

**Running title (4 words):** HLH variants in cSLE-MAS

**Full title: Hemophagocytic Lymphohistiocytosis Gene Variants in Childhood-Onset SLE with Macrophage Activation Syndrome**

**Authors:** Piya Lahiry (0000-0003-1427-4945)<sup>1</sup>, Sergey Naumenko (0000-0002-6218-7734)<sup>2</sup>, Madeline Couse<sup>3</sup>, Fangming Liao<sup>4</sup>, Daniela Dominguez (0000-0001-7001-2494)<sup>5</sup>, Andrea Knight (0000-0003-4278-9049)<sup>6</sup>, Deborah M. Levy (0000-0003-1023-9499)<sup>7</sup>, Melissa Misztal (0000-0002-1191-4364)<sup>8</sup>, Lawrence W.K. Ng (0000-0003-1758-4359)<sup>9</sup>, Linda T. Hiraki (0000-0002-6690-8148)<sup>10\*</sup>

**Keywords:** Macrophage Activation Syndrome (MAS), Pediatric systemic lupus erythematosus, Genetic studies, Whole Exome Sequencing (WES), Hemophagocytic Lymphohistiocytosis (HLH)

**Financial Support:** Dr. Hiraki - The Arthritis Society, Stars Career Development Award; Canada Research Chair in Genetics of Rare Systemic Inflammatory Diseases

**Author Information:**

<sup>1</sup>P. Lahiry, MD PhD FRCPC, Division of Rheumatology, SickKids, Toronto, Ontario, Canada;

<sup>2</sup>S. Naumenko, PhD, The Centre for Computational Medicine, Research Institute, SickKids, Toronto, Ontario, Canada;

<sup>3</sup>M. Couse, MSc, Genetics & Genome Biology, Research Institute, SickKids, Toronto, Ontario, Canada;

<sup>4</sup>F. Liao, MSc, Genetics & Genome Biology, Research Institute, SickKids, Toronto, Ontario, Canada;

<sup>5</sup>D. Dominguez, MD, MSc, Division of Rheumatology, SickKids, Toronto, Ontario, Canada;

<sup>6</sup>A. Knight, MD FRCPC, Division of Rheumatology and Research Institute, SickKids, Toronto, Ontario, Canada;

<sup>7</sup>D.M. Levy, MD MSc FRCPC, Division of Rheumatology, SickKids, Toronto, Ontario, Canada;

<sup>8</sup>M. Misztal, BSc, Genetics & Genome Biology, Research Institute, SickKids, Toronto, Ontario, Canada;

<sup>9</sup>L.W.K. Ng, BSc, Division of Rheumatology, SickKids, Toronto, Ontario, Canada;

<sup>10</sup>L.T. Hiraki, MD, FRCPC, ScD, Division of Rheumatology, SickKids; Genetics & Genome Biology, Research Institute, SickKids, Toronto, Ontario, Canada;

**Disclosures:** The authors have no disclosures or conflicts of interest to declare

**\*Corresponding Author:**

Linda T. Hiraki, MD, FRCPC, ScD

Clinical Scientist, Division of Rheumatology

Genetics & Genome Biology, The Peter Gilgan Centre for Research and Learning (PGCRL)

SickKids, 686 Bay Street, Toronto, Ontario, Canada, M5G 0A4, Email: [linda.hiraki@sickkids.ca](mailto:linda.hiraki@sickkids.ca)

**Statement of Ethics and Consent:** REB approval was obtained for this study (REB #1000035186); patient consent was not applicable for this study.

## ABSTRACT

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

**Methods** We enrolled patients from the Lupus Clinic at SickKids, Toronto. Demographic and clinical features were extracted from the lupus 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 (read-depth:70-118X) or whole-genome sequencing. Non-MAS patients had whole-genome sequencing (read-depth:37-40X). In 16 HLH genes we prioritized low-frequency (minor allele frequency (MAF) <0.05) exonic non-synonymous variants. We compared the proportion of patients with and without MAS carrying HLH variants (Fisher's exact test,  $P < 0.05$ ). MAFs were compared to an ancestrally-matched general population (TOPMed and gnomAD).

**Results** The study included 81 patients, 19 with MAS. We identified 47 unique low-frequency non-synonymous HLH variants. There was no difference in the proportion of MAS and non-MAS patients carrying  $\geq 1$  HLH variants (37% versus 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 MAS patients carrying non-synonymous HLH genetic variants compared to patients without

Accepted Article

MAS. This is the first study to examine the frequency of HLH genetic variants in relation to MAS among cSLE patients. Future studies are required to validate our findings.

## INTRODUCTION

Macrophage activation syndrome (MAS) is a potentially fatal hyperinflammatory condition characterized by fever, hepatosplenomegaly, and cytopenias.<sup>1</sup> The hallmark of MAS is excessive proliferation and activation of macrophages and T-lymphocytes resulting in hypercytokinemia or 'cytokine storm'.<sup>1</sup> The precise etiology of MAS is unknown which makes risk prediction and timely recognition difficult, delaying treatment and management.

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

MAS is also known as secondary hemophagocytic lymphohistiocytosis (HLH), due to clinical and laboratory similarities with primary or familial HLH.<sup>1</sup> Familial HLH (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 sJIA patients with MAS had at least one heterozygous fHLH genetic variant.<sup>4</sup> 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 cSLE patients with and without MAS.

## PATIENT AND METHODS

*Study Populations.* Our study patients were diagnosed and followed for cSLE (<18y) at The Hospital for Sick Children (SickKids). All cSLE patients met  $\geq 4$  American College of Rheumatology (ACR) and/or the Systemic Lupus International Collaborating Clinics (SLICC) classification criteria for SLE.<sup>5,6</sup> The non-MAS cSLE group underwent whole genome sequencing (WGS) for suspected monogenic lupus due to: [1] young age at cSLE diagnosis (<11y), [2] consanguinity and/or [3] multiple affected first-degree relatives. MAS was diagnosed in cSLE patients based on Pediatric Rheumatologist expert opinion (LH, DL, ES). Fifteen MAS patients underwent whole exome sequencing (WES) only, four had WGS and two had both WES and WGS. The MAS patients 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), SLE and MAS clinical and laboratory features (Supplementary Method).

We applied the preliminary diagnostic criteria for MAS proposed by the [1] Paediatric Rheumatology European Society (PReS) Lupus Working Group in cSLE,<sup>2</sup> [2] the recursive partitioning decision rule for MAS versus active cSLE<sup>7</sup> and [3] familial HLH criteria (HLH-2004)<sup>8</sup> to our MAS cSLE cohort and 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 Illumina HiSeq 2500 platform with a read depth of 70-118x. The patients with WGS underwent paired-end sequencing on the Illumina HiSeq X platform with a read depth of 37-40x. Variants were called according to GATK best practices<sup>9</sup> and annotated with ANNOVAR at The Centre for Applied Genomics (TCAG), 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 (MEGA) or the Global Screening Array (GSA). Ancestry was genetically inferred from these arrays using the ADMIXTURE<sup>10</sup> software with HRC (Haplotype Reference Consortium) 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*.<sup>11</sup> We then restricted to low frequency (minor allele frequency [MAF] <0.05) variants according to the gnomAD referent database. We further restricted to 'non-synonymous' 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>).<sup>12</sup>

The pathogenic effects of each non-synonymous variant were predicted using CADD (Combined Annotation Dependent Depletion), which integrates prediction programs (SIFT, GERP++, PolyPhen, CPG distance, GC content) to create an unbiased tool.<sup>13</sup> We considered CADD PHRED-scaled scores  $\geq 10$  as deleterious.

*Statistical Analysis.* We calculated counts and proportions for categorical variables and median and interquartile ranges (IQR) for continuous variables. We compared characteristics between MAS and non-MAS cohorts using two-tailed Fisher's exact tests for categorical variables and Kruskal-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 two-tailed Fisher's 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 at <https://gnomad.broadinstitute.org/>) or from TOPMed (accessed November 19, 2020 at <https://bravo.sph.umich.edu/freeze8/hg38/>) using two-tailed Fisher's exact test ( $P < 0.005$ ).

Institutional Research Ethics Board (REB) approval was obtained prior to initiation of the study (REB #1000035186).

## RESULTS

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

Ancestry was genetically inferred in 78 patients (96%) and in the three 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%) and 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 biopsy confirmed lupus nephritis and 20% had neuropsychiatric lupus (Table 1). The majority were ANA positive, 78% had hematological involvement and 68% had hypocomplementemia. Fevers, photosensitive rash, leukopenia, and hypocomplementemia were more common in MAS patients 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 1). The median ferritin level in the MAS patients was 2212 ug/L (IQR: 1035-4566). In addition to hyperferritinemia, the other common laboratory abnormalities



in MAS patients were elevated lactose dehydrogenase (LDH) (100% had values > 567 units/L), elevated aspartate aminotransferase (AST) (100% had values > 40 unit/L), hypertriglyceridemia (76% had values > 1.78 mmol/L) and cytopenia of  $\geq 2$  cell lines (79% had leukopenia, anemia and/or thrombocytopenia) (Supplementary Table 1). Only one of the 10 MAS patients with bone marrow aspiration had evidence of macrophage hemophagocytosis.

When applying the MAS classification criteria, all patients in the MAS cohort met the PRS Lupus Working Group in cSLE preliminary diagnostic criteria for MAS<sup>2</sup> and none met the HLH\_2004 criteria.<sup>8</sup> Based on the decision rule to differentiate MAS versus active cSLE, all met the criteria for MAS.<sup>14</sup>

In the total cohort, 60% of cSLE patients carried  $\geq 1$  low-frequency (MAF<0.05) exonic HLH-variant and 32% carried  $\geq 2$  low-frequency exonic HLH-variants. Filtering to non-synonymous HLH-variants, 46% of patients carried  $\geq 1$  HLH-variant and 16% carried  $\geq 2$  HLH-variants. All non-synonymous HLH-variants were independent (LD  $r^2 > 0.8$ ). In two patients with both WES and WGS, no difference was found in variant frequency between platforms.

There was no difference in the proportion of MAS versus non-MAS patients carrying  $\geq 1$  (37% versus 47%,  $P=0.44$ ) or  $\geq 2$  (16 % versus 16%,  $P=1.00$ ) exonic non-synonymous HLH-variants (Table 2). No difference found in the number of variants per gene in either cohort (data not shown). When further restricted to pathogenic variants (CADD scores  $\geq 10$ ) there was no difference in the proportion of patients with MAS versus non-MAS (47% versus 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: OR 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 MAS patients; 67 variants in 39 non-MAS patients). Of those variants, 47 were non-synonymous (11 variants in 7 MAS patients, Table 3; 36 variants in 30 non-MAS patients, Supplementary Table 2). Sixty-eight percent of these variants have been previously reported in fHLH patients but none are pathogenic.

One patient in the MAS group carried two heterozygous variants in the *LYST* gene (Table 3). In the non-MAS group, two patients carried two heterozygous variants in the *AP3B1* gene and one patient carried two heterozygous variants in the *LYST* gene (Supplementary Table 2). 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 and Supplementary Table 2).

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, 4 variants' MAFs were higher compared to the general population (ancestrally-specific, when available) (Supplementary Table 2). 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 multi-ethnic cSLE cohort we found no difference in the proportion of patients with MAS who carried an HLH-variant compared to cSLE patients 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 cSLE patients with and without MAS.

Our results are contrary to prior studies of HLH-variants and MAS in sJIA and influenza. A study of 14 sJIA patients with MAS found 36% (n=5) carried HLH-variants compared to 14% (4/29) of sJIA without MAS ( $P=0.098$ ).<sup>4</sup> In a case-series of H1N1 influenza cases with MAS, 36% (5/14) carried HLH-gene variants.<sup>15</sup> Our conflicting observations likely reflect sampling differences, differences in the number of analyzed genes and variants and potentially 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.<sup>2</sup> Our study cohort had a younger age at cSLE diagnosis (median age of 9) compared with prior lupus studies,<sup>2,3</sup> which can be explained by the use of the available non-MAS comparator group selected for WGS based on young age of cSLE onset. The MAS features of our patients were similar to those cSLE patients with MAS in the literature.<sup>2,16</sup> Hypocomplementemia was more common in the MAS group, which has been previously reported in MAS.<sup>2,16</sup>

We identified 47 unique non-synonymous low-frequency HLH-variants in our study population, three of which have been reported in prior studies of MAS in H1N1 and sJIA.<sup>4,15</sup> In H1N1-infected patients, the heterozygous *LYST* variant (rs146591126) was more frequent compared to the general population.<sup>15</sup> This variant was found in one of the 19 MAS cSLE patients and its frequency was not different compared to the ancestrally-matched general population. The other two previously reported variants in MAS in H1N1 (rs115330112 in *LYST*)<sup>15</sup> and in sJIA (rs35037984 in *UNC13D*)<sup>4</sup> 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 sJIA patients these two 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 investigators (LH, DL, ES).<sup>3</sup> 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.<sup>4,15</sup> Our non-MAS comparator group was comprised of patients suspected of monogenic forms of cSLE. This non-MAS comparator group was 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.<sup>2,3</sup> Our study focused on 16 known HLH genetic variants in unrelated probands.<sup>11</sup> 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.<sup>4,15</sup> We focused on exonic regions of the HLH-gene variants, precluding examination of non-coding or structural variations such as copy number variants (CNVs), since studies have demonstrated that most disease-causing variants are found in exonic regions.<sup>17</sup> 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 and possibly diminishing our power to detect more HLH-variants in the MAS cohort.<sup>17</sup> However, in patients with both WES and WGS there was 100% concordance in non-synonymous variant frequencies, suggesting the sequencing platform difference between both cohorts had minimal impact on our conclusions.

Our study has several strengths. This was the first study focusing on HLH genetic variants and MAS in cSLE patients. We studied a large multi-ethnic 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 multi-ethnic study of cSLE patients did not have a difference in the proportion of HLH-variants carried in MAS cSLE compared to non-MAS cSLE patients. Additional studies of large independent cohorts are required to validate our findings, using methods to interrogate both coding and non-coding regions of the genome.

## REFERENCES

1. Henderson LA, Cron RQ. Macrophage Activation Syndrome and Secondary Hemophagocytic Lymphohistiocytosis in Childhood Inflammatory Disorders: Diagnosis and Management. *Paediatr Drugs* 2020;**22**:29-44. (review).
2. Parodi A, Davi S, Pringe AB, et al. Macrophage activation syndrome in juvenile systemic lupus erythematosus: a multinational multicenter study of thirty-eight patients. *Arthritis Rheum* 2009;**60**:3388-99.
3. Borgia RE, Gerstein M, Levy DM, Silverman ED, Hiraki LT. Features, Treatment, and Outcomes of Macrophage Activation Syndrome in Childhood-Onset Systemic Lupus Erythematosus. *Arthritis Rheumatol* 2018;**70**:616-24.
4. Kaufman KM, Linghu B, Szustakowski JD, et al. Whole-exome sequencing reveals overlap between macrophage activation syndrome in systemic juvenile idiopathic arthritis and familial hemophagocytic lymphohistiocytosis. *Arthritis Rheumatol* 2014;**66**:3486-95.
5. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997;**40**:1725.
6. Petri M, Orbai AM, Alarcon GS, et al. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. *Arthritis Rheum* 2012;**64**:2677-86.
7. Gerstein M, Borgia RE, Dominguez D, et al. Predicting Macrophage Activation Syndrome in Childhood-onset Systemic Lupus Erythematosus Patients at Diagnosis. *J Rheumatol* 2021;**48**:1450-7.
8. Henter JI, Horne A, Arico M, et al. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer* 2007;**48**:124-31.
9. Van der Auwera GA, Carneiro MO, Hartl C, et al. From FastQ data to high confidence variant calls: the Genome Analysis Toolkit best practices pipeline. *Curr Protoc Bioinformatics* 2013;**43**:11 0 1- 0 33.
10. Alexander DH, Novembre J, Lange K. Fast model-based estimation of ancestry in unrelated individuals. *Genome Res* 2009;**19**:1655-64.
11. Schulert GS, Zhang K. Genetics of acquired cytokine storm syndromes. In: Cron RQ, Behrens EM, eds. *Cytokine Storm Syndrome*. Switzerland: Springer; 2019:113-29.
12. Machiela MJ, Chanock SJ. LDlink: a web-based application for exploring population-specific haplotype structure and linking correlated alleles of possible functional variants. *Bioinformatics* 2015;**31**:3555-7.
13. Rentzsch P, Witten D, Cooper GM, Shendure J, Kircher M. CADD: predicting the deleteriousness of variants throughout the human genome. *Nucleic Acids Res* 2019;**47**:D886-D94.
14. Gerstein M, Borgia RE, Dominguez D, et al. Predicting macrophage activation syndrome (MAS) in childhood-onset systemic lupus erythematosus (cSLE) patients at diagnosis. *J Rheumatol* 2020.
15. Schulert GS, Zhang M, Fall N, et al. Whole-Exome Sequencing Reveals Mutations in Genes Linked to Hemophagocytic Lymphohistiocytosis and Macrophage Activation Syndrome in Fatal Cases of H1N1 Influenza. *J Infect Dis* 2016;**213**:1180-8.

16. Gorelik M, Torok KS, Kietz DA, Hirsch R. Hypocomplementemia associated with macrophage activation syndrome in systemic juvenile idiopathic arthritis and adult onset still's disease: 3 cases. *J Rheumatol* 2011;**38**:396-7.
17. Barbitoff YA, Polev DE, Glotov AS, et al. Systematic dissection of biases in whole-exome and whole-genome sequencing reveals major determinants of coding sequence coverage. *Sci Rep* 2020;**10**:2057.

**Table 1. Demographic, Clinical and Laboratory Features of childhood-onset systemic lupus erythematosus (cSLE) patients with and without macrophage activation syndrome (MAS)**

	MAS (n= 19)	non-MAS (n= 62)	<i>p</i> <sup>a</sup>
<b>Sex: Female [n (%)]</b>	16 (84)	53 (85)	1.00
<b>Median age of cSLE diagnosis, yrs (IQR)</b>	12.25 (8.60-14.66)	8.57 (7.00-10.00)	<u>1.82E-03</u>
<b>Median duration of follow-up, yrs (IQR)</b>	3.15 (1.50-5.90)	7.39 (4.22-9.82)	<u>9.89E-04</u>
<b>Ancestry: [n (%)]<sup>b</sup></b>			
European	2 (11)	19 (31)	
East Asian	6 (32)	7 (11)	
South Asian	4 (21)	5 (8.1)	0.08
African	2 (11)	7 (11)	
Amerindian	0	5 (8.1)	
Admixed	5 (26)	19 (31)	
<b>SLE Clinical features:</b>			
Fever	18 (95)	18 (29)	<u>1.00E-03</u>
Malar rash	13 (68)	49 (79)	0.36
Photosensitive rash	0	27 (44)	<u>2.00E-04</u>
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
Nephritis <sup>c</sup>	12 (63)	20 (32)	0.03
Mesangial (Class II) LN	1 (8)	2 (10)	1.00
Membranous (Class V) LN	1 (8)	7 (35)	0.20
Proliferative (Class III and IV) LN	11 (92)	15 (75)	0.37
Mixed - membranous/proliferative LN	1 (8)	3(15)	1.00
Neuropsychiatric (delirium, psychosis or seizures)	3 (16)	13 (21)	0.75
Headaches	3 (16)	15 (24)	0.54
<b>SLE Laboratory features:</b>			
<b>Hematological<sup>d</sup></b>			
Leukopenia	18 (95)	30 (48)	<u>1.70E-03</u>
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)	<u>2.00E-04</u>
<b>SLE autoantibodies:</b>			
ANA	19 (100)	60 (97)	1.00
Anti-dsDNA	18 (95)	39 (63)	0.01
Anti-Sm	10 (53)	23 (37)	0.29
Anti-phospholipid <sup>e</sup>	9 (47)	26 (42)	0.79
LAC	1 (11)	10 (38)	0.22



<b>ACL</b>	<b>9 (100)</b>	<b>22 (85)</b>	<b>0.55</b>
------------	----------------	----------------	-------------

<sup>a</sup>  $P < 0.005$  is statistically significant and underlined; Fisher's exact test was used for sex, presence of features and antibodies; Kruskal-Wallis test was used for age and duration of follow-up; Logistic regression was used for the ancestry group.

<sup>b</sup> Inferred Ancestry – using the 2M MEGA array or GSA genotyped-data; else, self-reported ethnicity used.

<sup>c</sup> % based on total Nephritis patients.

<sup>d</sup> Hematological cut-offs based on SLICC criteria.

<sup>e</sup> % based on total Anti-phospholipid; Antiphospholipid is comprised of ACL and/or LAC.

cSLE: childhood-onset systemic lupus erythematosus; MAS: macrophage activation syndrome; LN: lupus nephritis; LAC: lupus anticoagulant; ACL: anticardiolipin.

**Table 2. HLH low-frequency variants in childhood-onset systemic lupus erythematosus (cSLE) patients with and without macrophage activation syndrome (MAS)**

	MAS (n =19)	Non-MAS (n= 62)	<i>P</i>
<b>Patients with ≥1 HLH variants [n (%)]</b>			
<b>Exonic</b>	10 (53)	39 (61)	0.44
<b>Non-synonymous</b>	7 (37)	30 (47)	0.44
<b>Patients with ≥2 HLH variants [n (%)]</b>			
<b>Exonic</b>	4 (21)	22 (35)	0.28
<b>Non-synonymous</b>	3 (16)	10 (16)	1.00
<b>Patients with CADD score ≥ 10</b>	9 (47)	33 (53)	0.79

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

**Table 3. HLH low-frequency non-synonymous variants in childhood-onset systemic lupus erythematosus (cSLE) patients with macrophage activation syndrome (MAS)**

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

<sup>a</sup> dbSNP database <<https://www.ncbi.nlm.nih.gov/snp/>>.

<sup>b</sup> CADD PHRED-scaled score <<https://cadd.gs.washington.edu/>>.

<sup>c</sup> Calculations for MAF in: MAS = # of alleles in cohort/(2\*19); non-MAS = # of alleles in cohort/(2\*62).

<sup>d</sup> Ancestry-specific referent minor allele frequency (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.

<sup>e</sup> NR = Variant not reported in dbSNP or referent population of gnomAD or TOPMed.

<sup>f</sup> Same variant found in MAS and non-MAS cohorts (see Supplementary).

HLH: hemophagocytic lymphohistiocytosis; cSLE: childhood-onset systemic lupus erythematosus; MAS: macrophage activation syndrome; Chr: Chromosome; Ref/Alt: reference/alternative; SNP: single nucleotide polymorphism; AA: amino acid; CADD: CADD: combined annotation dependent depletion; MAF: minor allele frequency; ADM – admixed, AFR – African, EAS – East Asian, EUR – European (non-Finnish), SAS – South Asia.