negative in CD3+ ATLL cell of all 14 cases, irrespective of the STAT3 mutation (Supplementary Fig 4). The results showed that the two ATLL patients with STAT3 mutations did not express phosphorylated STAT3 and were judged to be at least in the poor prognosis group, as previously reported [12].

### *3.6. Risk stratification of smoldering ATLL with skin eruptions according to genetic alterations for progression free survival (PFS)*

In addition to OS, progression-free survival (PFS) was analyzed in a manner similar to OS. For clinical indicators and genomic abnormalities, univariate analysis was performed for PFS, and age > 70 years and mutations in the transducin (beta)-like 1X-linked receptor 1 gene (TBL1XR1) were selected (Fig. 4**,** Supplementary Table 5 and 8). Multivariate analysis of age over 70 years and TBL1XR1 mutation showed significant differences only for TBL1XR1 mutation (Table 2-2**)**. On the other hand, the association between mutations and clinical factors was examined in patients with and without TBL1XR1 mutations. The results showed a correlation between blood LDH

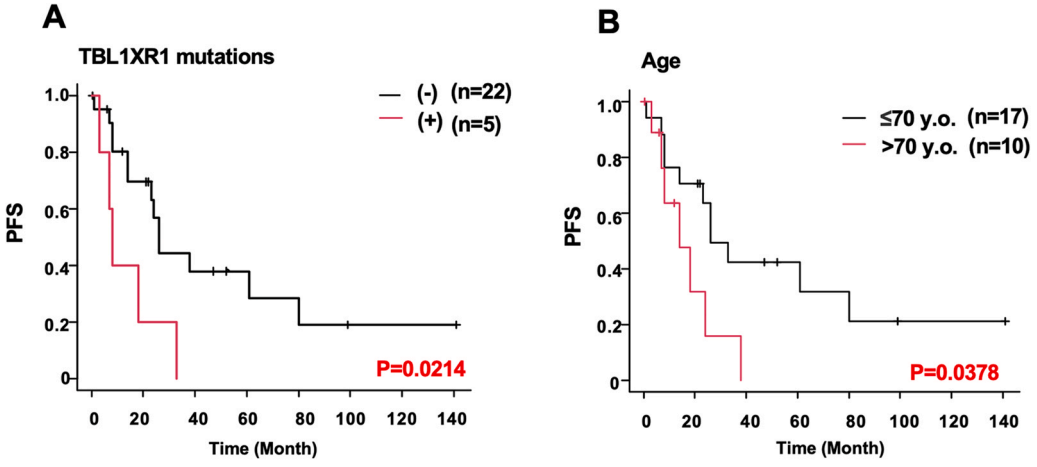


**Fig. 4.** Survival curves showing progression-free survival (PFS) for the two factors identified by univariate analysis. A. PFS with and without TBL1XR1 mutation are shown. **B**. PFS according to age above 70 years and below 70 years are shown.

levels below normal and the TBL1XR1 mutation, but no correlation between the mutation and other factors (Supplementary Table 9). Since mutations in TBL1XR1 are reported as loss-of-function or activation of the β-catenin signaling pathway [23], further functional analysis is needed.

## 4. Discussion

In this study, through genomic analysis of smoldering ATLL patients with skin lesions, we examined the correlation between patient prognosis with respect to skin lesions and genomic abnormalities. Although it has been previously reported that the shape of the skin lesion is a prognostic factor for all types of ATLL, there was no correlation between prognosis and the type of skin eruption in a study limited to smoldering-type ATLL. A genome-wide study of genomic alterations and other clinical factors in smoldering ATLL patients with skin lesions identified age > 70 years and STAT3 mutations as the predominant aggravating factors in multivariate analysis of overall survival (OS). Furthermore, TBL1XR1 mutations were the only aggravating factor in progression-free survival (PFS).

In the present analysis, STAT3 mutation was identified as a poor prognostic factor for smoldering ATLL with skin lesions using univariate and multivariate analyses. In a study by Kataoka et al. [9], STAT3 mutation was identified as a favorable prognostic factor when more than the entire ATLL genome was analyzed; however, this may be due to the high frequency of mutations in smoldering ATLL. In contrast, one of the manuscripts examined STAT3 mutations and phosphorylated STAT3 expression in 116 ATLL [12]. The results showed that STAT3 mutations did not correlate with prognosis, and that phosphorylated STAT3 expression was identified as a favorable prognostic factor for smoldering ATLL. However, in LGL, NK-CLL, and anaplastic large cell lymphoma (ALCL), STAT3 mutations activate the JAK/STAT signaling pathway as a gain of function, all of which have been shown to be involved in tumor growth as oncogenes with activated phosphorylated STAT3 [19–21]. The Y640F mutation is a major STAT3 mutation in ALCL and ATLL, and in vitro and in vivo experiments have demonstrated that STAT3 with the Y640F mutation acts as an oncogene in ALCL [21]. In this study, we also examined skin lesion sections from two cases with STAT3 mutations and 12 cases without mutations for phosphorylated STAT3 by immunohistochemistry and found no expression of phosphorylated STAT3 in CD3 positive ATLL cells in all 14 cases. Two STAT3 mutations, H147Y and I659L, are mutants that have not been functionally analyzed in other publications. However, the mean survival time of the two patients with this mutation was 17.5 months, compared to 44 months for the 12 patients without the mutation. Although not significantly different (p=0.058), the two patients with the STAT3 mutation had shorter survival and poorer prognosis (Supplementary Table 7). On the other hand, two models have been proposed in previous paper [12]: a pSTAT3 expression-dependent model of progression to acute ATLL and a pSTAT3-independent model of progression to lymphomatous ATLL. Although the present results show only a trend due to the small number of cases, it was inferred that patients with STAT3 mutation and pSTAT3 non-expression have a worse prognosis. Since the results for ATLL were different in each study, further analysis of many cases and a detailed investigation of the relationship between genomic mutations and STAT3 phosphorylation are needed. In addition, individual STAT3 mutations should be introduced into HTLV-1-infected cell lines and the properties of these cells should be examined.

This study examined the relationship between the type of skin eruption associated with smoldering ATLL and its prognosis, based on genomic abnormalities. Unfortunately, owing to the small number of patient samples in this study, the previously reported prognostic correlation between the type of skin eruption and the prognosis of patients with ATLL could not be obtained. However, the relationship between smoldering ATLL with skin lesions as a whole and genomic abnormalities identified age > 70 years and STAT3 mutations as factors associated with a worse prognosis. In the patch type, which is considered to have good prognosis, neither STAT3 nor PRKCB mutations, which were identified as prognostic factors in this study, were identified. Therefore, although it is necessary to examine a larger number of specimens, we believe that there is a trend towards this finding. Determining the prognosis of many patients with smoldering ATLL using genomic analysis of skin eruptions is likely to lead to treatment selection in the future. We would like to increase the number of cases in order to continue our study.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jdermsci.2023.02.001.

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