Systemic lupus erythematosus: pathogenesis, diagnosis, and treatment

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Familial and Sporadic Lupus Nephritis Compared: Genetics, Clinical Characteristics, Histology, and Renal Outcome

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Abstract

Systemic lupus erythematosus is an autoimmune disease which is thought to have a significant genetic contribution in its aetiology. Either susceptibility alleles identified in GWAS, such as in those involved in monocyte/macrophage function, or rare variants may play a role in familial disease. Therefore, the aim of our study was to compare patients with familial and sporadic lupus nephritis (LN) with respect to clinical parameters, serology, histological class, activity and chronicity indices (AI and CI), the number of glomerular monocytes/macrophages, and the contribution of known lupus susceptibility polymorphisms.

Our cohort consisted of 154 patients of which 16 patients had a first-degree relative with LN. Age, sex, ancestry, progression to advanced renal impairment, serology, histological class, AI, CI, and glomerular CD16 and CD68 counts were determined. Also, we calculated a polygenic risk score based on the number of selected lupus susceptibility alleles carried.

We found that patients with familial LN more often had juvenile onset disease (50% vs 22%, respectively; *P*=0.03), were more often male (44% vs 12% male, respectively; *P*=0.004), had a higher frequency of progressing to advanced renal impairment (25% vs 7%, respectively; *P*=0.03) and had different ancestral backgrounds than patients with sporadic LN (*P*=0.002). The serology was not different, neither was the distribution among the histological classes, the AI and CI, and the number of glomerular CD16 (0.9 vs 1.4, respectively; *P*=0.23) and CD68 (10.1 vs 6.2, respectively; *P*=0.12) positive cells. Familial LN patients did not have a statistically significant higher polygenic risk score than patients with sporadic LN.

In conclusion, although we did find a worse renal outcome in familial LN compared to sporadic LN, we did not find a difference in histological parameters or genetic background. Therefore, the cause of the observed differences remains unknown. Whole exome sequencing in families with multiple affected members to search for rare variants may provide new leads for future research.

Introduction

Systemic Lupus Erythematosus (SLE) is considered to be the prototypic autoimmune disease with aberrations throughout the immune system resulting in diverse clinical manifestations. Lupus nephritis (LN) is one of the most severe clinical manifestations of SLE, with an estimated 10-15% of patients progressing to end-stage renal disease (ESRD).[1 2] Renal damage is the overall most important predictor of mortality in SLE patients.[3 4]

Epidemiologic studies suggest a significant contribution of genetic factors in the aetiology of SLE. Disease concordance in SLE is higher in monozygotic twins (25-50%) than in dizygotic twins (2%) and there is a high sibling risk ratio (λs) of 20-29.[5-7] Although most studies found similar clinical presentations in patients with familial and sporadic SLE,[8-12] one small study in children reported an increase in all-cause mortality in familial SLE.[13] However, other studies did not show a difference in outcome.[14 15]

The genetics involved in familial SLE might include a clustering of multiple common risk alleles in families, or the presence of a rare variant with a large effect, such as in *DNASE1*.[16] Linkage analysis, candidate gene studies and genome wide-association studies (GWAS) have led to the identification of several candidate polymorphisms, including those effecting monocyte/macrophages function.[17 18] Also, monocytes/macrophages have been suggested to play a role in the pathogenesis of SLE in general,[19] and in LN in particular.[20 21]

The aim of this research was to explore the differences between familial and sporadic LN with respect to clinical parameters, serology, histological class, glomerular influx of monocytes/macrophages, and the contribution of known lupus susceptibility polymorphisms.

Methods

Study population

From July 2010 to January 2012, 160 patients with LN were recruited from three designated clinical centres. Inclusion criteria were as follows; a definite diagnosis of SLE in accordance with the American College of Rheumatology (ACR) revised classification criteria,[22] biopsy-proven LN, and the ability to provide written informed consent. Enquiry into the family history resulted in four additional cases with biopsy-proven LN, leading to a total of 164 cases. In families with clustering of LN, unaffected family members were also recruited where available (Figure 1). All study work was conducted in accordance with the requirements of the Helsinki Declaration and this study was approved by the Outer South East London and the London City Road and Hampstead Research Ethics Committees.

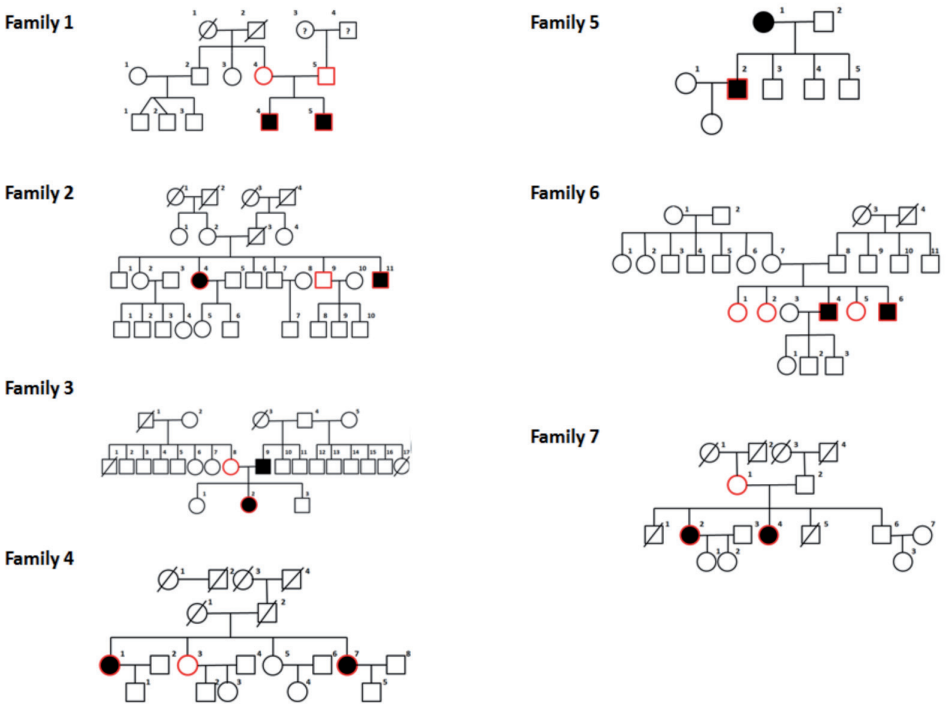

Figure 1. Families with lupus nephritis in two first degree family members
Seven families with clustering of lupus nephritis within our study cohort. Circles indicate females and squares indicate males. Filled circles/squares are probands. A red outline indicates that DNA was available for genotyping. A stoke through the circle/square signifies that the person has died. Family 1 and 5 are of South Asian ancestry, families 2, 3, 4, and 7 are of African ancestry and family 6 is of East Asian ancestry.

Clinical variables

Clinical variables explored in this study included gender, age at diagnosis of nephritis, family history of SLE, and ancestral background. Ancestry was self-reported by patients recruited to the study. To avoid bias due to population stratification in assessment of the frequency of susceptibility polymorphisms, Multidimensional Scaling was carried out using PLINK to identify outliers from the main ancestral groups.[23]

Autoantibody profiles were performed in all patients at the designated clinical centres including ANA (anti-nuclear antibodies), anti-double stranded DNA, anti-Ro (SS-A), anti-RNP (ribonucleoproteins) and anti-Sm (Smith antigen) antibodies using a standardized counterimmunoelectrophoresis.

Long-term renal outcomes in LN patients were assessed using the National Kidney

Foundation Kidney Disease Outcomes Quality Initiative scoring system.[24] Patients with Stage 4, severe reduction in glomerular filtration rate (GFR) (15-29 mL/min) and Stage 5, kidney failure (GFR<15 or dialysis) were classified as having advanced renal impairment.

Renal histology

Paraffin-embedded renal biopsy tissue was available from 77% (n=126) of LN patients recruited to this study. Biopsies were traced back to the time of the patients' original diagnosis of LN (n=107) or when this was not possible biopsies taken at the onset of a new nephritis flare before induction immunosuppression was commenced were obtained (n=19). Biopsies were reclassified independently by two renal histopathologists as per the 2004 ISN/RPS classification system.[25 26] Discrepancies in classes were resolved during a consensus meeting. For purpose of analyses, cases with class III or IV LN combined with class V LN were considered as class III or IV LN. In addition, the activity and chronicity indices (AI and CI) were obtained from the original pathology report and were available for 51 cases (all class III or IV LN). Furthermore, immunohistochemical staining was performed for CD16 and CD68. Slides were deparaffinised and subjected to antigen retrieval (Tris/EDTA buffer). After blocking endogenous peroxidase, the sections were incubated with either mouse anti-human CD16 (MS1085; Thermo Scientific, Waltham, MA, USA) for 2 hours, or mouse anti-human CD68 (KP-1; Dako, Glostrup, Denmark) for 1 hour. Sections were then counterstained with haematoxylin. Once mounted and dried, the slides were scanned and the number of CD16 and CD68 positive cells in the glomerular tuft was counted (viewer software: 3DHISTECH Pannoramic Viewer or Philips Digital Pathology Solution). Results are presented as average number of positive cells per glomerular tuft in a biopsy. During analysis, slides with < 7 glomeruli present were excluded, leaving 105 biopsies for ISN/RPS classification, 69 biopsies for CD16 analysis (only cases with either class III or IV LN were stained) and 91 biopsies for CD68 analysis.

Genotyping by ImmunoChip

Genomic DNA was extracted from thawed frozen whole blood using the GenElute™ Blood Genomic DNA Kit (Sigma Aldrich, St Louis, MO, USA) as per the manufacturer's instructions. Genotyping was performed using the Illumina ImmunoChip. Analysis and genotype calling was performed using Illumina GenomeStudio software. Four patients' genotyping results did not meet quality control standards leaving 160 patients results suitable for analysis.

A polygenic risk score was calculated using 20 common nucleotide polymorphisms (SNPs)

Table 1. Lupus susceptibility polymorphisms included in polygenic risk score

Gene	SNPs
HLA region	rs3135394, rs9271366
IRF5	rs2070197, rs10954213
IRAK1	rs2269368
PTPN22	rs2476601
ITGAM	rs1143679
IRF7	rs4963128
IRF8	rs2280381
NCF2	rs10911363
STAT4	rs7574865
IKZF1	rs4917014
IFIH1	rs1990760
TNFAIP3	rs6920220, rs5029939
TNFSF4	rs2205960
ETS1	rs6590330
BLK	rs2736340
BANK1	rs10516487
LYN	rs7829816

that represent confirmed SLE susceptibility loci (Table 1). Inclusion of SNPs that may in linkage disequilibrium with one another was avoided. The polygenic risk score was assessed by two methods, a simple polygenic risk score and a weighted polygenic risk score. The simple polygenic risk score (count genetic risk score, cGRS) was calculated by counting the number of risk allele carried by an individual. In the weighted polygenic risk score (wGRS), the risk allele is weighted by the logarithmic odds ratio (log OD) for that allele. The overall wGRS is the sum of the log OD for each individual risk allele included in the score divided by the number of alleles.

Statistical analysis

Categorical variables were compared using Pearson's Chi-squared ($\chi 2$) test, except in instances where expected counts were <5, when a Fisher's exact test was used. Continuous variables with a normal distribution were described as mean with standard deviation (SD) and continuous variables without a normal distribution as median with an interquartile range (IQR). Student's *t*-tests or Mann Whitney *U* tests were used to compare continuous variables that were normally or non-normally distributed, respectively. Correlations were

tested with Spearman's rank correlation test. A value of $P \leq 0.05$ was considered to be significant. Analyses were carried out using SPSS Statistics 20.0 (IBM, Armonk, NY).

Results

Demographics of overall patient cohort

Twenty-six patients reported a family history of SLE. Of these, 16 patients had a first-degree family history, defined as having a parent, sibling or children affected with LN. All self-reported first degree family relatives were confirmed clinically. Five patients reported a second degree family history and five a third degree family history. Only patients with a first-degree family member with LN were considered as familial cases. The remaining ten patients with a positive family history were excluded from the analyses, as they were considered as neither familial nor sporadic, leaving 154 patients for analysis. Of these 154 patients 130 (84%) patients were female and 24 (16%) were male. The mean age at diagnosis of LN was 26.0 ± 11.2 years. The mean disease duration was 11.7 ± 7.3 years. Twenty-five percent (n=38) of the study group were of juvenile onset as defined by diagnosis of nephritis before 18 years of age.

Forty-two percent (n=64) of the cohort were of European origin, predominantly from the United Kingdom. Thirty-two percent (n=49) were of African ancestry, the majority being of Afro-Caribbean descent and others from Nigeria, Ghana, Sierra Leone and Uganda. Fourteen percent (n=21) were of South Asian extraction, all from India and Pakistan. Eight percent (n=12) were East Asian, from China, Vietnam and Singapore. Five percent (n=8) were classified as outliers from the 4 main ancestral groups.

Comparison of familial and sporadic LN

Of the cases with familial LN 56% (n=9) were female as compared to 88% (n=121) of sporadic cases (P=0.004). Familial cases were younger, although just not statistically different (17 vs 26 years, P=0.07). However, 50% (n=8) of familial cases had juvenile onset disease as opposed to 22% (n=30) in the sporadic group (P=0.03). The distribution among the different ancestries was different in familial and sporadic LN (P= 0.001) due to a relatively high percentage of patients of African descent in the familial group and the absence of familial cases of European descent. Twenty-five percent (n=4) of familial LN cases had progressed to advanced renal disease while 7% (n=9) had done so in the sporadic patient cohort (P=0.03). The autoantibody profile did not differ significantly between familial and sporadic cases (Table 2).

Table 2. Patient sociodemographics, clinical, laboratory and histologic features in familial and sporadic lupus nephritis

	Familial nephritis (n=16)	Sporadic nephritis (n=138)	*P*-value
Female (% (n))	56% (9)	88% (121)	0.004[a]
Age at diagnosis (years) (Median (IQR) (n))	17 (15) (15)	26 (13) (127)	0.07[b]
Juvenile (<18 y) onset (% (n))	50% (8)	22% (30)	0.03[c]
European (% (n))	0%	46% (64)	-
African (% (n))	69% (11)	28% (38)	-
South Asian (% (n))	19% (3)	13% (18)	-
East Asian (% (n))	13% (2)	7% (10)	-
Outliers from main ancestral groups (% (n))	-	6% (8)	0.002[c]
Duration of follow-up (years) (Median (IQR) (n))	13 (9) (15)	10 (10.5) (125)	0.03[b]
Progression to advanced renal impairment (% (n))	25% (4)	7% (9)	0.03[a]
ANA (% (n))	94% (15/16)	97% (132/136)	0.43[a]
Anti-dsDNA (% (n))	67% (10/15)	71% (96/135)	0.77[a]
Anti-Ro (% (n))	40% (6/15)	36% (46/128)	0.76[c]
Anti-Sm (% (n))	36% (5/14)	18% (23/128)	0.15[a]
Anti-RNP (% (n))	43% (6/14)	36% (46/128)	0.61[c]
Class I (% (n))	0%	2% (2/95)	-
Class II (% (n))	0%	5% (5/95)	-
Class III (% (n))	40% (4/10)	27% (26/95)	-
Class IV-S (% (n))	20% (2/10)	30% (28/95)	-
Class IV-G (% (n))	30% (3/10)	22% (21/95)	-
Class V (% (n))	10% (1/10)	13% (13/95)	0.87[c]
Activity index (Mean ± SD (n))	10.4 ± 5.1 (7)	9.1 ± 3.8 (44)	0.42[d]
Chronicity index (Median (IQR) (n))	3.0 (1.0) (7)	3.0 (2.0) (44)	0.87[b]
Glomerular CD16 count (Median (IQR) (n))	0.9 (1.3) (6)	1.4 (2.3) (63)	0.23[b]
Glomerular CD68 count (Median (IQR) (n))	10.1 (16.2) (9)	6.2 (12.6) (82)	0.12[b]

P-values were assessed with [a] Fisher's exact test, [b] Mann-Whitney U test, [c] Pearson Chi-square test or [d] Student's t-test. ANA, anti-nuclear antibody; anti-dsDNA, anti-double stranded DNA antibody; anti-RNP, anti-ribonucleoprotein antibody; anti-Sm, anti-Smith; IQR, interquartile range; SD, standard deviation.

Histopathology

The distribution among the ISN/RPS classes was similar in familial and sporadic cases (Table 2). Also, the AI and CI were not different. The median number of glomerular CD68 positive cells was 10.1 in familial cases and 6.2 in sporadic cases, but this was not significantly different (*P*=0.12). CD16 staining showed the opposite result with 0.9 positive cells in familial cases and 1.4 positive cells in sporadic cases, but this was also not significantly different (*P*=0.23). In addition, within class III and IV LN, there was no difference in the number of glomerular CD68 positive cells (*P*=0.49) between familial and sporadic cases.

Overall, we did see a difference in the number of glomerular CD68 positive cells between classes, with class I, II and V LN having few CD68 positive cells and class IV LN having the most (Figure 2). There was no difference in the number of glomerular CD68 positive cells between IV-S and IV-G (*P*=0.93), in contrast to previous literature.[27] The number of CD68 positive cells was correlated with the AI (r=0.49, *P*=0.000).

Polygenic risk scores

Given the varying frequencies of risk alleles in different ancestral groups, polygenic risk scores were compared on an ancestry-by-ancestry basis, in which ancestry outliers were

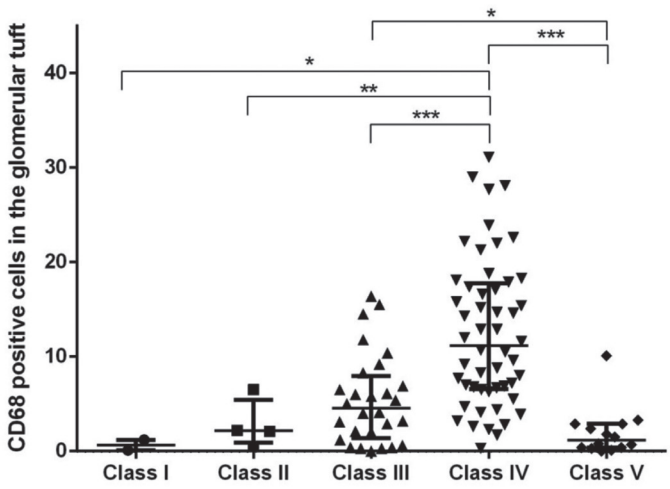

Figure 2. Number of glomerular CD68 positive cells in relation to the ISN/RPS class
Average number of CD68 positive cells in the glomerular tuft in relation to the ISN/RPS class. *P≤0.05; **P≤0.01, ***P≤0.001

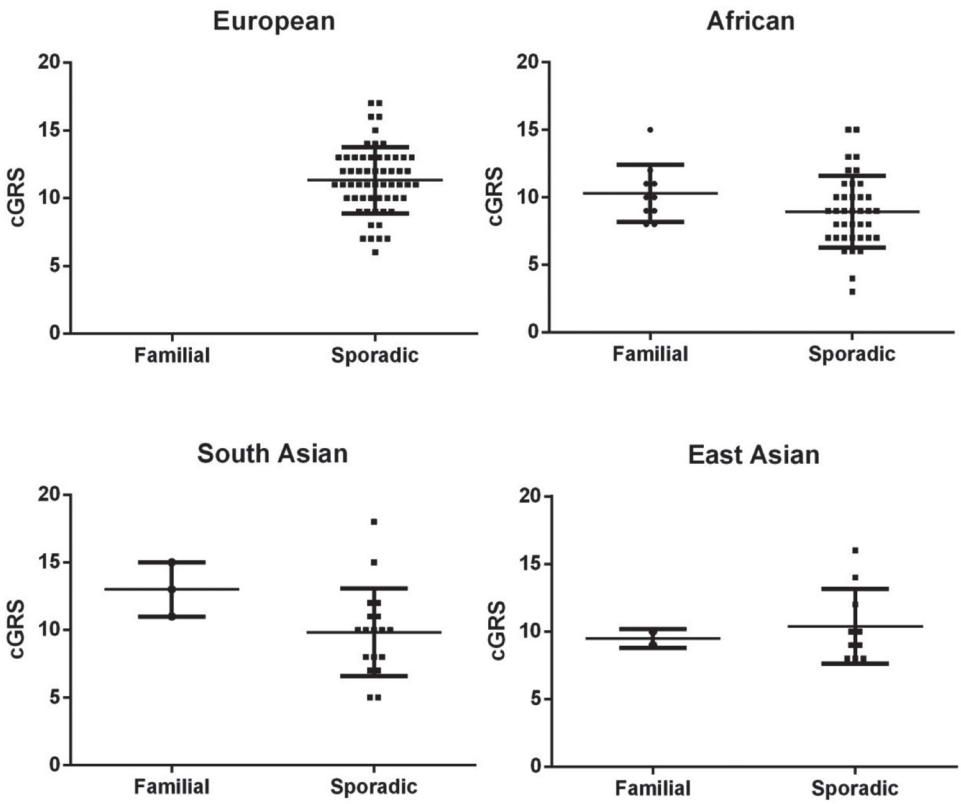

Figure 3. A comparison of polygenic risk score of lupus susceptibility alleles in familial and sporadic lupus nephritis patients of different ancestries

None of the comparisons between familial and sporadic lupus nephritis cases show a statistically significant difference.

excluded. European LN patients had the highest mean cGRS at 11.3 ± 2.5 while African patients had the lowest mean score at 9.2 ± 2.6 (*P*=0.000). South Asian and East Asian patients had similar mean cGRS, 10.3 ± 3.3 and 10.3 ± 2.5 respectively.

Mean cGRS in African familial cases was 10.3 ± 2.1 in comparison to 8.9 ± 2.7 in sporadic African patients (*P*=0.14). South Asian familial patients' mean cGRS was 13.0 ± 2.0 while the sporadic patients mean score was 9.8 ± 3.2 (*P*=0.12). East Asian familial mean cGRS was 9.5 ± 0.71 and 10.4 ± 2.8 in sporadic patients (*P*=0.67). There were no cases of familial nephritis in patients of European ancestry to enable a comparison of familial and sporadic disease (Figure 3).

Mean wGRS in African familial cases was 0.69 ± 0.14 as compared to 0.59 ± 0.18 in sporadic

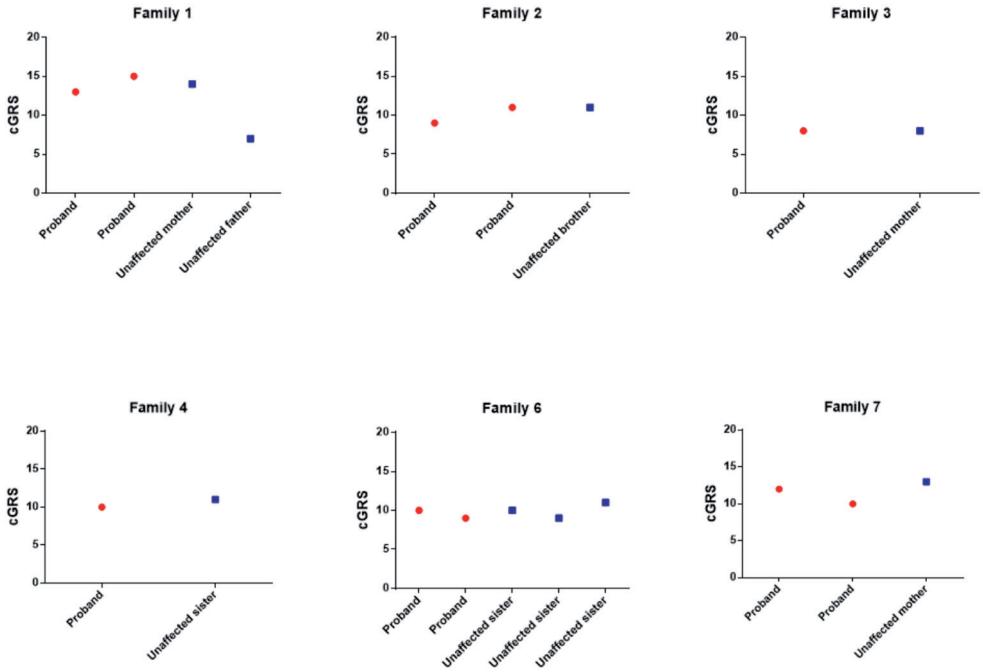

Figure 4. Polygenic risk score in probands and unaffected family members.
A comparison of cGRS (count genetic risk score) in probands and unaffected family members in families with clustering of lupus nephritis. Family 1 is of South Asian ancestry, families 2, 3, 4, and 7 are of African ancestry and family 6 is of East Asian ancestry. Of family 5 there was no DNA available of unaffected family members. Circles indicate family members with LN, squares indicate unaffected family members.

patients (*P*=0.16). South Asian familial patients had a mean wGRS of 0.83 ± 0.13 while sporadic patients of this ancestry scored 0.66 ± 0.22 (*P*=0.22). East Asian familial cases scored 0.60 ± 0.03 in comparison to 0.63 ± 0.18 in sporadic disease (*P*=0.848).
When both mean cGRS and wGRS of probands were compared to their unaffected relatives, their scores were found to be similar (Figure 4).

Discussion

We found that patients with familial LN were more often male, younger, had a different ancestral background and progressed to advanced renal impairment more often than patients with sporadic LN. However, we did not find a difference in their antibody profile, the distribution among the ISN/RPS classes, or the number of CD16 and CD68 positive

cells in the glomeruli. Furthermore, familial LN patients did not have more risk alleles than sporadic LN patients.

When comparing clinical characteristics, the familial patients in our cohort were younger than the sporadic patients, although this did not reach statistical significance (*P*=0.07). Nevertheless, familial cases did have juvenile onset disease more often (*P*=0.03). In a small study in paediatric SLE in Saudi Arabia familial cases were found to be younger[13]: 6.8 years old in familial patients and 10.2 years old in sporadic patients. However, this was not confirmed in other, larger, studies from France,[9] the US (multiracial)[11] and China.[12] Similar to other studies,[10 11] the antibody profile was not different in familial and sporadic cases. However, there was a clear difference in racial distribution between familial and sporadic cases.

We showed that familial patients have a worse renal outcome than sporadic patients. One other study addressing renal outcome did not find a difference.[14] However, in that study patients with a general family history of autoimmune disease and not SLE specifically were included. Since a significantly larger proportion of SLE patients have a family history of autoimmune disease in general than of SLE specifically, this may account for this difference.[28] A likely explanation for the difference in renal outcome would have been that renal disease was more severe in familial cases. We did not, however, find a difference in the distribution among the ISN/RPS classes or in activity or chronicity index. Because of the possible role of monocytes/macrophages in the pathogenesis of LN[20 21] and identified SLE susceptibility alleles involving monocyte/macrophage function, we investigated if there was a difference between familial and sporadic cases in the number of glomerular CD16 or CD68 positive cells. However, there were no statistically significant differences. This could be related to the number of cases included, or because changes are functional rather than numerical.

With regard to the polygenic risk score, there was minimal difference in the outcomes of our analysis whether cGRS or wGRS were used, presumably due to the modest odds ratios of most lupus susceptibility alleles. European LN patients in general had the highest polygenic risk scores. A possible explanation is that the majority of variants tested were identified in GWAS of SLE patients of European ancestry. However, many of these loci have been confirmed in East Asian populations and had similar effect sizes in both European and East Asian populations. [29-31] Nevertheless, SLE is known to be more prevalent in patients of African, Asian and Hispanic descent than in those of European ancestry.[32-34] In addition to being more frequent, the clinical phenotype of SLE is usually more severe in non-Europeans with younger onset disease and higher frequency of disease manifestations such as LN.[35-39] These observations indicate potential genetic heterogeneity for SLE between populations.

Due to a paucity of GWAS data in South Asian and African populations, it is unknown if these susceptibility alleles studied here even confer a higher risk of SLE in these populations.
When examining the polygenic risk score in familial LN, a trend was seen towards higher risk scores in African and South Asian familial cases as compared to sporadic disease but these did not reach statistical significance. Interestingly, in families with clustering of LN, probands and unaffected relatives had a similar GRS. This also argues against an accumulation of susceptibility alleles in familial cases as a cause of LN.
There are a number of factors to take into consideration when interpreting these results. First, our cohort consists of a relatively small number of patients, in particular when studying common variants with low effect sizes in different ancestries. In addition, follow up was 3 years longer in familial cases than in sporadic cases. However, it is unlikely that this explains the observed difference in renal outcome. Furthermore, the susceptibility alleles studied here were selected from GWAS data of SLE patients versus controls, and not from GWAS data of SLE patients with LN versus SLE patients without LN. Some of these alleles, however, have recently also been identified as LN predisposing loci.[40] Other complexities when studying genetics are the possibility of incomplete penetrance, and gene-gene and gene-environment interactions.
In summary, we report that patients with familial LN progressed to advanced renal impairment more often than patients with sporadic LN. Furthermore, familial LN patients were more often male, younger and had a different ancestral background. However, patients did not differ with respect to the histological severity of LN at presentation as determined by the ISN/RPS classification and activity and chronicity indices, and the count of CD16 and CD68 positive cells in the glomerulus. Also, familial LN cases did not show increased clustering of SLE susceptibility alleles. Therefore, the cause of the differences between familial and sporadic LN remains unknown. Performing whole exome sequencing on families with multiple affected members may identify variants for further exploration and may eventually lead to identification of factors involved.

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