An investigation of germline variants of *HAVCR2* in subcutaneous panniculitis-like T-cell lymphoma and related lesions in a North American population

Subcutaneous panniculitis-like T-cell lymphoma (SPTCL) is a clonal expansion of αβ cytotoxic T cells involving subcutaneous adipose tissue, first described by Gonzalez *et al.* in 1991.[1] Patients present with subcutaneous nodules, systemic symptoms, and up to 20% of cases are associated with an autoimmune condition.[2] SPTCL patients often respond to immunomodulator therapy.[3] The pathological and clinical features of SPTCL may show overlapping histological features with lobular panniculitis (LP). Features favoring LP include lack of prominent rimming of adipocytes, admixed B lymphocytes, plasma cells, plasmacytoid dendritic cells, and the absence of clonal T-cell gene rearrangement (TCR). Nonetheless, there are a significant number of cases that on morphologic grounds alone can be very difficult for even expert hematopathologists to definitively classify. These findings have led to the designation of these borderline cases as "atypical lobular lymphocytic panniculitis" or atypical lobular panniculitis (ALP).[4-6]

Hepatitis A Virus Cellular Receptor 2 (*HAVCR2/TIM3*) encodes a transmembrane protein expressed by T cells and natural killer cells that acts as a negative immune checkpoint inhibitor.[7,8] Recently, the presence of germline inactivating / hypomorphic variants of *HAVCR2* (p.Y82C, p.I97M and p.T101I) have been linked to SPTCL primarily in patients of Asian ethnicity.[7,9-12] Patients with these variants have a higher risk of developing HLH. Most published studies are from Asian countries, with one large study from France.[7,9-12] To date, there have been no studies evaluating *HAVCR2* germline variants from North America, and mutational studies of related lesions have not been reported. We investigated the incidence of *HAVCR2* germline variants in a mixed-ethnicity North American cohort of patients with SPTCL and other pathological conditions manifesting morphologically as LP, and correlated their presence with available clinical, pathological, and molecular features.

A total of 35 cases of SPTCL, 10 cases of LP, 7 cases of ALP, and 17 cases of primary cutaneous γ / δ T-cell lymphoma (PCGD-TCL) were retrieved from the consultation files of the Hematopathology Section of the National Cancer Institute, National Institutes of Health, Bethesda, MD, USA, between 2001 and 2020. (See Table 1 for case characteristics.) All cases were reviewed by 3 hematopathologists (ESJ, SP, JC) who provided a consensus diagnosis. The diagnostic features that distinguish SPTCL from classical LP are summarized in *Online Supplementary Table S1*. ALP was diagnosed when a case did not fit neatly into either group by these criteria. Available clinical information was obtained from referring physicians. This study was approved by the Institutional Review Board of the National Cancer Institute.

HAVCR2 p.Y82C, p.I97M, and p.T101I mutational analyses were performed using custom designed PrimePCR ddPCR Assays (BIO-RAD, Hercules, CA, USA). We identified *HAVCR2* variants in 9/35 SPTCL cases (26%), 3/7 ALP cases (44%), 0/10 LP cases, and in 0/17 PCGD-TCL cases (0%). Among the 9 positive SPTCL cases, 5 were homozygous for the p.Y82C mutation. Two additional cases were heterozygous for p.Y82C, including one with a co-existing p.T101I heterozygous mutation. The remaining 2 patients were heterozygous for a p.I97M mutation. There were no significant differences between the histological features of *HAVCR2* variant or wild-type SPTCL. Figure 1 illustrates one of the SPTCL cases with a heterozygous p.I97M variant, showing an atypical lobular panniculitic-like infiltrate characterized by adipocyte rimming by atypical T cells. None of the 9 SPTCL patients with *HAVCR2* variants had a history of autoimmune disease; one patient had low level serum ANA autoantibody at the time of diagnosis. Three out of 9 variant positive patients presented with hemophagocytic lymphohistiocytosis (HLH), while 0/27 wild-type patients presented with HLH. Recurrent cutaneous lesions were noted in both *HAVCR2* variant patients (3/9, 33%) and *HAVCR2* wild-type patients (6/26, 23%). Clonal TCRγ rearrangements were detected in 34/35 cases of SPTCL. Consistent with a recent report,[13] we did not detect *HAVCR2* variants in any of 17 cases of PCGD-TCL.

We did not detect *HAVCR2* variants in any of 10 cases of LP, but did identify *HAVCR2* variants in 3 cases diagnosed as ALP. The first patient was a 47-year-old female of Southeast Asian origin homozygous for the p.Y82C variant (Figure 2). This patient had no significant past medical history and presented with multiple subcutaneous nodules with intermittent fever, leukopenia, and positive anti-neutrophil autoantibody (titer 1:80). The patient responded to immunomodulator therapy but relapsed. The biopsies before and after treatment showed similar morphology including focal adipocyte rimming by T lymphocytes, admixed plasma cell and plasmacytoid dendritic cell infiltrate, and areas of fat necrosis (Figure 2). Oligoclonal TCRγ was reported in 2/3 biopsies and a polyclonal pattern was seen in a third (*data not shown*). The presence of admixed reactive cells, minimal cytological atypia, and clonality studies favored

Table 1. Summary of patients' clinical and pathological characteristics.

Characteristic, N (%)	SPTCL *HAVCR2* WT N=26	SPTCL *HAVCR2* VAR N=9	Lobular panniculitis N=10	Atypical lobular panniculitis N=7
Female/male	16/10	5/4	8/2	6/1
Ethnicity	7 AA, 3 C, 3 H, 1 AI, 13 U	5 AAPI, 1 C, 1 AA/C mix, 2 U	10 ND	1 C, 1 Asian, 5 ND
Median age in years (range)	39.5 (2-92)	27 (16-55)	46 (2-70)	54 (13-68)
Single nodule	7 (27)	3 (33)	5 (50)	3 (33)
Multiple nodules	19 (73)	6 (67)	5 (50)	4 (67)
Upper/lower extremity	19 (72)	4/8 (50)	9 (90)	4 (67)
Trunk/head/neck	10 (38)	4/8 (50)	5 (50)	5 (71)
Fever	7 (27)	8 (89)	0 (0)	2 (29)
HLH, clinical	0 (0)	3 (33)	0 (0)	0 (0)
Autoimmune disease, clinical/laboratory	6 (23)	1 (11)	0 (0)	2 (29)
Clinical recurrence	6 (23)	3 (33)	1 (10)	2 (29)
TCR monoclonality	24/25 (96)	3/7 (43)	2/8 (25)	1 (14)
HAVCR2 variant p.Y82C hom p.Y82C het p.Y82C het/T101I het p.T101I hom p.I97M hom p.I97M het	NA - - - - - -	9/35* (26) 5 1 1 0 0 2	0 (0) 0 0 0 0 0 0	3 (43) 1 0 0 1 1 0
Cases with somatic mutations, TSO500 gene panel	1/11	2/5	ND	0/5

*Total subcutaneous panniculitis-like T-cell lymphoma (SPTCL) patients. HLH: hemophagocytic lymphohistiocytosis; AA: African American; C: Caucasian; H: Hispanic; AI: American Indian; AAPI: Asian American and Pacific Islander; U: Unknown; hom: homozygous; het: heterozygous; N: number; NA: not applicable; ND: not determined; WT: wild-type; VAR: variant.

a diagnosis of ALP.

The second patient was homozygous for the p.T101I variant (*Online Supplementary Figure S1A-F*). This 61-year-old Caucasian female had a history of dermatomyositis and presented with new onset of subcutaneous nodules, weight loss, and fever. The patient initially responded to immunomodulator therapy but relapsed. Histological sections of initial and relapse biopsies showed a panniculitic pattern with a mixed infiltrate of lymphocytes, plasma cells and histiocytes. There was focal rimming of the adipocytes by $CD8^+$ T cells with karyorrhexis. TCRγ was clonal in one biopsy (*Online Supplementary Figure S1F*), but polyclonal in 2 subsequent lesions. The subsequent biopsies at one and three years did not show features of SPTCL and were polyclonal for TCRγ. Although the clinical presentation and clonal TCR were of concern for SPTCL, the absence of a reproducible clone favored ALP. The availability of several biopsies in this case, over a period of three years was essential in the final interpretation of this case.

The third patient was homozygous for the p.I97M variant (*Online Supplementary Figure S1G-L*). This patient was a 56-year-old female of unknown ethnicity with no past medical history, who presented with pink patches on the right buttock that grew rapidly in size. There were no systemic symptoms. Histological sections of the biopsies showed lobular panniculitis with a mixed infiltrate composed of lymphocytes and many plasma cells. There was abundant nuclear karyorrhexis and focal rimming of the adipocytes by mildly atypical $CD8^+$ T cells, which was of concern for SPTCL. TCRγ was polyclonal. The overall findings favored ALP.

Next Generation Sequencing (NGS) using the Illumina TSO500 panel was performed on 16 SPTCL with available material (11 *HAVCR2* wild-type, 5 *HAVCR2* p.Y82C mutant) and 5 ALP (4 *HAVCR2* wild-type, 1 with *HAVCR2* p.T101I homozygous mutant). Only 3/16 SPTCL cases were found to have somatic mutations (*Online Supplementary Table S2*). One case, a *HAVCR2* p.I97M mutant case, had 2 somatic mutations of uncertain significance (VUS), *CREBBP* p.V1802M (VAF = 13%) and *BCORL1* p.P1416L (VAF = 10%),

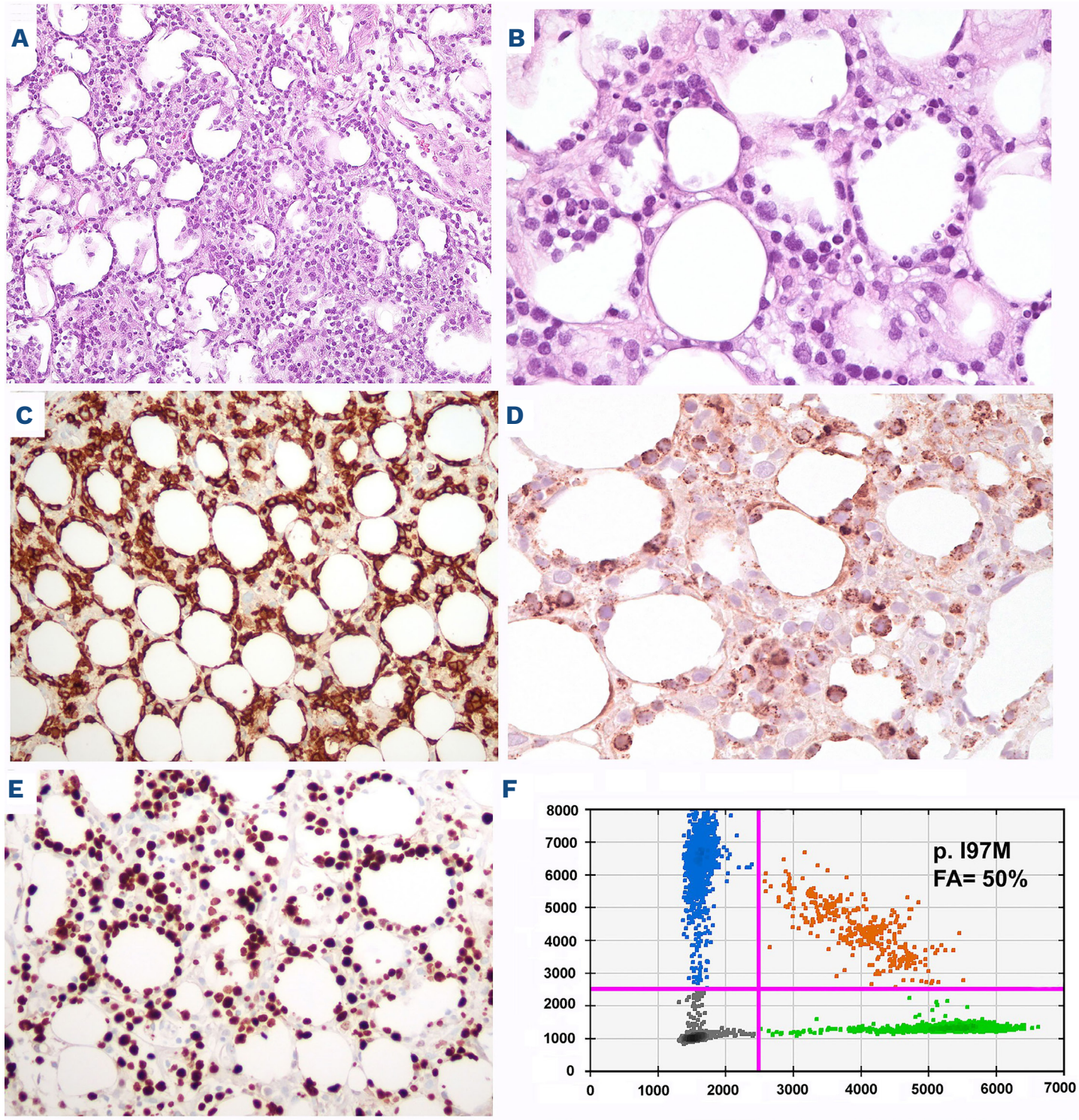

Figure 1. Representative case of subcutaneous panniculitis-like T-cell lymphoma with *HAVCR2* germline variant. (A) The subcutaneous tissue shows a prominent infiltrate of atypical lymphocytes that extend into the interstitial space beyond the individual fat cells (H&E, 100X). (B) The atypical cells have enlarged nuclei with presence of apoptotic debris (H&E, 400X). (C) A stain for CD8 highlights prominent rimming by the atypical lymphoid cells (IHC; 400X). (D) The atypical cells show strong staining for Granzyme B (IHC, 400X). (E) KI-67 shows a high proliferation rate in the atypical cells, which rim the fat spaces (IHC, 400X). (F) ddPCR detected *HAVCR2* p.I97M heterozygous germline variant. T-cell gene rearrangement studies were clonal (not shown). FA: fractional abundance; H&E: Hematoxylin&Eosin staining; IHC: immunohistochemistry.

while the second, also a *HAVCR2* p.I97M mutant case, had a somatic *KMT2D* p.L804del (VAF = 9%) VUS. The third case was *HAVCR2* wild-type and had a likely pathogenic *TET2* p.C1135fs*7 (VAF = 2.7%) variant. In all 3 cases, the VAF were roughly in agreement with the estimated tumor percentages, consistent with somatic clonal expansion of SPTCL tumor cells. However, in the third case, we cannot exclude the possibility that the *TET2* mutation was associated with clonal hematopoiesis.

In summary, our study is the first report from a single North American institution to assess the presence of *HAVCR2* variants in patients with SPTCL and additional pathological diagnoses with panniculitic features. Perhaps not unexpectedly, none of the PCGD-TCL cases with panniculitic features were found to have *HAVCR2* mutations.[13] Consistent with prior reports,[7,9-12] a subset of our SPTCL patients were found to harbor *HAVCR2* germline variants, mostly biallelic, as previously reported in patients of South Asian, East Asian, and Polynesian origin. With 2 exceptions, African American, Hispanic, and Caucasian patients with SPTCL did not carry any of the 3 previously reported inactivating / hypomorphic germline variants. The 2 exceptional cases included a mixed ethnicity patient of African American and Caucasian descent who

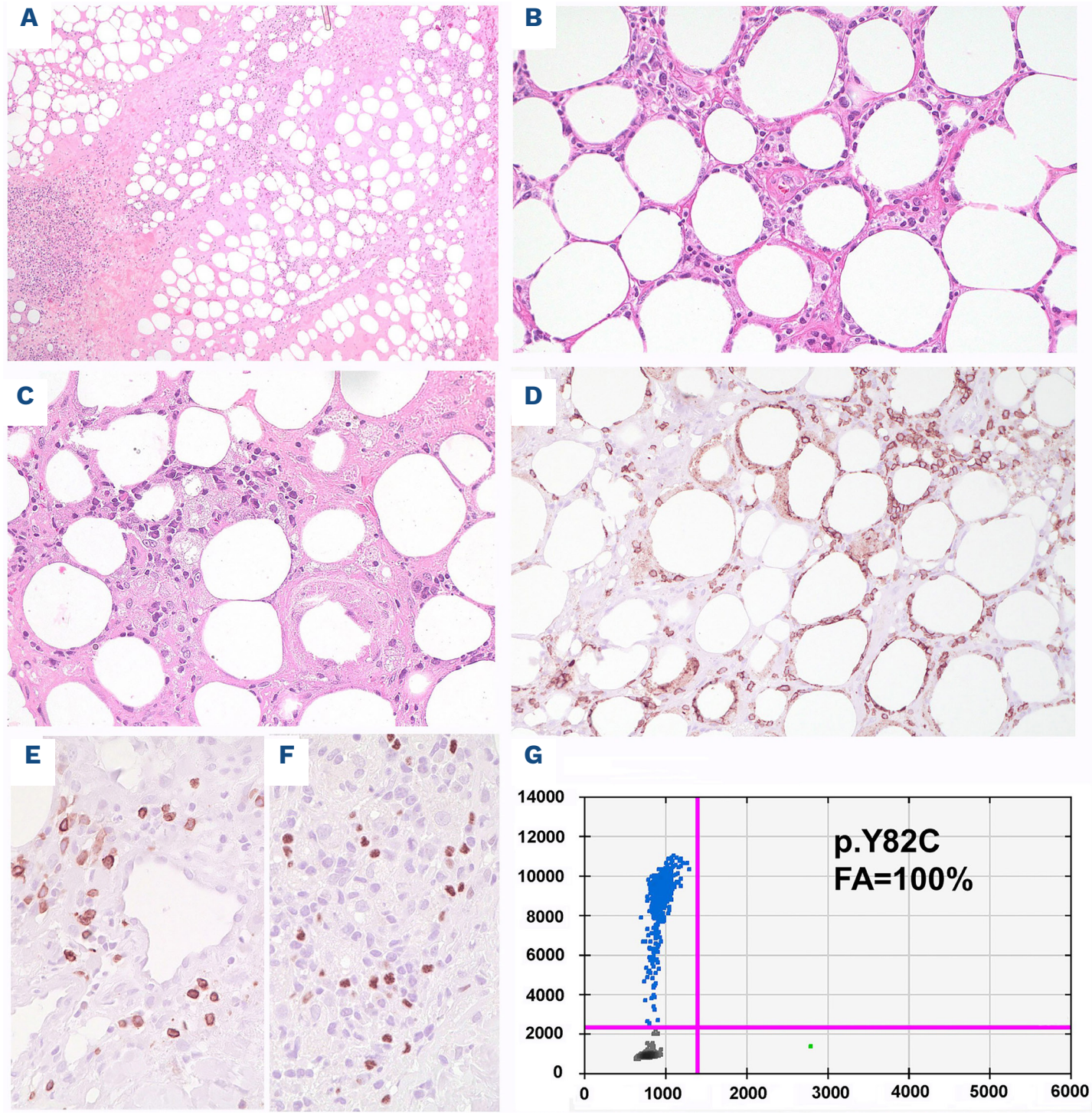

Figure 2. Representative case of atypical lobular panniculitis with *HAVCR2* germline variants. (A) Biopsy obtained from a 47-year-old female (Case 1) with atypical lobular panniculitis (ALP) shows a patchy lymphoplasmacytic infiltrate involving the adipose tissue (H&E, 20X). There is fibrinoid change with relatively sparse cellularity. (B) Focal areas show more numerous lymphoid cells with focal rimming of fat spaces (H&E, 200X). (C) Focal clusters of plasma cells are present (H&E, 400X). (D) A stain for CD8 highlights T cells rimming fat spaces (IHC, 400X). (E) Plasma cells are stained by CD79a (IHC, 400X). (F) TCF-4 stains plasmacytoid cells, favoring a diagnosis of ALP (IHC, 400X). (G) ddPCR detected homozygous *HAVCR2* p.Y82C germline variant. T-cell gene rearrangements were oligoclonal (not shown). FA: fractional abundance; H&E: Hematoxylin&Eosin staining; IHC: immunohistochemistry.

carried a p.I97M germline variant, and a Caucasian patient who carried a p.I97M germline variant. Similarly, a recent study of 37 European patients with SPTCL showed a small number of European patients harboring the p.I97M mutation.[10] The fact that the more commonly reported p.Y82C variants were not found in the non-Asian populations in our study, nor in the majority of the European patients studied, is in keeping with the much lower prevalence of this germline variant in non-Asian populations. Moreover, our data suggest that the somatic mutational burden of SPTCL, regardless of *HAVCR2* germline status, is extremely low, consistent with other studies that have reported rare mutations in genes involved in epigenetic modification and/or signaling pathways.[11,14]

An unexpected finding was the identification of 3 cases of "atypical lobular panniculitis" with homozygous *HAVCR2* germline variants. Long-term clinical follow-up could not be obtained in these cases. Nonetheless, these cases suggest that a fraction of cases that are diagnosed as ALP may have predisposing *HAVCR2* variants, and raises the possibility that these cases may be a *forme fruste* of SPTCL, which has also been suggested in prior studies.[4-6] In borderline cases, identification of germline mutations in *HAVCR2* may have clinical relevance, and this suggests

the need for long-term follow-up in these patients. Finally, NGS testing for secondary somatic mutations may be useful in evaluating these patients.

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Contributions

JC planned and performed the work, analyzed data, and wrote the manuscript. LX and JK performed the work, analyzed data, and reviewed the manuscript. YY performed the work and reviewed the manuscript. H-WW and SP analyzed data and reviewed the manuscript. EJ analyzed data, provided the photomicrographs, and reviewed the manuscript. MR planned the work, analyzed data, and wrote the manuscript.

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Data-sharing statement

All data are available on request to the corresponding author.

References

1. Gonzalez CL, Medeiros LJ, Braziel RM, Jaffe ES. T-cell lymphoma involving subcutaneous tissue. A clinicopathologic entity commonly associated with hemophagocytic syndrome. Am J Surg Pathol. 1991;15(1):17-27.
2. Huppmann AR, Xi L, Raffeld M, Pittaluga S, Jaffe ES. Subcutaneous panniculitis-like T-cell lymphoma in the pediatric age group: a lymphoma of low malignant potential. Pediatr Blood Cancer. 2013;60(7):1165-1170.
3. Swerdlow SH, Campo E, Harris NL, et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, revised 4th ed. Vol 2. Lyon (France). IARC/WHO Press; 2017.
4. Bosisio F, Boi S, Caputo V, et al. Lobular panniculitic infiltrates with overlapping histopathologic features of lupus panniculitis (lupus profundus) and subcutaneous T-cell lymphoma: a conceptual and practical dilemma. Am J Surg Pathol. 2015;39(2):206-211.
5. Magro CM, Schaefer JT, Morrison C, Porcu P. Atypical lymphocytic lobular panniculitis: a clonal subcutaneous T-cell dyscrasia. J Cutan Pathol. 2008;35(10):947-954.
6. LeBlanc RE, Tavallaee M, Kim YH, Kim J. Useful parameters for distinguishing subcutaneous panniculitis-like T-cell lymphoma from lupus erythematosus panniculitis. Am J Surg Pathol. 2016;40(6):745-754.
7. Gayden T, Sepulveda FE, Khuong-Quang DA, et al. Germline HAVCR2 mutations altering TIM-3 characterize subcutaneous panniculitis-like T cell lymphomas with hemophagocytic lymphohistiocytic syndrome. Nat Genet. 2018;50(12):1650-1657.
8. Sabatos CA, Chakravarti S, Cha E, et al. Interaction of Tim-3 and Tim-3 ligand regulates T helper type 1 responses and induction of peripheral tolerance. Nat Immunol. 2003;4(11):1102-1110.
9. Polprasert C, Takeuchi Y, Kakiuchi N, et al. Frequent germline mutations of HAVCR2 in sporadic subcutaneous panniculitis-like T-cell lymphoma. Blood Adv. 2019;3(4):588-595.
10. Sonigo G, Battistella M, Beylot-Barry M, et al. HAVCR2 mutations are associated with severe hemophagocytic syndrome in subcutaneous panniculitis-like T-cell lymphoma. Blood. 2020;135(13):1058-1061.
11. Koh J, Jang I, Mun S, et al. Genetic profiles of subcutaneous panniculitis-like T-cell lymphoma and clinicopathological impact of HAVCR2 mutations. Blood Adv. 2021;5(20):3919-3930.
12. Moonla C, Polprasert C, Komvilaisak P, et al. Germline HAVCR2 mutations and their relation to the clinical spectrum of subcutaneous panniculitis-like T-cell lymphoma and hemophagocytic lymphohistiocytosis: results from a multicenter study and meta-analysis. Haematologica. 2023;108(10):2743-2752.
13. Daniels J, Doukas PG, Escala MEM, et al. Cellular origins and genetic landscape of cutaneous gamma delta T cell lymphomas. Nat Commun. 2020;11(1):1806.
14. Li Z, Lu L, Zhou Z, et al. Recurrent mutations in epigenetic modifiers and the PI3K/AKT/mTOR pathway in subcutaneous panniculitis-like T-cell lymphoma. Br J Haematol. 2018;181(3):406-410.