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TESI DI DOTTORATO

Alternate allele counts associate with ancestry, age at disease onset, organ involvement and disease severity in juvenile systemic lupus erythematosus

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Abstract

Background: Juvenile systemic lupus erythematosus (jSLE) is more severe when compared to adult-onset disease, with higher disease activity, more organ damage and less favourable outcomes. Genetic factors have been suggested to influence age at disease onset, disease phenotypes and severity of SLE.

Aims: This study explored associations between genetic burden, approximated through alternate allele counts (AAC), and age at disease onset, sex, ancestry, disease activity/severity, organ involvement and treatments in jSLE.

Methods: 289 participants from the UK jSLE Cohort Study were studied applying a panel sequencing approach covering 62 genes/genomic regions. Clinico-demographic features, disease activity and treatments were recorded. Panel-wide AAC and gene-level alternate allele scores (GAAS) were generated by counting and weighting alternate alleles considering their *in silico* predicted impact on gene function. Correlation analyses were applied to investigate associations between genetic burden, ancestry and age at diagnosis. Generalised linear models, adjusted for ancestry, sex and family history of autoimmune/inflammatory diseases, were used to interrogate associations with organ involvement and disease activity/severity.

Results: A weak inverse correlation was found between age at diagnosis and AAC ($R=-0.15$, $p=0.01$) that was primarily driven by a moderate inverse correlation in South Asians ($R=-0.28$, $p<0.001$). Black African/Caribbean patients exhibited higher AAC compared to remaining ancestral groups ($p<0.001$). Constitutional ($p<0.001$), renal ($p=0.001$), haematological ($p=0.001$) and neuropsychiatric ($p=0.03$) involvement and treatment intensity ($p<0.001$) were associated with AAC. GAAS associated with clinical variables, including severity of neuropsychiatric (*ACP5, TYK2*, *RNASEH2A*, $p<0.001$; RASGRP3, $p=0.04$) and renal (*ACP5*, *ITGAM*, *LYN*, $p<0.001$; *TNFAIP3*, $p=0.007$) involvement.

Conclusions: Genetic variability likely contributes to early disease expression in South Asian patients. GAAS associate with organ involvement and disease activity/severity across ancestries. Observations from this study argue for genetic risk assessment and future patient stratification towards personalised treatment and care.

1. Background

1.1 Juvenile Systemic Lupus Erythematosus

1.1.1 Introduction

Systemic lupus erythematosus (SLE) is a severe, chronic, multisystem autoimmune and autoinflammatory disease characterized by relapsing-remitting episodes of widespread inflammation (1)(2). It can affect virtually any organ system, most commonly involving the skin, musculoskeletal system, haematological compartment, kidneys, and central nervous system. SLE is clinically heterogeneous, encompassing a broad spectrum of manifestations ranging from mild disease to life-threatening organ involvement, and is marked by unpredictable flares and periods of remission of variable duration.

Juvenile systemic lupus erythematosus (jSLE) is defined by disease onset before the age of 18 years and accounts for approximately 15-20% of all SLE cases (3)(4)(5). Compared with adult-onset disease, jSLE is associated with a more severe clinical phenotype, characterized by higher disease activity at presentation, greater burden of organ involvement and damage, and a greater need for aggressive immunosuppressive therapy (6)(7).

Mortality remains increased in SLE compared with the general population, with standardized mortality ratios (SMR) of approximately 2.2 across all age groups (8). Notably, children and adolescents with SLE experience substantially higher mortality, with an SMR of approximately 6.5, representing a threefold increase compared with adult patients (8). These observations underscore the critical importance of early recognition and timely diagnosis of jSLE to enable prompt therapeutic intervention, limit irreversible organ damage, improve long-term outcomes, and reduce disease-associated mortality.

1.1.2. Epidemiology

The reported incidence of jSLE ranges from 0.36 to 2.5 per 100,000 children per year, with a prevalence between 1.89 and 34.1 per 100,000. Incidence rates vary considerably across geographic regions, largely reflecting differences in the ethnic composition of populations, with higher rates observed among non-Caucasian groups (9)(10).

Ethnicity also influences disease phenotype, severity, and outcomes. In particular, patients of Black African/Caribbean ancestry exhibit a higher prevalence of renal involvement and are more likely to

receive intensive immunosuppressive therapies, including cyclophosphamide and rituximab, compared with Caucasian patients (11)(12). In addition, non-Caucasian patients tend to develop jSLE at a younger age than Caucasian patients (11).

The peak age of onset of jSLE is between 12 and 14 years. The disease shows a clear female predominance; however, this sex disparity is less pronounced in jSLE than in adult-onset SLE, with reported female-to-male ratios of approximately 5:1 in jSLE compared with 9:1 in adults (13).

1.1.3 Pathogenesis

The etiopathogenesis of SLE remains incompletely understood and is considered multifactorial, resulting from complex interactions between genetic susceptibility, epigenetic regulation, hormonal influences, and environmental triggers (14)(15).

Compared with adult-onset disease, genetic factors appear to play a more prominent role in jSLE, as suggested by earlier disease onset, increased clinical severity, a less marked female predominance, and the occurrence of lupus-like phenotypes in childhood associated with inherited disorders (16)(17)(18). Familial aggregation further supports a strong genetic contribution, with siblings of affected patients exhibiting a markedly increased risk of disease and higher concordance rates observed in monozygotic compared with dizygotic twins (19)(20)(21). Genetic predisposition in jSLE reflects alterations in genes involved in immune regulation, which may either directly cause disease in rare monogenic forms or increase susceptibility to autoimmunity in genetically complex disease (22)(23). In the latter scenario, additional environmental, hormonal, epigenetic, and immunoregulatory factors are required for clinical disease expression (24)(25).

Regardless of the underlying genetic architecture, both monogenic and genetically complex jSLE are characterised by profound dysregulation of the immune system, involving both innate and adaptive immune responses (26).

A central pathogenic mechanism in jSLE is the dysregulation of the innate immune system, particularly sustained activation of the type I interferon (IFN) pathway (27)(28)(29). Elevated IFN-α levels are a hallmark of SLE and contribute to chronic immune activation and persistent inflammation (30). Type I IFNs are produced predominantly by innate immune cells, including plasmacytoid dendritic cells, monocytes, macrophages, neutrophils, and epithelial cells, in response to nucleic acid-containing stimuli (31). Persistent IFN signalling amplifies immune responses and promotes loss of tolerance (31).

Additional innate immune abnormalities further contribute to disease pathogenesis. Neutrophils from patients with jSLE exhibit increased susceptibility to apoptosis and enhanced propensity for NETosis,

a specialised form of inflammatory cell death characterised by the release of neutrophil extracellular traps (NETs) composed largely of chromatin (32). NETs represent a major source of extracellular autoantigens and are thought to contribute to autoantibody production and immune complex formation. Excessive NET formation and/or impaired NET clearance have been described in jSLE, reinforcing their pathogenic role in genetically predisposed individuals (32). Cells of the monocyte-macrophage lineage, which are critical for the clearance of immune complexes and apoptotic debris, also show functional impairment, including reduced phagocytic capacity and increased production of type I IFNs, further amplifying inflammatory responses (33)(34).

Dysregulation of the adaptive immune system represents another hallmark of jSLE. The humoral immune response is particularly prominent, as reflected by the production of a broad spectrum of autoantibodies, predominantly directed against nuclear antigens (35)(36). These autoantibodies are generated by activated plasmablasts and plasma cells, likely driven by excessive innate immune activation. In parallel, patients with jSLE exhibit reduced numbers of regulatory B cells and increased levels of B-cell-activating cytokines, such as B-lymphocyte stimulator (BLyS), indicating profound disturbances in B-cell homeostasis (37).

Cellular adaptive immunity is also markedly altered (15). Circulating $CD4^+$ T cells are skewed towards differentiated effector phenotypes, including effector memory and terminally differentiated effector memory subsets, with a relative reduction in naïve T cells (38)(39). Activated T cells preferentially produce pro-inflammatory cytokines, such as interleukin-17A (IL-17A), while showing impaired production of interleukin-2 (IL-2)(40). These abnormalities have important clinical implications, as IL-17A-producing cells have been detected in target organs, including the kidneys of patients with lupus nephritis, contributing to tissue inflammation and damage (41)(42). Conversely, reduced IL-2 availability may disrupt the balance between effector and regulatory T cells and impair cytotoxic $CD8^+$ T-cell function, further promoting immune dysregulation in jSLE (43).

1.1.4. Clinical presentation

JSLE is a highly heterogeneous autoimmune/inflammatory disease that may present with a broad spectrum of clinical manifestations and disease severity, potentially affecting every organ or system (44)(45). At disease onset, the most frequently reported symptoms are constitutional features, including fatigue, fever, and weight loss, which are non-specific and overlap with those of more prevalent conditions such as chronic fatigue syndrome (11). This clinical overlap contributes to diagnostic delay, increasing the risk of under-recognition and subsequent irreversible organ damage.

Additional non-specific manifestations commonly observed at presentation include oral ulcers, myalgia, arthralgia, lymphadenopathy, headache, and neuropsychiatric symptoms such as mood disorders and headaches (11). Among objective clinical findings, arthritis (typically non-erosive), malar rash, and renal involvement represent the most common signs at diagnosis.

Mucocutaneous manifestations are reported in approximately 60-80% of patients and encompass malar rash, photosensitivity, nasal or oral ulcers, cutaneous vasculitis, and alopecia (46). Other dermatological features, including Raynaud's phenomenon, livedo reticularis, subacute cutaneous lupus erythematosus, discoid lesions, and lupus panniculitis, may also occur but are more frequently observed in adult-onset disease (46).

Renal involvement represents one of the most severe and clinically relevant manifestations of jSLE (47)(48)(49). Overall, 50-80% of affected children develop urinary abnormalities or impaired renal function during the disease course. The clinical spectrum of lupus nephritis ranges from asymptomatic urinary findings to arterial hypertension, nephrotic syndrome, and progressive renal failure. Renal disease is independently associated with increased mortality risk and greater long-term damage accrual (47)(48)(49). Kidney biopsy is recommended in patients presenting with deteriorating renal function, significant proteinuria, haematuria, or arterial hypertension, as histological characteristics according to the ISN/RPS 2003 classification not only provide prognostic information but also directly inform therapeutic decision-making (50).

Neuropsychiatric (NP) involvement is more prevalent and often more severe in jSLE compared with adult-onset SLE (51)(52). Clinical manifestations include seizures, psychosis, cerebrovascular events, peripheral nervous system involvement, headache, cognitive dysfunction, and mood or anxiety disorders (51)(52). The presence of NP disease is associated with higher disease activity, poorer overall prognosis, and increased mortality (53).

Haematological abnormalities are observed in more than 70% of patients with jSLE and occur more frequently than in adult-onset SLE (54). These manifestations encompass a wide spectrum, including autoimmune haemolytic anaemia, leukopenia, lymphopenia, and immune-mediated thrombocytopenia (54). Nearly two-thirds of patients present with at least one cytopenia at diagnosis or even prior to the formal recognition of SLE. Idiopathic thrombocytopenic purpura represents a classical example of an early haematological presentation, with antinuclear antibody (ANA) positivity and positive type I IFN signature identified as risk factors for subsequent lupus development (55)(56). Autoimmune haemolytic anaemia, in particular, is associated with increased disease severity and greater damage accrual (57).

Gastrointestinal (GI) involvement in jSLE is heterogeneous and may occur as a direct manifestation of disease activity, as a consequence of treatment-related adverse effects, or more rarely in the context of macrophage activation syndrome (MAS)(58)(59)(60). Primary disease-related GI manifestations include autoimmune hepatitis and lupus enteritis, whereas drug-related complications comprise toxic hepatitis associated with non-steroidal anti-inflammatory drugs or methotrexate, and gastritis related to glucocorticoid therapy. Hepatosplenomegaly and hypertransaminasemia may reflect MAS. Additional GI features include non-specific abdominal pain, oesophageal dysmotility, serositis, and pancreatitis (58)(59)(60).

Cardio-pulmonary involvement is less common in jSLE compared with adult-onset SLE (61). Pericarditis and myocarditis represent the most frequently reported cardiac manifestations, while coronary artery and valvular involvement are rare in the paediatric population. Pleural effusions, pleuritis, acute and chronic pneumonitis, and pulmonary infiltrates constitute the main pleuro-pulmonary manifestations observed in affected children (62).

MAS is a rare but life-threatening complication of systemic inflammatory diseases, including SLE (63). The presence of unexplained persistent fever and cytopenia, in association with hyperferritinemia, hypertransaminasemia and/or hypofibrinogenemia should prompt a high index of suspicion for MAS, allowing for timely diagnosis and prompt initiation of appropriate treatment (63).

1.1.5 Laboratory examinations

Laboratory investigations play a crucial role in supporting the diagnosis of jSLE and in monitoring disease activity over time (11)(44)(64). Baseline and follow-up assessments typically include a full blood count to identify cytopenias, together with a direct Coombs test to evaluate immune-mediated haemolysis. Biochemical investigations encompass liver, renal, and pancreatic function tests, which may reflect disease involvement or treatment-related toxicity (11)(44)(64).

Inflammatory markers are routinely assessed, with erythrocyte sedimentation rate (ESR) commonly elevated during disease flares, whereas C-reactive protein (CRP) levels are often normal unless a concomitant infection is present (65). Complement levels, particularly C3 and C4, are frequently reduced during periods of increased disease activity and are widely used as biomarkers of flare (66). Additional investigations include coagulation studies and serum ferritin, the latter being especially relevant when MAS is suspected (63). Measurement of immunoglobulin levels (IgG, IgA, and IgM) may reveal polyclonal hypergammaglobulinaemia, reflecting immune system activation.

Immunological evaluation represents a cornerstone of jSLE assessment and includes the detection of antinuclear antibodies (ANA), anti-dsDNA antibodies, and extractable nuclear antigen (ENA) antibodies, such as anti-Sm, anti-RNP, anti-Ro, and anti-La (67). Screening for antiphospholipid antibodies, anti-C1q antibodies, and antineutrophil cytoplasmic antibodies (ANCA) is also performed (67). ANCA testing is particularly valuable in the differential diagnosis of lupus-related renal and systemic manifestations. Moreover, ANCA positivity, when observed in conjunction with specific clinical features such as lupus nephritis and hypocomplementemic urticarial vasculitis, may raise suspicion for an underlying monogenic condition, including DNASE1L3 deficiency (68). Assessment of renal involvement requires dedicated investigations, including urinalysis to detect proteinuria, haematuria, and urinary casts, as well as quantification of proteinuria using the urine albumin-to-creatinine ratio (69).

1.1.6 Diagnosis

The broad clinical heterogeneity characterising jSLE poses significant diagnostic challenges (3)(44). In addition, the absence of pathognomonic clinical manifestations or laboratory biomarkers means that the diagnosis of jSLE is supported by the application of classification criteria. To date, no classification criteria have been specifically developed or validated for jSLE, and all currently available criteria sets are derived predominantly from adult-onset SLE cohorts (70)(71)(72).

The 1997 revised American College of Rheumatology (ACR) classification criteria remain the most widely used and historically accepted framework for SLE classification (70). These criteria consist of 11 clinical and laboratory items, with classification as SLE requiring the fulfilment of at least four criteria. Clinical criteria include malar rash, discoid lupus, photosensitivity, oral or nasal ulcers, non-erosive arthritis, serositis, renal involvement, and seizures or psychosis. Laboratory criteria comprise haematological disorders, namely haemolytic anaemia with reticulocytosis, leukopenia, lymphopenia, or thrombocytopenia, together with ANA positivity and the presence of at least one among anti-dsDNA antibodies, anti-Sm antibodies, or antiphospholipid antibodies.

The Systemic Lupus International Collaborating Clinics (SLICC) classification criteria, published in 2012, have demonstrated higher sensitivity than the ACR criteria in the paediatric population (71)(73)(72). These criteria include 17 items, divided into clinical and immunological domains, with classification requiring at least four fulfilled criteria, including at least one from each domain. Clinical criteria encompass acute and chronic cutaneous lupus, non-scarring alopecia, oral or nasal ulcers,

arthritis, serositis, renal disease, neurological manifestations, and haematological involvement. Immunological criteria include ANA positivity, anti-dsDNA and anti-Sm antibodies, antiphospholipid antibodies, low complement levels, and a positive direct Coombs test. Notably, the SLICC criteria allow classification of SLE in the presence of histologically confirmed lupus nephritis together with ANA or anti-double-stranded DNA antibody positivity. In both the ACR-1997 and SLICC-2012 criteria, all items are equally weighted.

More recently, the 2019 EULAR/ACR SLE classification criteria were developed to improve sensitivity (72). This system includes seven clinical and three immunological domains, with a weighted scoring system in which a total score of at least 10 points is required for classification. Key innovations include the use of ANA positivity as a mandatory entry criterion, the inclusion of fever among the clinical items, and the assignment of different weights to individual criteria. However, studies evaluating the performance of the 2019 EULAR/ACR criteria in jSLE have not demonstrated superiority over the ACR or SLICC criteria, particularly in prepubertal patients and at disease onset (74)(75). A major limitation in the paediatric setting is the requirement for ANA positivity as an entry criterion, which may contribute to diagnostic delay in ANA-negative patients.

Overall, the ACR and SLICC criteria appear to provide the best sensitivity and specificity, respectively, in jSLE (76)(77). Nevertheless, it is essential to recognise that these criteria were developed for classification rather than diagnostic purposes and have not been formally validated in paediatric populations. Therefore, the diagnostic evaluation of suspected jSLE should always involve paediatric rheumatology expertise to ensure timely and accurate diagnosis.

1.1.7. Treatment

In line with the treat-to-target (T2T) strategy, the primary goals of treatment in jSLE are to induce disease remission or, when this is not achievable, to attain and maintain a low disease activity state (LDAS), while minimising disease flares, preventing irreversible organ damage, and reducing long-term disability, morbidity, and mortality (78)(79)(80).

Owing to the low prevalence of jSLE, the conduct of adequately powered randomised clinical trials remains challenging, resulting in limited high-quality evidence and disease-specific management guidelines. Consequently, substantial variability exists in therapeutic approaches across countries, regions, and individual centres, highlighting the need for internationally agreed, evidence-based recommendations (81)(82). In this context, international collaboration is essential to ensure equity in

standards of care and harmonisation of clinical practice, as exemplified by the Single Hub and Access point for paediatric Rheumatology in Europe (SHARE) initiative (64).

In parallel, advances in the understanding of the molecular and immunological pathophysiology of SLE have enabled the development of personalised and target-directed therapies (3). Recent years have witnessed unprecedented progress in the therapeutic landscape, including the approval of biologic agents such as belimumab and anifrolumab and the emergence of innovative approaches like chimeric antigen receptor (CAR) T-cell therapy (83)(84)(85). Although jSLE management remains largely informed by adult-onset SLE data, an expanding body of paediatric-specific evidence increasingly supports the use of biologic and targeted therapies, particularly in patients with refractory disease or severe multi-organ involvement.

Glucocorticoids

Systemic glucocorticoids are the most frequently used first-line agents in jSLE due to their rapid anti-inflammatory effects (86)(87). Oral prednisone, at doses of 1-2 mg/kg/day, is commonly employed in patients with mild to moderate disease activity. In cases of severe disease, major organ involvement, or acute flares, intravenous high-dose (1-2 mg/kg) methylprednisolone or pulses (30 mg/kg/dose max 1g/dose for 3-5 days) may be administered at presentation. However, prolonged glucocorticoid exposure is associated with significant adverse effects, including Cushingoid features, hyperglycaemia, dyslipidaemia, secondary osteoporosis, and growth impairment (88). For this reason, steroid-sparing immunosuppressive therapies should be introduced early when adequate disease control cannot be achieved.

Hydroxychloroquine

Hydroxychloroquine is an antimalarial agent widely used alone in the management of mild SLE, and is generally well tolerated. Given its favourable safety profile, hydroxychloroquine is recommended for routine use in all jSLE patients, unless contraindicated (64). Although its precise mechanism of action remains incompletely understood, it is known to inhibit endosomal Toll-like receptors (TLR3, TLR7, and TLR9), thereby reducing IFN-α production (89). Retinal toxicity represents a potential adverse effect, although the risk is low and primarily associated with prolonged exposure to high doses or pre-existing ocular disease (89).

Disease-modifying anti-rheumatic drugs (DMARDs)

When persistent disease activity precludes glucocorticoid tapering, or in case of moderate-severe disease activity at presentation, the addition of a disease-modifying anti-rheumatic drug (DMARD) is recommended to achieve sustained disease control and minimise steroid-related toxicity (90)(91).

Mycophenolate mofetil (MMF) is an immunosuppressive agent that inhibits inosine monophosphate dehydrogenase, thereby impairing de novo purine synthesis and selectively suppressing T- and B-lymphocyte proliferation. MMF is a well-tolerated steroid-sparing agent and represents the most commonly used first-line immunomodulatory therapy in many countries (92). In recent years, MMF has increasingly replaced cyclophosphamide as first-line induction therapy for lupus nephritis. Evidence from a Cochrane review suggests that MMF may be associated with higher rates of complete remission in proliferative lupus nephritis compared with cyclophosphamide, with an acceptable toxicity profile (93).

Azathioprine is another commonly used steroid-sparing immunosuppressive agent (94)(92). Its mechanism of action involves inhibition of RNA, DNA, and protein synthesis, resulting in suppression of both B- and T-cell function and reduced antibody production. Genetic screening for thiopurine S-methyltransferase (TPMT) deficiency is recommended prior to treatment initiation, as azathioprine is contraindicated in patients with absent enzyme activity and requires careful monitoring in those with reduced activity due to the risk of myelotoxicity (95).

Cyclophosphamide is primarily reserved for remission induction in life-threatening disease or severe major organ involvement, particularly lupus nephritis, vasculitis, and neuropsychiatric lupus (90)(91). As an alkylating agent, it inhibits DNA replication, leading to lymphocyte depletion and suppression of B- and T-cell responses. Its use is limited by significant adverse effects, including myelosuppression, gonadal toxicity, haemorrhagic cystitis, and alopecia.

Methotrexate is infrequently used in jSLE and is generally restricted to patients with predominant musculoskeletal or mucocutaneous manifestations, where it exerts its immunomodulatory effects mainly through inhibition of dihydrofolate reductase and modulation of purine metabolism, leading to reduced lymphocyte proliferation and cytokine production (90)(91).

Biological and targeted therapies

Advances in the understanding of the immunopathogenesis of SLE, particularly the central role of B cells and type I IFN pathways, have driven the development of biologic and targeted therapies that

are increasingly relevant in these patients, especially in patients with refractory disease or severe organ involvement (3).

Belimumab, a monoclonal antibody targeting B-lymphocyte stimulator (BLyS), is currently the only biologic disease-modifying antirheumatic drug formally approved for use in jSLE (≥5 years) (83)(96)(97)(98). Its efficacy in reducing disease flares, enabling glucocorticoid tapering, and maintaining disease control has been demonstrated in clinical trials, real-world cohorts, and long-term extension studies across adult and paediatric populations.

Rituximab, a chimeric anti-CD20 monoclonal antibody, despite not being formally approved for SLE, remains widely used in jSLE and is recommended in international guidelines for refractory disease (99)(100)(101). Real-world data support its effectiveness, particularly in lupus nephritis and in patients with persistent serological activity (102)(103)(104). More potent B-cell-depleting strategies, including type II anti-CD20 antibodies such as obinutuzumab, as well as plasma cell-targeting approaches, are emerging as promising options, although paediatric evidence remains limited and largely derived from ongoing trials (105)(106).

Beyond B-cell-directed therapies, targeting the type I IFN pathway has represented a major advance in SLE treatment (15). Anifrolumab, a monoclonal antibody against the IFN-α receptor, is approved for adult SLE and is currently under investigation in paediatric populations, with early reports suggesting potential benefit, particularly in cutaneous disease (84)(107)(108)(109).

In parallel, intracellular signalling inhibitors have emerged as promising therapeutic options in SLE by targeting key pathways involved in immune activation and inflammation. Janus kinase (JAK) inhibitors, through blockade of multiple cytokine signalling cascades have demonstrated encouraging efficacy in adult SLE, particularly for mucocutaneous and musculoskeletal manifestations (110)(111). Although paediatric experience remains limited, early case series and ongoing mechanistic studies support a growing interest in their potential application to jSLE (112)(113).

Similarly, calcineurin inhibitors (CNIs) have a well-established role in the management of lupus nephritis, exerting a dual mechanism of action by inhibiting T-cell activation via the calcineurin pathway and stabilising the podocyte cytoskeleton. While traditional CNIs such as tacrolimus have demonstrated efficacy, concerns regarding nephrotoxicity have driven the development of newer agents with improved safety profiles, including voclosporin, which has shown significant benefit in adult lupus nephritis (114)(115)(101)(100)(116). Although not yet approved for use in children, these

agents are currently under evaluation in adolescent populations, further expanding the therapeutic landscape of jSLE (NCT05337124, NCT05288855).

Finally, highly innovative immunotherapeutic approaches such as CD19-directed chimeric antigen receptor (CAR) T-cell therapy have demonstrated sustained drug-free remission in adults with severe, treatment-refractory SLE, with emerging evidence of similar responses in adolescents(117)(118)(119)(120)(121). While these strategies are potentially transformative, their complexity, cost, and the need for long-term safety data currently limit their use to specialised centres.

Multitarget therapeutic strategies in lupus nephritis

Lupus nephritis is the most frequent severe manifestation of jSLE and a major determinant of long-term morbidity and mortality. Despite high initial remission rates with conventional induction regimens, renal relapses remain common, highlighting the limitations of the traditional sequential treatment approach based on glucocorticoids combined with MMF or cyclophosphamide, followed by maintenance therapy(122)(123)(124). In recent years, treatment strategies in adult lupus nephritis have evolved towards a multitarget induction approach, combining glucocorticoids with mycophenolate mofetil and either belimumab or calcineurin inhibitors, or low-dose cyclophosphamide and belimumab. This strategy has been incorporated into major international guidelines, including those from EULAR, KDIGO, and the ACR (101)(125)(126). Although not yet formally approved for jSLE, increasing clinical experience suggests that multitarget regimens may also be effective in paediatric lupus nephritis, allowing improved disease control and more rapid glucocorticoid tapering without significant safety concerns (127)(128). These observations support further evaluation of combination strategies in jSLE within both clinical practice and future paediatric trials.

1.1.8 Disease monitoring

jSLE is characterised by marked clinical heterogeneity and an unpredictable disease course, with alternating phases of remission and disease flares. Consequently, regular and systematic assessment of disease activity is central to optimal patient management, in line with the T2T strategy (129)(130). Patients should be reviewed at regular intervals, with each visit including a detailed clinical history and a comprehensive physical examination aimed at identifying both active disease manifestations

and early signs of flare. In addition, the impact of disease on quality of life should be routinely assessed using validated child health and well-being questionnaires.

Objective quantification of disease activity is essential for evaluating treatment effectiveness and guiding therapeutic decisions. To this end, validated disease activity indices should be applied at every clinical review. The most widely used instruments in both clinical practice and clinical trials include the Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K)(131), the British Isles Lupus Assessment Group (BILAG) score (132), and the Physician Global Assessment (PGA)(133). In parallel, annual assessment of cumulative organ damage using the SLICC/ACR Damage Index is recommended (134)(135).

SLEDAI-2K

SLEDAI-2K is a validated composite index for the assessment of disease activity in SLE (131). It comprises 24 clinical and laboratory items, each weighted according to the type of manifestation, although not according to severity. The index captures activity across multiple organ systems, including neuropsychiatric, mucocutaneous, cardiopulmonary, renal, haematological, musculoskeletal, and vascular domains, as well as the presence of fever, immunological abnormalities such as anti-dsDNA antibodies, and low complement levels.

BILAG

The BILAG index is an organ-based disease activity assessment tool grounded in the principle of the physician's intention to treat. Its paediatric adaptation, the paediatric BILAG-2004 (pBILAG-2004), has been specifically validated for use in children with SLE (132).

For each clinical encounter, disease activity is assessed across individual organ systems and graded using a five-tier alphabetical scale (A-E):

- **A**: severe disease activity requiring urgent treatment escalation;
- **B**: moderate disease activity requiring close monitoring and often treatment adjustment;
- **C**: stable, low-level disease activity not requiring treatment modification;
- **D**: inactive disease with previous organ involvement;
- **E**: no current or previous involvement.

Numerical values are assigned to each category (A = 9, B = 3, C = 1, D/E = 0), allowing calculation of a global pBILAG-2004 score. According to BILAG definitions, a flare is classified as severe ("A

flare") when a domain previously scored B-E worsens to A, and as moderate ("B flare") when a domain progresses from C-E to B.

Physician Global Assessment

The Physician Global Assessment is a subjective measure of overall disease activity based on the clinician's integrated evaluation of symptoms, physical findings, and laboratory results (133). It is typically scored on a visual analogue or numerical scale ranging from 0 (no disease activity) to 3 (severe disease activity) and is most informative when used in conjunction with objective disease activity indices such as SLEDAI-2K.

SLICC/ACR Damage Index

The SLICC/ACR Damage Index is a validated tool designed to quantify irreversible organ damage accrued since disease onset, irrespective of ongoing disease activity (134)(135). The index encompasses 17 organ systems, including mucocutaneous, cardiovascular, pulmonary, renal, neuropsychiatric, musculoskeletal, haematological, gastrointestinal, and malignancy-related damage. Each item is scored according to the presence and extent of permanent damage, with higher scores reflecting greater cumulative burden. This index is typically assessed annually and provides valuable information on long-term outcomes, treatment effectiveness, and prognosis, supporting longitudinal monitoring and clinical decision-making in patients with jSLE.

1.2 Genetic architecture of SLE and disease heterogeneity

Despite convergence on shared immunopathogenic pathways, jSLE is characterised by marked clinical heterogeneity (136). Differences in age at onset, disease severity, organ involvement, and long-term outcomes suggest that variability in genetic background plays a central role in shaping individual disease phenotypes (137)(22)(18). The genetic architecture of SLE encompasses both rare, highly penetrant variants and a large number of common susceptibility alleles, whose combined effects contribute to disease risk and heterogeneity (138)(139).

1.2.1 (Ultra-)rare variants and monogenic lupus

A small but clinically relevant proportion of SLE cases (3.5-15%) are caused by rare or ultra-rare pathogenic variants in single genes, resulting in monogenic forms of lupus or lupus-like disease (138)(140)(141)(142). These cases are more frequently observed in paediatric populations, particularly among patients with very early disease onset, severe phenotypes, or atypical clinical features (143).

Monogenic lupus arises from defects in genes involved in key immune regulatory processes, including nucleic acid sensing and clearance, complement activation, apoptotic pathways, and immune tolerance (144)(145). Although monogenic forms account for only a minority of SLE cases, their study has been instrumental in elucidating fundamental disease mechanisms (144)(145). Importantly, many of the pathways disrupted in monogenic lupus overlap with those implicated in genetically complex SLE, supporting the concept that rare variants represent extreme examples of dysregulation within shared pathogenic networks.

1.2.2 Common genetic variants and polygenic susceptibility

In the majority of patients, SLE develops as a genetically complex disease driven by the cumulative impact of multiple common genetic variants, each conferring a modest increase in disease risk (5)(2)(136). Genome-wide association studies have identified hundreds of SLE-associated loci, predominantly affecting genes involved in innate and adaptive immune responses, cytokine signalling, immune complex handling, and host defence mechanisms (146)(147)(148)(149)(150)(151)(152).

The contribution of common variants to disease heritability is substantial and appears to be particularly pronounced in jSLE, where patients often exhibit a higher overall genetic load compared with adult-onset disease (153)(154). The human leukocyte antigen (HLA) region represents the strongest genetic risk locus and shows marked ancestry-specific associations with both disease susceptibility and clinical manifestations (155)(156)(157)(158). Together, these findings underscore

the polygenic nature of SLE and highlight the importance of considering cumulative genetic risk rather than individual variants in isolation.

1.2.3 Genetic burden and risk modelling in SLE

In recent years, innovative genetic approaches have identified previously unrecognized SLE-associated variation (159). A large multi-ancestral study including individuals of East Asian, European, and admixed American ancestries applied multitrait analyses across 13 autoimmune and inflammatory diseases, confirming known and identifying novel SLE risk loci (159). Given the potential cumulative effect of common variants, genetic variability has been proposed as a tool to predict disease outcomes and organ involvement in SLE through genetic risk scores (GRS), polygenic risk scores (PRS), and genome- or gene-specific alternate allele counts (AAC).

GRS aggregate the effects of selected variants previously associated with SLE, whereas PRS capture the cumulative contribution of thousands of variants across the genome derived from GWAS, not all of which are individually linked to SLE; AAC, instead, consider all variants within a given genomic region, regardless of prior disease association (160). Several GRS models have been proposed, showing potential for patient stratification (18). Consistent with earlier studies, Dominguez et al. reported an inverse association between non-HLA GRS and age at disease onset, while HLA GRS correlated with older age at diagnosis (17). Webber et al. found that both HLA and non-HLA genetic load were associated with lupus nephritis risk, with stronger (though non-significant) effects in jSLE compared with adult-onset SLE (161). Misztal et al. further showed that jSLE patients with monogenic disease do not differ in GRS from non-carriers, supporting the concept that rare deleterious variants alone may drive early-onset disease (142).

Using PRS approaches, associations between both HLA and non-HLA scores and reduced estimated glomerular filtration rate were demonstrated, suggesting a role for PRS in LN prediction and monitoring (162). Similarly, higher PRS in genetically complex jSLE and aSLE compared with controls have been described, whereas PRS in monogenic jSLE were comparable to those of healthy individuals, indicating distinct genetic architectures between monogenic and polygenic disease (163).

Despite these promising findings, several limitations hinder clinical implementation. Most GWAS underpinning PRS and GRS have been conducted in European populations, reducing accuracy in other ancestries and raising equity concerns in genomic medicine (163)(164)(164). Additionally, current models do not account for gene-environment interactions, epigenetic effects, or lifestyle factors (165)(166). Finally, a substantial proportion of SLE heritability remains unexplained (167)(168).

2. Aims

This study investigated overall patterns of genetic variability around previously reported SLE-associated genes and risk loci in a multi-ancestral cohort, with a focus on cumulative alternate allele burden, assessing the relationship between alternate allele counts (AAC), age at disease onset, sex, ancestry, organ involvement clinical severity, and treatments. It furthermore explored associations between gene-level alternate allele scores (GAAS), organ involvement and disease severity.

3. Methods

This study follows the STREGA (Strengthening the Reporting of Genetic Association Studies) reporting guidelines (169).

3.1 Study Cohort

A total of 315 patients from the UK jSLE Cohort Study were initially enrolled in this study (137). The UK jSLE Cohort Study includes two distinct cohorts: an established jSLE cohort, comprising patients diagnosed with jSLE from 1995 to the present day (retrospective data), and a prospective jSLE cohort, encompassing all newly diagnosed jSLE patients (since 2006) enrolled during the ongoing study period (prospective data). For this specific study, patient data were collected up to September 2022. All participants fulfilled the ACR 1997 classification criteria for SLE (≥ 4 items) (136) and were diagnosed before their 18th birthday. We were not able to perform a formal power analysis prior to this study, due to the absence of studies using a comparable design in this specific disease area. Furthermore, sample size was pre-defined accessing a large national cohort in a rare disease area. Written patient assent/consent and/or parental consent was obtained as appropriate. The study received ethical approval from the National Research Ethics Service Northwest (REC_06/Q1502/77). Research was carried out in accordance with the declaration of Helsinki.

3.2 Data collected

Comprehensive patient data were collected and analysed in the following areas: 1) Demographic information, encompassing age at diagnosis, sex, self-reported ancestry, and family history of autoimmune/inflammatory diseases in first-grade relatives (SLE, systemic connective tissue diseases/CTD, rheumatoid arthritis/RA, endocrinopathies); 2) Disease activity, assessed at each study visit using the pBILAG-2004 (138) and the SLEDAI-2K score, and damage at the last visit through the SLICC/ACR standardised damage index (SDI) (174); 3) Prospectively collected clinical data, including malar rash, discoid lupus, photosensitivity, oral and/or nasal ulcers, non-erosive arthritis, serositis, nephritis, neurological, haematological, and/or immunological involvement, as per the 1997 ACR criteria for SLE; and 4) treatments. Patients were categorised in the “intensive treatment” group if, throughout the disease course, they received either cyclophosphamide, rituximab or belimumab, and/or two or more conventional DMARDs, excluding hydroxychloroquine, simultaneously for ≥2 consecutive visits, and in the “non-intensive treatment” group, when a maximum of one conventional DMARD, including hydroxychloroquine, was used at a time (Table 1, Supplementary Table 1). Ancestry inference was not performed in this study primarily because the available data were limited

to 62 genes/genomic target regions (exonic regions, exon:intron junctions), which represent only a small fraction of the genome and are typically under strong functional constraint. A more detailed rationale is provided in the discussion.

Considering all patient visits, we assigned a “severity” value to each pBILAG organ/system domain based on the highest score ever recorded: “severe involvement” was attributed to any organ or system that received a score of 'A' at any visit, “moderate involvement” was assigned if the domain's highest recorded score was 'B', “mild involvement” was determined if the most severe score ever given was 'C'. Lastly, “never involved” was the designation for any organ or system that received an 'E' score across all visits.

3.3 Gene panel selection

Sequencing targets were selected based on a literature search (2018) targeting: 1) genes associated with known Mendelian forms of SLE/SLE-like disease, and 2) SLE-associated risk alleles previously identified through GWAS. As a result, 62 genes/genomic regions were selected for sequencing, including exons and exon:intron junctions, as well as previously reported SLE-associated risk alleles, as described previously (141).

3.4 Target sequencing and variant identification

Sequence capture probes (NimbleGen/Roche) were designed to target exonic regions and exon:intron junctions of pre-selected genes (Supplementary Table 2). Sequencing libraries were prepared from genomic DNA, hybridized to the probes and then sequenced with 150bp paired-end reads using Illumina MiSeq technology (Illumina). Demultiplexing, adaptor and quality trimming (Cutadapt v1.2.1, Sickle v1.2) of reads was performed (175,176). Polymerase Chain Reaction (PCR) duplicates were identified and excluded from the dataset using Picard (177). Sequencing data were aligned to the human reference genome (hg38) using bwa (178) and variants were subsequently detected with Genome Analysis Toolkit (GATK) Software (178,179). Variant calling was performed jointly across samples using the GATK Haplotype Caller. GATK base quality score recalibration was applied. Variants were then filtered using the GATK Variant Filtration tool. Single nucleotide polymorphisms (SNPs) were removed if they met any of the following criteria: Quality by Depth (QD) < 5.0, Quality Score (QUAL) < 30.0, Strand Odds Ratio (SOR) > 3.0, (Fisher Strand) FS > 60.0, Mapping Quality (MQ) < 40.0, Mapping Quality Rank Sum (MQRankSum) < -12.5, Read Position Rank Sum (ReadPosRankSum) < -8.0. Variants passing the filtering thresholds were annotated using SNP Effect

(SnpEff) (180) and database of SNP (dbSNP) (181). Finally, data were extracted from the Variant Call Format (vcf) file using the R packages *VariantAnnotation* (182) and *vcfR* (183). HLA class I regions were excluded from this project, as the high genetic variability within these regions undermines the accuracy of standard variant calling methods. Genotype (GT) data were extracted and converted into a matrix where genotypes were numerically encoded based on alternate allele counts: homozygous reference (0/0) = 0, heterozygous (0/1 or 1/0) = 1, and homozygous alternate (1/1) = 2. Missing genotypes (./.) were excluded from downstream analysis.

3.5 Alternate allele counts

Genotype calls were converted to reflect the number of alternate alleles per SNP (0, 1, or 2), and samples with missing genotype data across any of the 4100 exonic SNPs were excluded to ensure complete-case analysis. This meant 238/289 (82.4%) patients were retained for this analysis as they had genotype information for all 4100 SNPs contained in the genome fractions studied and complete clinical information.

Functional annotations (ANN), extracted from the INFO field of the VCF file, were used to assign biological impact weights: MODIFIER and LOW = 1, MODERATE = 2, and HIGH = 3. For each individual, the alternate allele count at each SNP was multiplied by its corresponding ANN weight, and these values were summed to produce a weighted AAC score. The weighted AAC scores were merged with the following 21 demographic and clinical variables: age at diagnosis; severity of constitutional, mucocutaneous, neuropsychiatric, musculoskeletal, cardiorespiratory, gastrointestinal, ophthalmic, renal and haematological involvement (pBILAG domains); presence of malar rash, discoid lupus, photosensitivity, oral or nasal ulcers, non-erosive arthritis, serositis, nephritis, neurologic, haematological and immunological disorders (1997 ACR classification criteria) and treatment received (“non-intensive” versus “intensive”). As mentioned above, for each pBILAG organ/system domain, patients were stratified into four mutually exclusive severity categories based on the highest score ever recorded during follow-up: “severe involvement” (maximum score = A), “moderate involvement” (maximum score = B), “mild involvement” (maximum score = C), and “never involved” (only E scores across all visits). For the 1997 ACR classification criteria, patients were categorised as “yes” (feature ever present) or “no” (feature never present) (Supplementary Table 1).

To identify associations between AAC and clinical phenotypes, generalized linear models (GLMs) with a Poisson distribution were used. Each model included ancestry, sex, and family history of

autoimmune disease as covariates. P-values from the GLMs were adjusted for multiple testing using the Benjamini-Hochberg false discovery rate (FDR) method. In addition, Pearson correlation analyses were performed to evaluate relationships between AAC and continuous clinical outcomes, including age at diagnosis, the highest SLEDAI and numeric BILAG scores ever recorded during follow-up, and the SLICC-SDI at the latest visit. Correlation tests were also stratified by ancestry to explore subgroup-specific associations, with FDR correction applied across tests. All methods can be consulted in detail and fully reproduced in the publicly available R Markdown document https://github.com/CBFLivUni/jSLE_paper/blob/main/scripts/AAC_calculation_and_analysis.Rmd.

3.6 Gene-level alternate allele scores

For each SNP and individual, a mutation burden score was calculated by multiplying the alternate allele count by the assigned annotation severity value (as described above). Mutation burden scores were then aggregated at the gene level by summing scores across all variants within a gene for each individual. This resulted in a matrix of 62 Gene-level alternate alelle scores (GAAS) for all patients, where rows corresponded to individuals and columns to genes. Note that SNPs present across all patients were 3764 of the sequenced 4100 SNPs (91.8%), and the total number of patients included was 289 (those with complete clinical data). To visualize sample stratification, PCA was performed on the transposed gene-level score matrix using the prcomp() function. The first two principal components (PC1 and PC2) were visualized using ggplot2, with patient samples coloured by ancestry. To identify gene-level associations with clinical features, generalized linear models (GLMs) with a Poisson distribution were fitted for each gene against a panel of 21 curated clinical variables, corresponding to the same set of demographic and clinical features used in the AAC analyses. Each model included additional covariates to adjust for ancestry, sex, and family history of autoimmune diseases. Genes previously identified as pseudogenes, antisense transcripts, or non-coding RNAs (n=26) were excluded from testing. Statistical significance of the clinical predictor was assessed using chi-square tests from the model ANOVA. P-values were corrected for multiple testing using the Benjamini and Hochberg method. Models with adjusted p-values <0.05 were considered significant.

4. Results

4.1 Cohort characteristics

Of the 315 jSLE patients sequenced, 289 (91.7%) were included in the GAAS analysis by excluding SNPs lacking sequencing data across all patients as well as patients with incomplete demographic/clinical information. For the AAC analysis, only patients with complete sequencing information for all 4100 SNPs contained in the genome portions studied were included, resulting in 238/289 (82.4%) patients being analysed (Figure 1A).

Demographic and clinical characteristics of the two sub-cohorts were comparable (Table 1; Supplementary Table 1). The subsequent description of demographics focuses on the larger cohort subjected to the GAAS analysis.

The median age at diagnosis in this cohort was 12.8 years (interquartile range/IQR 10.3-14.5) (Figures 1B), and most patients were female (83.7%). Patients of European ancestry represented 47.7% of the study population; 24.2% were South Asian, 16.6% African/Caribbean, 3.5% East Asian, and 8.0% were of “other” Asian ancestry (Figure 1B). 18% of participants had a family history of autoimmune/inflammatory diseases in first-degree relatives, including SLE (39.6%), systemic CTD (47.2%), endocrinopathies (39.6%) and RA (22.6%). Notably, damage measured by SLICC-SDI was low across the cohort, with a median score of 0 (IQR 0-1). Almost half of the participants (44.6%) received “intensive” treatment throughout their disease course (Table 1).

Table 1: Demographic and clinical information on the study cohort.

	jSLE patients included in the alternate allele count analysis (n=238)	**jSLE patients included in the gene-level score analysis (n=289)**
Age at diagnosis, years (median [IQR])	13.0 [10.7-14.5]	12.8 [10.3-14.5]
Sex, n (%)		
Female	198 (83.2)	242 (83.7)
Male	40 (16.8)	47 (16.3)
Ancestry, n (%)		
European	114 (47.9)	138 (47.7)
African/Caribbean	38 (16.0)	48 (16.6)
South Asian	57 (23.9)	70 (24.2)
East Asian	9 (3.8)	10 (3.5)
Other Asian	20 (8.4)	23 (8.0)
Family history autoimmune diseases, n (%)	**48 (20.2)**	**53 (18.3)**
SLE	18 (37.5)	21 (39.6)
Systemic CTD	22 (45.8)	25 (47.2)
Endocrinopathies	19 (39.6)	21 (39.6)
RA	11 (22.9)	12 (22.6)
Highest SLEDAI score during follow-up (median [IQR])	20 (17-24)	20 (17-24)
Highest numerical pBILAG score during follow-up (median [IQR])	21 (13-32)	21 (13-31)
SLICC SDI at last visit (median [IQR])	0 [0-1]	0 [0-1]
Treatments, n (%)*		
Intensive	109 (45.8)	129 (44.6)
Non-intensive	129 (54.2)	160 (55.4)

jSLE, juvenile systemic lupus erythematosus; IQR, interquartile range; CTD, connective tissue disease; RA, rheumatoid arthritis; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index; pBILAG, paediatric British Isles Lupus Assessment Grade 2004; SLICC SDI, SLICC/ACR Damage Index.

*"Intensive treatment": patients, throughout the disease course, received either cyclophosphamide, rituximab or belimumab, and/or two or more conventional DMARDs, excluding hydroxychloroquine, simultaneously for ≥ 2 consecutive visits. "Non-intensive treatment": a maximum of one conventional DMARD, including hydroxychloroquine, was used at a time.

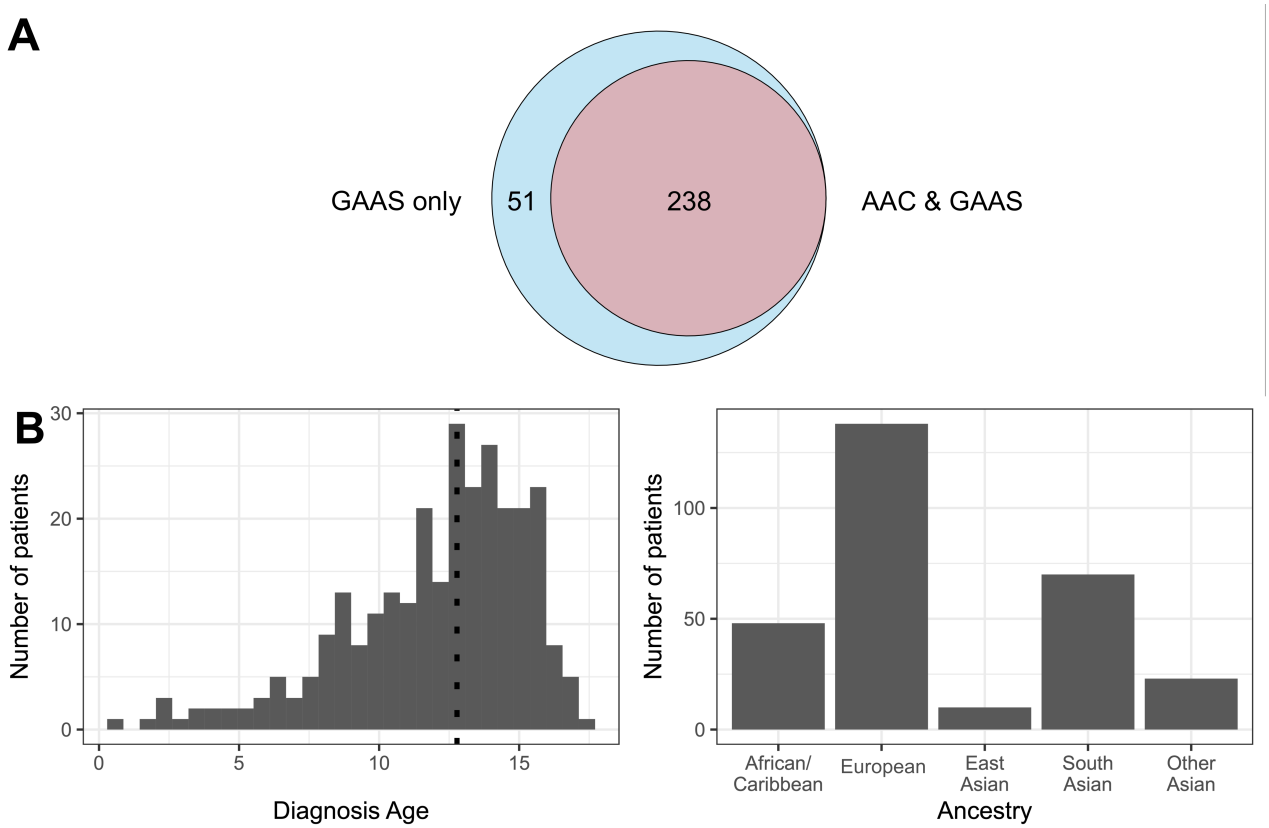

Figure 1. Demographic characteristics of the jSLE patient sub-cohorts. A) Venn diagram displaying the overlap of study participants between the gene-level alternate allele scores (GAAS) (blue circle) and alternate allele counts (AAC) (pink circle) analysis groups. Out of the 289 patients included in the GAAS analysis, (238, 82.4%) were included in both GAAS and AAC analyses, and no patients were included exclusively in the AAC analysis. B) Demographic characteristics of jSLE patients included in the GAAS. The left panel illustrates the age distribution in years at the time of diagnosis; the blue dotted line indicates the median age at diagnosis. The right panel displays the ancestral composition.

4.2 Alternate allele counts inversely correlate with age at diagnosis

Based on previous studies suggesting that jSLE patients with early disease onset experience a higher genetic burden (17), we investigated the possible relationship between AAC and age at diagnosis. A weak inverse correlation between age at diagnosis and AAC was noted (R=-0.15, p=0.012, Figure 2A). Correlation analyses within each ancestral group revealed a moderate inverse correlation between age at diagnosis and AAC among South Asian participants (R=-0.34, p=0.047) but no significant correlation was found within the remaining ancestral groups (Figure 2B).

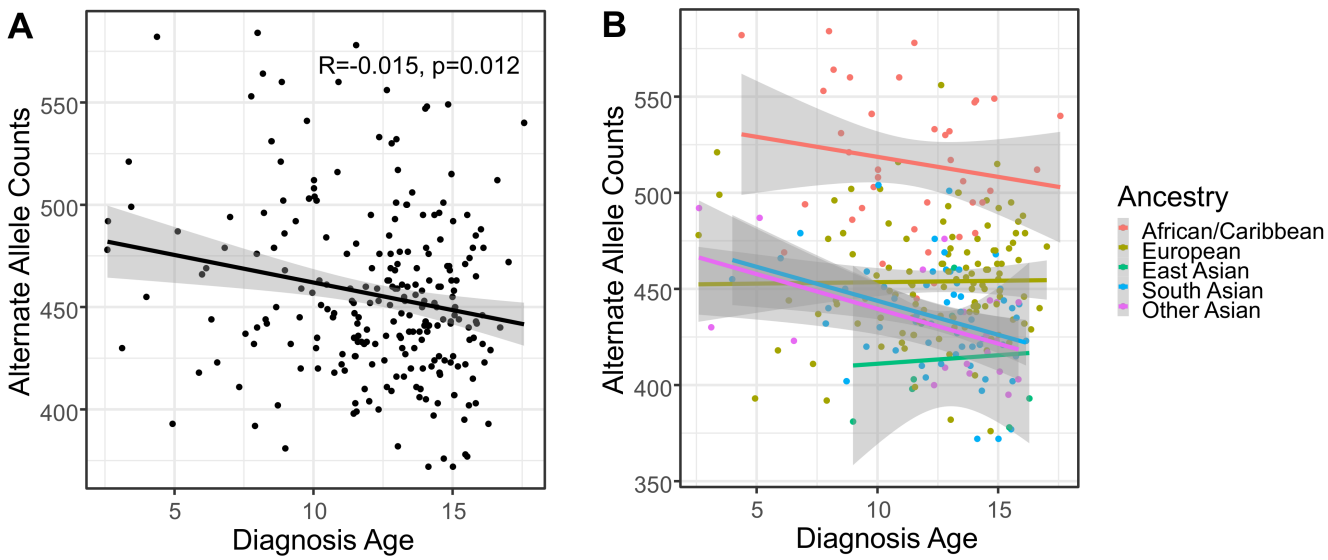

Figure 2. Alternate allele counts (AAC) inversely correlate with age at disease onset. A) In a multi-ancestral jSLE cohort, age at diagnosis (in years) and AAC are inversely correlated (R=-0.015, p=0.012). Data points represent the number of alternate alleles in individual jSLE patients; a trend line indicates the direction and strength of the relationship. The grey shaded area indicates the 95% confidence interval. B) Analysis of the relationship between AAC and age at disease onset across the five different ancestral groups, reveals a moderate inverse correlation between age at onset and AAC among South Asian jSLE patients (R=-0.34, p=0.047). Each data point represents an individual sample, colour-coded by ancestry: African/Caribbean (red), European (gold), East Asian (green), South Asian (blue), and "Other" Asian (purple). Trend lines indicate the direction and strength of the relationships. The grey shaded areas represent the 95% confidence intervals.

4.3 Alternate allele counts are higher in patients of African/Caribbean ancestry

When comparing AAC across ancestries, higher scores were recorded in patients of African/Caribbean ancestry compared to patients of other ancestral groups ($p<0.001$) (Figure 3A). No significant differences were detected between male and female participants across ancestral groups. Principal Component Analysis of GAAS across all 62 genes/loci included in this study showed that patients of African/Caribbean ancestry cluster separately from other ancestries (Figure 3B).

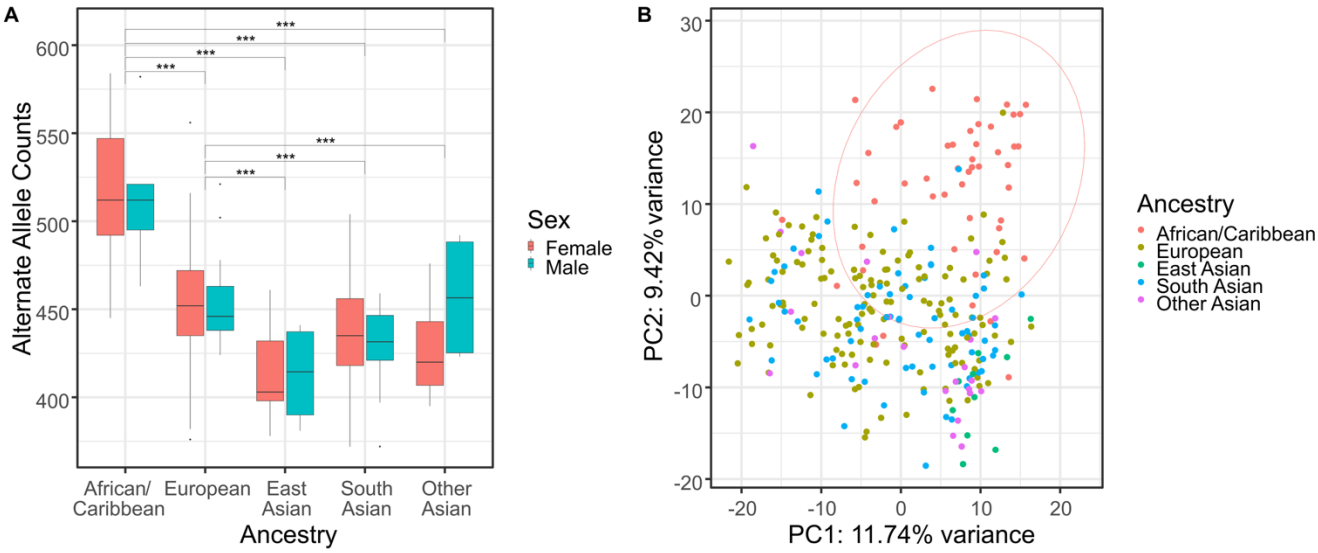

Figure 3. Alternate allele counts (AAC) reveal differences among ancestries. A) Distribution of AAC stratified by ancestry and sex, showing higher AAC in patients of African/Caribbean ancestry, with no differences observed between male and female participants across ancestral groups. Sex distribution across ancestry groups: African females 33 (87%), males 5 (13%); European females 101 (89%), males 13 (11%); East Asian females 5 (56%), males 4 (44%); South Asian females 43 (75%), males 14 (25%); other Asian background females 16 (80%), males 4 (20%). Adjusted p-values are displayed (***$p\leq0.001$). Box plots display interquartile ranges (IQR) and median values. Whiskers extend to 1.5-fold IQR, with outliers as individual data points. Ancestral groups are labelled along the x-axis: African/Caribbean, European, East Asian, South Asian and "Other" Asian. Sexes are separated by colour as indicated. B) Principal Component Analysis (PCA) of GAAS highlighting the distribution across different ancestral groups. Each dot signifies an individual data sample, with colour coding ancestry African/Caribbean, European, South Asian, and other Asian background. The first principal component (PC1) accounts for 11.74% of the total variance, while the second principal component (PC2) captures 9.42%. The points of African/Caribbean participants cluster largely separated from the other ancestries. The distinct clustering of African/Caribbean participants is encircled in red.

4.4 Alternate allele counts associate with organ involvement but not with disease activity or damage

GLM analyses (Supplementary Table 3) identified relationships between AAC and severity of pBILAG-2004 defined constitutional ($p<0.001$), renal ($p=0.0016$), haematological ($p=0.0016$) and neuropsychiatric ($p=0.03$) involvement, and the presence of non-erosive arthritis ($p=0.004$) and malar rash ($p=0.03$). We did not observe correlations between AAC and highest SLEDAI scores ($R=0.044$, $p=0.5$; Supplementary Figure 1A), highest pBILAG scores ($R=0.041$, $p=0.53$; Supplementary Figure 1B) or SLICC-SDI at last visit ($R=0.032$, $p=0.62$; Supplementary Figure 1C).

4.5 Gene-level alternate allele scores associate with the severity of organ involvement

We found associations between 18 clinical variables and 13 genes (Supplementary Table 4, Figure 4). Neuropsychiatric severity associated with GAAS of the genes *Acid Phosphatase 5 (ACP5)* ($p<0.001$), *Rat Sarcoma Guanyl Releasing Protein 3* (*RASGRP3)* ($p=0.04$), *Ribonuclease H2 subunit A* (*RNASEH2A)* ($p<0.001$) and *Tyrosine Kinase 2* (*TYK2)* ($p<0.001$) (Figure 5A). Renal severity associated with GAAS of *ACP5* ($p<0.001$), *Integrin Subunit Alpha M* (*ITGAM)* ($p<0.001$), *Lck/Yes Novel Tyrosine Kinase (LYN)* ($p<0.001$), and *Tumor Necrosis Factor Alpha-Induced Protein 3* (*TNFAIP3)* ($p=0.007$) (Figure 5B). Haematological involvement severity associated with GAAS of *ACP5* ($p=0.003$), *Deoxyribonuclease 1* (*DNASE1)* ($p=0.003$), *RNASEH2A* ($p<0.001$), and *Ubiquitin Conjugating Enzyme E2 L3* (*UBE2L3)* ($p=0.03$) (Supplementary Figure 2A). Cardiorespiratory severity associated with GAAS of *Interferon Regulatory Factor 8* (*IRF8)* ($p=0.02$), *ITGAM* ($p<0.001$) and *UBE2L3* ($p=0.04$) (Supplementary Figure 2B), while gastrointestinal severity associated with *ITGAM* ($p<0.001$) and *PX Domain Containing Serine/Threonine Kinase Like* (*PXK)* ($p<0.001$) (Supplementary Figure 2C).

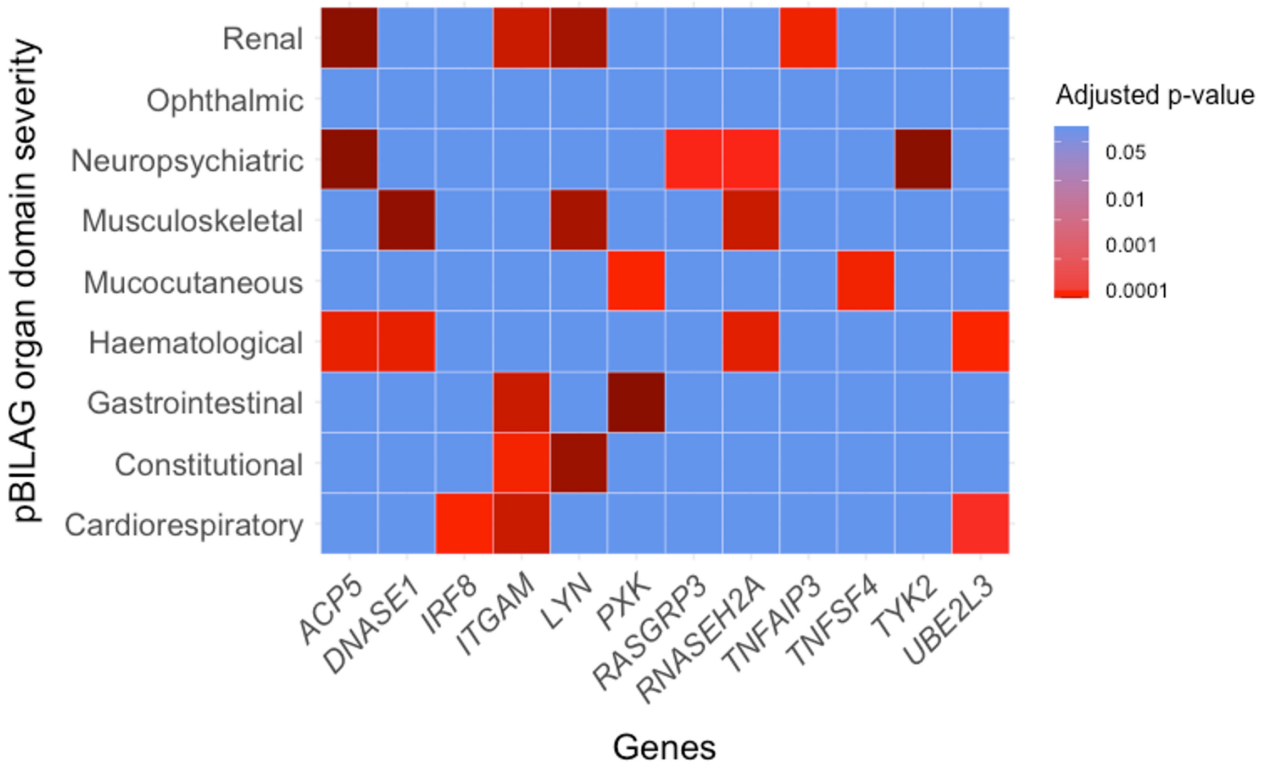

Figure 4. Associations between pBILAG organ domain severity and gene-level alternate allele scores (GAAS). Heatmap showing adjusted p-values of associations between clinical severity and GAAS, including the 12 genes displaying correlations between GAAS and pBILAG domain severity. Each cell represents a specific gene (x-axis) and pBILAG organ domain severity (y-axis), with colour intensity indicating the strength of the association. Darker red shades denote lower p-values, while blue shades indicate non-significant associations. The adjusted p-value thresholds are categorized as follows: 0.0001 (darkest red), 0.001, 0.01, 0.05 (lightest red) and >0.05 (blue).

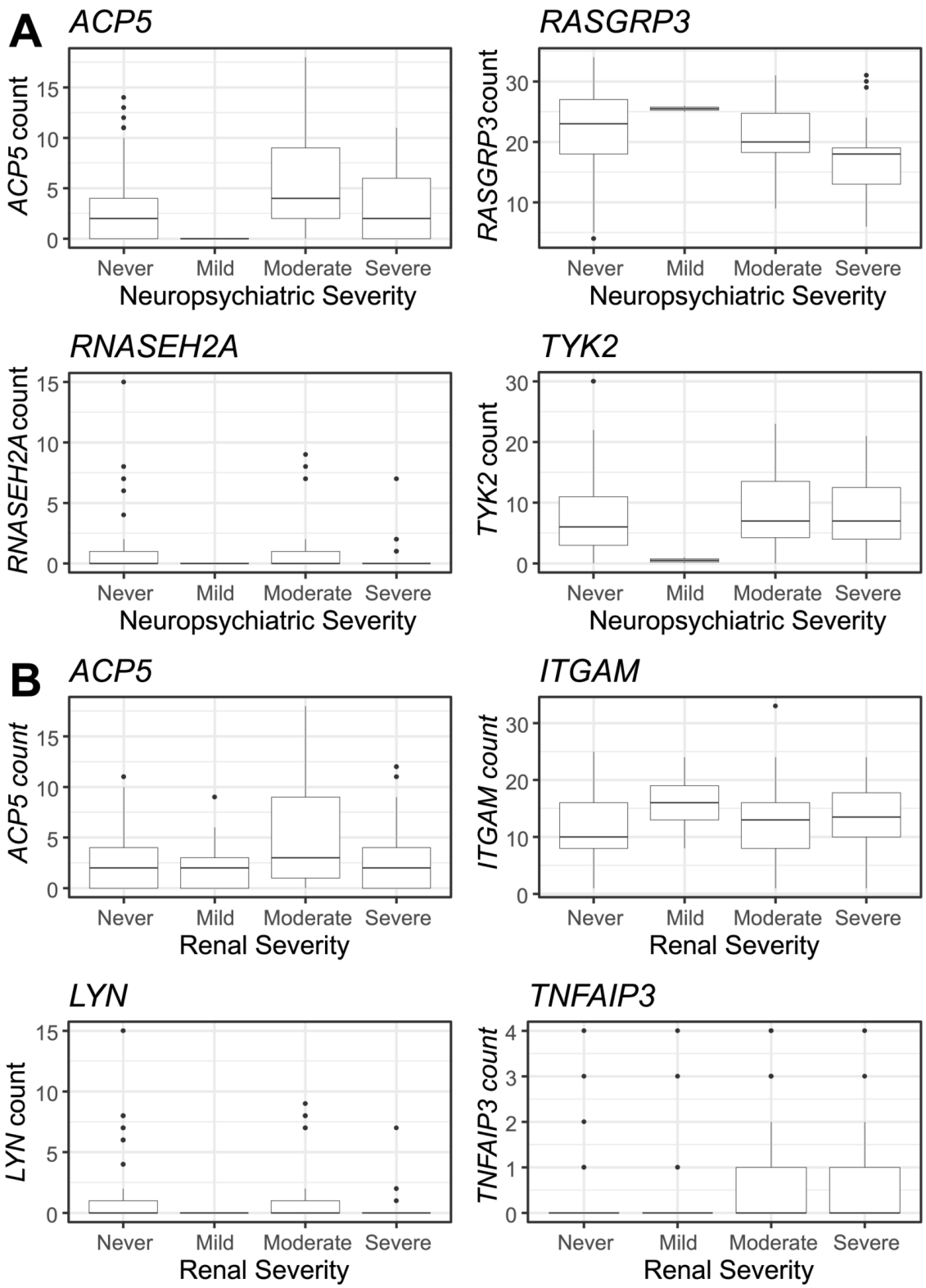

Figure 5. Associations between severity of neuropsychiatric and renal involvement, and gene-level alternate allele scores (GAAS). A) Neuropsychiatric severity significantly associates to ACP5 ($p<0.001$), RASGRP3 ($p=0.04$), RNASEH2A ($p<0.001$) and TYK2 ($p<0.001$) GAAS. B) Renal severity significantly associates to ACP5 ($p<0.001$), ITGAM ($p<0.001$), LYN ($p<0.001$), TNFAIP3 ($p=0.007$) GAAS.

4.6 Alternate allele counts and gene-level alternate allele scores associate with treatment intensity

Overall, patients in the "intensive" treatment group had significantly higher AAC compared to participants receiving "non-intensive" treatment ($p<0.001$, Figure 6A). Participants from all ancestral groups, except European patients, receiving "intensive" treatment exhibited higher AAC compared to those receiving "non-intensive" treatment, although differences were only significant for African/Caribbean participants ($p=0.001$) (Figure 6B), consistent with their overall higher disease burden. Additionally, GAAS of *ITGAM* ($p<0.001$), *LYN* ($p<0.001$), *PXK* ($p<0.001$) and *RNASEH2A* ($p<0.001$) associated with increased treatment intensity (Figure 6C).

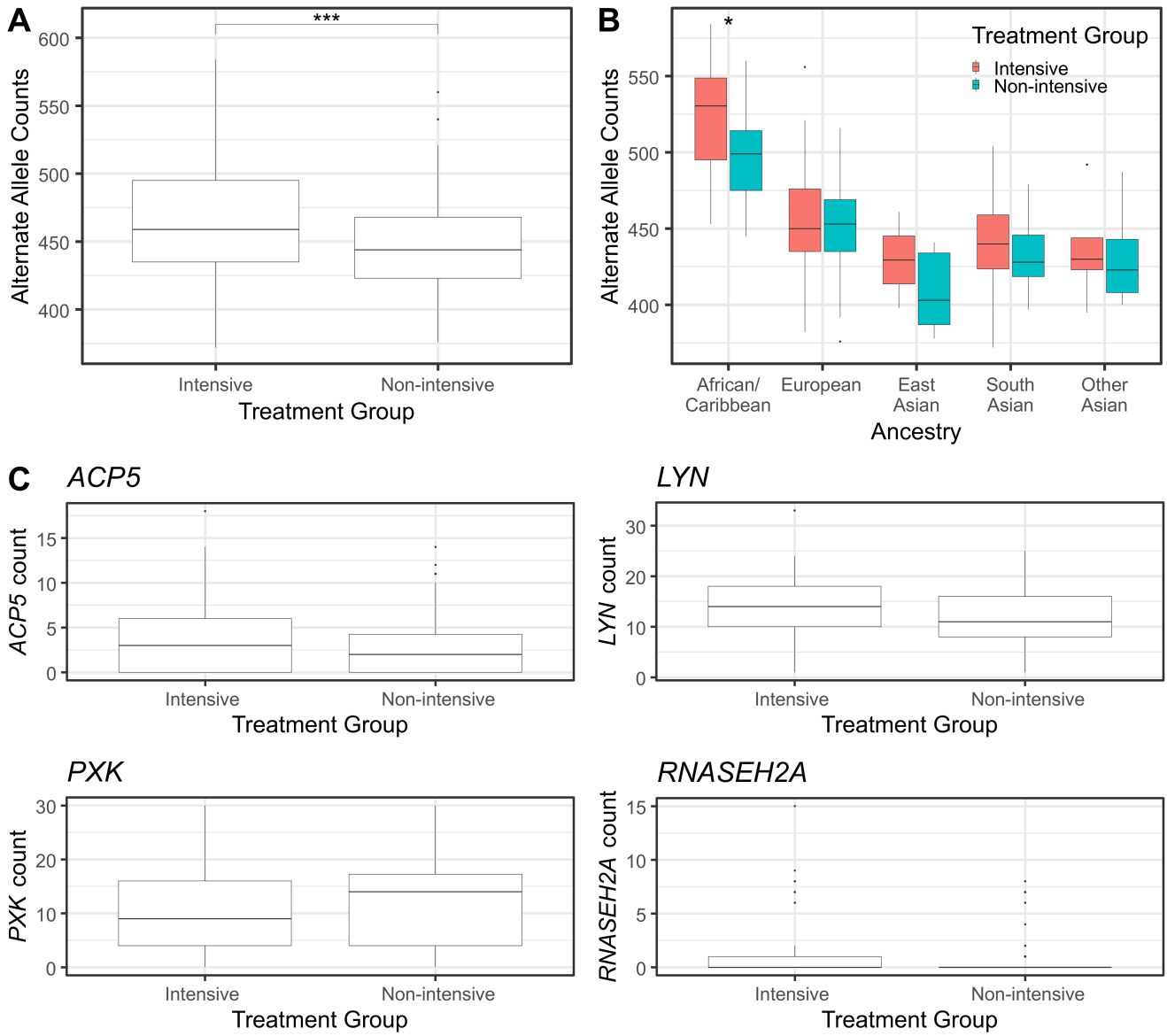

Figure 6. Alternate allele counts (AAC) and gene-level alternate allele scores (GAAS) associate and with treatment intensity. A) Relationship between AAC and treatment intensity across ancestries, showing significantly higher AAC in the "intensive treatment" group compared to the "non-intensive treatment" group (***p≤0.001). Box plots display interquartile ranges (IQR) and median values. Whiskers extend to 1.5-fold IQR, with outliers as individual data points. B) Box plot illustrating the relationship between alternate allele count (AAC) and two different treatment groups («intensive» and «non-intensive») across the five ancestral categories, showing higher AAC in patients requiring intensive treatment (in red) compared to those receiving non-intensive treatment regimens (in blue) among African/Caribbean jSLE patients (*p≤0.05). Box plots display interquartile ranges (IQR) and median values. Whiskers extend to 1.5-fold IQR, with outliers as individual data points. C) Box plots showing the distribution of significant associations between GAAS and treatment choices. Treatment intensity was significantly associated with ITGAM (p<0.001), LYN (p<0.001), PXK (p<0.001), and RNASEH2A (p<0.001) GAAS. Box plots display interquartile ranges (IQR) and median values. Whiskers extend to 1.5-fold IQR, with outliers as individual data points.

4.7 Alternate allele counts correlate with the subset alternate allele counts generated from previously published SNPs

A strong correlation was observed between the originally calculated AACs and the subset AACs, which were calculated using the 15 previously reported SLE-associated SNPs that were also captured by our sequencing panel ($R=0.47$, $p<0.001$; Supplementary Figure 3A). However, when including previously reported SNPs only, an inverse correlation between AAC and age at diagnosis was not observed ($R=-0.08$, $p=0.964$; Supplementary Figure 3B). When stratifying by ancestry, European and African/Caribbean ancestral groups followed similar trends when comparing the two AACs, though significance was lost ($R=0.05$, $p=1$ and $R=-0.08$, $p=1$, respectively). In East Asians, a moderate but non-significant inverse correlation emerged ($R=-0.48$, $p=0.965$), while no correlation was observed in South Asian patients ($R=-0.05$, $p=1$) or those of "other" Asian ancestry ($R=-0.07$, $p=1$) (Supplementary Figure 3C).

5. Discussion

In adult-onset SLE, associations between genetic variability, age at onset, ancestry, and disease severity have been established (18,184,185). Preliminary reports suggest that, also in jSLE, increased genetic impact associates with early disease onset and high disease activity, especially in patients of African/Caribbean ancestry (17,18). This study represents the most comprehensive analysis currently available in the age group, linking genetic variability across 62 SLE-associated genes/genomic regions with demographic and clinical variables, as well as treatment. Although all patients were recruited from study centers within a single nation, the cohort was multi-ancestral, with an expected "SLE-typical" over-representation of "non-European" ancestry, when compared to national UK census data (11,153) which allowed investigation of genetic disparities across ancestral groups.

The identified inverse correlation between AAC and age at diagnosis is in agreement with previous smaller studies, adding weight to the hypothesis that genetic burden contributes to early disease onset in jSLE (17,18,184). In this study, the inverse correlation between AAC and age at onset was mainly driven by participants of South Asian descent. South Asian jSLE patients represented 24.2% of the study population, while other non-European ancestries were less represented. Therefore, the relatively small sample size among these ancestries likely limited the statistical power to detect similar correlations. For example, a trend was observed in the African/Caribbean group, though it did not reach significance.

While Webb *et al.* reported an inverse correlation between genetic impact, age at disease-onset and severity in jSLE patients of African descent in the United States of America (18), we failed to identify associations between AAC and age at onset in the UK's African/Caribbean sample population. Differences may be explained by the more extensive sequencing panel approach chosen here (4100 vs. 19 SNPs) and the relatively small sample sizes across both cohorts (289 vs. 111 jSLE patients). Nevertheless, this study confirmed increased overall AAC in jSLE patients of African/Caribbean ancestry (compared to other ancestral groups) which may contribute to a lower threshold for SLE development, an overall earlier age of onset, more severe disease and less favourable outcomes (80). An association between ancestry, disease severity and the need for intensive treatment, particularly among African/Caribbean patients, has been previously established in cohorts consisting of mostly adult-onset SLE patients (79) (187). However, findings from this study not only confirm this pattern in a paediatric multi-ancestral cohort, but they also link ancestry and phenotype-related differences with increased overall genetic variability around SLE-related genes/loci. Although expected to some extent based on previous studies reporting SNP-associations (188,189), here reported associations between overall genetic variability, organ involvement (especially renal involvement and *ACP5*, *ITGAM*, *LYN* and *TNFAIP3*) and treatment intensity underscore the importance of understanding

genetic factors and their role in shaping organ-specific disease features for the development of individualised therapeutic approaches. Lastly, although SLE patients of Asian ancestry have previously been suggested to experience an increased genetic risk in adult-onset SLE cohorts (190), this study, when compared to other ancestries, did not detect higher AAC in children of Asian descent. This may be due to the relatively small sample size, differences in the sequencing panels used across studies, and/or possible differences between juvenile- and adult-onset SLE (37).

In addition to age at onset, AAC associated with the presence of certain clinical features. High AAC associated with severity of renal involvement. This is of particular interest, because LN centrally impacts on overall disease severity, associates with the use of "intensive" treatments, and contributes to SLE-associated mortality (184,192). Notably, a recent GWAS also reported a correlation between the development of LN and the number of SLE risk variants (184). Moreover, a large Canadian study including both juvenile- and adult-onset SLE patients (1237 participants/572 jSLE) found associations between SLE susceptibility loci and the risk of developing LN (161).

Although previous studies in predominantly adult-onset SLE cohorts suggested increased damage accrual in patients with high genetic risk scores (185), this study did not detect correlations between AAC and SLICC-SDI scores. This may be the result of overall low SLICC-SDI scores across the study cohort (median: 0, IQR: 0-1), which could be attributed to SLICC-SDI having been developed and validated for adult SLE patients, emphasizing aspects of potentially lower relevance to children (including malignancy and diabetes mellitus) (134).

Key goals of genetic profiling across autoimmune/inflammatory diseases are the prediction of organ involvement, disease outcomes and/or treatment responses (193). Among various associations identified, we found organ domains uniquely associated to GAAS of specific genes, such as *TNFAIP3* with renal severity and *TYK2* with neuropsychiatric severity. Additionally, in some cases, shared associations were observed; for example, GAAS of *ACP5* were associated with neuropsychiatric, renal and haematological severity.

Several genes associated with specific organ involvement patterns are of particular interest as they have previously been linked with genetically determined SLE-like diseases and/or are targets of already available molecular interventions. For instance, recessive mutations in *ACP5*, encoding for the tartrate-resistant acid phosphatase (TRAP), associate with spondyloenchondrodysplasia (SPENCD). In line with organ domains associated with GAAS in this study, SPENCD is characterised by increased IFN expression, central nervous system involvement, haematological manifestations, vasculitis and renal involvement (194). Furthermore, genetic variability affecting *RNASEH2A* associated with neurological and haematological involvement and severity. This gene has previously been linked with the neurodegenerative disease Aicardi-Goutières syndrome (AGS), another

interferonopathy characterised by early-onset encephalopathy and lupus-like features (195). Notably, both SPENCD and AGS patients show (at least limited) improvement in response to JAK/STAT inhibitors that are used to control IFN expression (196), suggesting that genetic profiling may not only predict organ involvement but also inform future treatment choices. Additionally, the tyrosine kinase encoding *TYK2* gene is another IFN signalling related gene associated with neuropsychiatric severity and the occurrence of discoid lupus. The pivotal role of TYK2 in the JAK/STAT pathway and IFN expression prompted the development of TYK2 modulators (deucravacitinib, brepocitinib). While approval for the treatment of SLE is pending, deucravacitinib was effective and safe in adult SLE patients (197). The tyrosine kinase encoding *LYN* gene was associated with disease severity affecting the renal pBILAG organ domain. The Lyn kinase plays a key role in regulating B lymphocyte signalling; mice carrying gain-of-function variants in *Lyn* exhibit circulating autoantibodies and severe autoimmune glomerulonephritis (198). A genome-wide association study in IgA nephropathy identified *LYN* as a risk locus (199), and a recent study in severe COVID-19 found *LYN* to be associated with progression of kidney damage (200). Notably, the Lyn/Bcr-Abl tyrosine kinase inhibitor bafetinib was efficacious and safe in acute lymphocytic leukaemia, refractory/relapsing B cell lymphocytic leukaemia and prostate cancer (201). Finally, the observed association between *TNFAIP3* GAAS and renal severity is particularly interesting because heterozygous loss-of-function mutations in *TNFAIP3* have recently been linked with haploinsufficiency A20 (202). Patients affected by this autoinflammatory condition can exhibit SLE-like phenotypes (153,203), and some can develop LN in the presence of autoantibodies and a pronounced type I IFN signature (203). Findings from this and another recent study (204) therefore suggest that variants in the *TNFAIP3* may affect renal involvement and outcomes in SLE. Taken together, associations between GAAS and organ involvement/severity may inform future personalised jSLE management, including genetic risk assessment and patient stratification towards individualised treatment and care. Such an approach could, for example, entail increased monitoring frequency for renal function, proteinuria and blood pressure elevation or the choice of more aggressive immunosuppressive treatment in jSLE patients with elevated GAAS in *ACP5*, *ITGAM*, *LYN*, or *TNFAIP3*. Indeed, similar approaches have already been introduced in cancer medicine (205). This is the first study to show that high genetic risk scores (AAC) in jSLE patients associate with the need for “intensive” treatment. While only reaching statistical significance among African/Caribbean jSLE patients, this association remained across all ancestral groups, except European participants. While the reason for this remains unclear and requires further investigation, genetic risk has been reported “lower” among European patients as compared to other ancestries (206,207), which also associates with “milder” disease courses and better outcomes (208).

Differences between allele counts and age at disease onset observed between AAC, measuring overall genetic variation, and the subset AAC generated from previously reported SLE-associated SNPs, highlight the impact of (ancestry- and potentially age-related biased) SNP selection in genetic studies. A large proportion of previously published SLE-associated SNPs, more precisely 148/330 (44.8%), were exclusively identified in East Asian populations (151). Previous studies overall had limited representation of, e.g., South Asian or African/Caribbean jSLE patients, notably, two of the three most represented ancestries in the dataset presented here (respectively 23.9/24.2% and 16/16.6% of the cohort). Analyses restricted to a subset of previously published SLE-associated SNPs may therefore have resulted in a loss of signal, particularly in underrepresented populations (such as South Asian patients). This underscores the importance of inclusive and appropriately matched genomic datasets, as limiting analyses to previously reported variants may skew associations, particularly in multi-ancestral cohorts.

A key strength of this study is its focus on genetic variability across SLE-associated genes and regions rather than limiting to specific previously identified risk alleles (usually from GWAS). While GWAS have been instrumental in identifying individual SNPs associated with SLE risk, or protection from disease or complications, the approach taken in this study provides a broader and, potentially, less biased assessment of genetic burden by capturing the combined effect of multiple alternate alleles. This perspective may offer insights into disease heterogeneity and severity, particularly in multi-ancestral populations, and could complement existing analytic approaches by providing an alternative or additional measure of variability and its impact on disease expression and/or phenotype. This is reflected in the design of the sequencing panel, which was focused on exons, exon–intron junctions, and regions around several previously reported risk alleles (153). Consequently, a large proportion of sequences analysed here are located in coding or nearby regulatory regions, rather than in intergenic areas where many GWAS hits are found. This choice aligned with our aim to assess gene-level variation rather than replicate known SNP-level associations.

While this study highlights the potential of genetic assessment in guiding and personalising clinical management for jSLE patients, several limitations require to be acknowledged. Despite representing one of the largest jSLE cohorts available, the overall sample size remains relatively small, especially when compared to studies in adult-onset SLE (17,185). This limitation is particularly evident in subgroup analyses comparing sex across ancestries, where the number of male patients, especially those of African or East Asian descent, was low. Additionally, although the multi-ancestral composition of the cohort supports broader relevance, variability in genetic backgrounds, healthcare systems, drug availability and socio-economic factors may influence the generalizability of findings to other populations. Furthermore, the selection of

genes/loci was based on a literature review conducted in 2018. Consequently, SLE-associated genes identified more recently, such as *SAT1, P2RY8* and *DOCK11* (209–211), were not included. Another potential limitation may be related to the use of self-reported ancestry data. While this approach may introduce some degree of misclassification, it remains widely accepted when comprehensive genomic data are not available, as it has been shown to have good concordance with genetically inferred ancestry (212).

6. Conclusions

This study underscores the important role of genetic variability in jSLE, demonstrating its key contribution to early disease expression - particularly in jSLE patients of South Asian ancestry - as well as organ involvement, disease severity and the need for more intensive treatment. By capturing a broader spectrum of genetic variability rather than focusing on previously reported SLE-associated risk alleles, the approach taken in this study may provide new insights into how cumulative genetic impact can influence disease heterogeneity. Prospective studies in larger, unrelated multi-ancestral cohorts are required to validate these findings and further assess the need for more aggressive treatments in patients exhibiting high GAAS in genes associated with specific manifestations.

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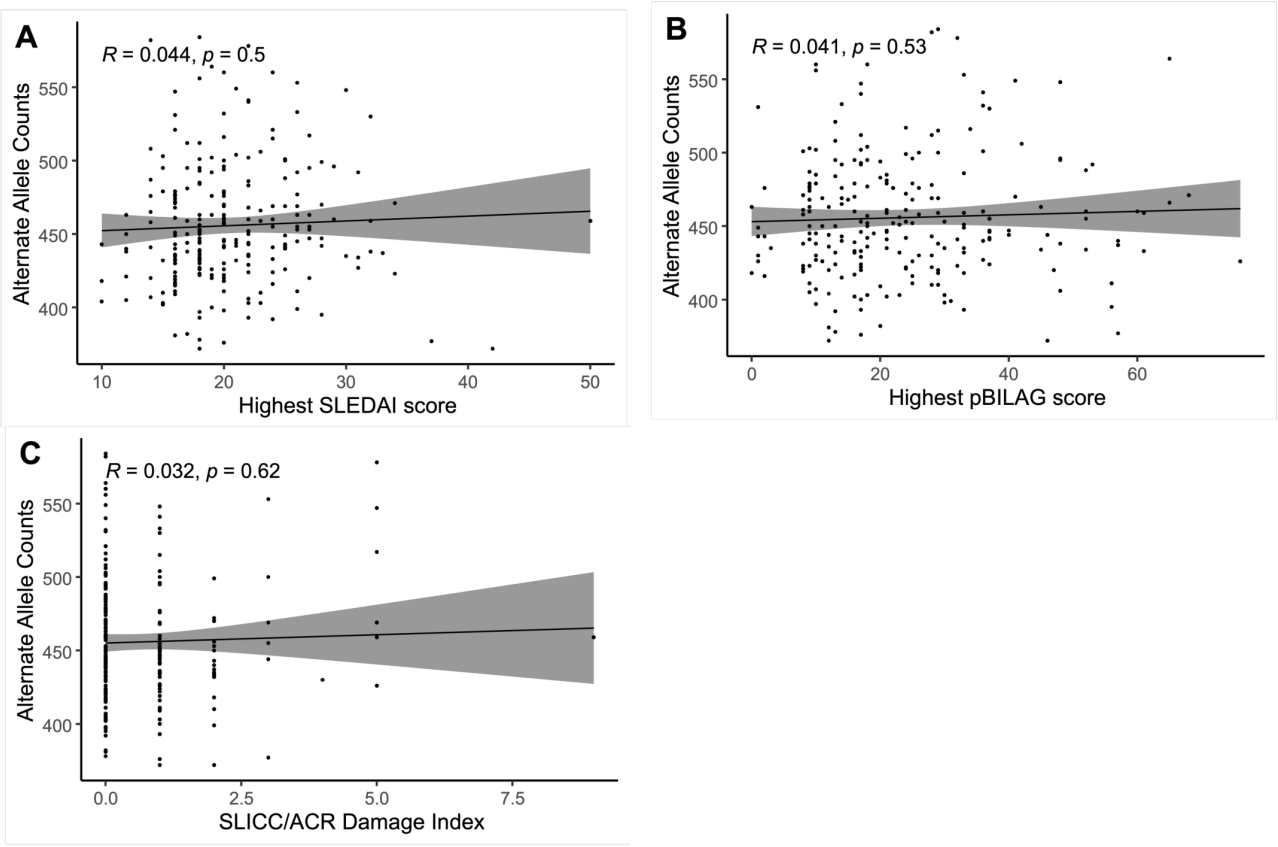
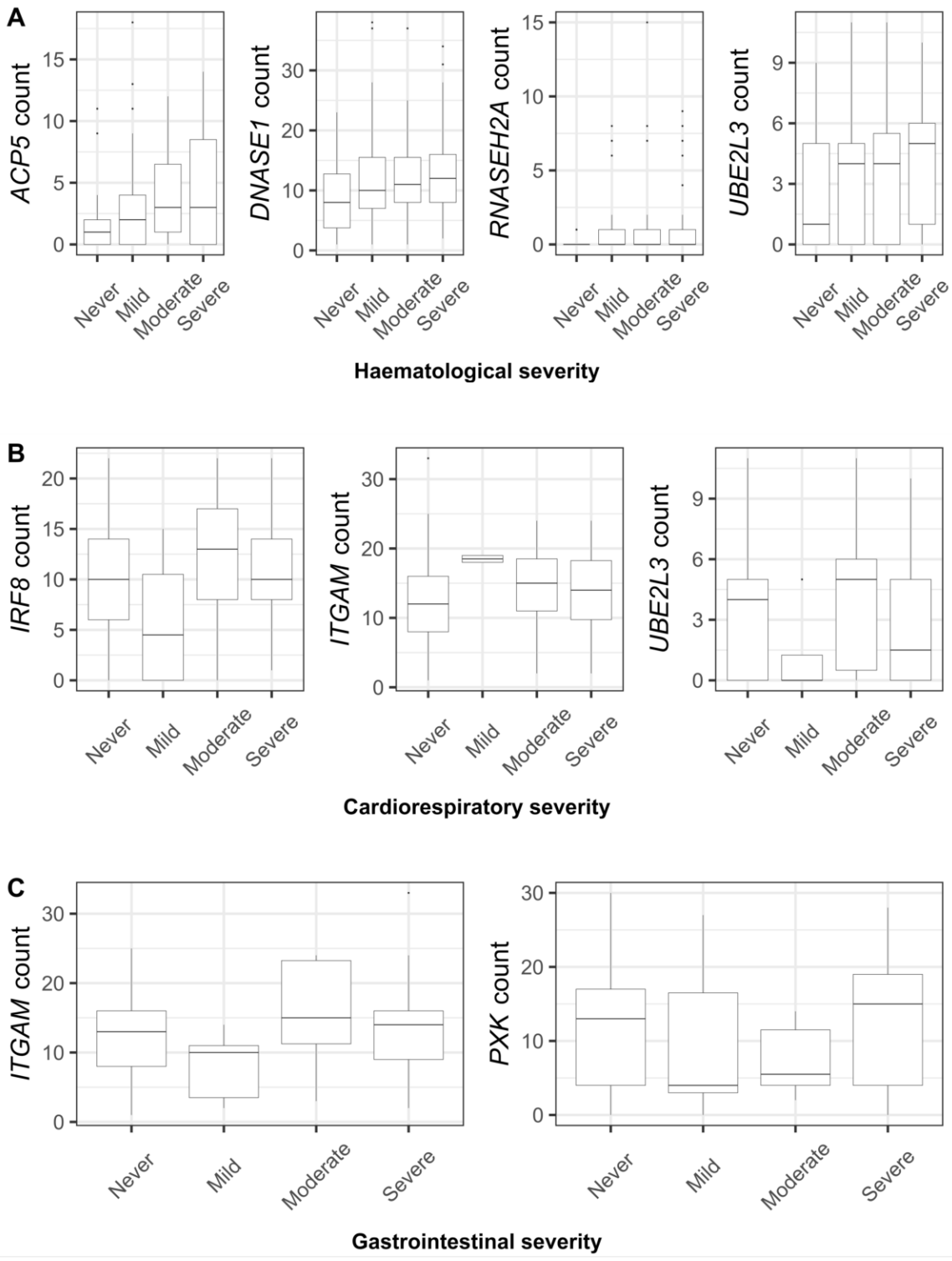
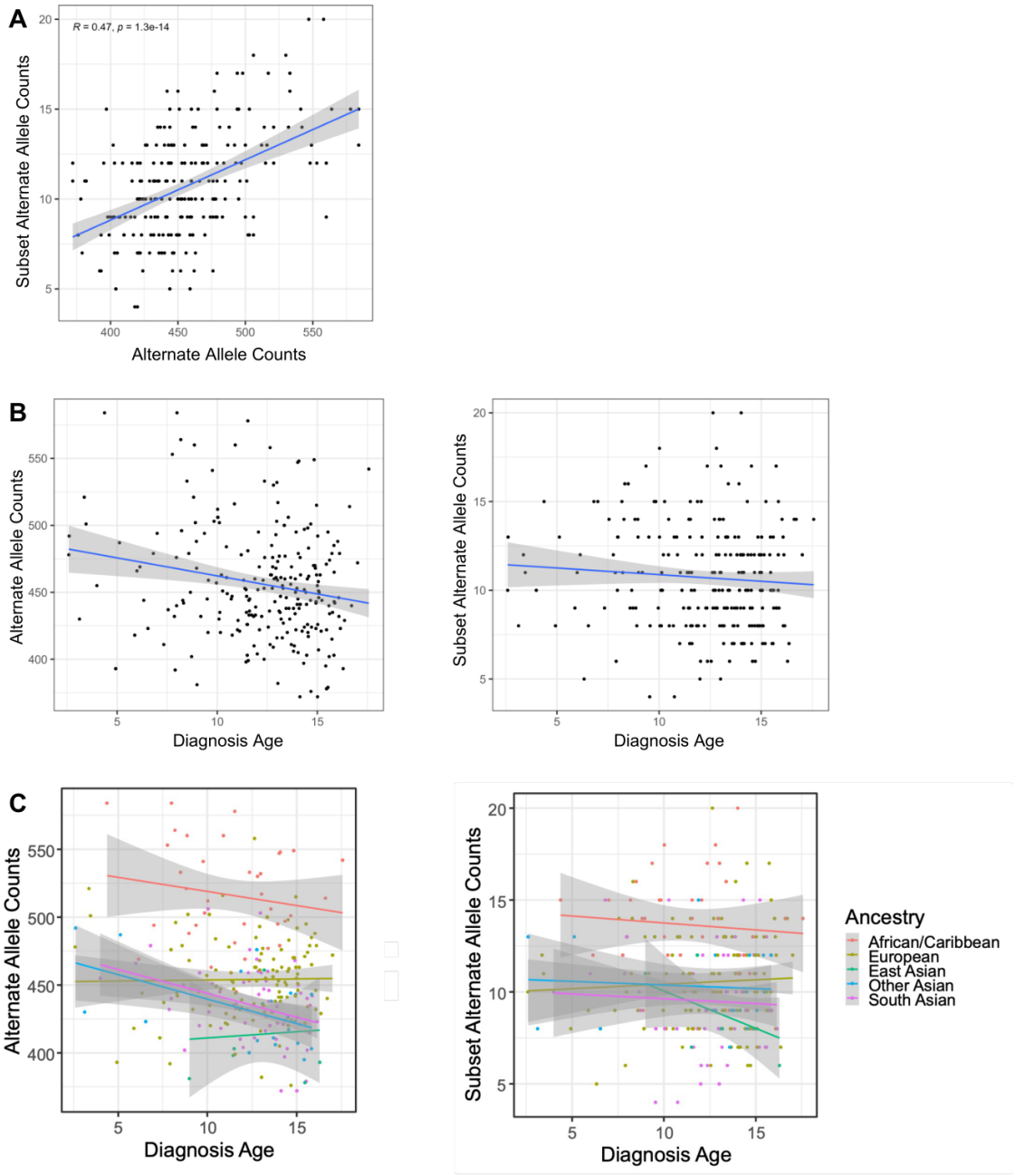
8. Supplementary Figures



Supplementary Figure 1. Alternate allele counts (AAC) do not correlate with disease activity and damage accrual. A) Scatter plot examining the correlation between the highest SLEDAI score registered and AAC, indicating a weak positive correlation with no statistical significance (R=0.081, p=0.19). **B)** Scatter plot examining the correlation between the highest pBILAG score registered and AAC, indicating a weak positive correlation with no statistical significance (R=0.032, p=0.61). **C)** Scatter plot examining the correlation between the SLICC/ACR Damage Index (SDI) and AAC, indicating a weak negative correlation with no statistical significance (R=-0.072, p=0.26). The trend line is presented with a 95% confidence interval in grey shading.



Supplementary Figure 2. Gene-level alternate allele scores (GAAS) associate to organ/system severity. A) Haematological severity significantly associates to *ACP5* (p=0.003), *DNASE1* (p=0.003), *RNASEH2A* (p<0.001), *UBE2L3* (p=0.03) GAAS. **B)** Cardiorespiratory severity significantly associates to *IRF8* (p=0.02), *ITGAM* (p<0.001) and *UBE2L3* (p=0.04) GAAS. **C)** Gastrointestinal severity significantly associates to *ITGAM* (p<0.001) and *PXK* (p<0.001) GAAS.



Supplementary Figure 3. Alternate Allele Counts (AAC) correlated with the subset AAC generated from previously published SNPs. A) Scatter plot demonstrating a strong positive correlation between AAC and the subset AAC based on previously published SNPs only across the study cohort (R=0.47, p<0.001). **B)** Scatter plot depicting the inverse correlation between AAC and age at diagnosis in the entire study cohort (R=-0.15, p=0.012) (left panel), and scatter plot showing the relationship between AAC generated from previously published SLE-associated SNPs and age at diagnosis, with no significant correlation detected (right panel). For both panels, data points represent the number of alternate alleles in individual jSLE patients, and a trend line indicates the direction and strength of the relationship. The grey shaded area indicates the 95% confidence interval. **C)** Scatter plot showing the inverse correlation between AAC and age at diagnosis, stratified by ancestry (left panel), and **s**catter plot displaying the correlation between AAC generated from previously reported SLE-associated SNPs only and age at diagnosis, with the same stratification by ancestry. No significant inverse correlation was observed. Each data point represents an individual sample, colour-coded by ancestry: African/Caribbean (red), European (gold), East Asian (green), South Asian (blue), and "Other" Asian (purple). Trend lines indicate the direction and strength of the relationships. The grey shaded areas represent the 95% confidence intervals.

9. Supplementary Tables

Supplementary Table 1. Clinical features of the study cohort (pBILAG score domains and ACR criteria)

	JSLE patients included in the alternate allele counts (AAC) analysis (n=238)	JSLE patients included in the gene-level alternate score (GAAS) analysis (n=289)
pBILAG domain involvement		
Constitutional involvement, n (%)	**151 (63.5)**	**183 (63.3)**
Severe	39 (25.8)	45 (24.6)
Moderate	59 (39.1)	77 (42.1)
Mild	53 (35.1)	61 (33.3)
Mucocutaneous involvement, n (%)	**213 (89.5)**	**259 (89.6)**
Severe	54 (25.3)	62 (23.9)
Moderate	108 (50.7)	138 (53.3)
Mild	51 (24.0)	59 (22.8)
Neuropsychiatric involvement, n (%)	**54 (22.7)**	**71 (24.6)**
Severe	18 (33.3)	23 (32.4)
Moderate	34 (63.0)	46 (64.8)
Mild	2 (3.7)	2 (2.8)
Musculoskeletal involvement, n (%)	**207 (87.0)**	**251 (86.9)**
Severe	12 (5.8)	17 (6.8)
Moderate	104 (50.2)	128 (51.0)
Mild	91 (44.0)	106 (42.2)
Cardiorespiratory involvement, n (%)	**57 (23.9)**	**67 (23.2)**
Severe	18 (31.6)	24 (35.8)
Moderate	35 (61.4)	39 (58.2)
Mild	4 (7.0)	4 (6.0)
Gastrointestinal involvement, n (%)	**40 (16.8)**	**46 (15.9)**
Severe	18 (45.0)	21 (45.7)
Moderate	16 (40.0)	18 (39.1)
Mild	6 (15.0)	7 (15.2)
Ophthalmic involvement, n (%)	**17 (7.2)**	**21 (7.3)**
Severe	5 (29.4)	6 (28.6)
Moderate	4 (23.5)	7 (33.3)
Mild	8 (47.1)	8 (38.1)
Renal involvement, n (%)	**190 (79.9)**	**231 (79.9)**
Severe	49 (25.8)	58 (25.2)
Moderate	118 (62.1)	144 (62.3)
Mild	23 (12.1)	29 (12.5)

Haematological involvement, n (%) Severe Moderate Mild	**218 (91.6)** 77 (35.3) 48 (22.1) 93 (42.6)	**269 (93.1)** 91 (33.8) 59 (22.0) 119 (44.2)
ACR criteria fulfilled		
Malar rash, n (%)	205 (86.1)	251 (86.8)
Discoid lupus, n (%)	76 (32.0)	91 (31.5)
Photosensitivity, n (%)	132 (55.5)	168 (58.1)
Oral/nasal ulcers, n (%)	157 (66.0)	186 (64.4)
Non-erosive arthritis, n (%)	199 (83.6)	242 (83.7)
Serositis, n (%)	84 (35.3)	101 (34.9)
Lupus nephritis, n (%)	128 (53.8)	159 (55.0)
Neurologic involvement, n (%)	40 (16.8)	50 (17.3)
Haematological involvement, n (%)	205 (86.1)	254 (87.9)
Immunological involvement, n (%)	224 (94.1)	274 (94.8)

JSLE, juvenile systemic lupus erythematosus; pBILAG, paediatric British Isles Lupus Assessment Grade 2004; ACR, American College of Rheumatology; n, number of patients

Supplementary Table 2: Sequencing panel.

Gene Name	*Variants investigated*	*Genomic location & effect*	*Pathway involved*	*Disease association*
"Genetic SLE"				
ACP5	Entire gene		Nucleic acid metabolism and processing	SPENCD
ADAR	Entire gene		Nucleic acid metabolism and processing	AGS
C1QA	Entire gene		IC clearance	SLE-like disease
C1QB	Entire gene		IC clearance	SLE-like disease
C1QC	Entire gene		IC clearance	SLE-like disease
C1R	Entire gene		IC clearance	SLE-like disease
C1S	Entire gene		IC clearance	SLE-like disease
C2	Entire gene		IC clearance	SLE-like disease
C3	Entire gene		IC clearance	SLE-like disease
C4A	Entire gene		IC clearance	SLE-like disease
C4B	Entire gene		IC clearance	SLE-like disease
DNASE1	Entire gene		Nucleic acid metabolism and processing	AGS, ChLE
DNASE1L3	Entire gene		Nucleic acid metabolism and processing	AGS, ChLE
FAS	Entire gene		Apoptosis	ALPS
FASLG	Entire gene		Apoptosis	ALPS
IFIH1	Entire gene		Nucleic acid metabolism and processing	AGS
PEPD	Entire gene		IC clearance	SLE-like disease
RNASEH2A	Entire gene		Nucleic acid metabolism and processing	AGS, ChLE
RNASEH2B	Entire gene		Nucleic acid metabolism and processing	AGS, ChLE
RNASEH2C	Entire gene		Nucleic acid metabolism and processing	AGS, ChLE
SAMHD1	Entire gene		Nucleic acid metabolism and processing	AGS, ChLE
TMEM173	Entire gene		Nucleic acid metabolism and processing	SAVI
TREX1	Entire gene		Nucleic acid metabolism and processing	AGS, ChLE

SLE risk alleles				
AFF1	rs340630	Intron variant	B and T cell signalling	SLE
PRDM1 - ATG5 intergenic region	rs548234	Intron variant	Autophagy	RA, SLE
	rs742108	Intron variant		SLE
	rs6568431	Intron variant		SLE
ATG5	rs2245214	Intron variant	Autophagy	SLE
	rs573775	Intron variant		SLE
BANK1	rs4522865	Intron variant	Immune cell signalling	SLE
	rs10028805	Intron variant		CLL, SLE
	rs17266594	Intron variant, T allele		SSc, RA, SLE
	rs10516487	Missense variant, R61H		SSc, RA, SLE
	rs3733197	Missense variant, A383T		RA, AITD, SSc, SLE
BLK	rs2736332	Intergenic variant	Immune cell signalling	SLE
	rs7812879	Intergenic variant		pSS, SLE
	rs2254546	Upstream regulatory region variant		Kawasaki, SLE
	rs2736340	Upstream regulatory region variant		AITD, Kawasaki, pSS, SLE
	rs13277113	Upstream gene variant		pSS, RA, AITD, SLE
	rs922483	5' UTR variant, T allele		SLE
	rs2736345	Intron variant		SLE
	rs2618476	Intron variant		SLE
	rs1478897	Intron variant		SLE
CSK	rs34933034	Intron variant, A allele	Immune cell signalling	SLE
	rs2289583	Intron variant		SLE
CTLA4	rs231775	missense variant, G allele	Immune cell signalling	HT, T1D, SLE
ETS1	rs6590330	Intergenic variant, A allele	Immune cell signalling	SLE
	rs1128334	3' UTR variant		SLE
	rs7941765	downstream gene variant		SLE
FCGR2A	rs6671847	Intron variant	IC clearance	SLE
	rs1801274	Missense variant, H166R		SLE
FCGR2B	rs1801274	missense variant	IC clearance	SLE
	rs1801274	missense variant		
IFIH1	rs2111485	intergenic variant	TLR/IFN signalling	T1D, SLE
	rs1990760	Missense variant, A946T		SLE

	rs10930046	Missense variant, H460R		SLE
	rs13023380	Intron variant, A allele		SLE
IKZF1	rs11185602	intron variant	Immune cell signalling	SLE
	rs4917014	upstream gene variant, T allele		pSS, SLE
	rs11185603	upstream gene variant		SLE
	rs10276619	downstream gene variant		SLE
IL10	rs3024505	downstream gene variant	Immune cell signalling	CD, IBD, pSS, SLE
	rs3024493	intron variant		SLE
	rs1800872	upstream gene variant, -592C>A		SLE
	rs1800871	upstream gene variant, -819C>T		SLE
	rs1800896	upstream gene variant, -1082G>A		SLE
	rs3122605	intergenic variant, G allele		SLE
IL21	rs907715	intron variant, G allele	Immune cell signalling	SSc, SLE
IRAK1	rs5986948	intergenic variant	TLR/IFN signalling	SLE
	rs1059702	missense variant, S196F		HT, RA, SSc, SLE
	rs1734787	non coding transcript exon variant		SLE
IRF5	rs729302	intergenic variant, A allele	TLR/IFN signalling	RA, SLE
	rs4728142	upstream gene variant, A allele		RA, SSc, SLE
	rs2070197	3 prime UTR variant		SLE
	rs10954213	3 prime UTR variant		SLE
	rs12537284	intergenic variant		SLE
IRF6	rs12531711	intron variant	TLR/IFN signalling	SLE
IRF7	rs12802200	non coding transcript exon variant	TLR/IFN signalling	SLE
	rs12805435	downstream gene variant		SLE
	rs1061502	missense variant, T/C		SLE
	rs58688157	5 prime UTR variant		SLE
	rs113478424	upstream gene variant, CTTAGCTATTGCTC/-		SLE
	rs3757387	upstream gene variant		SS, RA, SLE
	rs35000415	intron variant		SLE
	rs10488631	upstream gene variant		SLE

	rs12539741	intron variant		SLE
IRF8	rs13332649	downstream gene variant	Neutrophil and monocyte signalling, Type 1 IFN signalling	SLE
	rs11648084	upstream gene variant		SLE
	rs11644034	missense variant, A/G		SLE
	rs10521318	upstream gene variant		IBD, SLE
ITGAM	rs34572943	intron variant	IC clearance	SLE
	rs1143679	missense variant, G/A, R77H		SLE
	rs35472514	intron variant		SLE
	rs9937837	intron variant		SLE
	rs9888739	intron variant		SLE
	rs11150610	intron variant		SLE
	rs11574637	missense variant, T/C		AML, SLE
LYN	rs6983130	intron variant	B cell signalling	SLE
	rs7829816	intron variant		SLE
MECP2	rs5986948	intergenic variant	Immune cell signalling	SLE
	rs17435	intergenic variant, T allele		SLE
	rs1734787	non-coding transcript exon variant		SLE
MIR146A	rs2431697	intergenic variant	TLR/IFN signalling	SLE
	rs2431098	intergenic variant		SLE
	rs57095329	upstream variant, G allele		SLE
MSH5	rs409558	5 prime UTR variant	B cell signalling	SLE
	rs3131379	intron variant		SLE
	rs3130490	non-coding transcript exon variant		SLE
NCF1	rs201802880	missense variant, R90H	Autophagy	SLE
NCF2	rs13306575	missense variant	Autophagy	SLE
	rs17849502	missense variant, H389Q		SLE
	rs17849501	synonymous variant		SLE
PTPN22	rs6679677	upstream gene variant	Immune cell signalling	RA, T1D, CD, JIA, SLE
	rs2476601	missense variant, R620W		RA, T1D, CD, JIA, HT, SLE
PRKCB	rs16972959	intron variant	NFkB signalling	SLE
PXK	rs4681677	intron variant, G allele	Immune cell signalling	SLE
	rs6445975	intron variant		SLE
	rs9311676	regulatory region variant		SLE

RASGRP3	rs13385731	intron variant	B cell signalling	SLE
SLC15A4	rs1059312	synonymous variant	NFkB signalling	SLE
	rs11059919	intron variant		SLE
	rs10847697	synonymous variant		SLE
	rs1385374	intron variant		SLE
STAT4	rs12612769	intron variant	Immune cell signalling	SLE
	rs3821236	intron variant		SSc, SLE
	rs11889341	intron variant		SS, RA, JIA, SLE
	rs6736175	intron variant		SLE
	rs7574865	intron variant, T allele		SSc, RA, SS, SLE
	rs7582694	intron variant		SLE
TLR7	rs3853839	3 prime UTR variant, G allele	TLR/IFN signalling	SLE
TNFAIP3	rs5029939	intron variant, TT>A polymorphism	NFkB signalling	SS, SLE
	rs2230926	missense variant		RA, SSc, SLE
	rs6932056	intergenic variant		SLE
	rs58721818	intergenic variant		SSc, RA, SLE
TNIP1	rs7708392	intron variant	NFkB signalling	SLE
	rs6889239	intron variant		SLE
	rs10036748	intron variant		SLE
TNFSF4	rs2205960	intergenic variant, T allele	Immune cell signalling	SLE
	rs76413021	intergenic variant		SLE
	rs704840	intergenic variant		SSc, SLE
	rs10912578	intergenic variant		SLE
	rs10753074	intergenic variant		SLE
	rs4916342	intergenic variant		SLE
TYK2	rs11085727	intron variant	TLR/IFN signalling, T cell signalling	SLE
	rs2304256	missense variant		T1D, SLE
UBE2L3	rs131654	intron variant	NFkB signalling	SLE
	rs140490	intron variant, T allele		SLE
	rs5754217	intron variant		SLE
	rs7444	3 prime UTR variant		SLE
	rs3747093	upstream gene variant		SLE

ACP5: Acid Phosphatase 5, Tartrate Resistant; ADAR: Adenosine Deaminase RNA Specific; AFF1: AF4/FMR2 Family Member 1; AITD: Autoimmune thyroid disease; ATG5: Autophagy Related 5; BANK1: B Cell Scaffold Protein with Ankyrin Repeats 1; BLK: BLK Proto-Oncogene, Src Family Tyrosine Kinase; C1QA: Complement C1q A Chain; C1QB: Complement C1q B Chain; C1QC: Complement C1q C Chain; C1R: Complement C1r ; C1S: Complement C1s; C2: Complement C2; C3: Complement C3; C4A: Complement C4A; C4B: Complement C4B; CSK: C-Terminal Src Kinase;

CTLA4: Cytotoxic T-Lymphocyte Associated Protein 4; DNASE1: Deoxyribonuclease 1; DNASE1L3: Deoxyribonuclease 1 Like 3; ETS1: ETS Proto-Oncogene 1, Transcription Factor; FAS: Fas Cell Surface Death Receptor; FASLG: Fas Ligand; HT: Hashimoto Thyroiditis; FCGR2A: Fc Fragment of IgG Receptor 2A; FCGR2B: Fc Fragment of IgG Receptor 2B; IBD: Inflammatory bowel disease; IFIH1: Interferon Induced with Helicase C Domain 1; IKZF1: IKAROS Family Zinc Finger 1; IL10: Interleukin 10; IL21: Interleukin 21; IRAK1: Interleukin 1 Receptor Associated Kinase 1 ; IRF5: Interferon Regulatory Factor 5; IRF6: Interferon Regulatory Factor 6; IRF7: Interferon Regulatory Factor 7; IRF8: Interferon Regulatory Factor 8; ITGAM: Integrin Subunit Alpha M; JIA: Juvenile Idiopathic Arthritis; LYN: LYN Proto-Oncogene, Src Family Tyrosine Kinase; MECP2: Methyl-CpG Binding Protein 2; MIR146A: MicroRNA 146a; MSH5: MutS Homolog 5; NCF1: Neutrophil Cytosolic Factor 1; NCF2: Neutrophil Cytosolic Factor 2; PEPD: Peptidase D; PRDM1: PR/SET Domain 1;PTPN22: Protein Tyrosine Phosphatase Non-Receptor Type 22; PRKCB: Protein Kinase C Beta; pSS: primary Sjögren's syndrome; PXK: PX Domain Containing Serine/Threonine Kinase Like; RASGRP3: RAS Guanyl Releasing Protein 3; 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; SAVI: STING-associated vasculopathy with onset in infancy; SLE: Systemic Lupus Erythematosus; SLC15A4: Solute Carrier Family 15 Member 4; SPENCD: Spondyloenchondrodysplasia; SS: Sjögren's syndrome; SSc: systemic sclerosis; T1D: Type 1 diabetes; STAT4: Signal Transducer and Activator Of Transcription 4; TLR7: Toll Like Receptor 7; TNFAIP3: TNF Alpha Induced Protein 3; TNIP1: TNFAIP3 Interacting Protein 1; TNFSF4: TNF Superfamily Member 4; TMEM173: Stimulator of Interferon Response CGAMP Interactor 1; TREX1: Three Prime Repair Exonuclease 1; TYK2: Tyrosine Kinase 2; UBE2L3: Ubiquitin Conjugating Enzyme E2 L3.

Supplementary Table 3. Associations between alternate allele counts and the 21 demographic/clinical variables included in the generalised linear model.

Variable	P-value	Adjusted p-value
Age at diagnosis	5.64E-09	**5.93E-08**
Constitutional severity	3.55E-06	**2.48E-05**
Mucocutaneous severity	0.12	0.20
Neuropsychiatric severity	0.008	0.02
Musculoskeletal severity	0.02	0.05
Cardiorespiratory severity	0.02	0.05
Gastrointestinal severity	0.39	0.52
Ophthalmic severity	0.15	0.23
Renal severity	5.91E-05	**0.0003**
Haematological severity	0.0006	**0.002**
Malar rash	0.02	0.05
Discoid lupus	0.88	0.88
Photosensitivity	0.06	0.13
Oral/nasal ulcers	0.46	0.52
Non-erosive arthritis	0.0009	**0.003**
Serositis	0.11	0.20
Nephritis	0.47	0.52
Neurologic involvement	0.67	0.71
Haematological involvement	0.21	0.30
Immunological involvement	0.43	0.52
Treatment Intensity	6.80E-12	**1.43E-10**

Supplementary Table 4. 13 genes showed significant correlations between gene-level alternate allele scores and clinical features/severity or treatment choices.

Variable	*ACP5*	*DNASE1*	*IRF8*	*ITGAM*	*LYN*	*PEPD*	*PXK*	*RASGRP3*	*RNSSEH2A*	*TNFAIP3*	*TNFSF4*	*TYK2*	*UBE2L3*
Diagnosis Age	NS	NS	0.02240831	NS	0.01065484	NS	NS	NS	NS	NS	NS	NS	NS
Constitutional Severity	NS	NS	NS	0.01210467	3.17E+09	NS	NS	NS	NS	NS	NS	NS	NS
Mucocutaneous Severity	NS	NS	NS	NS	NS	NS	0.0252778	NS	NS	NS	0.00813828	NS	NS
Neuropsychiatric Severity	1.46E+08	NS	NS	NS	NS	NS	NS	0.04284439	0.0001746	NS	NS	1.16E+08	NS
Musculoskeletal Severity	NS	1.45E+09	NS	NS	5.45E+08	NS	NS	NS	0.00026318	NS	NS	NS	NS
Cardiorespiratory Severity	NS	NS	0.02448327	0.00024176	NS	NS	NS	NS	NS	NS	NS	NS	0.04497508
Gastrointestinal Severity	NS	NS	NS	0.00024176	NS	NS	4.80E+07	NS	NS	NS	NS	NS	NS
Renal Severity	1.46E+08	NS	NS	0.00026373	5.07E+09	NS	NS	NS	NS	0.00712858	NS	NS	NS
Haematological Severity	0.00338154	0.00328468	NS	NS	NS	NS	NS	NS	0.00127586	NS	NS	NS	0.02683156
Malar Rash	0.03799275	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Discoid Lupus	NS	NS	NS	NS	3.17E+09	0.00672523	NS	NS	NS	NS	NS	0.0282559	NS
Photosensitivity	2.64E+09	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Oral/Nasal Ulcers	NS	NS	0.03942069	NS	NS	NS	1.69E+07	NS	NS	NS	NS	NS	NS
Non-Erosive Arthritis	NS	1.45E+09	0.03942069	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Serositis	NS	9.36E+08	NS	NS	NS	NS	1.69E+07	NS	NS	NS	NS	NS	NS
Nephritis	0.03192245	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Treatment Intensity	NS	NS	NS	2.99E+09	4.22E-03	NS	NS	NS	5.38E+09	NS	NS	NS	NS