

Original article

Panel sequencing links rare, likely damaging gene variants with distinct clinical phenotypes and outcomes in juvenile-onset SLE

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Abstract

Objectives. Juvenile-onset systemic lupus erythematosus (jSLE) affects 15–20% of lupus patients. Clinical heterogeneity between racial groups, age groups and individual patients suggests variable pathophysiology. This study aimed to identify highly penetrant damaging mutations in genes associated with SLE/SLE-like disease in a large national cohort (UK JSLE Cohort Study) and compare demographic, clinical and laboratory features in patient sub-cohorts with ‘genetic’ SLE vs remaining SLE patients.

Methods. Based on a sequencing panel designed in 2018, target enrichment and next-generation sequencing were performed in 348 patients to identify damaging gene variants. Findings were integrated with demographic, clinical and treatment related datasets.

Results. Damaging gene variants were identified in ~3.5% of jSLE patients. When compared with the remaining cohort, ‘genetic’ SLE affected younger children and more Black African/Caribbean patients. ‘Genetic’ SLE patients exhibited less organ involvement and damage, and neuropsychiatric involvement developed over time. Less aggressive first line treatment was chosen in ‘genetic’ SLE patients, but more second and third line agents were used. ‘Genetic’ SLE associated with anti-dsDNA antibody positivity at diagnosis and reduced ANA, anti-LA and anti-Sm antibody positivity at last visit.

Conclusion. Approximately 3.5% of jSLE patients present damaging gene variants associated with younger age at onset, and distinct clinical features. As less commonly observed after treatment induction, in ‘genetic’ SLE, autoantibody positivity may be the result of tissue damage and explain reduced immune complex-mediated renal and haematological

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involvement. Routine sequencing could allow for patient stratification, risk assessment and target-directed treatment, thereby increasing efficacy and reducing toxicity.

Key words: SLE, juvenile-onset, paediatric, childhood, jSLE, genetic, monogenic, stratification

Rheumatology key messages

- Using panel sequencing, rare variants predicted to be damaging were identified in 3.5% of jSLE patients.
- The majority of SLE-associated gene mutations detected affect type I interferon expression.
- ‘Genetic’ SLE associates with reduced persistent antibody positivity, less organ involvement, fewer disease flares and reduced damage.

Introduction

SLE is a complex autoimmune/inflammatory disease that can affect any organ system [1]. Approximately 15–20% of patients develop disease during childhood and are diagnosed with juvenile-onset SLE (jSLE) [2]. When compared with adult-onset SLE (aSLE), paediatric patients exhibit higher disease activity, more organ involvement and damage, and increased need for immunosuppressive treatment [3].

Variability in clinical presentations, treatment response and outcomes suggests that ‘SLE’ comprises a range of conditions [4]. Across all ages, an estimated 1–3% of SLE patients experience disease that is caused by mutations in single genes [5], and this discovery has improved our understanding of the pathophysiology of more common ‘classical’ SLE and explained shared characteristics between SLE and conditions caused by uncontrolled expression of type 1 interferons (T1IFN; ‘type 1 interferonopathies’), including Aicardi–Goutières syndrome (AGS), Singleton–Merten syndrome, and STING-associated vasculopathy with onset in infancy [6].

While the exact pathophysiology of SLE remains unknown [1], genetic heterogeneity contributes to clinical variation and inconsistent outcomes [7, 8]. Thus, detailed molecular phenotyping may allow for patient stratification towards individualized and target-directed treatment [4].

This study linked genetic factors with clinical phenotypes and outcomes in jSLE. Target enrichment followed by next-generation sequencing was chosen to screen for variants in lupus-associated genes in a large national cohort (UK JSLE Cohort Study). This paper introduces the sequencing platform, and presents data from the sub-cohort experiencing ‘genetic’ SLE.

Methods

Study cohort

Three hundred and forty-eight patients with jSLE enrolled in the UK JSLE Cohort Study were included [9]. JSLE patients were classified using the 1997 ACR SLE classification criteria [10] and ≤ 18 years at diagnosis. The study received ethical approval from the National Research

Ethics Service North West (REC 06/Q1502/77). Written informed patient assent/consent and parental consent was obtained to participate in the UK JSLE Cohort Study. Research was carried out in accordance with the Declaration of Helsinki.

Data collected

Demographic, clinical and serological information was analysed, including: (i) ACR-1997 scores; (ii) Systemic Lupus International Collaborating Clinics standardized damage index (SLICC-SDI) scores; (iii) The SLE Disease Activity Index 2000 (SLEDAI-2K); (iv) paediatric British Isles Lupus Assessment Grade 2004 numerical scores (pBILAG) with organ/system domains (alphabetical score A–E); (v) key laboratory findings ([Supplementary methods](#), available at *Rheumatology* online), and (vi) treatments received ([Supplementary methods](#), available at *Rheumatology* online).

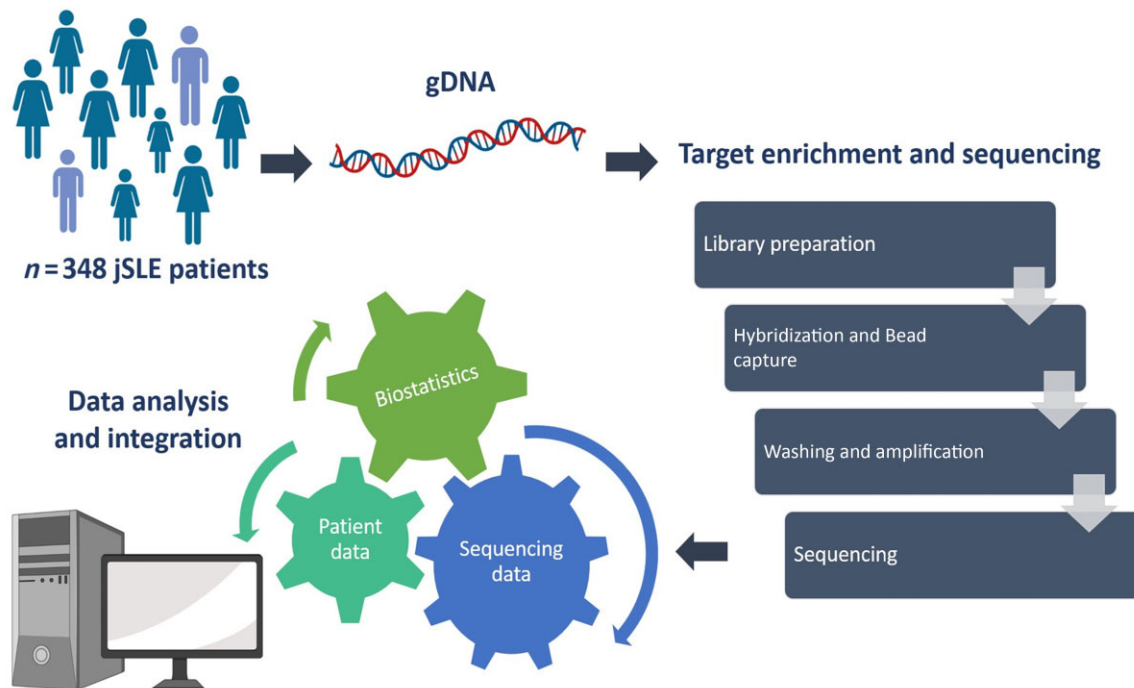
Gene panel selection

Sequencing targets were selected based on a literature search targeting known Mendelian disease, and risk alleles previously identified through GWAS. For this, a literature search was performed (March 2018) using PubMed, OMIM and Ensembl.

Target sequencing and variant identification

Sequence capture enrichment (NimbleGen/Roche, Switzerland) was followed by sequencing (MiSeq2500 technology, Illumina, San Diego, CA, USA). Demultiplexing, adaptor and quality trimming [Cutadapt v1.2.1 (<https://doi.org/10.14806/ej.17.1.200>), Sickle v1.2 (<https://github.com/najoshi/sickle>)] of reads was performed. Sequencing data were aligned using BWA (Burrows–Wheeler Alignment), and variants were subsequently detected, filtered and annotated with Genome Analysis Toolkit Software (GATK) (<https://doi.org/10.1101/gr.107524.110>) and SnpEFF (<https://doi.org/10.4161/fly.19695>) ([Fig. 1](#)). Variants were considered to be of ‘high impact’ when predicted to disrupt proteins encoded, cause truncation, loss-of-function (LOF) or to trigger decay ([Supplementary methods](#), available at *Rheumatology* online).

Fig. 1 Analytic workflow



gDNA: genomic DNA; jSLE: juvenile SLE. Created using BioRender.com.

Bioinformatic analysis

Publicly available databases were used in combination to predict pathogenicity of variants, including SIFT (Sorting Intolerant From Tolerant), polyphen-2, CADD (Combined Annotation Dependent Depletion), and REVEL (Rare Exome Variant Ensemble Learner) scores. Clinical associations were investigated using OMIM and ClinVar. SNPnexus was used to complete interpretation of sequence variants. Allele frequencies and counts were extracted from the 1000 Genomes Project Phase 3 and gnomAD genomes v3.1.1. Variant and reference allele counts within this cohort were subsequently compared with those of the control datasets using Fisher's exact test with false discovery rate correction of P -values to identify variants with significantly different frequencies between cohort and control groups.

Statistical analysis

Demographics and baseline characteristics were analysed using descriptive and summary statistics. The χ^2 test was used to compare frequencies between patient subgroups. Analyses were completed using GraphPad Prism 6 (GraphPad Software, La Jolla, CA, USA).

Results

Sequencing panel

Twenty-three genes associated with Mendelian forms of SLE/SLE-like disease, and 40 genomic regions/59 genes

covering >130 SLE-associated risk alleles were identified and included (Supplementary Table S1, available at *Rheumatology* online).

Demographic information

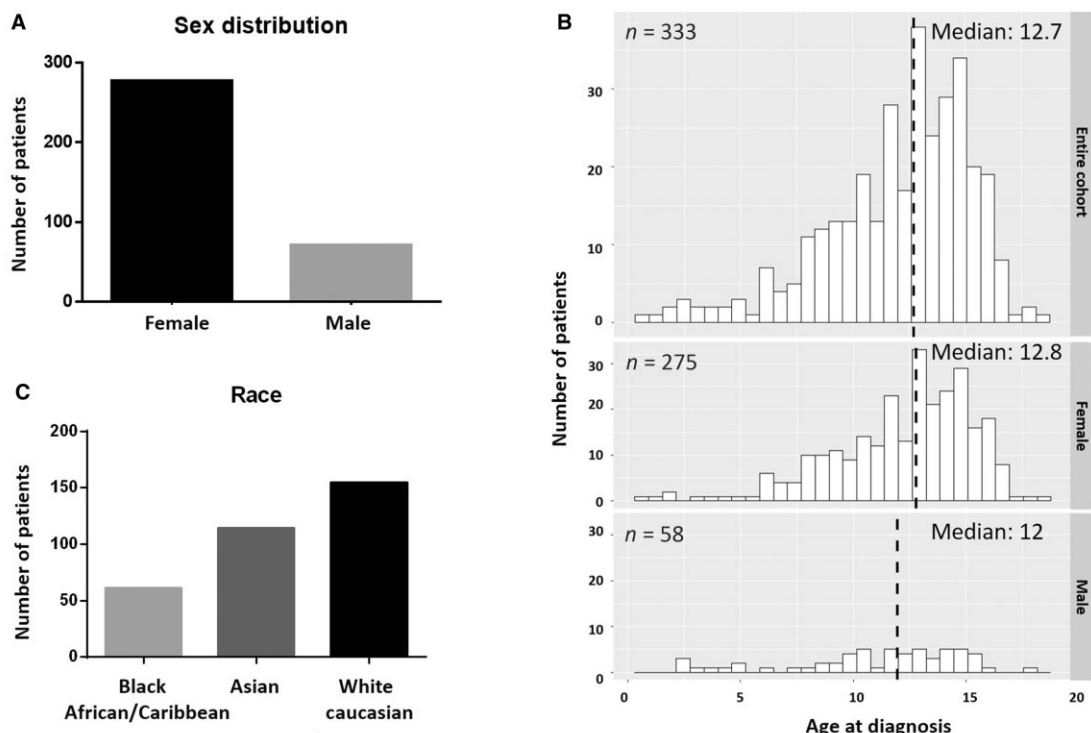
Three hundred and forty-eight jSLE patients were recruited to this study, of whom 82.6% ($n=275$) were girls. The mean age at disease onset was 12 years (SD: 3; 0.7–17.7). Two sub-cohorts were defined: (i) 'genetic' SLE including 12 patients (3.5%, below), and (ii) 'remaining' SLE patients (336; 96.5%).

When compared with the remaining UK jSLE cohort, 'genetic' SLE patients were characterized by a higher proportion of patients with Black African/Caribbean background (36.3% vs 18.2%, $P=0.004$) (Supplementary Table S2, available at *Rheumatology* online). Although the median age at disease onset was comparable between sub-cohorts, a higher proportion of patients with 'genetic' SLE developed disease around puberty (81.2% vs 56.5%, $P=0.0002$; Supplementary Table S2, available at *Rheumatology* online, Fig. 2).

Variant selection strategy

Using *in silico* (SnPEFF) screening for 'high impact' variants with low minor allele frequencies (MAF; <5%) delivered 78 candidate variants in 36 genes. This was followed by analysis of inheritance patterns using OMIM, ClinVar and SNPnexus. Patients with predicted 'high impact' variants and autosomal dominant (AD) inheritance were included in the study ($n=8$). Patients with variants

Fig. 2 Demographic composition of the study cohort



following autosomal recessive inheritance were included, if ≥ 1 additional ‘high impact’ mutations were present on the second allele (compound heterozygous) or ≥ 1 other gene associated with SLE within the same immunological pathway exhibited a ‘high impact’ mutation ($n=4$). This delivered 18 variants in 14 genes of 12 (3.5%) jSLE patients (Table 1).

Allele counts were calculated for all sub-selected variants and compared with allele frequencies in the general population (Supplementary Table S3, available at *Rheumatology* online). The frequency of rare, predicted damaging variants was significantly increased in the UK JSLE cohort for 8/18 variants, for three differences were not statistically significant [1] or comparable between the JSLE cohort and general populations. For 7/18 variants, no allele frequencies were available in the general population.

Notably, the most ‘common’ variant included had a reported MAF of 0.007 in the general population (gnomAD) (Supplementary Table S4, available at *Rheumatology* online).

Pathways affected

Four main signalling pathways were defined: (i) nucleic acid sensing and processing (42% of ‘genetic’ SLE), (ii) a combination of (i) and immune complex (IC) clearance (33%), (iii) immune cell signalling (17%), and (iv) the nuclear factor κ B (NF- κ B) pathway (8%; Fig. 3, Table 2 and Supplementary Table S5, available at *Rheumatology* online).

Nucleic acid sensing and processing

One patient carried a previously reported mono-allelic endonuclease LOF variant in *DNASE1* (rs201571412) [11].

Two patients had a structural interaction variant in *SAMHD1* (c.811C>A) affecting the HD [histidine (H) and Aspartate (D)] domain [12]. One additional variant was reported in these patients: one mono-allelic variant in *IRF7* (c.-283-1G>T), and *C1S* (rs117907409). One patient had a monoallelic mutation in *SAMHD1* (rs1400380009) affecting the aforementioned HD domain. A non-synonymous variant in *TREX1* (rs749323787), encoding the 3’ repair exonuclease three prime repair exonuclease 1, introduces a splice site. Another previously unreported frameshift variant affects the DNase domain (rs1331920811).

Mono-allelic mutations affecting RNASEH2 subunits were present in patients with additional variants in other genes. In a patient with an additional mutation in *TNFAIP3*, *RNASEH2A* (rs549586181), previously reported in AGS, resulted in frameshift and LOF [13]. One individual had a ‘stop’ mutation in *RNASEH2B* (c.184G>T) in addition to a mutation in *C3*. Another patient carried a missense mutation in *RNASEH2C* (rs759118175), previously associated with AGS (ClinVar), and mutations in *C3* (c.1303G>T) and *TNFSF4* (c.368_369delAG).

Immune complex clearance

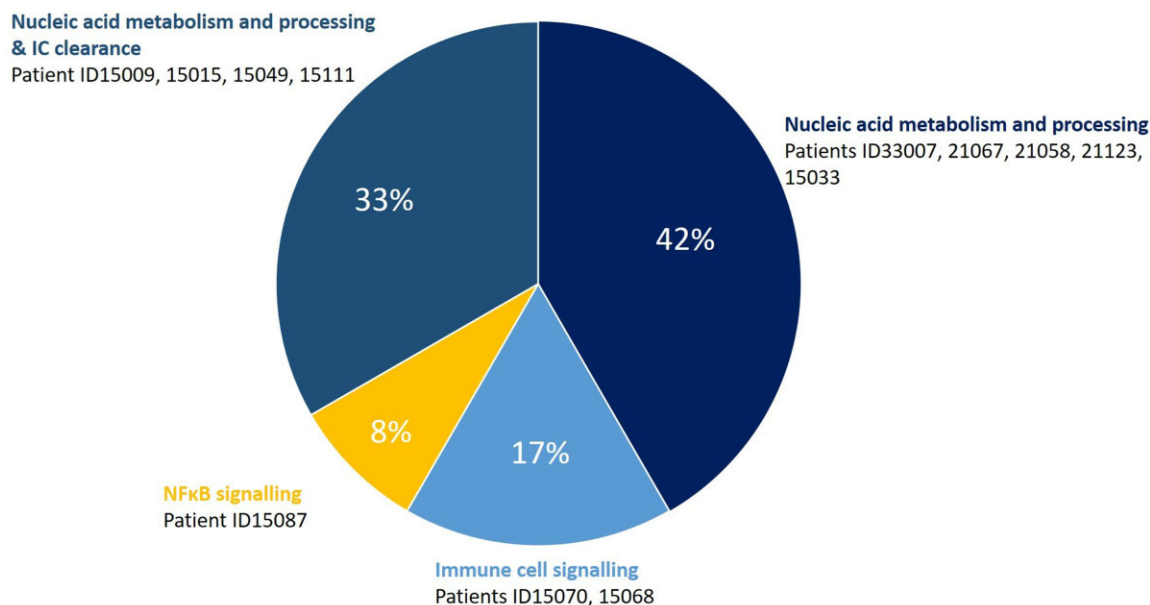
One patient with a homozygous variant in *SAMHD1* also exhibited a mono-allelic *C1S* variant (rs117907409) recently associated with ‘genetic’ SLE [14].

TABLE 1 Patients with 'genetic' SLE and disease-causing variants

Patient ID	Gene (variant)	Likely effects	Function affected/predicted effect (snpEFF, ClinVar, snpNexus and PubMed)	Zygoty	Inheritance pattern	Reference
15009	<i>C1S</i> (rs117907409)	Disease-causing	Deleterious	Heterozygous	AD	[14]
	<i>SAMHD1</i> (c.811C>A)	Disease-causing and/or -modifying	Impacts catalytic activity	Heterozygous	AD/AR	[12]
15015	<i>C3</i> (rs117793540)	Disease-causing and/or -modifying	Gain-of-function	Heterozygous	AD/AR	[33, 34]
	<i>TREX1</i> (rs133192081)	Disease-causing and/or -modifying	Loss-of-function	Heterozygous	AD/AR	[13]
15033	<i>SAMHD1</i> (c.811C>A)	Disease-causing and/or -modifying	Impacts catalytic activity	Heterozygous	AD	[12]
	<i>IRF7</i> (c.-283-1G>T)	Disease-modifying	Loss-of-function	Heterozygous	AR	—
15049	<i>C3</i> (c.4341C>A)	Disease-causing	Loss-of-function	Heterozygous	AD/AR	—
	<i>RNASEH2B</i> (c.184G>T)	Disease-modifying	Deleterious	Heterozygous	AR	—
15068	<i>BANK1</i> (rs928173624)	Disease-causing	Loss-of-function	Heterozygous	N/A	[16, 18]
15070	<i>PTPN22</i> (c.369 + 1G>T)	Disease-causing	<i>In silico</i> predicted 'high impact'	Heterozygous	AD	—
15087	<i>TNFAIP3</i> (rs776714084), <i>RNASEH2A</i> (rs549586181)	Disease-causing Disease-modifying	Loss-of-function Loss-of-function	Heterozygous Heterozygous	AD AR	— [13]
15111	<i>C3</i> (c.1303G>T)	Disease-causing	Loss-of-function	Heterozygous	AD/AR	—
	<i>TNFSF4</i> (c.368_369delAG)	Disease-modifying	Loss-of-function	Heterozygous	N/A	—
	<i>RNASEH2C</i> (rs759118175)	Disease-modifying	Loss-of-function	Heterozygous	AR	—
21058	<i>TREX1</i> (rs749323787)	Disease-causing	Loss-of-function	Heterozygous	AD/AR	[18]
21067	<i>SAMHD1</i> (rs1400380009)	Disease-causing	Impacts catalytic activity	Heterozygous	AD/AR	—
21123	<i>PEPD</i> (rs529315200)	Disease-modifying	Loss-of-function	Heterozygous	AR	[24]
33007	<i>DNASE1</i> (rs201571412)	Disease-causing	Loss-of-function	Heterozygous	AD	[11]

AD: autosomal dominant inheritance; AR: autosomal recessive inheritance, BANK1: B cell scaffold protein with ankyrin repeats 1; C1S: complement C1S; C3: complement C3; DNASE1: deoxyribonuclease 1; IRF7: interferon regulatory factor 7; N/A: not available; PEPD: peptidase D; PTPN22: protein tyrosine phosphatase non-receptor type 22; RNASEH2A: ribonuclease H2 subunit A; RNASEH2B: ribonuclease H2 subunit B; RNASEH2C: ribonuclease H2 subunit C; SAMHD1: SAM and HD domain containing deoxynucleoside triphosphate triphosphohydrolase 1; TNFAIP3: TNF- α induced protein 3; TNFSF4: TNF superfamily member 4; TREX1: three prime repair exonuclease 1.

Fig. 3 Stratification of gene variants by immunological pathway



Pie chart summarizing immunological pathways identified in 12 patients with ‘genetic’ SLE carrying 18 variants in 14 genes. Variants following autosomal inheritance were considered for the association with immunological pathways, and patients may carry additional disease-modifying alleles (summarized in [Table 1](#) and [Supplementary Tables S3 and S4](#), available at *Rheumatology* online). IC: immune complex; NF-κB: nuclear factor κB.

TABLE 2 Genetic variants and pathways affected

Pathway	Gene	Disease associated	Reference
IC clearance	<i>C1S</i>	SLE-like disease, aHUS, primary angioedema	[30]
	<i>C3</i>	SLE-like disease	[30]
Immune cell signalling	<i>PEPD</i>	SLE-like disease	[23]
	<i>BANK1</i>	AITD, CLL, SSc, RA, SLE	[38]
	<i>PTPN22</i>	RA, T1D, CD, JIA, HT, SLE	[15]
	<i>TNFSF4</i>	SSc, SLE	[37]
NF-κB signalling	<i>TNFAIP3</i>	SS, RA, SSc, SLE, A20 haploinsufficiency	[36]
Nucleic acid sensing and processing	<i>DNASE1</i>	AGS, ChLE	[6]
	<i>RNASEH2A</i>	AGS, ChLE	[13]
	<i>RNASEH2B</i>	AGS, ChLE	[13]
	<i>RNASEH2C</i>	AGS, ChLE	[13]
	<i>SAMHD1</i>	AGS, ChLE	[6]
	<i>TREX1</i>	AGS, ChLE	[6]
TLR/IFN signalling	<i>IRF7</i>	SLE	[28]

aHUS: atypical haemolytic uremic syndrome; BANK1: B cell scaffold protein with ankyrin repeats 1; C1S: complement C1S; C3: complement C3; ChLE: chilblain lupus erythematosus; DNASE1: deoxyribonuclease 1; HT: Hashimoto thyroiditis; IFN: (type 1) interferon; IC: immune complex; IRF7: interferon regulatory factor 7; NF-κB: nuclear factor κB; PEPD: peptidase D; PTPN22: protein tyrosine phosphatase non-receptor Type 22; RNASEH2A: ribonuclease H2 subunit A; RNASEH2B: ribonuclease H2 subunit B; RNASEH2C: ribonuclease H2 subunit C; SAMHD1: SAM and HD domain containing deoxynucleoside triphosphate triphosphohydrolase 1; T1D: type 1 diabetes; TLR: Toll-like receptor; TNFAIP3: TNF-α induced protein 3; TNFSF4: TNF superfamily member 4; TREX1: three prime repair exonuclease 1.

Three mono-allelic variants were identified in C3; one patient carried a variant predicted to result in a gain-of-function (GOF) (rs117793540). One variant introducing a stop codon (c.4341C>A) affecting the A2M (Alpha-2-Macroglobulin) receptor binding domain and one not previously described variant results in LOF (c.1303G>T) were found in this sub-cohort.

Immune cell signalling

The cytoplasmic lymphoid-specific phosphatase (Lyp, *PTPN22*), inhibits T-cell activation and is involved in the termination of antigen responses [15]. A previously undescribed non-synonymous mutation in *PTPN22* was predicted to mediate LOF.

One previously unreported LOF mutation in B-cell scaffold protein with ankyrin repeats 1 (*BANK1*; rs928173624) was identified in another patient [16].

A not previously reported LOF variant was found in the tumour necrosis factor superfamily 4 (*TNFSF4*) gene of one patient (c.368_369delAG) who carried additional mutations in C3 and *RNASEH2C*.

NF- κ B signalling pathway

An autoinflammatory disease resembling Behçet's disease was linked with mutations in *TNFAIP3* [17]. A mono-allelic missense *TNFAIP3* variant (rs776714084), predicted to cause LOF, was identified in one individual also carrying a *RNASEH2A* mutation.

Clinical and laboratory characteristics

Considering pBILAG organ domains, at diagnosis, smaller proportions of 'genetic' SLE patients exhibited 'severe' and 'moderate' (pBILAG A, B) or 'mild' (C) activity in the neuropsychiatric ($P=0.002$), cardiorespiratory ($P=0.0006$), gastrointestinal ($P=0.01$), musculoskeletal ($P=0.0005$), renal ($P=0.004$) and haematological ($P=0.004$) domains when compared with the remaining cohort. A smaller proportion of 'genetic' SLE patients exhibited 'mild' ophthalmic involvement ($P=0.009$; Table 3). Moreover, a lower proportion of patients had a high SLEDAI score (27.3% vs 41.5%; $P=0.03$).

At last visit, a higher proportion of 'genetic' SLE exhibited a 'high' SLEDAI score (SLEDAI ≥ 10 ; 27.3% vs 11.3%; $P=0.004$); 2/3 of these patients (15049 and 15111) sustained a 'high' SLEDAI score from the first to last visit. Moreover, 'genetic' SLE patients exhibited less 'severe' or 'moderate' activity in the pBILAG haematological ($P=0.008$) and musculoskeletal ($P=0.01$) domains, and a smaller proportion of patient had 'no activity' in the neuropsychiatric domain ($P=0.02$, Table 3). Lastly a higher proportion demonstrated a severe to moderate activity for the neuropsychiatric domain (18.2% vs 3.7%; $P=0.0016$) and an absence of activity for the haematological domain (72.7% vs 54.7%, $P=0.008$) in the 'genetic' SLE group (Table 3).

To assess disease progression, patients were followed over time. The median disease duration across the entire cohort was 9.1 (range: 2.8–22.6) years. Notably, median follow-up duration was comparable between sub-cohorts

['genetic SLE': 10.97 (range: 7.2–14.8) years; remaining cohort: 9.12 (range: 2.8–22.7) years].

Considering disease progression from first to last visits, fewer patients with 'genetic' SLE experienced 'deterioration' in the mucocutaneous and renal domains ($P=0.013$ and $P=0.007$, respectively) when compared with the remaining cohort. Persistent activity in the haematological domain was less common among 'genetic' SLE patients ($P=0.04$). Likely because of lower disease activity at diagnosis, 'improvement' in the neuropsychiatric ($P=0.004$) and gastrointestinal ($P=0.02$) domains was less common among patients with 'genetic' SLE when compared with the remaining cohort. More 'genetic SLE' patients experienced 'deterioration' in the neuropsychiatric and musculoskeletal domains ($P=0.0005$ and $P=0.03$, respectively; Supplementary Table S6, available at *Rheumatology* online).

At first visit, while ANA positivity did not vary between sub-cohorts, a higher proportion of 'genetic' SLE patients tested positive for anti-dsDNA antibodies ($P=0.02$), and fewer exhibited anti-Sm and anti-Ro antibodies ($P=0.03$ and $P=0.0004$, respectively; Table 4). Furthermore, 'genetic' SLE patients less frequently experienced anaemia [$P=0.0002$ (overall 18.2% vs 41.6%)] when compared with the remaining cohort (Table 4). No patient in the 'genetic' SLE group presented severe anaemia ($P=0.001$), which associated with the absence of mild and moderate leukopenia (0% vs 23.2%; $P<0.0001$). Notably, a higher proportion of 'genetic' SLE patients experienced severe thrombocytopenia ($P=0.003$), which was, however, caused by one individual patient.

At last visit, when compared with the remaining cohort, fewer 'genetic' SLE patients remained positive for ANA ($P<0.0001$), anti-Sm ($P<0.0001$), and anti-La ($P=0.004$) antibodies (Table 4). And a higher proportion of patients remained positive for dsDNA ($P<0.0001$) and anti-RNP antibodies ($P<0.0001$).

Next, we asked whether reduced organ involvement in 'genetic' SLE associated with more aggressive induction treatment. Notably, patients with 'genetic' SLE did not receive cytotoxic cyclophosphamide (CPM) as first-line treatment, while 7.7% of the remaining cohort did (Supplementary Table S7, available at *Rheumatology* online). Later in the disease course, however, fewer 'genetic' SLE patients were off steroid-sparing agents, while more third-line DMARDs were used as compared with the remaining cohort.

Discussion

Using a panel sequencing approach limited to genes chosen during the design stage in 2018, we identified rare damaging gene mutations in 3.5% of jSLE patients. This is above the 1–3% of SLE patients with 'genetic disease' previously reported across all ages [4]. The majority of rare, likely damaging variants identified in the UK JSLE cohort, were significantly more common than in the general population (8/18), not previously reported and therefore no data

TABLE 3 Clinical picture at diagnosis and last visit

	At diagnosis				At last visit				Test used
	Total	SLE patient with rare variants predicted to be damaging ('genetic' SLE)	Remaining SLE patients	P-value	Total	SLE patient with rare variants predicted to be damaging ('genetic' SLE)	Remaining SLE patients	P-value	
SLICC, median (IQR)	0.00 (0.00–3)	0.00 (0.00–1)	0.00 (0.00–3)	0.62	0.00 (0.00–12)	0.00 (0.00–1)	0.00 (0.00–12)	0.07	Mann–Whitney
SLEDAI, median (IQR)	8.00 (0–34)	6 (2–23)	8.00 (0–34)	0.22	2 (0–24)	2 (0–14)	2 (0–24)	0.77	Mann–Whitney
SLEDAI high (≥10), n (%)	140/341 (41)	3/11 (27.3)	137/330 (41.5)	0.026	40/338 (11.8)	3/11 (27.3)	37/327 (11.3)	0.004	χ^2
SLEDAI intermediate (5–9), n (%)	87/341 (25.5)	3/11 (27.3)	84/330 (25.4)	0.75	56/338 (16.6)	1/11 (9.1)	55/327 (16.8)	0.09	χ^2
SLEDAI mild (0–4), n (%)	114/341 (33.4)	5/11 (45.5)	109/330 (33)	0.06	242/338 (71.6)	7/11 (63.6)	235/327 (71.9)	0.22	χ^2
Missing information, n (%)	7 (2)	1 (8.3)	6 (1.8)	—	10 (2.9)	1 (8.3)	9 (2.7)	—	—
BILAG2004score, median (IQR)	7.00 (0–51)	5 (1–16)	7.00 (0–51)	0.52	1 (0–30)	2 (0–4)	1 (0–30)	0.70	Mann–Whitney
Active organ/system involvement, n (%)									
pBILAG constitutional domain									
A/B ('severe' and 'moderate' activity)	94/339 (27.7)	2/11 (18.2)	91/328 (27.7)	0.09	6/338 (1.8)	0/11 (0)	6/327 (1.8)	0.15	χ^2
C ('mild' activity)	40/339 (11.8)	2/11 (18.2)	38/328 (11.6)	0.24	18/338 (5.3)	0/11 (0)	18/327 (5.5)	0.01	χ^2
D and E (no activity)	205/339 (60.5)	7/11 (63.6)	198/328 (60.4)	0.56	314/338 (92.9)	11/11 (100)	296/327 (90.5)	0.002	χ^2
pBILAG mucocutaneous domain									
A/B ('severe' and 'moderate' activity)	122/339 (36)	4/11 (36.4)	118/328 (36)	1	38/338 (11.2)	1/11 (9.1)	37/327 (11.3)	0.64	χ^2
C ('mild' activity)	75/339 (22.1)	3/11 (27.3)	72/328 (21.9)	0.41	50/338 (14.8)	2/11 (18.2)	48/327 (14.7)	0.57	χ^2
D and E (no activity)	142/339 (41.9)	4/11 (36.4)	138/328 (42.1)	0.38	250/338 (74)	8/11 (72.7)	242/327 (74)	0.87	χ^2
pBILAG neuropsychiatric domain									
A/B ('severe' and 'moderate' activity)	30/339 (8.8)	0/11 (0)	30/328 (9.1)	0.002	14/338 (4.1)	2/11 (18.2)	12/327 (3.7)	0.0016	χ^2
C ('mild' activity)	2/329 (0.6)	0/11 (0)	2/329 (0.6)	0.32	3/338 (0.9)	0/11 (0)	3/327 (0.9)	0.32	χ^2

(continued)

TABLE 3 Continued

	At diagnosis				At last visit				Test used
	Total	SLE patient with rare variants predicted to be damaging ('genetic' SLE)	Remaining SLE patients	P-value	Total	SLE patient with rare variants predicted to be damaging ('genetic' SLE)	Remaining SLE patients	P-value	
D and E (no activity)	307/329 (93.3)	11/11 (100)	289 (87.8)	0.0004	321/338 (95)	9/11 (81.8)	305/327 (93.3)	0.02	χ^2
pBILAG musculoskeletal domain									
A/B ('severe' and 'moderate' activity)	93/339 (27.4)	4/11 (36.4)	89/328 (27.1)	0.17	17/338 (5)	0/11 (0)	17/327 (5.2)	0.02	χ^2
C ('mild' activity)	92/339 (27.1)	1/11 (9.1)	91/328 (27.7)	0.0005	51/338 (15.1)	1/11 (9.1)	49/327 (15)	0.19	χ^2
D and E (no activity)	154/339 (45.4)	6/11 (54.5)	148/328 (45.1)	0.16	270/338 (79.9)	10 (90.9)	255/327 (78)	0.01	χ^2
pBILAG cardiorespiratory domain									
A/B ('severe' and 'moderate' activity)	38/339 (11.2)	0/11 (0)	38/328 (11.6)	0.0004	6/338 (1.8)	0/11 (0)	6/327 (1.8)	0.15	χ^2
C ('mild' activity)	2/339 (0.6)	0/11 (0)	2/328 (0.6)	0.32	0/338 (0.0)	0/11 (0)	0/327 (0)	—	χ^2
D and E (no activity)	299/339 (88.2)	11/11 (100)	288/328 (87.8)	0.0004	332/338 (98.2)	11 (100)	321/327 (98.1)	0.15	χ^2
pBILAG gastrointestinal domain									
A/B ('severe' and 'moderate' activity)	19/339 (5.6)	0/11 (0)	19/328 (5.8)	0.01	5/338 (1.5)	0/11 (0)	5/327 (1.5)	0.15	χ^2
C ('mild' activity)	3/339 (0.9)	0/11 (0)	3/328 (0.9)	0.32	1/338 (0.3)	0/11 (0)	1/327 (0.3)	1	χ^2
D and E (no activity)	317/339 (93.5)	11/11 (100)	306/328 (93.3)	0.007	332/338 (95.4)	11/11 (100)	321/327 (98.1)	0.15	χ^2
pBILAG ophthalmic domain									
A/B ('severe' and 'moderate' activity)	2/339 (0.6)	0/11 (0)	2/328 (0.6)	0.32	1/338 (0.3)	0/11 (0)	1/327 (0.3)	1	χ^2
C ('mild' activity)	4/339 (1.2)	1/11 (9.1)	3/328 (0.9)	0.009	1/338 (0.3)	0/11 (0)	1/327 (0.3)	1	χ^2
D and E (no activity)	333/339 (98.2)	10 (90.9)	323/328 (98.5)	0.03	336/338 (99.4)	11/11 (100)	325/327 (99.4)	0.32	χ^2
pBILAG renal domain									
A/B ('severe' and 'moderate' activity)	102/339 (30)	3/11 (27.3)	99/328 (30.2)	0.64	52/338 (15.4)	1/11 (9.1)	51/327 (15.6)	0.13	χ^2

(continued)

TABLE 3 Continued

	At diagnosis				At last visit				Test used
	Total	SLE patient with rare variants predicted to be damaging ('genetic' SLE)	Remaining SLE patients	<i>P</i> -value	Total	SLE patient with rare variants predicted to be damaging ('genetic' SLE)	Remaining SLE patients	<i>P</i> -value	
C ('mild' activity)	26/339 (7.7)	0/11 (0)	26/328 (7.9)	0.004	26/338 (7.7)	1/11 (9.1)	25/327 (7.6)	0.80	χ^2
D and E (no activity)	211/339 (62.2)	8/11 (72.7)	203/328 (61.9)	0.17	260/338 (76.9)	9/11 (81.8)	251/327 (76.8)	0.38	χ^2
pBILAG haematological domain									
A/B ('severe' and 'moderate' activity)	90/339 (26.5)	4/11 (36.4)	86/328 (26.2)	0.13	24/338 (7.1)	0/11 (0)	24/327 (7.3)	0.007	χ^2
C ('mild' activity)	120/339 (35.4)	2/11 (18.2)	118/328 (36)	0.004	127/338 (37.6)	3/11 (27.3)	123/327 (37.6)	0.097	χ^2
D and E (no activity)	129/339 (38)	5/11 (45.5)	124/328 (37.8)	0.25	187/338 (55.3)	8/11 (72.7)	179/327 (54.7)	0.008	χ^2

P-values indicating statistical significance are displayed in bold italic. IQR: interquartile range.

TABLE 4 Laboratory findings at diagnosis and last visit

	At diagnosis				At last visit				Test used
	Total	SLE patient with rare variants predicted to be damaging ('genetic' SLE)	Remaining SLE patients	P-value	Total	SLE patient with rare variants predicted to be damaging ('genetic' SLE)	Remaining SLE patients	P-value	
Immunology									
ANA (when tested), n (%)	219/275 (79.6)	8/9 (88.9)	211/266 (79.3)	0.054	143/228 (62.7)	1/5 (20)	141/223 (63.2)	<0.0001	χ^2
Anti-dsDNA negative (0–15), n (%)	111/314 (35.4)	2/10 (20)	109/304 (35.8)	0.04	172/316 (54.4)	2/10 (20)	170/306 (55.5)	<0.0001	χ^2
Anti-dsDNA positive (15–>400), n (%)	203/314 (64.6)	8/10 (80)	195/304 (64.1)	0.02	144/316 (43.6)	8/10 (80)	136/306 (40.5)	<0.0001	χ^2
Anti-Sm, n (of 238 tested at first visit, of 200 tested at last visit) (%)	56/238 (23.5)	1/8 (12.5)	55/230 (23.9)	0.03	31/200 (15.5)	0/4 (0.0)	31/196 (15.8)	<0.0001	χ^2
Anti-RNP, n (of 238 tested at first visit, of 200 tested at last visit) (%)	74/238 (31.1)	2/8 (25)	72/230 (31.3)	0.34	44/200 (22)	2/4 (50)	42/196 (21.4)	<0.0001	χ^2
Anti-Ro, n (of 238 tested at first visit, of 200 tested at last visit) (%)	76/238 (31.9)	1/8 (12.5)	75/230 (32.6)	0.0004	47/200 (23.5)	1/4 (25)	46/196 (23.5)	0.87	χ^2
Anti-La, n (of 238 tested at first visit, of 200 tested at last visit) (%)	33/238 (13.9)	1/8 (12.5)	32/230 (13.9)	0.63	15/200 (7.5)	0/4 (0.0)	15/196 (7.6)	0.004	χ^2
C3, median (IQR)	0.94 (0.12–3.47)	0.82 (0.21–1.44)	0.95 (0.12–3.47)	0.38	1.06 (0.12–2.08)	0.9 (0.39–1.36)	1.07 (0.12–2.08)	0.08	Mann-Whitney
C4, median (IQR)	0.12 (0.01–1.95)	0.1 (0.01–0.28)	0.12 (0.01–1.95)	0.31	0.17 (0.01–0.66)	0.18 (0.01–0.22)	0.17 (0.03–0.66)	0.47	Mann-Whitney
Haemoglobin level, n (%)									
Severe anaemia (<8 g/dl)	31/309 (10)	0/11 (0)	31/298 (10.4)	0.001	15/300 (5)	0/8 (0.0)	15/292 (5.1)	0.02	χ^2
Moderate anaemia (8–8.9 g/dl)	24/309 (6.9)	1/11 (9.1)	23/298 (7.7)	0.80	4/300 (1.3)	0/8 (0.0)	4/292 (1.4)	0.32	χ^2
Mild anaemia (9–10.9 g/dl)	72/309 (23.3)	1/11 (9.1)	70/298 (23.5)	0.004	34/300 (11.3)	2/8 (25)	32/292 (11)	0.01	χ^2
Normal (>10.9 g/dl)	182/309 (58.9)	8/11 (72.7)	167/298 (56)	0.02	247/300 (82.3)	6/8 (75)	241/292 (82.5)	0.16	χ^2
White cell count, n (%)									
Severe leukopenia (<1.0 × 10 ⁹ /l)	0/307 (0.0)	0/10 (0.0)	0/297 (0.0)	1	0/302 (0.0)	0/9 (0.0)	0/293 (0.0)	1	χ^2
Moderate leukopenia (1–1.9 × 10 ⁹ /l)	6/307 (1.9)	0/10 (0.0)	6/297 (2)	0.15	2/302 (0.7)	0/9 (0.0)	2/293 (0.7)	0.32	χ^2
Mild leukopenia (2–3.9 × 10 ⁹ /l)	63/307 (20.5)	0/10 (0.0)	63/297 (21.2)	<0.0001	62/302 (20.5)	2/9 (22.2)	60/293 (20.5)	0.86	χ^2
Normal (>3.9 × 10 ⁹ /l)	238/307 (77.5)	9/10 (90)	229/297 (77.1)	0.01	238/302 (78.8)	6/9 (66.7)	232/293 (79.2)	0.056	χ^2
Platelets, n (%)									
Severe thrombocytopenia (<25 × 10 ⁹ /l)	4/305 (1.3)	1/9 (11.1)	3/295 (1)	0.003	0/301 (0.0)	0/8 (0.0)	0/292 (0.0)	1	χ^2
Moderate thrombocytopenia (25–49 × 10 ⁹ /l)	8/305 (2.6)	0/9 (0.0)	8/295 (2.7)	0.08	3/301 (1)	0/8 (0.0)	3/292 (1)	0.32	χ^2
Mild thrombocytopenia (50–149 × 10 ⁹ /l)	30/305 (9.8)	1/9 (11.1)	29/295 (9.8)	0.82	14/301 (4.6)	1/8 (12.5)	13/292 (4.4)	0.02	χ^2
Normal (>149 × 10 ⁹ /l)	263/305 (86.2)	7/9 (77.8)	256/295 (86.8)	0.09	284/301 (94.3)	7/8 (87.5)	277/292 (94.9)	0.08	χ^2
ESR, n (%)									
Severe elevation (>50 mm/h)	98/263 (37.3)	4/11 (36.4)	94/252 (37.3)	0.97	14/277 (5.0)	0/6 (0)	14/271 (5.2)	0.02	χ^2
Mild–moderate elevation (≥10, ≤50 mm/h)	112/263 (42.6)	5/11 (45.5)	107/252 (42.5)	0.67	130/277 (46.9)	3/6 (50)	127/271 (46.9)	0.67	χ^2
Normal (<10 mm/h)	53/263 (20.1)	1/11 (9.1)	52/252 (20.6)	0.02	133/277 (48)	3/6 (50)	130/271 (48)	0.78	χ^2

P-values indicating statistical significance are displayed in bold italic. IQR: interquartile range.

available, 1/18 variants was more common while failing to reach significance level, and two showed comparable frequencies. While results should be interpreted with caution because of the relatively small size of the cohort, and different sequencing approaches were used across studies, this suggests increased incidence and prevalence of 'genetic' SLE in the paediatric age group [18]. In agreement with previous reports, 'genetic' SLE patients exhibited mutations nucleic acid sensing and processing (42%), combination of nucleic acid sensing and processing with IC clearance (33%), immune cell signalling (17%) and NF- κ B (8%) signalling pathways. This translates to a majority (80%) of mutations affecting T1IFN production [19]. In agreement with previous reports [7], 'genetic' SLE was associated with an earlier disease onset, peaking in peri-pubertal patients [8], and less widespread organ involvement. As previously suggested by case reports and small case series, 'genetic' SLE more frequently affects young children (namely peripubertal onset) when compared with other forms of jSLE [7]. The relative over-representation of Black African/Caribbean patients in the 'genetic' (36.3%) when compared with the remaining SLE cohort (18.2%) further supports reports on genetic factors contributing to increased incidence of SLE in this racial group [4, 8, 20, 21].

While genetic factors in SLE are not limited to rare damaging variants, but include disease predisposing factors such as copy number variants, common nucleotide polymorphisms, and others [4, 20, 22], this study focussed on the group of jSLE patients experiencing disease caused by rare damaging variants, here referred to as 'genetic' SLE. The study identified a number of variants previously linked with AGS (9/348 patients, 2.6%), a type I interferonopathy that shares clinical and laboratory characteristics with SLE [6]. Why some patients develop AGS while others experience SLE remains unknown, but several 'genetic' SLE patients carried additional variants in SLE-associated genes (6/9, 66.7%) that may explain a shift from AGS to SLE phenotypes [6]. One patient exhibited a heterozygous 'stop' mutation in *PEPD*. Peptidase D is a prolidase involved in cleavage of dipeptides with a proline or hydroxyproline at their C-terminus. It contributes to collagen metabolism and matrix remodelling. Associations between prolidase deficiency and SLE-like disease and hypocomplementaemia have been reported [23, 24]. Thus, the identified heterozygous *PEPD* variant was classified as likely 'disease-modifying'. The cytoplasmic enzyme *SAMHD1* depletes deoxynucleoside triphosphates (dNTPs) necessary for viral cDNA synthesis and has nuclease activity against single-stranded DNA/RNA associated with its HD domain [25]. Damaging mutations in *SAMHD1* have been linked with AGS, familial chilblain lupus and 'genetic' SLE [26]. Three patients in this cohort exhibit mutations that likely impact on phosphohydrolase activity, and two carried additional variants in genes previously associated with 'genetic' SLE (*C1S*, *IRF7*). Notably, no patient with *SAMHD1* variants in this cohort had neurological, but two had severe mucocutaneous involvement. The absence of

neurological disease supports reports of mild delay or normal development, and increased skin disease and glaucoma in patients with *SAMHD1* mutations [27]. An additional variant in *IRF7* was present in one patient with a *SAMHD1* mutation. The *IRF7* transcription factor regulates T1IFN-dependent immune responses against DNA and RNA viruses. While the here-identified *IRF7* variant was not reported before, other variants in *IRF7* were linked with increased IFN- α serum levels in SLE [28]. Two mutations affecting the endonuclease subunit of *TREX1* were identified here. LOF mutations reducing DNase activity are associated with cytoplasmic accumulation of single-stranded DNA and 'genetic' SLE [14].

Gene variants affecting IC clearance can result in Toll-like receptor activation and T1IFN expression [29]. Defective IC clearance compromises immune tolerance and contributes to the production of autoantibodies, mainly directed against nuclear components [30]. Here, mutations affecting IC clearance were found in *C1S*, *C3* and *PEPD*. The *C1S* AD variant rs117907409 has been associated with 'genetic' SLE [14]. Deficiencies of C1r and C1s impact upon the classic complement pathway. C1s contributes to C4 and C2 activation, and formation of the C3-convertase enzyme complex [30]. Two patients had not previously reported predicted LOF variants in *C3* (c.4341C>A; c.1303G>T). Both experienced severe mucocutaneous involvement and high disease activity at diagnosis (SLEDAI: 23,12), mirroring reports of jSLE patients with low complement and high frequency of mucocutaneous manifestations [31]. One patient exhibited a GOF mutation in *C3* (c.2203C>T, rs117793540) predicted to cause hyper-activation of C3 through the alternative C3-convertase pathway, interfering with the anaphylatoxin-mediated response to infection. Previously, this mutation was reported in atypical haemolytic uremic syndrome (aHUS), C3 glomerulopathy [32], and increased risk of age-related macular degeneration [33, 34]. A significant proportion of aHUS patients exhibit C3 GOF mutations that associate with poor prognosis. The patient reported here did not experience renal involvement or haemolytic anaemia (yet). It remains unknown how many patients with C3 GOF mutations exhibit SLE-like disease in the absence of aHUS. Furthermore, patients reported in the literature developed aHUS in adulthood (>30 years of age), while the patient reported here is still now an adolescent/young adult [35]. The patient here is a carrier of an additional variant in *TREX1*, which may shift the phenotype to jSLE. One patient carried a predicted 'high impact' *TNFAIP3* variant. The *TNFAIP3* gene encodes the A20 protein, a negative regulator of the NF- κ B pathway. Loss-of-function mutations in *TNFAIP3* were reported in haploinsufficiency A20 (HA20), phenotypically resembling Behçet's disease. Recent reports suggest the HA20 clinical picture may be more variable, including SLE-like phenotypes [36]. Interestingly, the patient here carried an additional variant in *RNASEH2A* previously associated with AGS that may contribute to the SLE phenotype [13].

Monoallelic mutations in genes involved in T- and B-cell activation were observed in three patients. One patient carried a *PTPN22* mutation predicted to introduce a splice site abrogating protein function. Lymphoid-specific phosphatase (Lyp) is a key regulator of T cell activation, effector responses during infection, anti-tumour responses and autoimmunity. Risk alleles in *PTPN22* have been associated with SLE risk [15]. Another patient had a predicted LOF mutation in *TNFSF4*, accompanied by mutations in *C3* and *RNASEH2C*. The *TNFSF4* gene is implicated in T cell–antigen-presenting cell interactions, T/B cell proliferation, and adhesion of activated T lymphocytes to endothelial cells. Polymorphisms are associated with pathological T cell responses [37]. One patient carried a mutation in *BANK1*. *BANK1* is a positive regulator of B cell signalling, and variants in the gene are associated with SLE and other autoimmune/inflammatory disorders [38]. The here-identified variant was localized at the exon 2–intron 2 junction encoding a TIR domain involved in aggregation with MyD88, K63-linked polyubiquitination and IL-8 induction [16].

Antibody patterns across jSLE cohorts are of particular interest, as revised ACR/EULAR classification criteria include ‘ANA positivity’ as an entry criterion [39]. Compared with the remaining cohort, a higher proportion of ‘genetic’ SLE patients tested positive at diagnosis for anti-dsDNA antibodies that contribute to the pathogenesis through IC formation and T1IFN expression [40]. Antibodies against nuclear components can result from tissue damage and release of (auto-)antigens [41]. Type I interferonopathies and complement deficiencies involve pathological cytokine expression, sometimes in the absence of autoantibodies, resulting in tissue damage that, in turn, may trigger autoantibody production [4]. Thus, anti-dsDNA antibody production may be an early event in more common, ‘classical’ forms of SLE, contributing to disease expression, whereas in patients with ‘genetic’ SLE they may be secondary to tissue damage and reflect disease activity as previously reported in ‘classical’ SLE [42]. This is supported by the observation that, while comparable at diagnosis, ‘genetic’ SLE patients more frequently tested negative for ANA, anti-Sm and anti-La antibodies at last visit, which associated with reduced damage and fewer flares over time when compared with the remaining UK jSLE cohort.

In line with differential autoantibody patterns between sub-cohorts, and in agreement with previous reports, at diagnosis, ‘genetic’ SLE patients less frequently exhibited renal, neuropsychiatric and gastrointestinal involvement when compared with the remaining UK jSLE cohort, and deterioration in the renal domain and persistent significant activity in the haematological domain were less common [7]. In fact, persistent autoantibody positivity in the ‘remaining’ SLE cohort may contribute to, when compared with ‘genetic’ SLE, higher incidences and severity of renal and haematological involvement as autoantibodies have been linked to the pathophysiology and damage accrual in lupus nephritis, cytopenia and haemolysis [43].

Deterioration in the neuropsychiatric domain was more common in ‘genetic’ SLE when compared with the remaining patient cohort. Between diagnosis and last visit, two ‘genetic’ SLE patients developed neuropsychiatric involvement; both carried gene variants affecting genes involved in immune cell signalling and nucleic acid sensing and processing (*BANK1* and *DNASE1*). Though no *in vitro* data are available for the two mutations reported here, mutations in these genes were reported in SLE and AGS [6]. Development of neuropsychiatric symptoms may result from uncontrolled T1IFN expression as seen in AGS [44]. Generally, patients with AGS develop neurological symptoms early in life that are the result of pathological T1IFN expression, among others, caused by mutations in *TREX1* (60%), *SAMHD1* (15%), *IFIH1* and *DNASE1* [6]. In this cohort, six ‘genetic’ SLE patients exhibited six individual or combined variants affecting these genes; 33.3% (2/6) exhibited moderate or severe neuropsychiatric involvement by their last visit, in contrast to none of the remaining ‘genetic’ SLE and 3.6% of the remaining cohort. Recently, Blokland *et al.* reported a mechanism involving the AKT–dopamine signalling pathway, suggesting a mechanism for the involvement of *BANK1* in the BOLD (blood oxygen level-dependent) response to working memory and thus indirect impact on common disorders affecting brain function [45]. Moreover, *BANK1* was identified as a modulator of gut microbiota composition, which affects susceptibility to schizophrenia [46].

Considering that, across the available literature, the cumulative jSLE patient population experiences more renal and haematological involvement when compared with adult-onset SLE patients [1], jSLE patients without rare damaging gene variants (‘classical SLE’) appear to be primarily responsible for this. Notably, this is not caused by more aggressive first-line treatment in ‘genetic’ SLE. Initially, DMARD use (including rituximab) was comparable between groups, and ‘genetic’ SLE patients did not receive CPM as first-line agent, while 7.7% of the remaining SLE patients did. However, later during the disease course, ‘genetic’ SLE patients less frequently received ‘no treatment’, while more frequently requiring third-line DMARDs. This suggests that persisting inflammation and accumulation of tissue damage may trigger increased use of second- or third-line treatments, and that early effective treatment may prevent disease progression. This study investigates the largest number of jSLE patients to date targeting a comprehensive panel of genetic variants, but is limited by patient numbers and the *in silico* methodology. While for some variants *in vitro* data were available and considered for data interpretation, previously not reported variants were assessed using combined computational tools. Study design and ethics precluded investigation of family members, which may have assisted in identifying additional rare mutations and to assess the *de novo* character of variants [47]. The size of the ‘genetic’ and ‘remaining’ SLE cohorts varied and may limit statistical comparisons. Variants included were selected in 2018, based on literature available at

the time. As expected, in the meantime, additional rare variants were identified suggesting likely underestimation of the number of 'genetic SLE' patients [14, 18]. Furthermore, protein kinase C δ (*PKC δ*), a regulator of B cell survival and proliferation that has been associated with SLE-like disease, was not included in the panel [48].

Conclusions

In the UK, a minimum of 3.5% of JSLE patients experience genetic disease, exceeding the previously reported 1–3% across all age groups. Variants identified affect nucleic acid sensing and processing, IC clearance, and immune cell and NF- κ B signalling pathways. Notably, mutations previously associated with other autoimmune/inflammatory conditions were identified, suggesting that additional factors may shift phenotypes to SLE. Patients with 'genetic' SLE more frequently develop disease around puberty, exhibit distinct antibody patterns and dynamics, develop fewer flares and damage, exhibit less haematological and renal involvement, while developing neuropsychiatric involvement over time. Thus, achieving a molecular diagnosis aiding patient stratification will enable personalized treatment, prevention of damage, individualized disease-monitoring, patient education and (genetic) counselling.

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Data availability statement

Access to raw data will be considered by the UK JSLE Cohort Study board upon reasonable request. At the end of the wider study, sequencing datasets will be deposited in publicly available databases.

Supplementary data

Supplementary data are available at *Rheumatology* online.

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