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Hemophagocytic Lymphohistiocytosis Gene Variants in Childhood-Onset Systemic Lupus Erythematosus With Macrophage Activation Syndrome

Piya Lahiry[1], Sergey Naumenko[2], Madeline Couse[3], Fangming Liao[3], Daniela Dominguez[1], Andrea Knight[4], Deborah M. Levy[1], Melissa Misztal[3], Lawrence W.K. Ng[1], and Linda T. Hiraki[5]

ABSTRACT. ***Objective.*** Macrophage activation syndrome (MAS), a life-threatening complication of systemic lupus erythematosus (SLE), resembles familial hemophagocytic lymphohistiocytosis (HLH), an inherited disorder of hyperinflammation. We compared the proportion of patients with childhood-onset SLE (cSLE) with and without MAS who carried low-frequency HLH nonsynonymous variants.

Methods. We enrolled patients from the Lupus Clinic at SickKids, Toronto. Demographic and clinical features were extracted from the SLE database and ancestry was genetically inferred using multiethnic genotyping array data. Patients with MAS (based on expert diagnosis) underwent either paired-end whole-exome sequencing (WES; read depth: 70-118X) or whole-genome sequencing (WGS). Patients without MAS had WGS (read depth: 37-40X). In 16 HLH genes, we prioritized low-frequency (minor allele frequency [MAF] < 0.05) exonic nonsynonymous variants. We compared the proportion of patients with and without MAS carrying HLH variants (Fisher exact test, $P < 0.05$). MAFs were compared to an ancestrally matched general population (Trans-Omics for Precision Medicine [TOPMed] and Genome Aggregation Database [gnomAD]).

Results. The study included 81 patients with cSLE, 19 of whom had MAS. We identified 47 unique low-frequency nonsynonymous HLH variants. There was no difference in the proportion of patients with and without MAS carrying ≥ 1 HLH variants (37% vs 47%, $P = 0.44$). The MAS cohort did not carry more HLH variants when compared to an ancestrally matched general population.

Conclusion. In a single-center multiethnic cSLE cohort, we found no difference in the proportion of patients with MAS carrying nonsynonymous HLH genetic variants compared to patients without MAS. To our knowledge, this is the first study to examine the frequency of HLH genetic variants in relation to MAS among patients with cSLE. Future studies are required to validate our findings.

Key Indexing Terms: genetic studies, hemophagocytic lymphohistiocytosis, macrophage activation syndrome, pediatric systemic lupus erythematosus, whole-exome sequencing, whole-genome sequencing

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[1]P. Lahiry, MD, PhD, FRCPC, D. Dominguez, MD, MSc, D.M. Levy, MD, MSc, FRCPC, L.W.K. Ng, BSc, Division of Rheumatology, SickKids; [2]S. Naumenko, PhD, The Centre for Computational Medicine, Research Institute, SickKids; [3]M. Couse, MSc, F. Liao, MSc, M. Misztal, BSc, Genetics & Genome Biology, Research Institute, SickKids; [4]A. Knight, MD, FRCPC, Division of Rheumatology and Research Institute, SickKids; [5]L.T. Hiraki, MD, FRCPC, ScD, Division of Rheumatology, SickKids, and Genetics & Genome Biology, Research Institute, SickKids, Toronto, Ontario, Canada.

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Address correspondence to Dr. L.T. Hiraki, Clinical Scientist, Division of Rheumatology, Genetics & Genome Biology, The Peter Gilgan Centre for Research and Learning (PGCRL), SickKids, 686 Bay Street, Toronto, ON M5G 0A4, Canada. Email: linda.hiraki@sickkids.ca.

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Macrophage activation syndrome (MAS) is a potentially fatal hyperinflammatory condition characterized by fever, hepatosplenomegaly, and cytopenias.[1] The hallmark of MAS is excessive proliferation and activation of macrophages and T lymphocytes, resulting in hypercytokinemia or cytokine storm.[1] The precise etiology of MAS is unknown and makes risk prediction and timely recognition difficult, delaying treatment and management.

MAS is a complication of rheumatic diseases, infections, and malignancies.[1] Among pediatric rheumatic diseases, MAS is a well-recognized complication of systemic juvenile idiopathic arthritis (sJIA), affecting 30% to 40% of patients.[1] MAS is increasingly recognized as a complication of childhood-onset systemic lupus erythematosus (cSLE), affecting up to 10% of patients.[2,3]

MAS is also known as secondary hemophagocytic lymphohistiocytosis (HLH), due to clinical and laboratory similarities

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with primary or familial HLH (fHLH).[1] fHLH syndromes are genetic defects of excessive immune activation. Although fHLH and MAS share abnormalities of inflammatory pathways, treatments for these diseases differ. For fHLH, the definitive treatment is bone marrow transplant, whereas MAS is treated by addressing the underlying rheumatic, infectious, or malignant process.

Genetics may play a role in MAS etiology. Prior studies of MAS complicating sJIA demonstrated up to 36% of patients with sJIA with MAS had at least 1 heterozygous fHLH genetic variant.[4] These findings suggest HLH genetic variants may lower the threshold for MAS in patients with sJIA. No study to date has examined the role of HLH genetic variants and MAS in cSLE. The aim of this study was to compare the prevalence of HLH genetic variants between patients with cSLE with and without MAS.

METHODS

Study populations. Our study patients were diagnosed and followed for cSLE (age < 18 yrs) at The Hospital for Sick Children (SickKids). All patients with cSLE met ≥ 4 American College of Rheumatology and/or the Systemic Lupus International Collaborating Clinics classification criteria for SLE.[5,6] The non-MAS cSLE group underwent whole-genome sequencing (WGS) for suspected monogenic lupus due to (1) young age at cSLE diagnosis (age < 11 yrs), (2) consanguinity, and/or (3) multiple affected first-degree relatives. MAS was diagnosed in patients with cSLE based on the expert opinion of a consultant pediatric rheumatologist and the investigators (LTH, DML). Fifteen MAS patients underwent whole-exome sequencing (WES) only, 4 had WGS, and 2 had both WES and WGS. The patients with MAS who underwent WGS were suspected of monogenic lupus.

Demographic, clinical, and laboratory data were prospectively collected and extracted from the SickKids Lupus database and supplemented with medical record review. Demographic features included sex, age at cSLE diagnosis, duration of follow-up, ethnicity (based on Census Canada categories), and SLE and MAS clinical and laboratory features (Supplementary Methods, available with the online version of this article).

We applied the preliminary diagnostic criteria for MAS proposed by the (1) Paediatric Rheumatology European Society (PReS) Lupus Working Group in cSLE,[2] (2) recursive partitioning decision rule for MAS vs active cSLE,[7] and (3) fHLH criteria (HLH-2004)[8] to our MAS cSLE cohort, and we reported the number and proportion of patients satisfying each criterion.

Genotyping platforms and variant calling. The patients with WES underwent paired-end sequencing on the HiSeq 2500 (Illumina) platform with a read depth of 70X to 118X. The patients with WGS underwent paired-end sequencing on the HiSeq X (Illumina) platform with a read depth of 37X to 40X. Variants were called according to Genome Analysis Toolkit (GATK) Best Practices recommendations[9] and annotated with ANNOVAR (https://annovar.openbioinformatics.org) at The Centre for Applied Genomics, SickKids (annotation pipeline v26.2, v.26.5). Small variants were recalled and reannotated with gatk4/bcbio/VEP/vcfanno gemini. The majority of patients were also genotyped on the Multiethnic Genotyping Array or the Global Screening Array (Illumina). Ancestry was genetically inferred from these arrays using the ADMIXTURE software (dalexander.github.io/admixture/index.html) with the Haplotype Reference Consortium (haplotype-reference-consortium.org) as the referent. Concordance was compared with self-reported ethnicity. Patients were categorized into one of these ancestral groups: European, East Asian, South Asian, African, Amerindian, or admixed (patients with < 80% of genetic segments from a single ancestral group). If genetically inferred ancestry was not available, self-reported ethnicity was used.

Variant filtering. We focused on single-nucleotide exonic variants in the following 16 HLH-associated genes: *AP3B1*, *BLOC1S6*, *CD27*, *GATA2*, *ITK*, *LYST*, *MAGT1*, *NLRC4*, *PRF1*, *RAB27A*, *SH2D1A*, *SLC7A7*, *STX11*, *STXBP2*, *UNC13D*, *XIAP*.[11] We then restricted to low-frequency (minor allele frequency [MAF] < 0.05) variants according to the Genome Aggregation Database (gnomAD) referent database (https://gnomad.broadinstitute.org). We further restricted to "nonsynonymous" variants defined as missense, stop gain/loss, frameshift, or nonsense variants. In patients sequenced with both WES and WGS platforms, we compared concordance of variant frequencies. To determine if the HLH variants on the same chromosome or within the same gene were independent, we tested linkage disequilibrium (LD) using LDlink (https://ldlink.nci.nih.gov/?tab=ldmatrix).[12]

The pathogenic effects of each nonsynonymous variant were predicted using Combined Annotation Dependent Depletion (CADD), which integrates prediction programs (SIFT, GERP++, PolyPhen, CPG distance, GC content) to create an unbiased tool.[13] We considered CADD Phred-scaled scores ≥ 10 as deleterious.

Statistical analysis. We calculated counts and proportions for categorical variables and median and IQR for continuous variables. We compared characteristics between MAS and non-MAS cohorts using 2-tailed Fisher exact tests for categorical variables and Kruskall-Wallis test for continuous variables ($P < 0.005$).

For our main analysis, we compared the MAS and non-MAS cohorts for the proportion of patients carrying HLH variants using 2-tailed Fisher exact test ($P < 0.05$). We also tested the association between HLH variant carrier status and the odds of MAS in logistic regression univariate and multivariable models, adjusted for sex and ancestry categories.

Variant MAFs from the MAS and non-MAS cohorts were compared to the largest publicly available ancestry-specific population MAFs, from gnomAD v2.1.1 exome dataset (accessed November 19, 2020) or from Trans-Omics for Precision Medicine (TOPMed; accessed November 19, 2020, from: https://bravo.sph.umich.edu/freeze8/hg38) using 2-tailed Fisher exact test ($P < 0.005$).

Institutional Research Ethics Board (REB) approval was obtained prior to initiation of the study (REB #1000035186). Patient consent was not applicable for this study.

RESULTS

The study cohort included 81 patients with cSLE, 19 of whom had MAS. In the total cohort, the majority were female (85%), with a median age at cSLE diagnosis of 9 years (IQR 7-10.7 yrs). Patients in the MAS cohort had an older age at cSLE diagnosis compared to the non-MAS cohort (12.25 [IQR 8.57-14.66] yrs vs 8.57 [IQR 7.00-10.00] yrs, $P = 1.82 \times 10^{-3}$; Table 1). The non-MAS cohort had a longer duration of follow-up than the MAS cohort (7.39 [IQR 4.22- 9.82] yrs vs 3.15 [IQR 1.50-5.90] yrs, $P = 9.89 \times 10^{-4}$; Table 1).

Ancestry was genetically inferred in 78 patients (96%) and in the 3 additional patients, ancestry was based on the self-report (Table 1). The concordance of genetically inferred ancestry and self-reported ethnicity was high (> 96%). Overall, the majority of patients were admixed (30%) or European (26%). In the MAS cohort, the majority of patients were East Asian (32%) or admixed (26%); in the non-MAS cohort, the majority were European (31%) or admixed (31%). Ancestry distribution was not significantly different between the MAS and non-MAS cohorts ($P = 0.08$).

Overall, the most common clinical features were malar rash (77%) and arthritis (64%). Forty percent of patients had

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Table 1. Demographic, clinical and laboratory features of patients with cSLE with and without macrophage activation syndrome (MAS).

	MAS, n = 19	Non-MAS, n = 62	P[a]
Sex, female	16 (84)	53 (85)	> 0.99
Age of cSLE diagnosis, yrs	12.25 (8.57-14.66)	8.57 (7.00-10.00)	**1.82E-03**
Duration of follow-up, yrs	3.15 (1.50-5.90)	7.39 (4.22-9.82)	**9.89E-04**
Ancestry[b]			
European	2 (11)	19 (31)	0.08
East Asian	6 (32)	7 (11)	
South Asian	4 (21)	5 (8.1)	
African	2 (11)	7 (11)	
Amerindian	0	5 (8.1)	
Admixed	5 (26)	19 (31)	
SLE clinical features			
Fever	18 (95)	18 (29)	**1.00E-03**
Malar rash	13 (68)	49 (79)	0.36
Photosensitive rash	0	27 (44)	**2.00E-04**
Oral or nasal ulcers	7 (37)	26 (42)	0.79
Alopecia	10 (53)	29 (47)	0.79
Arthritis	10 (53)	42 (67)	0.28
Serositis (pericarditis, pleuritis, or peritoneal effusion)	5 (26)	11 (17)	0.51
LN[c]	12 (63)	20 (32)	0.03
Mesangial (class II)	1 (8)	2 (10)	1.00
Membranous (class V)	1 (8)	7 (35)	0.20
Proliferative (class III and IV)	11 (92)	15 (75)	0.37
Mixed (membranous/proliferative)	1 (8)	3(15)	1.00
Neuropsychiatric (delirium, psychosis, or seizures)	3 (16)	13 (21)	0.75
Headaches	3 (16)	15 (24)	0.54
SLE laboratory features			
Hematological[d]			
Leukopenia	18 (95)	30 (48)	**1.70E-03**
Thrombocytopenia	7 (37)	23 (37)	1.00
Lymphopenia	18 (95)	46 (57)	0.06
Coombs-positive hemolytic anemia	10 (53)	19 (31)	0.10
Hypocomplementemia	19 (100)	36 (58)	**2.00E-04**
SLE autoantibodies			
ANA	19 (100)	60 (97)	1.00
Anti-dsDNA	18 (95)	39 (63)	0.01
Anti-Sm	10 (53)	23 (37)	0.29
aPL[e]	9 (47)	26 (42)	0.79
LAC	1 (11)	10 (38)	0.22
aCL	9 (100)	22 (85)	0.55

Values are expressed as n (%) or median (IQR). Values in bold are statistically significant at $P < 0.005$. [a] Fisher exact test was used for sex, presence of features, and antibodies; Kruskall-Wallis test was used for age and duration of follow-up; logistic regression was used for the ancestry group. [b] Genetically inferred ancestry was determined using the 2M MEGA array or GSA genotyped data; else, self-reported ethnicity used. [c] Percent based on total patients with LN. [d] Hematological cut-offs based on SLICC criteria. [e] Percent based on total aPL. aCL: anticardiolipin antibody; ANA: antinuclear antibody; cSLE: childhood-onset systemic lupus erythematosus; LAC: lupus anticoagulant; LN: lupus nephritis; GSA: Global Screening Array; MEGA: Multi-Ethnic Genotyping Array; SLE: systemic lupus erythematosus; SLICC: Systemic Lupus International Collaborating Clinics.

biopsy-confirmed lupus nephritis and 20% had neuropsychiatric lupus (Table 1). The majority were antinuclear antibody positive, 78% had hematological involvement, and 68% had hypocomplementemia. Fevers, photosensitive rash, leukopenia, and hypocomplementemia were more common in patients with MAS compared to those without MAS.

MAS was diagnosed at SLE diagnosis in the majority of patients (84%), and 16% had MAS triggered by a documented viral infection. The most common MAS clinical feature was fever (95%, Supplementary Table S1, available with the online version of this article). The median ferritin level in the MAS patients was 2212 (IQR 1035-4566) µg/L. In addition to

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hyperferritinemia, the other common laboratory abnormalities in patients with MAS were elevated lactose dehydrogenase (LDH; 100% had values > 567 U/L), elevated aspartate aminotransferase (AST; 100% had values > 40 U/L), hypertriglyceridemia (76% had values > 1.78 mmol/L) and cytopenia of ≥ 2 cell lines (79% had leukopenia, anemia, and/or thrombocytopenia; Supplementary Table S1). Only 1 of the 10 patients with MAS with bone marrow aspiration had evidence of macrophage hemophagocytosis.

When applying the MAS classification criteria, all patients in the MAS cohort met the PReS Lupus Working Group cSLE preliminary diagnostic criteria for MAS[2] and none met the HLH-2004 criteria.[8] Based on the decision rule to differentiate MAS from active cSLE, all met the criteria for MAS.[7]

In the total cohort, 60% of patients with cSLE carried ≥ 1 low-frequency (MAF < 0.05) exonic HLH variant and 32% carried ≥ 2 low-frequency exonic HLH variants. Filtering to nonsynonymous HLH variants, 46% of patients carried ≥ 1 HLH variant and 16% carried ≥ 2 HLH variants. All nonsynonymous HLH variants were independent (LD r^2 > 0.8). In 2 patients with both WES and WGS, no difference was found in variant frequency between platforms (data not shown).

There was no difference in the proportion of patients with or without MAS carrying ≥ 1 (37% vs 47%, *P* = 0.44) or ≥ 2 (16% vs 16%, *P* = > 0.99) exonic nonsynonymous HLH variants (Table 2). No difference was found in the number of variants per gene in either cohort (data not shown). When further restricted to pathogenic variants (CADD scores ≥ 10), there was no difference in the proportion of patients with vs without MAS (47% vs 53%, *P* = 0.79; Table 2). The presence of a rare HLH variant increased the odds of MAS; however, this was not statistically significant (multivariable adjusted model: odds ratio 1.07, 95% CI 0.32-3.52, *P* = 0.91).

In the total cohort, 84 unique low-frequency exonic HLH variants were found in 50 patients (16 variants in 11 patients with MAS; 67 variants in 39 patients without MAS). Of those variants, 47 were nonsynonymous (11 variants in 7 patients with MAS [Table 3]; 36 variants in 30 patients without MAS [Supplementary Table S2, available with the online version of this article]). Sixty-eight percent of these variants have been previously reported in patients with fHLH but none are pathogenic.

One patient in the MAS group carried 2 heterozygous variants in the *LYST* gene (Table 3). In the non-MAS group, 2 patients carried 2 heterozygous variants in the *AP3B1* gene, and 1 patient carried 2 heterozygous variants in the *LYST* gene (Supplementary Table S2, available with the online version of this article). No patients were homozygous for HLH variants. No variant was shared between patients in the MAS cohort. In the non-MAS cohort, 4 variants were shared by 2 patients and 1 variant was shared by 3 patients. The *UNC13D* gene variant (rs35037984) was the only shared variant between both cohorts (Table 3; Supplementary Table S2).

In the MAS cohort, only the *STXBP2* variant had a higher MAF when compared to the MAF in the ancestrally specific general population (Table 3). In the non-MAS cohort, the MAFs of 4 variants were higher compared to the general population (ancestrally specific, when available; Supplementary Table S2, available with the online version of this article). In total, 5 rare variants (1 in the MAS cohort, 4 in the non-MAS cohort) were not identified in gnomAD or TOPMed.

DISCUSSION

In a multiethnic cSLE cohort, we found no difference in the proportion of patients with MAS who carried an HLH variant compared to patients with cSLE without MAS. Additionally, no significant difference was found in the frequency of HLH variants in patients with cSLE and MAS compared with ancestrally matched non-SLE populations (gnomAD or TOPMed). Our findings suggest that prevalence of known HLH variants is similar in patients with cSLE with and without MAS.

Our results are contrary to prior studies of HLH variants and MAS in sJIA and influenza. A study of 14 patients with sJIA with MAS found 36% (n = 5) carried HLH variants compared to 14% (4/29) of sJIA without MAS (*P* = 0.098).[4] In a case series of H1N1 influenza cases with MAS, 36% (5/14) carried HLH gene variants.[14] Our conflicting observations likely reflect sampling differences, differences in the number of analyzed genes and variants, and potential differences in MAS pathogenesis complicating sJIA and H1N1 compared to cSLE.

The SLE clinical and laboratory features in our cohort were comparable to prior studies.[2] Our study cohort had a younger age at cSLE diagnosis (median age of 9 yrs) compared with prior SLE studies,[2,3] which can be explained by the use of the available

Table 2. HLH low-frequency variants in patients with cSLE with and without macrophage activation syndrome (MAS).

	MAS, n = 19	Non-MAS, n = 62	*P*
Patients with ≥ 1 HLH variants			
Exonic	10 (53)	39 (61)	0.44
Nonsynonymous	7 (37)	30 (47)	0.44
Patients with ≥ 2 HLH variants			
Exonic	4 (21)	22 (35)	0.28
Nonsynonymous	3 (16)	10 (16)	> 0.99
Patients with CADD score ≥ 10	9 (47)	33 (53)	0.79

CADD: combined annotation dependent depletion; cSLE: childhood-onset systemic lupus erythematosus; HLH: hemophagocytic lymphohistiocytosis; MAS: macrophage activation syndrome.

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Table 3. HLH low-frequency nonsynonymous variants in patients with cSLE with macrophage activation syndrome (MAS).

Chr	Start Position	Gene	Ref/Alt Allele	SNP[a]	AA Change	CADD score[b]	MAS MAF[c]	Non-MAS MAF[c]	Referent Population[d]	
									MAF	Ancestry
1	235894174	*LYST*	T/C	rs140934482	K3006R	23.90	0.03	0	9.61E-04	EUR
1	235929555	*LYST*	G/A	rs146591126	T1982I	17.10	0.03	0	0.01	EUR
1	235969329	*LYST*	T/G	rs147756847	E1036A	21.70	0.03	0	9.03E-04	EAS
1	235922671	*LYST*	T/G	rs559751044	G2161A	1.00E-03	0.03	0	4.98E-03	EUR
2	32475063	*NLRC4*	A/T	rs61741169	W624R	24.1	0.03	0	1.80E-03	ADM
5	77335091	*AP3B1*	G/A	rs146624866	T813I	22.6	0.03	0	0.02	AFR
5	156641315	*ITK*	G/A	NR[e]	D147N	11.0	0.03	0	NR	EUR
10	72358845	*PRF1*	G/A	rs368524364	A211V	20.9	0.03	0	0.02	SAS
17	73836317	*UNC13D*	T/C	rs35037984	I283V	23.9	0.03	0	2.69E-03	ADM
17	73826491[f]	*UNC13D*	G/A	rs61754871	A928C, A909C, A10C	17.1	0.03	0.02	0.03	EUR
19	7711153	*STXBP2*	C/T	rs142105943	R459W, R470W, R456W, R154W	21.7	0.03	0	0	SAS

[a] dbSNP database: https://www.ncbi.nlm.nih.gov/snp/. [b] CADD Phred-scaled score: https://cadd.gs.washington.edu. [c] Calculations for MAF: MAS = no. of alleles in cohort/(2 × 19); non-MAS = no. of alleles in cohort/(2 × 62). [d] Ancestry-specific referent MAF of variant reported, unless cSLE cohort variant from admixed population; variants in referent population variant from gnomAD exome dataset or TOPMed accessed on November 19, 2020. [e] NR = variant not reported in dbSNP or referent population of gnomAD or TOPMed. [f] Same variant found in MAS and non-MAS cohorts (see Supplementary Material, available with the online version of this article). AA: amino acid; ADM: admixed; AFR: African; CADD: combined annotation dependent depletion; Chr: chromosome; cSLE: childhood-onset systemic lupus erythematosus; EAS: East Asian; EUR: European (non-Finnish); gnomAD: Genome Aggregation Database; HLH: hemophagocytic lymphohistiocytosis; MAF: minor allele frequency; Ref/Alt: reference/alternative; SAS: South Asian; SNP: single-nucleotide polymorphism; TOPMed: Trans-Omics for Precision Medicine.

non-MAS comparator group selected for WGS based on young age of cSLE onset. The MAS features of our patients were similar to those patients with cSLE with MAS in the literature.[2,15] Hypocomplementemia was more common in the MAS group, which has been previously reported in MAS.[2,15]

We identified 47 unique nonsynonymous low-frequency HLH variants in our study population, 3 of which have been reported in prior studies of MAS in H1N1 and sJIA.[4,14] In H1N1-infected patients, the heterozygous *LYST* variant (rs146591126) was more frequent compared to the general population.[14] This variant was found in 1 of the 19 patients with cSLE with MAS, and its frequency was not different compared to the ancestrally matched general population. The other 2 previously reported variants in MAS in H1N1 (rs115330112 in *LYST*)[14] and in sJIA (rs35037984 in *UNC13D*)[4] were found in our non-MAS cSLE cohort and did not have higher frequency compared to the general population. This suggests that unlike MAS in H1N1-infected or in patients with sJIA, these 2 variants in *UNC13D* and *LYST* are unlikely to have pathogenic roles in MAS complicating cSLE.

Our study findings should be considered in light of some limitations. Currently, there is no gold standard for MAS diagnosis and, as such, MAS diagnosis was based on expert opinion by pediatric rheumatologists. MAS cases were independently reviewed by pediatric rheumatologists, and in the event of disagreement, consensus was reached among the pediatric rheumatology consultant and the investigators (LTH, DML).[3] MAS misclassification would diminish our power to detect a difference in HLH variant frequency between the MAS and non-MAS groups. We had limited power (< 50%) to detect a difference between the MAS and non-MAS cohorts. However, our sample size was larger than prior studies investigating the role of HLH variants in MAS.[4,14] Our non-MAS comparator group comprised patients suspected of monogenic forms of cSLE, which potentially enriched for rare variants. When reviewing clinical characteristics of this non-MAS cSLE population, apart from the younger age of cSLE diagnosis, the prevalence of clinical and laboratory SLE features were comparable to other cSLE-reported cohorts.[2,3] Our study focused on 16 known HLH genetic variants in unrelated probands.[11] Although our gene list was more comprehensive than prior MAS genetic studies, the lack of trio analyses meant we could not identify novel variants or genes for MAS in cSLE.[4,14] We focused on exonic regions of the HLH gene variants, precluding examination of noncoding or structural variations such as copy number variants, since studies have demonstrated that most disease-causing variants are found in exonic regions.[16] Our MAS and non-MAS cohorts were mostly sequenced on different platforms. Read depth uniformity differences between WGS and WES may have resulted in more variants identified by WGS in the non-MAS cohort compared to WES in the MAS cohort, MAS cohort, which possibly diminished our power to detect more HLH variants in the MAS cohort.[16] However, in patients with both WES and WGS, there was 100% concordance in nonsynonymous variant frequencies, suggesting the sequencing platform difference between both cohorts had minimal impact on our conclusions.

Our study has several strengths. To our knowledge, this is the first study focusing on HLH genetic variants and MAS in patients with cSLE. We studied a large multiethnic cSLE patient cohort with detailed genetic sequencing and phenotypic data. We also included an ancestrally diverse population and validated self-reported ethnicity with genetically inferred ancestry.

In conclusion, this large multiethnic study of patients with cSLE did not have a difference in the proportion of HLH

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variants carried in patients with cSLE with MAS compared to those without MAS. Additional studies of large independent cohorts are required to validate our findings, using methods to interrogate both coding and noncoding regions of the genome.

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ONLINE SUPPLEMENT

Supplementary material accompanies the online version of this article.

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