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

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Abstract

Germline *HAVCR2* mutations are frequently detected in subcutaneous panniculitis-like T-cell lymphoma (SPTCL) patients with/without hemophagocytic lymphohistiocytosis (HLH) but factors associated with variable manifestations remain undetermined. To evaluate clinical variations and associated factors in SPTCL and/or HLH with/without *HAVCR2* mutations, we performed direct sequencing of *HAVCR2* exon 2 using DNA from patients with SPTCL or idiopathic HLH/HLH-like systemic illnesses, defined by HLH alone without secondary causes. The systematic review and individual patient data (IPD) level meta-analysis which included the present and previously published studies reporting *HAVCR2* mutations in SPTCL with/without HLH populations was subsequently conducted using random-effects meta-analysis and multivariate logistic regression. Among 34 patients enrolled, ten of 28 SPTCL patients developed HLH/HLH-like systemic illnesses. Six cases with *HAVCR2*^{Y82C} mutation manifested with HLH without panniculitis. Male sex (P=0.03) and age <18 years (P=0.04) were associated with HLH, corresponding to the inverse correlation between age and HLH-2004 score (r=-0.40; P=0.02). Homozygous *HAVCR2*^{Y82C} mutation was more common in the presence of HLH compared with the absence (75.0% *vs*. 44.4%; P=0.02). Using IPD from the present and the other three eligible cohorts (N=127), male sex, heterozygous and homozygous/compound heterozygous *HAVCR2* mutations were associated with HLH by the adjusted odds ratio of 2.93 (95% confidence interval [CI]: 1.22-7.06), 4.77 (95% CI: 1.05-21.63) and 8.48 (95% CI: 2.98-24.10), respectively. Patients with male sex and/or germline *HAVCR2* mutations showed an increased risk of developing HLH. Younger patients tended to manifest with HLH, while older patients typically presented with SPTCL with less frequent HLH/HLH-like systemic illnesses.

Introduction

Subcutaneous panniculitis-like T-cell lymphoma (SPTCL) is a rare subtype of T-cell non-Hodgkin lymphoma classically presenting with subcutaneous nodules. The pathological hallmark of SPTCL is an adipocyte rimming by atypical lymphoid cells expressing CD3, CD8, T-cell intracytoplasmic antigen 1 (TIA-1) and T-cell receptor β F1 (BF1). Recently, germline mutations of hepatitis A virus cellular receptor 2 (*HAVCR2*) gene have been discovered to be an important predisposing factor for primary SPTCL, while secondary SPTCL could be associated with several conditions, including viral infections and autoimmune diseases.[1]

Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening syndrome commonly observed in patients with SPTCL as well as germline *HAVCR2* mutations.[2,3] These mutations result in misfolding of T-cell immunoglobulin and mucin-domain containing-3 (TIM-3) protein, leading to persistent immune activation and cytokine release, which are responsible for the pathogenesis of HLH. In general, HLH is often secondary to several conditions, including autoimmune diseases, cancers, and infections. Nonetheless, HLH without identifiable primary cause or idiopathic HLH has been observed.[4] Germline *HAVCR2* variants in this entity have never been explored. For clinical studies, HLH is customarily diagnosed by fulfilling the HLH-2004 classification criteria,[5] but in clinical practice, patients with high-grade fever, cytopenias and presence of hemophagocytic activity in bone marrow (BM) can be presumptively diagnosed as having hemophagocytic syndrome (HPS). These clinical phenotypes should be categorized as HLH-like systemic illnesses where treatments are urgently required.

The previously reported germline *HAVCR2* mutations included p.Y82C, p.I97M and p.T101I substitutions.[1-3] Although the pathogenic consequences of homozygous or compound heterozygous *HAVCR2* mutations have been described, the effects of heterozygous *HAVCR2* mutations on clinical manifestations of SPTCL and associated HLH remain undetermined. SPTCL shows a wide array of presentations and can be observed in both male and female sex, and in all age groups, ranging from children, young adults, to the elderly. Factors that influence the phenotypic variations are not yet well defined. Therefore, this multicenter study aims to explore the associations between clinical manifestations of SPTCL and/or HLH/HLH-like systemic illnesses and several factors, including *HAVCR2* mutational statuses, sex and age groups. In order to enhance better understanding of the clinical spectrum of the rare disorder, a systematic review and meta-analysis which incorporates data from previously published studies reporting *HAVCR2* mutations in SPTCL/HLH populations is also conducted.

Methods

Patients and study design

Patients with SPTCL with/without HLH or patients with idiopathic HLH were enrolled from nine hematology centers in Thailand from January 2009 to June 2022 (*Online Supplementary Appendix; Online Supplementary Figure S1*). Idiopathic HLH included patients manifested with HLH alone without any conditions contributing to HLH, i.e., infections, autoimmune disorders, and malignancies. Pathological tissues designating SPTCL/HLH were revalidated by the hematopathologists. SPTCL clonality was identified by immunohistochemistry staining and T-cell receptor gene rearrangement analyses, as previously described.[1] HLH was defined by the HLH-2004 criteria.[5] Those incompletely fulfilling the criteria but being clinically consistent with HLH (i.e., fever, cytopenias, serum ferritin ≥500 μg/L, and the presence of hemophagocytosis in BM) were accounted for 'HLH-like systemic illnesses'.[3] The study protocol was ethically approved by the Institutional Review Boards of the respective institutions.

Sanger sequencing to detect germline *HAVCR2* exon 2 mutations was performed in all subjects using DNA extracted from bone marrow (BM) or peripheral blood. The designed primer could cover pathogenic *HAVCR2* variants of p.Y82C, p.I97M and p.T101I. Whole exome sequencing (WES) following the standard pipeline (Novogene Co. Ltd., Beijing, China) was explored in the selected cases (NovaSeq 6000 platform, Illumina Inc., San Diego, CA). The methodology on *HAVCR2* sequencing and WES is fully outlined in the *Online Supplementary Appendix* and the *Online Supplementary Figures S2, S3*.

Due to the disease rarity, a systematic review and meta-analysis of previously published series of SPTCL with/without HLH/HLH-like systemic illnesses, including one from our group,[1] combined with our present cohort, was performed. The prespecified individual patient data (IPD) level meta-analysis would be analyzed if the included studies provided sufficient outcomes per each individual. The primary outcome was the risk factors associated with HLH/HLH-like systemic illnesses, including *HAVCR2* mutations, sex and age at diagnosis. The prevalence of *HAVCR2* mutations was the secondary outcome. This systematic review and meta-analysis were proceeded following the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines and the International Prospective Register of Systematic Reviews (PROSPERO) registration (CRD42022341310).[6] Study search and selection, data extraction, and quality assessment of the included studies are fully described in the *Online Supplementary Appendix*.

Statistical analysis

For the present cohort, STATA version 15.1 (StataCorp, Col-

lege Station, TX) and descriptive statistics were employed, considering $P<0.05$ for statistical significance. For the conventional meta-analysis, Comprehensive Meta-Analysis version 3.0 (Biostat Inc., Englewood, NJ) was applied. The pooled odds ratios (pOR) or pooled differences in means (pMD) with 95% confidence intervals (CI) for risk factors associated with HLH/HLH-like systemic illnesses were estimated by the DerSimonian and Laird random-effects meta-analysis. The weighted mean prevalence of *HAVCR2* mutations and HLH/HLH-like systemic illnesses was also reported. The influence of factors on the synthesized estimates were determined by meta-regression analysis, if appropriate.[7] The publication bias and inter-study heterogeneity were assessed systematically (*Online Supplementary Appendix*).

For the IPD level meta-analysis, the risk factors associated with HLH/HLH-like systemic illnesses were determined by logistic regression. Multivariate models were developed by adjusting for covariates with $P<0.1$ in univariate models and stepwise backward logistic regression to select the final models. The adjusted odds ratios (aOR) were estimated alongside 95% CI.

Table 1. Clinical characteristics of the patients in our present cohort (N=34)

Clinical characteristics	Value
Participants, N	34
Median age in years (IQR)	20.5 (12-32)
Male sex, N (%)	12 (35.3)
Clinical manifestation, N (%)	
HLH/HLH-like systemic illnesses alone	6 (17.6)
HLH/HLH-like systemic illnesses with SPTCL	10 (29.4)
SPTCL alone	18 (52.9)
Treatment, N (%)	
Chemotherapy	14 (41.2)
Immunosuppressive drugs	20 (58.8)
Relapse, N (%)	10 (29.4)
Deceased, N (%)	2 (5.8)
Germline *HAVCR2*^{Y82C} mutational status, N (%)	
Homozygous *HAVCR2*^{Y82C} mutation	20 (58.8)
Heterozygous *HAVCR2*^{Y82C} mutation	7 (20.6)
No *HAVCR2*^{Y82C} mutation	7 (20.6)

HLH: hemophagocytic lymphohistiocytosis; IQR: interquartile range; SPTCL: subcutaneous panniculitis-like T-cell lymphoma.

Results

Analysis of our present cohort

Among the 34 cases enrolled, 28 cases presented with SPTCL with/without HLH/HLH-like systemic illnesses, while six cases presented with idiopathic HLH/HLH-like systemic illnesses. The median age of the patients at the time of diagnosis was 20.5 years (interquartile range [IQR], 12-32), with female sex predominance (64.7%). Homozygous *HAVCR2*^{Y82C} mutation was detected in 20 cases (58.8%), heterozygous *HAVCR2*^{Y82C} mutation in seven cases (20.6%), while no *HAVCR2*^{Y82C} mutation was detected in 7 cases (20.6%; Table 1; *Online Supplementary Table S1*). No *HAVCR2* p.I97M or p.T101I mutations were detected in our cohort.

Patients younger than 18 years showed more HLH/HLH-like systemic illnesses than those 18 years or older. There was a higher proportion of male patients in patients who had HLH/HLH-like symptoms compared to those who did not (56.3% *vs*. 16.7%; $P=0.03$). In patients with HLH/HLH-like events, the frequency of homozygous *HAVCR2*^{Y82C} mutation was significantly higher than those without HLH/HLH-like systemic illnesses (75.0% *vs*. 44.4%; $P=0.02$; Table 2).

When analyzing HLH-2004 score according to the HLH-2004 criteria, there was an inverse correlation between patient age and HLH-2004 score ($r=-0.40$; $P=0.02$; Figure 1A). Patients harboring *HAVCR2*^{Y82C} mutations were younger than those with no *HAVCR2*^{Y82C} mutation (median age of 15 *vs*. 19 *vs*. 36 years for homozygous mutation, heterozygous mutation, and no *HAVCR2*^{Y82C} mutation, respectively; $P=0.02$). Patients with *HAVCR2*^{Y82C} mutations had higher median HLH-2004 scores than those with no *HAVCR2*^{Y82C} mutation (median HLH-2004 score of 4 for homozygous mutation, 5 for heterozygous mutation, and 0 for no *HAVCR2*^{Y82C} mutation; $P=0.003$; *Online Supplementary Table S2*). There were no clinical factors associated with disease relapse and mortality rates.

WES was done in one heterozygous *HAVCR2*^{Y82C} mutation case (case no. 23) and in five homozygous *HAVCR2*^{Y82C} mutation cases with HLH/HLH-like systemic illnesses (case no. 2, 3, 12, 18, and 20). There were no other pathogenic *HAVCR2* mutations in a case with heterozygous *HAVCR2*^{Y82C} mutation. In the five cases with a homozygous *HAVCR2*^{Y82C} mutation, although no mutations in other familial HLH genes were identified, mutated genes associated with primary immune deficiency disease (PIDD) and dysregulated immune activation or proliferation (DIAP)[8] were observed in all cases (*Online Supplementary Table S3; Online Supplementary Figure S4*).

Systematic review and conventional meta-analysis

Online databases including MEDLINE, Embase and Cochrane Library were systematically searched between the inception date of each database and July 15, 2022. Our present cohort was integrated into 312 results from the initial search. After removing 60 duplicates, 237 studies were excluded by screening through the titles and abstracts. Three of 16 studies were excluded due to studying in the same populations with other more mature studies. Thirteen studies (6 cohorts and 7 case reports) were eli-

gible for the systematic review (Table 3).[1-3,9-17] The PRISMA flow diagram for study screening and the selection process is illustrated in the *Online Supplementary Figure S5*. The risks of bias for cohort studies were evaluated by the Newcastle-Ottawa scale as outlined in the *Online Supplementary Table S4*.[18]

Of 13 studies, six cohorts with at least ten participants were finally included in the meta-analysis, representing 224 patients with SPTCL with/without HLH/HLH-like systemic illnesses and six idiopathic HLH/HLH-like systemic illnesses.[1-3,9-10] From which, 207 cases had been documented for both statuses in HLH/HLH-like systemic illnesses and *HAVCR2* mutation. Overall, the pooled prevalence of HLH/HLH-like systemic illnesses was 31.9% (95% CI: 18.2-49.7; I^2=81%), while the pooled prevalence of *HAVCR2* mutation was 51.6% (95% CI: 30.3-72.4; I^2=87%). *HAVCR2*^{Y82C} variant was the most

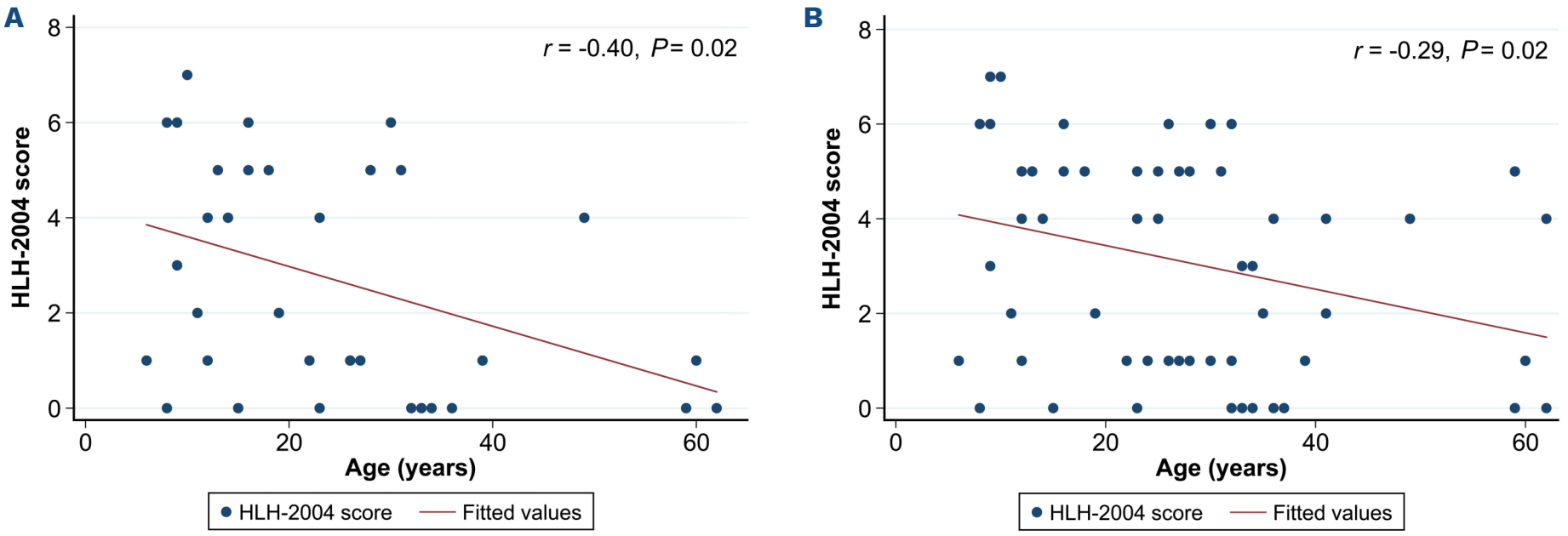


Figure 1. Correlation of HLH-2004 score and age in our present cohort (A, N=34) and the analysis of individual patient data from 4 cohorts (B, N=127). HLH: hemophagocytic lymphohistiocytosis.

Table 2. Demographic and mutation data by hemophagocytic lymphohistiocytosis (HLH)/HLH-like systemic illnesses status.

Parameters	Total	Presence of HLH/HLH-like systemic illnesses	Absence of HLH /HLH-like systemic illnesses	*P*
Our present cohort				
Participants, N	34	16	18	-
Age in years, median (IQR)	20.5 (12-32)	16 (11-26)	27 (15-36)	0.07
Age <18 years old, N (%)	15 (44.1)	10 (62.5)	5 (27.8)	0.04
Male sex, N (%)	12 (35.3)	9 (56.3)	3 (16.7)	0.03
Mutation, N (%) No *HAVCR2*^{Y82C} mutation Heterozygous *HAVCR2*^{Y82C} mutation Homozygous *HAVCR2*^{Y82C} mutation	 7 (20.6) 7 (20.6) 20 (58.8)	 0 (0) 4 (25.0) 12 (75.0)	 7 (38.9) 3 (16.7) 8 (44.4)	0.02
Analysis of individual patient data from 4 cohorts				
Participants, N	127*	52	71	-
Age in years, median (IQR)	27 (16-41)	23 (12-33)	32 (22-46)	0.005
Male sex, N (%)	44 (35.8)	26 (50.0)	18 (25.4)	0.005
Mutation, N (%) No *HAVCR2* mutation Heterozygous *HAVCR2* mutation Homozygous/compound heterozygous *HAVCR2* mutation	 43 (35.5) 12 (9.9) 66 (54.6)	 6 (11.5) 5 (9.6) 41 (78.9)	 37 (53.6) 7 (10.1) 25 (36.2)	<0.001

HLH: hemophagocytic lymphohistiocytosis; IQR: interquartile range. *There were 4 cases with unknown HLH status and 4 cases with unknown mutational status. In which, there were 2 cases with unknown both HLH and mutational statuses.

commonly detected (54.5%; 95% CI: 29.1-77.8; I^2=89%). The weighted mean age at diagnosis was 30.4 years (95% CI: 25.3-35.5; I^2=8%) and approximately one third of the patients were men (34.8%; 95% CI: 26.8-43.7; I^2=0%). Regarding histopathology, necrosis was a typical feature found in BM among the overall population (44.0%; 95% CI: 32.5-56.1; I^2=0%). However, granulomatous inflammation was less frequently observed in patients with *HAVCR2* mutation, compared with those in the *HAVCR2* wild-type group (9.6% *vs.* 49.0%; pOR=0.06; 95% CI: 0.01-0.53; I^2=0%; *P*=0.01). The pooled data on participant characteristics are displayed in the *Online Supplementary Tables S5, S6*.

Notably, patients with HLH/HLH-like systemic illnesses harbored *HAVCR2* mutations substantially higher than those without HLH/HLH-like systemic illnesses (76.6% *vs.* 33.5%; pOR=6.75; 95% CI: 1.65-27.64; I2=56%; *P*=0.008), particularly homozygous/compound heterozygous mutation (71.2% *vs.* 31.3%; pOR=4.67; 95% CI: 1.07-20.35; I^2=65%; *P*=0.04; Figure 2A, B; *Online Supplementary Table S7*) and *HAVCR2*^{Y82C} variant (75.5% *vs.* 35.3%; pOR=7.06; 95% CI: 1.05-47.51; I^2=65%; *P*=0.04). Compared with no *HAVCR2* mutation, heterozygous *HAVCR2* mutation showed a tendency to be associated with HLH/HLH-like systemic illnesses (pOR=6.41; 95% CI: 0.94-43.58; I^2=0%; *P*=0.06; Figure 2C; *Online Supplementary Tables S7, S8*), despite its low prevalence (7.5%; 95% CI: 2.8-18.6; I^2=54%) among studies. Furthermore, the meta-regression confirmed a positive correlation between allelic numbers of *HAVCR2* mutation and occurrence of HLH/HLH-like systemic illnesses (*P*=0.01; Figure 3). Male sex was also correlated with HLH/HLH-like systemic illnesses (pOR=2.97; 95% CI: 1.28-6.85; I^2=3%; *P*=0.01; Figure 2D). The pOR of *HAVCR2* mutational statuses and male sex as risk factors for the presence of HLH/HLH-like systemic illnesses are illustrated as the forest plots in Figure 2.

Patients with HLH/HLH-like systemic illnesses tended to develop the disease at a younger age, compared with those without HLH/HLH-like systemic illnesses (26.5 *vs.* 32.7 years), but did not reach statistical significance (pMD=-6.02 years; 95% CI: -15.40 to 3.35; I^2=54%; *P*=0.21; *Online Supplementary Table S7*). Nonetheless, by excluding Polprasert *et al.*[1] which comprised only one patient younger than 18 years old as a sensitivity analysis, patients with HLH/HLH-like systemic illnesses manifested their

Table 3. Key characteristics of the studies reporting *HAVCR2* mutational statuses in patients with subcutaneous panniculitis-like T-cell lymphoma and/or hemophagocytic lymphohistiocytosis (HLH)/HLH-like systemic illnesses which were included in the systematic review.

Study (Year)	Study design	Total participants N	Male sex N (%)	Median age at diagnosis (IQR)	*HAVCR2* mutations N (%)	*HAVCR2*^{Y82C} mutation N (%)	Homozygous/ compound heterozygous mutation N (%)	Heterozygous mutation N (%)	HLH/HLH-like systemic illnesses N (%)	Presence of SPTCL N (%)
Gayden *et al.* (2018)	RCS	27	11/27 (40.7)	22 years (1-90)	16/27 (59.3)	13/27 (48.1)	15/27 (55.6)	1/27 (3.7)	17/27 (6.3)	27/27 (100)
Polprasert *et al.* (2019)	RCS	13	4/13 (30.8)	32 years (5-59)	11/13 (84.6)	11/13 (84.6)	11/13 (84.6)	0/13 (0)	5/13 (38.5)	13/13 (100)
Cheng *et al.* (2020)	RCS	33	NA	NA	6/33 (18.2)	6/33 (18.2)	4/33 (12.1)	2/33 (6.1)	3/33 (9.1)	33/33 (100)
Sonigo *et al.* (2020)	RCS	70*	15/70 (21.4)	42 years (1-90)	13/53 (24.5)	8/53 (15.1)	13/53 (24.5)	0/53 (0)	12/68 (17.6)	70/70 (100)
Wegehaupt *et al.* (2020)	CR	1	1/1 (100)	17 years	1/1 (100)	0/1 (0)	1/1 (100)	NA	1/1 (100)	1/1 (100)
Bauman *et al.* (2021)	CR	2	1/2 (50.0)	21 months (18-24)	0/2 (0)	NA	NA	NA	1/2 (50.0)	2/2 (100)
Chaweephisal *et al.* (2021)	CR	3	2/3 (66.7)	12 years (9-14)	3/3 (100)	3/3 (100)	3/3 (100)	0/3 (0)	3/3 (100)	3/3 (100)
Frederiks *et al.* (2021)	CR	1	0/1 (0)	14 years	1/1 (100)	1/1 (100)	1/1 (100)	NA	0/0 (0)	1/1 (100)
Koh *et al.* (2021)	RCS	53**	16/53 (30.2)	32 years (8-74)	25/49 (51.0)	25/49 (51.0)	21/49 (42.9)	4/49 (8.2)	14/47 (29.8)	53/53 (100)
LeBlanc *et al.* (2021)	CR	1	0/1 (0)	34 years	0/1 (0)	NA	NA	NA	1/1 (100)	1/1 (100)
Sheng *et al.* (2021)	CR	1	1/1 (100)	40 years	0/1 (0)	NA	NA	NA	1/1 (100)	1/1 (100)
Tromp *et al.* (2022)	CR	1	1/1 (100)	30 years	1/1 (100)	0/1 (0)	1/1 (100)	NA	1/1 (100)	0/0† (0)
Present cohort (2023)	RCS	34	12/34 (35.3)	20.5 years (12-32)	27/34 (79.4)	27/34 (79.4)	20/34 (58.8)	7/34 (20.6)	16/34 (47.1)	28/34 (82.4)

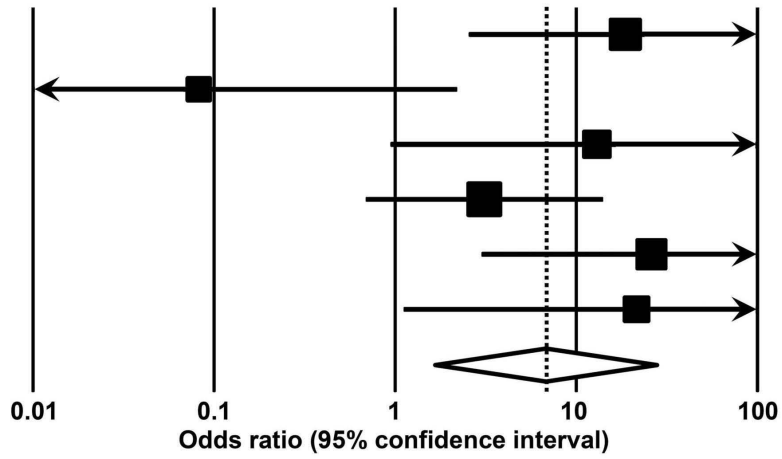
CR: case report; HLH: hemophagocytic lymphohistiocytosis; IQR: interquartile range; NA: not available; RCS: retrospective cohort study; SPTCL, subcutaneous panniculitis-like T-cell lymphoma. *Only 53 cases had available data on *HAVCR2* mutational status. **There were 4 cases with unknown HLH status and 4 cases with unknown mutational status. In which, there were 2 cases with unknown both HLH and mutational statuses. †Omental panniculitis was found in the reported case without detectable monoclonal T-cell receptor gene rearrangement.

A

Any *HAVCR2* mutation

Study	No. of predisposed cases/ total participants	Weight (%)	Odds ratio (95% confidence interval)
Gayden *et al* (2018)	16/27	19.24	18.67 (2.55-136.41)
Polprasert *et al* (2019)	11/13	11.60	0.08 (0.003-2.19)
Cheng *et al* (2020)	6/33	15.01	13.00 (0.95-178.77)
Sonigo *et al* (2020)	13/53	23.01	3.11 (0.69-14.01)
Koh *et al* (2021)	24/47	18.01	26.00 (3.00-225.20)
Present cohort	27/34	13.13	21.52 (1.12-415.35)
Total	**97/207**	**100.00**	**6.75 (1.65-27.64)**

Heterogeneity: df = 5, I^2 = 55.65%, P = 0.046

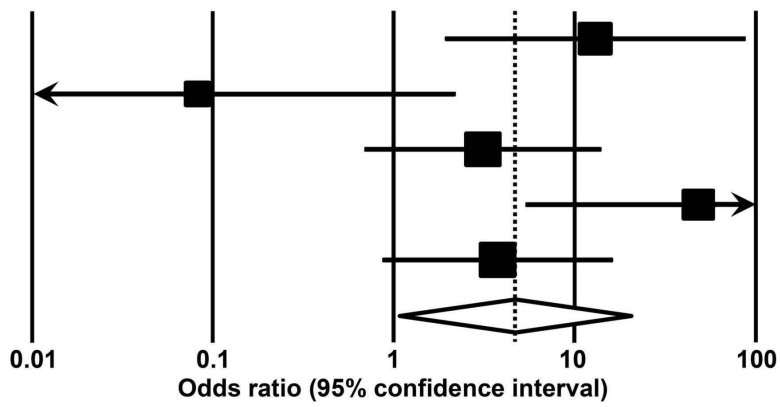


B

Homozygous/compound heterozygous *HAVCR2* mutation

Study	No. of predisposed cases/ total participants	Weight (%)	Odds ratio (95% confidence interval)
Gayden *et al* (2018)	15/27	20.76	13.00 (1.92-87.99)
Polprasert *et al* (2019)	11/13	12.36	0.08 (0.003-2.19)
Sonigo *et al* (2020)	13/53	23.95	3.11 (0.69-14.01)
Koh *et al* (2021)	20/47	18.66	48.29 (5.36-435.13)
Present cohort	20/34	24.27	3.75 (0.87-16.22)
Total	**79/174**	**100.00**	**4.67 (1.07-20.35)**

Heterogeneity: df = 4, I^2 = 65.49%, P = 0.02

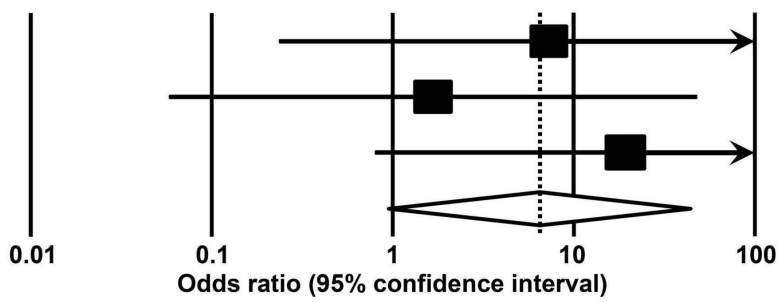


C

Heterozygous *HAVCR2* mutation *versus* *HAVCR2* wild-type

Study	No. of predisposed cases/ total participants	Weight (%)	Odds ratio (95% confidence interval)
Gayden *et al* (2018)	1/12	31.16	7.29 (0.23-225.89)
Koh *et al* (2021)	4/27	32.62	1.67 (0.06-47.83)
Present cohort	7/14	36.22	19.29 (0.80-466.24)
Total	**12/53**	**100.00**	**6.41 (0.94-43.58)**

Heterogeneity: df = 2, I^2 = 0.00%, P = 0.58



D

Male sex

Study	No. of predisposed cases/ total participants	Weight (%)	Odds ratio (95% confidence interval)
Gayden *et al* (2018)	12/27	24.73	2.63 (0.50-13.73)
Polprasert *et al* (2019)	4/13	10.08	0.42 (0.03-5.71)
Koh *et al* (2021)	15/47	38.33	3.13 (0.84-11.65)
Present cohort	12/34	26.86	6.43 (1.32-31.37)
Total	**43/121**	**100.00**	**2.97 (1.28-6.85)**

Heterogeneity: df = 3, I^2 = 3.29%, P = 0.38

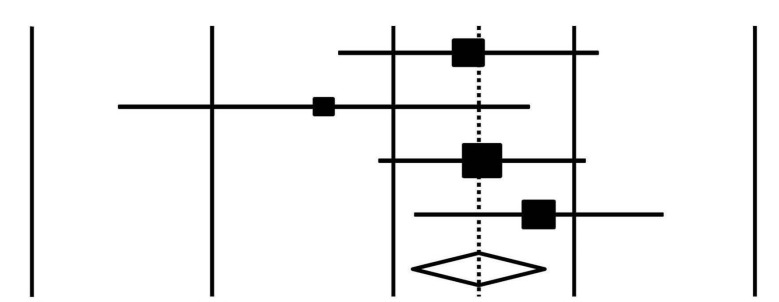


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Figure 2. Association of *HAVCR2* mutational statuses, male sex and hemophagocytic lymphohistiocytosis (HLH)/HLH-like systemic illnesses. The forest plots demonstrate the pooled odds ratios with 95% confidence intervals of any *HAVCR2* mutations (A), homozygous/compound heterozygous *HAVCR2* mutation (B), heterozygous *HAVCR2* mutation in comparison with *HAVCR2* wild-type (C), and male sex (D) in significant associations with presence of HLH/HLH-like systemic illnesses among patients with subcutaneous panniculitis-like T-cell lymphoma or idiopathic HLH.

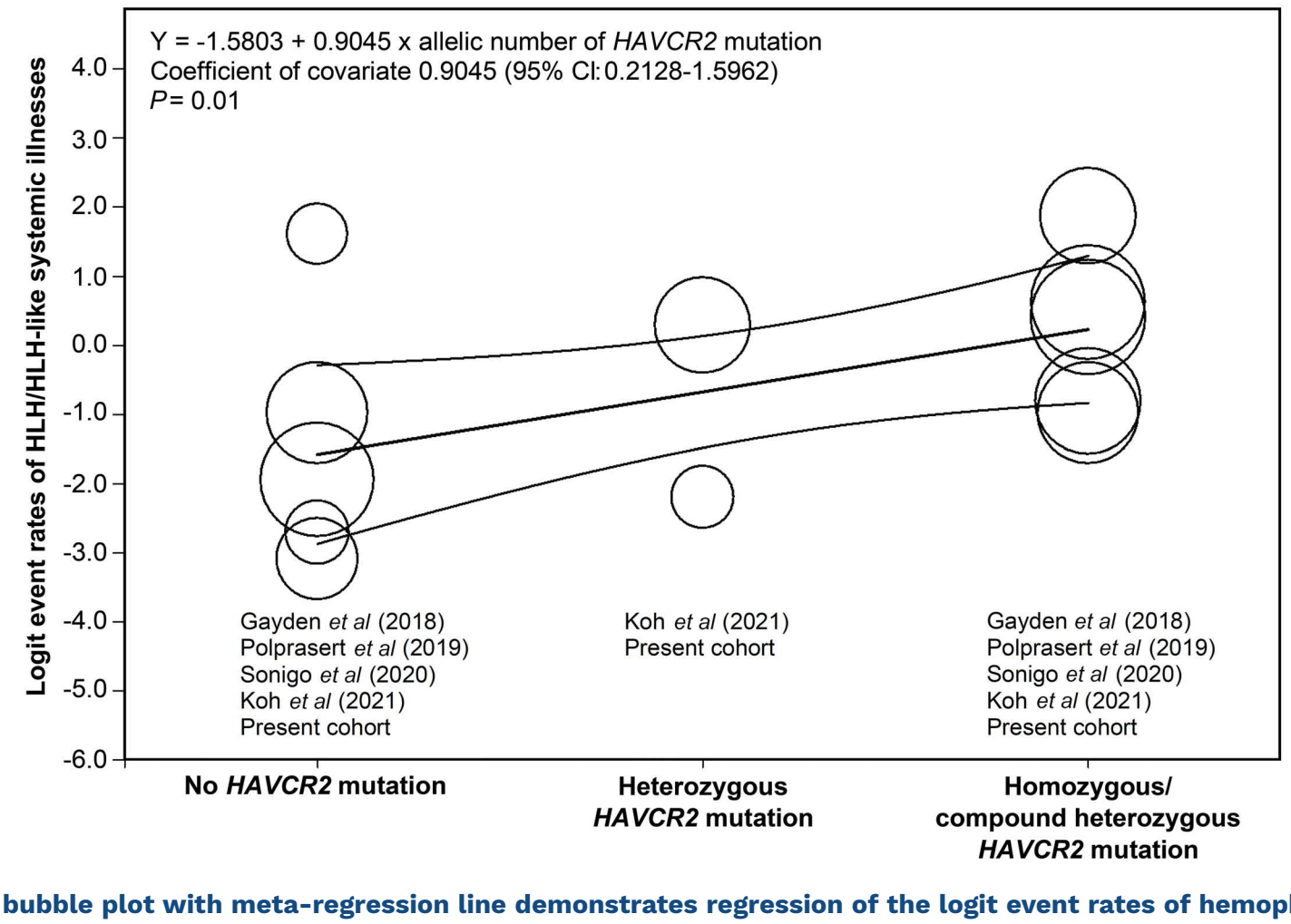

Figure 3. The bubble plot with meta-regression line demonstrates regression of the logit event rates of hemophagocytic lymphohistiocytosis (HLH)/HLH-like systemic illnesses on allelic numbers of *HAVCR2* mutation. CI: confidence interval.

disease phenotypes significantly earlier than those without HLH/HLH-like systemic illnesses (pMD=-10.31 years; 95% CI: -17.04 to -3.57; I^2=0; *P*=0.003). Since fewer than ten studies were aggregated in the meta-analysis, evaluation of the publication bias was not indicated.

Individual patient data level meta-analysis

Three previously published cohorts provided individual data of their participants.[1-3] We therefore conducted the IPD level meta-analysis using data from those three studies and our present cohort, resulting in a total of 127 cases. Based on 123 cases with *HAVCR2* mutational statuses available, it was found that 65 cases harbored homozygous *HAVCR2* mutations (63 cases with p.Y82C and 2 cases with p.I97M), two cases harbored compound heterozygous *HAVCR2* mutations (p.Y82C with p.T101I and p.Y82C with p.I97M), 12 cases harbored heterozygous *HAVCR2* mutations (11 cases with p.Y82C and 1 case with p.I97M), while 44 cases showed no *HAVCR2*^{Y82C} mutation. Diagram of case distribution based on SPTCL/HLH phenotypes and mutational statuses is shown in the *Online Supplementary Figure S6*.

Patients who manifested HLH/HLH-like systemic illnesses were younger than patients without HLH/HLH-like systemic illnesses (median age of 23 *vs*. 32 years; *P*=0.005). There were more men in cases with HLH/HLH-like systemic illnesses than without HLH/HLH-like systemic illnesses (50.0% *vs*. 25.4%; *P*=0.005). The homozygous/compound heterozygous *HAVCR2* mutation group showed more HLH/HLH-like systemic illnesses than the others (78.9% *vs*. 9.6% [heterozygous] *vs*. 11.5% [no mutation]; *P*<0.001; Table 2).

Since the correlation between age at diagnosis and HLH-2004 score could not be estimated by the conventional meta-analysis due to lack of reported data based on age groups, we re-evaluated this outcome in the IPD level meta-analysis. Remarkably, an inverse correlation between age at diagnosis and HLH-2004 score was demonstrated (*r*=-0.29; *P*=0.02; Figure 1B). Patients with no *HAVCR2*^{Y82C} mutation were significantly older (*P*<0.001) and associated with lower HLH-2004 scores (*P*=0.002; *Online Supplementary Table S2*).

By the multivariate analysis, male sex (aOR=2.93; 95% CI: 1.22-7.06; *P*=0.02), heterozygous *HAVCR2* mutation (aOR=4.77; 95% CI: 1.05-21.63; *P*=0.04) and homozygous/compound heterozygous *HAVCR2* mutation (aOR=8.48; 95% CI: 2.98-24.10; *P*<0.001) were significantly associated with HLH/HLH-like systemic illnesses (Table 4).

Table 4. Risk factors associated with hemophagocytic lymphohistiocytosis (HLH)/HLH-like systemic illnesses by univariate and multivariate analyses using the individual patient data from 4 cohorts (N=127)

Factors	Presence of HLH/HLH-like systemic illnesses (%)	Univariate		Multivariate	
		OR (95% CI)	*P*	aOR (95% CI)	*P*
Age					
≥18 years	33.3	1	Ref	1	Ref
<18 years	63.9	3.54 (1.57-7.98)	0.002	2.33 (0.95-5.76)	0.07
Sex					
Female	32.9	1	Ref	1	Ref
Male	59.1	2.94 (1.37-6.31)	0.006	2.93 (1.22-7.06)	0.02
HAVCR2 mutation					
No mutation	14.0	1	Ref	1	Ref
Heterozygous mutation	41.7	4.40 (1.05-18.51)	0.04	4.77 (1.05-21.63)	0.04
Homozygous/compound heterozygous mutation	62.1	10.11 (3.74-27.37)	<0.001	8.48 (2.98-24.10)	<0.001

aOR: adjusted odds ratio; CI: confidence interval; HLH: hemophagocytic lymphohistiocytosis; OR: odds ratio; Ref: reference.

Discussion

SPTCL is a unique hematologic malignancy that is associated with germline *HAVCR2* mutations (25-60%)[2,9] and concomitant systemic inflammation which may be as severe as HLH. In this multicenter study, we demonstrated that patients with SPTCL-associated and idiopathic HLH/HLH-like systemic illnesses were associated with a younger age group, male sex, and the presence of germline *HAVCR2* mutation. The results were substantiated by the systematic review and IPD level meta-analysis which included all existing cohorts with available data.

Although *HAVCR2* mutation is a congenital mutation, SPTCL with or without HLH can occur later in life. In addition, a positive family history in SPTCL and idiopathic HLH is unusual. Therefore, additional factors, such as infections, additional somatic mutations, or vaccines, are generally required to trigger disease manifestations.[1] Furthermore, our preliminary WES data suggest possible roles of other concomitant immunoregulatory gene mutations in the development of HLH/HLH-like systemic illnesses, but the known variants in the genes causing familial HLH (i.e., perforin 1 [*PRF1*], UNC-13 homolog D [*UNC13D*], RAS-related protein Rab-27A [*RAB27A*], syntaxin 11 [*STX11*] and syntaxin binding protein 2 [*STXBP2*] genes) were not observed. In this study, older patients were more likely to present with SPTCL alone, while younger patients usually had SPTCL with concurrent HLH/HLH-like systemic illnesses or even HLH/HLH-like systemic illnesses alone. The earlier onset of illnesses was associated with homozygous or compound heterozygous *HAVCR2* mutations. This strong genetic predisposition may make them more susceptible to developing HLH.

The germline *HAVCR2* mutation in patients with HLH/HLH-like systemic illnesses without clinical panniculitis has never been reported. In our cohort, there were six patients who manifested with idiopathic HLH/HLH-like systemic illnesses without SPTCL and no evidence of infections or other malignancies. Five of the six cases harbored a homozygous *HAVCR2*^{Y82C} mutation, while one case showed a heterozygous *HAVCR2*^{Y82C} mutation. Patients with a *HAVCR2* mutation may have clinical manifestations similar to other familial HLH. Most familial HLH occurs at a very young age of approximately 1 year, and germline mutations in *PRF1, UNC13D, RAB27A, STX11* and *STXBP2* genes were responsible for this entity.[4,8] The germline *HAVCR2* mutation is probably the first genetic factor that predisposes the patients to develop late childhood- and young adult-onset familial HLH. In our series, two fatal cases with HLH were treated with chemotherapy, while five cases with HLH who received only immunosuppression survived (*Online Supplementary Table S1*). Hence, the detection of *HAVCR2* mutation in the patients with unknown causes of HLH/HLH-like systemic illnesses is crucial since chemotherapy might be avoided in this setting to prevent serious complications of cytotoxic agents, particularly BM suppression. Due to the fact that germline *HAVCR2* mutations led to persistent immune activation,[2] immunosuppressive agents should provide potential therapeutic benefits for these patients. As supported by our cohort, most patients (58.8%), with or without HLH, responded well to corticosteroids and/or cyclosporine.

Based on this multicenter study and IPD level meta-analysis, we proposed the age-dependent clinical spectrum from HLH/HLH-like systemic illnesses with or without SPTCL in children and young adults to SPTCL alone in elderly patients. Of note, the age-dependent effect on the phenotype of HLH/HLH-like systemic illnesses was masked by a study from Polprasert *et al.*[1] which comprised mainly adult pa-

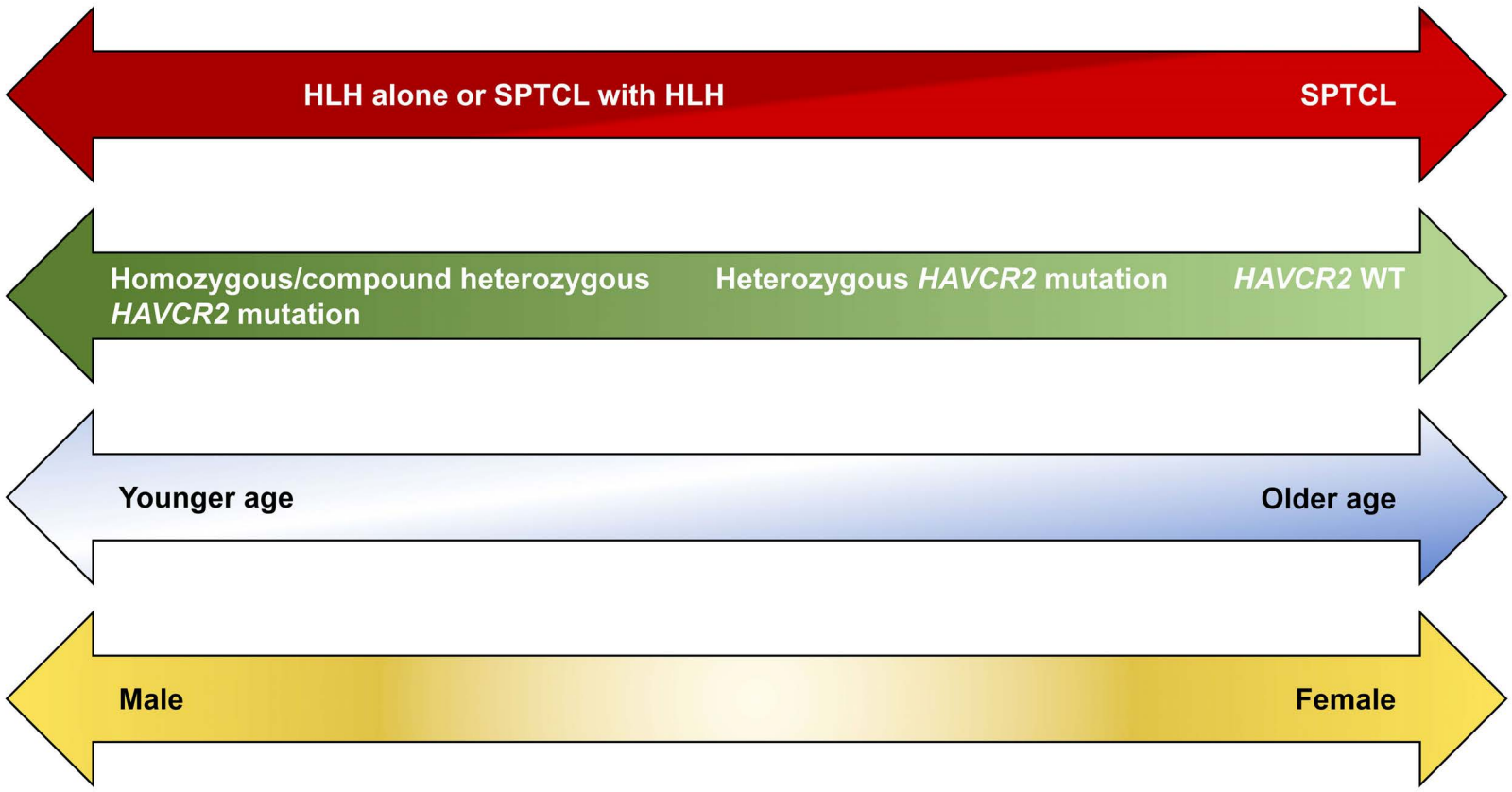

Figure 4. Proposed differences in genetic predisposition and clinical manifestation between age groups and sex. HLH: hemophagocytic lymphohistiocytosis; SPTCL: subcutaneous panniculitis-like T-cell lymphoma; WT: wild-type.

tients in the conventional meta-analysis but could demonstrate its significance when analyzed at the IPD level. The presence of HLH is also more prevalent in male patients and patients with a homozygous *HAVCR2* mutation (Figure 4). Although a heterozygous *HAVCR2* mutation was significantly associated with an increased risk of HLH at the IPD level (Table 4), supported by the meta-regression in which the occurrence of HLH increased when the allelic number of *HAVCR2* mutation increased (Figure 3), the small number of cases per study limited its interpretation in the conventional meta-analysis.

As a limitation of the retrospective study, we could not exclude the presence of internal organ panniculitis in our six patients with idiopathic HLH/HLH-like systemic illnesses since the computed tomography scan of the whole abdomen was not performed among those without abdominal symptoms, although omental panniculitis has been reported in a patient with a germline *HAVCR2* mutation presenting with HLH without skin lesion.[17] Additionally, we could not demonstrate any factors associated with disease relapse or mortality, possibly due to the heterogeneity of the treatments among cohorts. WES was not performed in the patients with no *HAVCR2* mutation to identify other potential genetic alterations contributing to HLH/HLH-like systemic illnesses. Nevertheless, data on other additional immune regulatory diversities from our study were small and therefore deserved further exploration in a larger study.

In conclusion, the clinical manifestations of patients with SPTCL and associated HLH/HLH-like systemic illnesses are sex- and *HAVCR2* mutation-dependent. The presence of HLH/HLH-like systemic illnesses is more common in younger patients, while SPTCL alone is more prevalent in the older age group.

Disclosure

No conflicts of interest to dislcose.

Contributions

CM designed and performed the systematic review and meta-analysis, analyzed data and wrote the manuscript. CP collected samples and clinical data, designed and performed the systematic review and meta-analysis, analyzed data, wrote the manuscript, and conceptualized the overall research. PK, TR, PB, PR, KP, SarP, UB, DS provided samples and clinical data. SuK and KW wrote the manuscript. SiK extracted DNA and performed PCR. NT performed the meta-analysis and wrote the manuscript. SamP and CC provided samples, clinical data and wrote the manuscript. NS conducted histopathological studies. PR supervised, conceptualized the research, and wrote the manuscript. All authors read and approved the final manuscript.

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Data-sharing statement

WES results have been deposited in the European Genome-phenome Archive under accession number: EGAS00001006740.

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