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

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Yoon Yang: performed the work, reviewed the manuscript

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Elaine Jaffe: analyzed data, provided photomicrographs, and reviewed the manuscript

Mark Raffeld: planned the work, analyzed data and wrote the manuscript

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Running title: *HAVCR2* variants in SPTCL and LP

Key words: SPTCL, LP, PCGD-TCL, ALP, *HAVCR2*

Subcutaneous panniculitis-like T-cell lymphoma (SPTCL) is a clonal expansion of $\alpha\beta$ 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 are 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 gamma/delta 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 between 2001 and 2020. All cases were reviewed by three hematopathologists (ESJ, SP, JC) and a consensus diagnosis rendered. The diagnostic features that distinguish SPTCL from classical LP are summarized in Supplemental Table S1. ALP was diagnosed when a case did not fit cleanly 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). 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 coexisting p.T101I heterozygous mutation. The remaining two patients were heterozygous for 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. 3/9 variant positive patients presented with hemophagocytic lymphohistiocytosis (HLH), while 0/27 wildtype 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 TCR gamma (TCR γ) 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 three 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 cells and plasmacytoid dendritic cells infiltrate, and areas of fat necrosis (Figure 2). Oligoclonal TCR γ was reported in two of three biopsies and a polyclonal pattern was seen in a third (not shown). The presence of admixed reactive cells, minimal cytological atypia, and clonality studies favored a diagnosis of ALP.

The second patient was homozygous for the p.T101I variant (Supplemental Figure S1, A-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. TCR γ was clonal in one biopsy (Supplemental Figure S1, F), but polyclonal in two subsequent lesions. The subsequent biopsies at one and three years did not show features of SPTCL and were polyclonal for TCR γ . Although the clinical presentation and clonal TCR were concerning for SPTCL, the absence of a reproducible clone favored ALP. The availability of several biopsies in this case, over a period of 3 years was essential in the final interpretation of this case.

The third patient was homozygous for the p.I97M variant (Supplemental Figure S1, G-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 concerning for SPTCL. TCR γ 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* wildtype, 5 *HAVCR2* p.Y82C mutant) and 5 ALP (4 *HAVCR2* wildtype, 1 with *HAVCR2* p.T101I homozygous mutant). Only 3/16 SPTCL cases were found to have somatic mutations (Supplemental Table S2). One case, a *HAVCR2* p.I97M mutant case, had two somatic mutations of uncertain significance (VUS), *CREBBP* p.V1802M (VAF = 13%) and *BCORL1* p.P1416L (VAF = 10%), while the second, also a *HAVCR2* p.I97M mutant case, had a somatic *KMT2D* p.L804del (VAF = 9%) VUS. The third case was *HAVCR2* wildtype and had a likely pathogenic *TET2* p.C1135fs*7 (VAF = 2.7%) variant. In all three cases, the VAFs 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 two exceptions, African American, Hispanic, and Caucasian patients with SPTCL did not carry any of the three previously reported inactivating/hypomorphic germline variants. The two exceptional cases included a mixed ethnicity patient of African American and Caucasian descent who 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 three 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 raise 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 suggests the need for long term follow-up in these patients. NGS testing for secondary somatic mutations may be useful in evaluating these patients.

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Table 1. Summary of patients' clinical and pathological characteristics

	SPTCL <i>HAVCR2</i> WT n = 26	SPTCL <i>HAVCR2</i> 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, y (range)	39.5 (2-92)	27 (16-55)	46 (2-70)	54 (13-68)
Single nodule	7/26 (27%)	3/9 (33%)	5/10 (50%)	3/7 (33%)
Multiple nodules	19/26 (73%)	6/9 (67%)	5/10 (50%)	4/7 (67%)
Upper/lower extremity	19/26 (72%)	4/8 (50%)	9/10 (90%)	4/7 (67%)
Trunk/Head/neck	10/26 (38%)	4/8 (50%)	5/10 (50%)	5/7 (71%)
Fever	7/26 (27%)	8/9 (89%)	0/10 (0%)	2/7 (29%)
HLH (clinical)	0/26 (0%)	3/9 (33%)	0/10 (0%)	0/7 (0%)
Autoimmune disease (Clinical/ laboratory)	6/26 (23%)	1/9 (11%)	0/10 (0%)	2/7 (29%)
Clinical recurrence	6/26 (23%)	3/9 (33%)	1/10 (10%)	2/7 (29%)
TCR monoclonality	24/25 (96%)	3/7 (43%)	2/8 (25%)	1/7 (14%)
<i>HAVCR2</i> variant	NA	9/35 (26%)	0/10 (0%)	3/7 (43%)
p.Y82C hom	-	5	0	1
p.Y82C het	-	1	0	0
p.Y82C het/T101I het	-	1	0	0
p.T101I hom	-	0	0	1
p.I97M hom	-	0	0	1
p.I97M het	-	2	0	0
Cases with somatic mutations (TSO500 gene panel)	1/11	2/5	ND	0/5

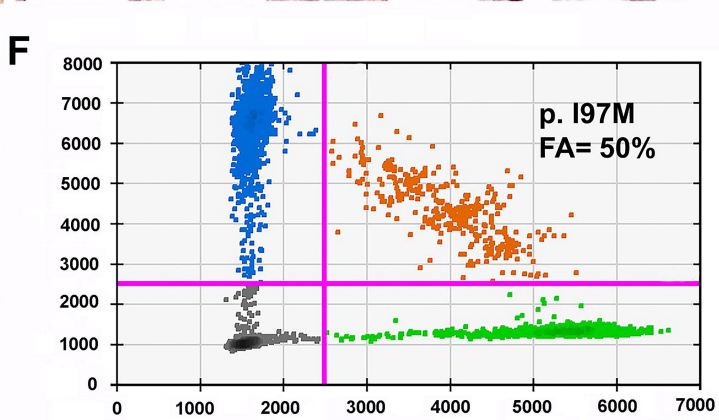
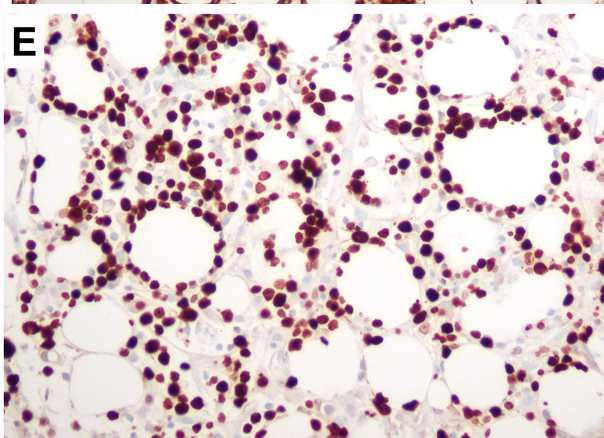
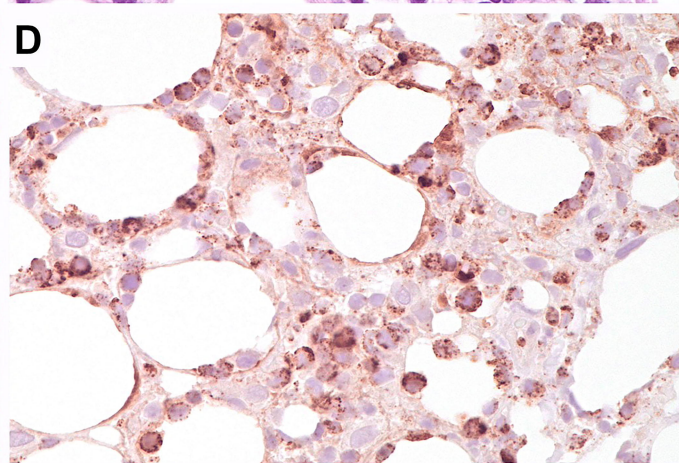
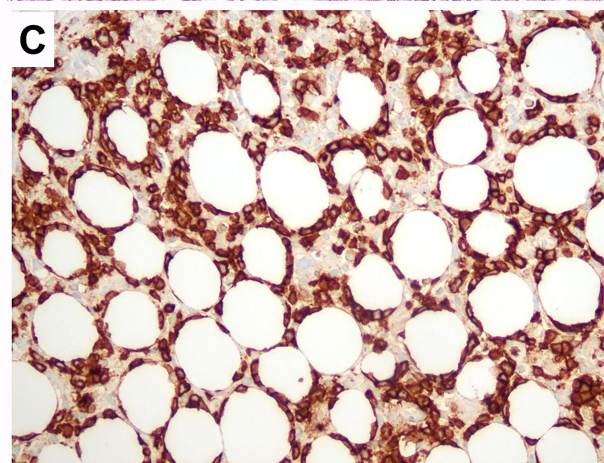
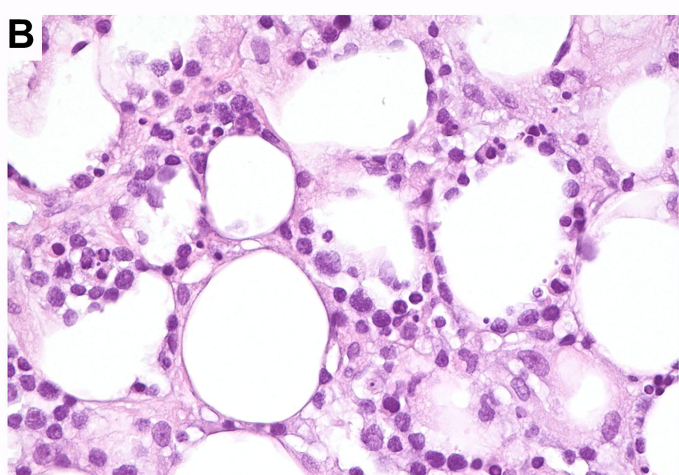
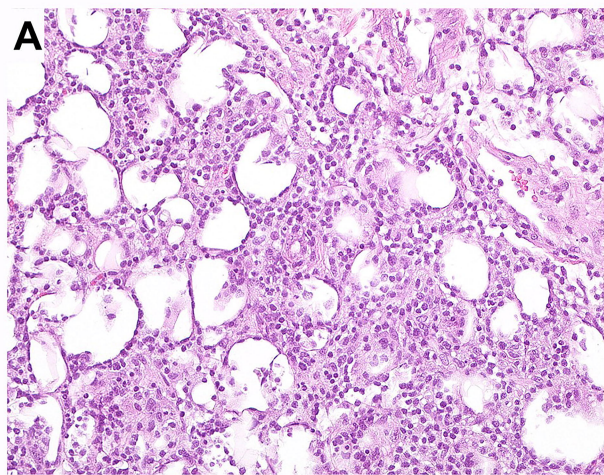
Abbreviations: SPTCL = subcutaneous panniculitis-like T-cell lymphoma; HLH = hemophagocytic lymphohistiocytosis; AA = African American; C = Caucasian; H = Hispanic; AI = American Indian; AAPI = Asian American and Pacific Islander; U = Unknown; hom = homozygous; het = heterozygous; NA = not applicable, ND = not determined; WT = Wild Type; VAR = variant.

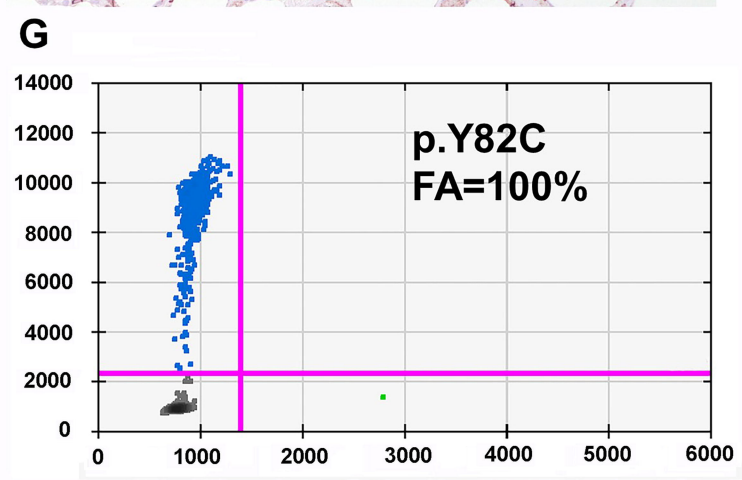
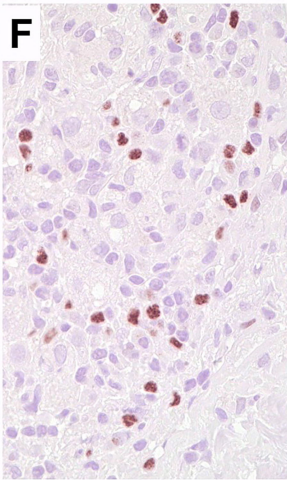
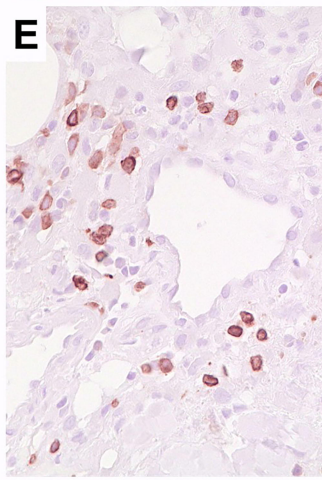
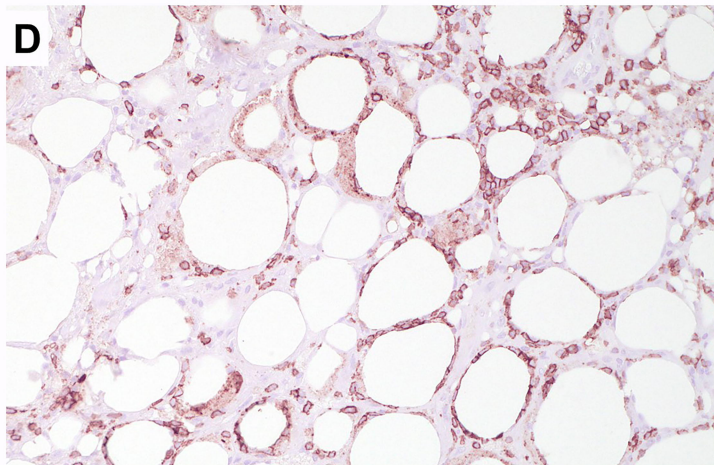
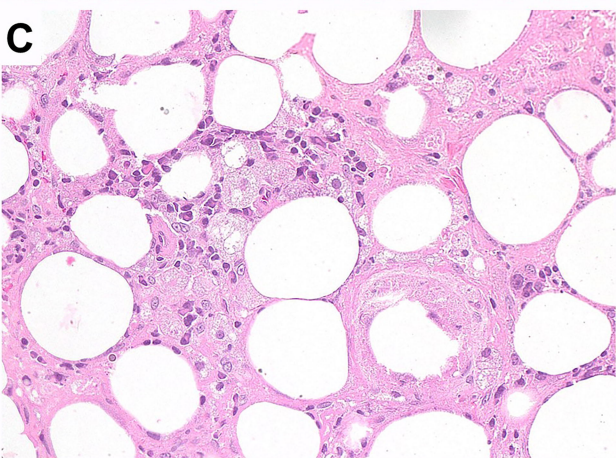
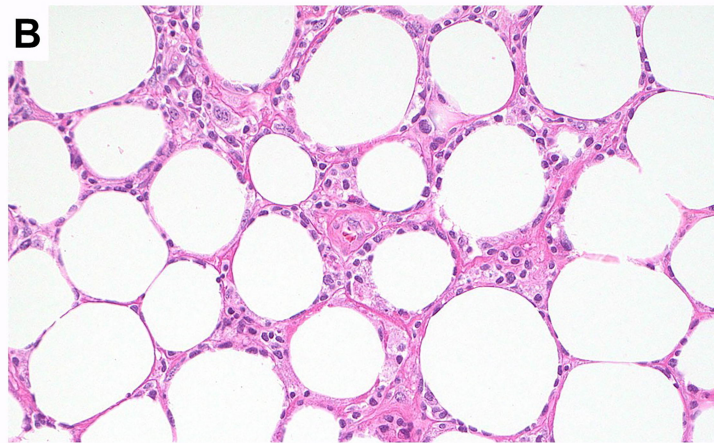
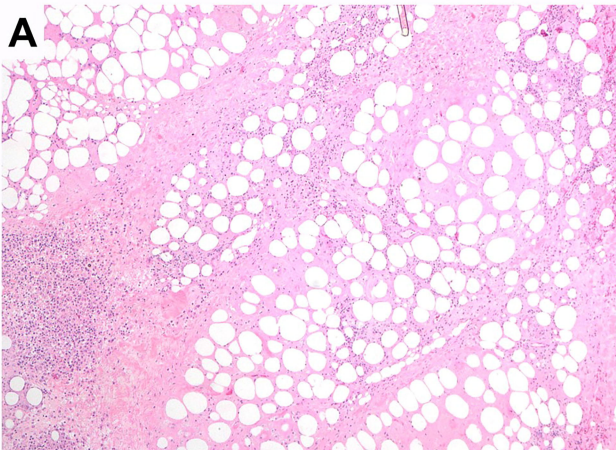
FIGURE LEGENDS

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.197M heterozygous germline variant. T-cell gene rearrangement studies were clonal (not shown).

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).





SUPPLEMENTAL TABLES AND FIGURES

Table S1. Characteristic features distinguishing SPTCL from Lobular Panniculitis

	<u>SPTCL</u>	<u>Lobular Panniculitis</u>
Clinical Presentation	<p>Multiple subcutaneous nodules with progression (skin ulceration uncommon)</p> <p>Systemic symptoms (fever, malaise, cytopenia)</p> <p>Autoimmune disease or positive autoantibodies</p> <p>HLH in subset of patients</p>	<p>Subcutaneous nodules</p> <p>Usually no systemic symptoms of fever, malaise, cytopenia</p> <p>Autoimmune disease or positive autoantibodies</p> <p>Usually, no HLH</p>
Morphology	T lymphocytes are predominantly CD8+ $\alpha\beta$ cytotoxic cells	Mixture of CD4+ and CD8+ T cells
	Fat rimming by tumor cells with cytological atypia	Rare to absent
	Karyorrhexis	Rare to absent
	Low or absent reactive cells (B lymphocytes, plasma cells, plasmacytoid dendritic cells,	Admixed reactive cells (B lymphocytes, plasma cells, plasmacytoid dendritic cells
Genetics		
TCR monoclonality	Yes	Negative or Oligoclonal
<i>HAVCR2</i> variants	Yes	No
Somatic mutations	Uncommon (only in 10-20%)	No

Table S2. HAVCR2 and clonality status of cases with detected somatic mutations

CASE NUMBER	DIAGNOSIS	SOMATIC MUTATIONS*	VAF	INTER- PRETATION	HAVCR2 STATUS (VAR/WT)**	TRG
Case 22	SPTCL	<i>TET2</i> ; p.C1135fs*7	2.7%	P	WT	Clonal
Case 33 [^]	SPTCL	<i>KMT2D</i> ; p.L804delinsPHLSPQPEEL	9%	VUS	VAR: p.I97M het	Polyclonal
Case 35	SPTCL	<i>BCORL1</i> ; p.P1416L <i>CREBBP</i> ; p.V1802M	10% 13%	VUS VUS	VAR: p.I97M het	Clonal

*Next Generation sequencing was performed using TruSight® Oncology 500 (TSO500, Illumina®) kit. Sequencing was performed using a NextSeq 550Dx system (Illumina®). Proprietary TruSight Oncology 500 and TruSight Oncology 170 v1.0 local applications (Illumina®) were used for alignment and variant calling. Resulting vcf file is uploaded to the QIAGEN Clinical Insight (QCI, QIAGEN) for filtering and annotation. This assay has a limit of detection of 1-3% VAF for both small nucleotide variants and 3-5% VAF for indels to 25 nucleotides.

***HAVCR2* c.245A>G, p.Y82C (COSM3683806), c.291A>G, p.I97M and c.302C>T, p.T101I mutational analysis were performed using the custom designed PrimePCR ddPCR Assays (BIO-RAD, Hercules, CA) on a BIO-RAD QX200 droplet digital PCR (ddPCR) system. The presence of mutation and the fractional abundance (FA) of the mutant allele was determined with QuantaSoft v.1.7 (BIO-RAD).

[^]Case was sequenced by submitter. VAF = variant allele frequency, VAR = variant, WT = Wild Type, het = heterozygous, TRG = T-cell Receptor Gamma Chain, P = Pathogenic, VUS = variant of uncertain significance

Figure S1. Atypical lobular panniculitis with *HAVCR2* germline variants, cases 2 and 3.

Case 2 (Panels A-F): Biopsy from a 61-year-old female shows atypical lobular panniculitis-like infiltrate separated by intact fibrous septae (A, H&E 20X). On high power, a mixed lymphohistiocytic infiltrate is present (B, H&E 200X). Focally, adipocytes are rimmed by perforin+ cytotoxic T cells (C, H&E 200x; D, Perforin immunostain, 200x). ddPCR detected homozygous *HAVCR2* p.T101I variant (E). T-cell gene rearrangement studies were clonal (F), but polyclonal in two other biopsies in two subsequent biopsies (not shown).

Case 3 (Panels G-L): Biopsy from a 56-year-old female shows an atypical lobular panniculitis-like infiltrate (G, H&E 20x). On high power, reactive plasma cells are seen (H, H&E 200X). There is focal adipose rimming by Granzyme+ cytotoxic T cells (I, H&E 200x; J, Granzyme immunostain 200x). ddPCR detected homozygous *HAVCR2* p.I97M germline variant (K). T-cell gene rearrangements were polyclonal (L).

