Diversity and pattern of inheritance of autoantibodies in families with multiple cases of systemic lupus erythematosus

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Abstract
The pattern of inheritance of autoantibodies in eight families chosen from a pool of 110 families of patients with systemic lupus erythematosus (SLE) is described. In all the eight families at least two members were already affected by SLE. In total, 19 patients and 43 first degree relatives were examined.

The inheritance of a large set of antinuclear antibodies (for example, DNA, Sm, RNP, Ro, La, histones) and 16/6 idiotype seemed to be related to some unknown genetic factors but not related to HLA. The presence of numerous antinuclear autoantibodies in the serum of a subject was not necessarily associated with overt disease. The incidence of the 16/6 idiotype among patients and their relatives was low.

It is not yet clear whether the 'autoantibody burden' is greater in families with multiple cases of SLE than in families with single cases.

In almost all known autoimmune diseases there is a tendency for these conditions to run in families. This may occur as a greater incidence of a given disorder in relatives of patients than in a control group,[1–4] or as an increased incidence of other autoimmune diseases in family members of patients with specific disorders.[5 6]

The appearance of systemic lupus erythematosus (SLE) in identical twins was reported in 1951 by Davis and Gutridge,[7] and five years later Glagov and Gechman diagnosed SLE in both a mother and her daughter.[8]

In 1964 Leonhardt published the results of a larger study which compared 113 patients with SLE and about 500 of their relatives with a group of 324 subjects consisting of patients' spouses and their relatives.[9] When summarising his study, Leonhardt again suggested the existence of a genetic tendency towards this autoimmune disease.

Winchester and Núñez-Roldán studied 33 families with two or more cases of lupus and found a parent offspring concordance in 15.[10] They estimated that the susceptibility to lupus is determined by at least four independently segregating genes, some of which seem to have a dominant mode of inheritance.

Not only do autoimmune diseases themselves develop with an increased incidence among family members but laboratory findings related to these diseases are also found with an increased incidence in asymptomatic relatives of patients known to have autoimmune disorders. As already mentioned, Leonhardt described hypergammaglobulinaemia in asymptomatic siblings of patients with SLE,[1] and linked this finding to the hereditary state related to the development of the disease itself. In 1960 Holman and Deicher reported hypergammaglobulinaemia in 11 of 57 relatives of patients with SLE belonging to 18 families, and a borderline (upper limit of normal) level in an additional 10 relatives.[11] Furthermore, they found that 14% of the asymptomatic relatives had serological findings commonly detected in patients with rheumatoid arthritis, compared with 6% in the control group. Simultaneously, Pollack et al reported the presence of 'antinuclear factor' in 19 of 36 first degree relatives of 12 patients with SLE and in five of 14 second degree relatives.[12] These positive findings were significantly more prevalent among female relatives (17 of 26 women (65%) than among male relatives (seven of 24 men (29%)). The control group included 50 patients with other diseases, all unrelated to SLE, of whom only one was found to be antinuclear factor positive, and 40 healthy subjects, all negative. In a later study an antinuclear antibody was found in 47 first degree relatives of 43 patients with SLE and in 10 of 70 second degree relatives.[13] Overt autoimmune disease (SLE or scleroderma) developed in three families, among relatives who were asymptomatic at the time of the detection of autoantibodies. An increased prevalence of anticardiolipin antibodies among lupus family members has also been reported by Mackworth-Young et al.[14]

This study aimed at describing eight families with multiple cases of SLE and determining their genetics and diversity of serological manifestations.

Patients and methods
PATIENTS AND FAMILIES
The eight families were chosen from a pool of 110 Mexican families of patients with SLE. In all the families at least two members were already affected by SLE. In each family an effort was made to analyse serum samples from as many first degree members as possible. In total, 19 patients and 43 first degree relatives were examined.

CONTROLS
Serum samples of 38 controls were screened for the different autoantibody specificities. The cut off point for each autoantibody was determined. Values equal to or above the mean of the normal value plus three standard deviations were considered positive. Values between the

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mean of the control value plus two standard deviations and the control value plus three standard deviations were considered borderline.

AUTOANTIBODY DETERMINATIONS

Titres of the following autoantibodies were determined by enzyme linked immunosorbent assay (ELISA). Antibodies against calf thymus single stranded DNA (ssDNA; denatured DNA), double stranded DNA (dsDNA; Sigma Chemicals, St Louis, MO, USA) were measured as previously described.[15] Ninety six well flat bottomed ELISA plates (Dynatech Atlanta, GA, USA) were sequentially coated with 50 µg/ml of poly-L-lysine (Sigma), the antigen in question (2 µg/well), and, after washing, poly-L-glutamate (50 µg/ml). After two hours of blocking with 2% bovine serum in TRIS buffer saline the plates were washed and serial dilutions of serum samples were added and incubated for two hours. Then goat antihuman immunoglobulin conjugated with alkaline phosphatase (Sigma) was applied. Absorbance was measured at 405 nm directly from microtitre wells with a Titertek spectrophotometer.

Antibodies to histones[16] and anticardiolipin antibodies were detected as detailed elsewhere.[15 17] Polystyrene plates were coated overnight with 5 µg/ml cardiolipin (Sigma) in ethanol solution until evaporation. The plates were blocked, diluted serum samples were applied, and the enzymatic reaction took place as detailed above.

Antinuclear antibodies (Sm, RNP, SSA, SSB) were detected using autoantigens extracted from fresh thymus cells.[18] The plates were coated with 5 µg/ml of the antigen and the procedure was carried out as described above.

Antinuclear antibodies and antibodies to dinitrophenyl, extractable nuclear antigens, tRNA, and mitochondria were also screened for, as described in detail previously.[18]

HLA AND COMPLEMENT STUDIES
Factor B
For factor B typing[19] EDTA plasma samples were electrophoresed in agarose gels in 0·05 M barbital buffer (pH 8·6) containing calcium lactate (1·8 mol/l). Patterns were identified by immunofixation with goat antihuman factor B (Atlantic Antibodies, Scarborough, ME, USA).

C2 typing
C2 typing was by isoelectric focusing in polyacrylamide gels.[20] Patterns were developed with an agarose gel overlay containing antibody sensitised sheep erythrocytes and fresh normal human serum diluted 1:90 in isotonic veronal buffered saline (10^{-3} M Mg^{2+} and $1·5 \times 10^{-4}$ M Ca^{2+}), pH 7·4, containing 0·1% gelatin.

C4 typing
For C4 typing plasma samples were desialated by incubation with *Clostridium perfringens* type VI neuraminidase (Sigma Chemicals) at an enzyme concentration of 10 U/ml plasma, for 15

hours at 4°C while dialysing against 0·1 M phosphate buffer, pH 6·8, containing 0·005 M Na_2EDTA.[21] The structural variants were detected by electrophoresis of desialated plasma in agarose gels and immunofixation with goat antihuman antiserum (Atlantic Antibodies). Half-null haplotypes were detected by crossed immunoelectrophoresis of desialated plasma samples.[22]

Nomenclature
The nomenclature used for the genetic polymorphisms of C4 conforms to the International System for Human Gene Nomenclature.[23] The specific C4 allele designation is that agreed at the 4th international workshop for the genetics of complement, 1982.[24] To date, six common variants (C4A*1–6) and one null variant (C4A*Q0) have been detected at the locus A of C4, and three common variants (C4B*1–3) and one null variant (C4B*Q0) at the locus B. In addition, a large number of rare variants have been identified at both loci.

Complement haplotypes are written in the following order: factor B (BF), C2, C4A, and C4B. Thus the complotype SC31 contains the alleles BF*S, C2*C, C4A*3, and C4B*1.

Glyoxalase I typing
Haemolysates were used for glyoxalase I typing in cellulose acetate electrophoresis in a 0·003 M TRIS, 0·03 M barbituric acid, 0·2 mM mercaptoethanol, 0·4 mM $MgCl_2$ buffer, pH 8·0, at 200 V for one hour. Plates were stained first with 0·02 M glutathione and 0·34 M methylglyoxal in a 0·1 mM 3-(4,5-dimethylthiazolyl-2-(2,5-diphenyl)tetrazolium bromide, 0·69 mM 2,4-dichlorophenolindophenol in a 0·1 M TRIS hydrocloride buffer at pH 7·8.[25]

HLA typing
HLA-A, B, and C antigens were identified by the standard National Institutes of Health lymphocyte microcytotoxicity assay using 140 antisera to define 19 A locus, 28 B locus, and six C locus antigens. HLA-DR typing was done with 70 well characterised alloantisera using the technique of the Oxford (seventh international histocompatibility) workshop.[26]

SA-1 idiotype determination
The SA-1 idiotype is akin to the 16/6 idiotype described by us.[27] Therefore, hereinafter we will refer to the SA-1 as the 16/6 idiotype. An ELISA was used for the 16/6 idiotype determination as described previously.[28] Briefly, plates were coated with 75 ml (10 µg/ml) of rabbit anti-16/6 idiotype serum in 50 mM sodium bicarbonate buffer at pH 9·6 for 12–16 hours at 4°C. The plates were washed three times with phosphate buffered saline (PBS) and blocked with 200 µl/well of PBS with 1% bovine serum albumin for two hours at room temperature. The plates were washed three times with PBS. Duplicate 75 µl serum samples were diluted 1:2000 in PBS in the wells and incubated for
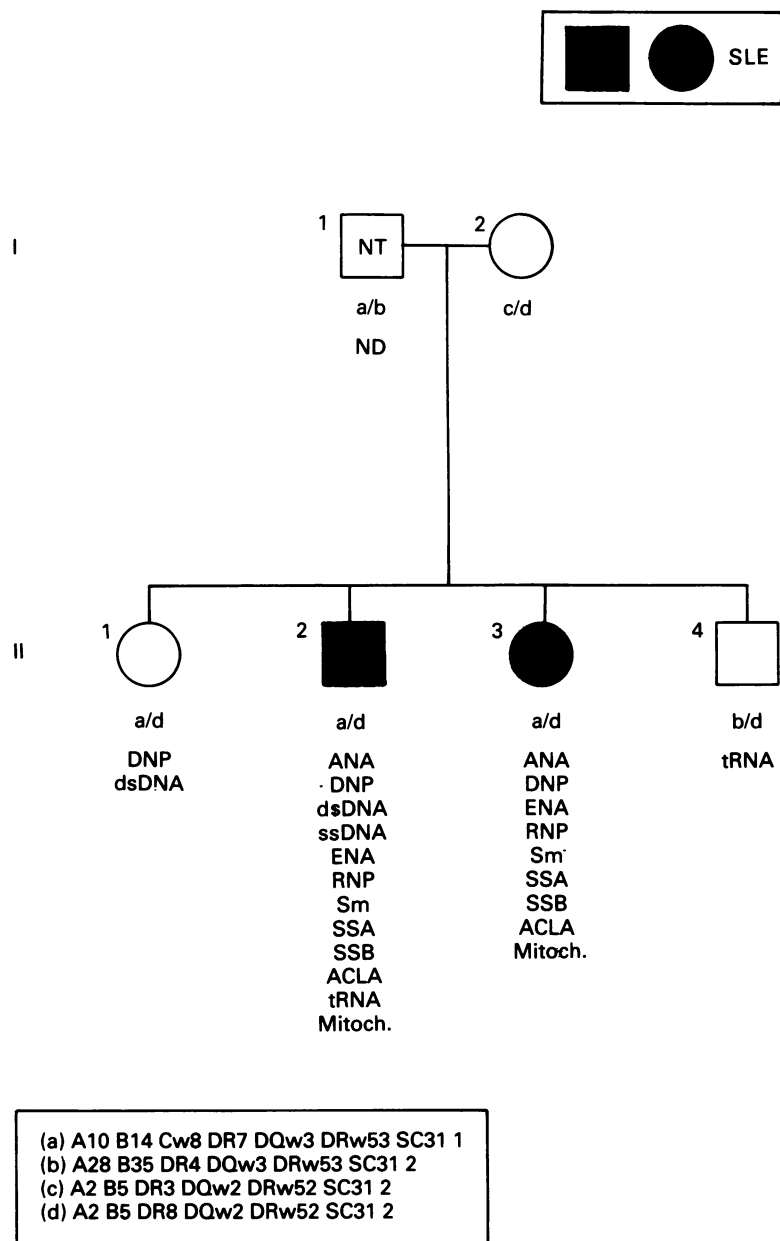
Figure 1 (family 1) shows a family in which SLE seemed to emerge as spontaneous mutation in two children—a boy and a girl (patients Nos II.2 and II.3), both with the same HLA type. Yet their sister (II.1) with the same HLA, antibodies to DNP and dsDNA has not developed the variety of autoantibodies as the female patients II.3.

mitochondria, the presence of the 16/6 idiotype, and HLA typing were recorded. In each case the complement phenotypes were also determined.

An analysis of the antinuclear presentation in these families exemplifies the diversity of auto-antibodies present.

Figure 2 (family 2) shows an interesting marriage between a mother with SLE and a father with serum autoantibodies. Possibly, the father might have developed autoantibodies by sharing the same household with the mother, as has been discussed elsewhere.[29] Their son had antibodies to dsDNA only, and borderline values of antinuclear antibodies and antibodies to DNA (data not shown), but his sister with the same HLA typing was free from disease and

Figure 1   Family No 1. ND indicates autoantibodies were not determined. ANA = antinuclear antibodies; dsDNA = antibodies to double stranded DNA; ssDNA = antibodies to single stranded DNA; ENA = antibodies to extractable nuclear antigens; ACLA = anticardiolipin antibodies; Mitoch = antibodies to mitochondria.

two hours at 37°C. The plates were washed three times with PBS, and then was added 75 μl of polyvalent goat antihuman serum conjugated with alkaline phosphatase (Sigma Chemicals), diluted (1:1500) in free PBS with 1% bovine serum albumin, and incubation was carried out for 12–16 hours at 4°C. The plates were washed three times with PBS, 75 μl of *p*-nitrophenyl phosphate disodium (Sigma) in 50 mM carbonate buffer, pH 9·6, containing 2 mM MgCl$_2$, was added, and incubation was carried out for one hour at 37°C. The plates were read at 405 nm using a Titertek ELISA reader.

Results

Figures 1–8 depict the pedigrees of eight families with multiple cases of SLE, in whom antinuclear autoantibodies, anticardio-lipin antibodies, antibodies to tRNA and to

Figure 2   Family No 2. For abbreviations see fig 1.

I

1 □——2 ○
a/b   c/d
          ND

● SLE

II

1 □   2 ○   3 □   4 ●   5 ●   6 ●   7 ●⁄
a/d   a/d   a/d   b/d   b/d   b/d   ND
         ANA   ND   DNP   ANA   ANA
         DNP   DNP   ANA   ANA
         dsDNA   DNP   DNP
         ENA   dsDNA   dsDNA
         RNP   ENA   ssDNA
         Mitoch.   ENA
                                             Sm

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(a) A2 Bw35 Cw3 DR2 DQw1 DRw52 SC31 1
(b) A2 Bw35 Cw3 DR5 DQw2 DRw52 SC42 2
(c) A2 B12 DR4 DQw3 DRw52 SC30 2
(d) A19 B16 DR4 DQw3 DRw52 SC01 2
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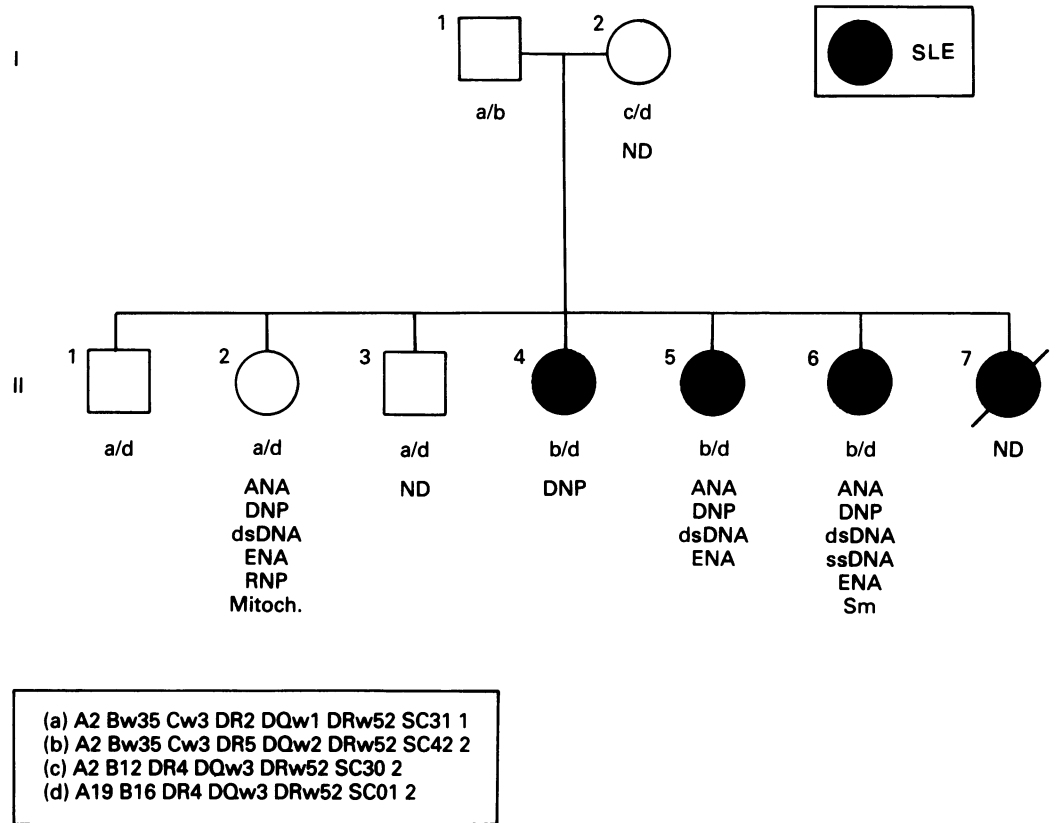
Figure 3   Family No 3. One of the four patients with SLE died before any study could be performed. Serum samples from the mother (I. 2) and one male sibling (II.5) were not determined (ND) for autoantibodies nor for the 16/6 idiotype. For abbreviations see fig 1.
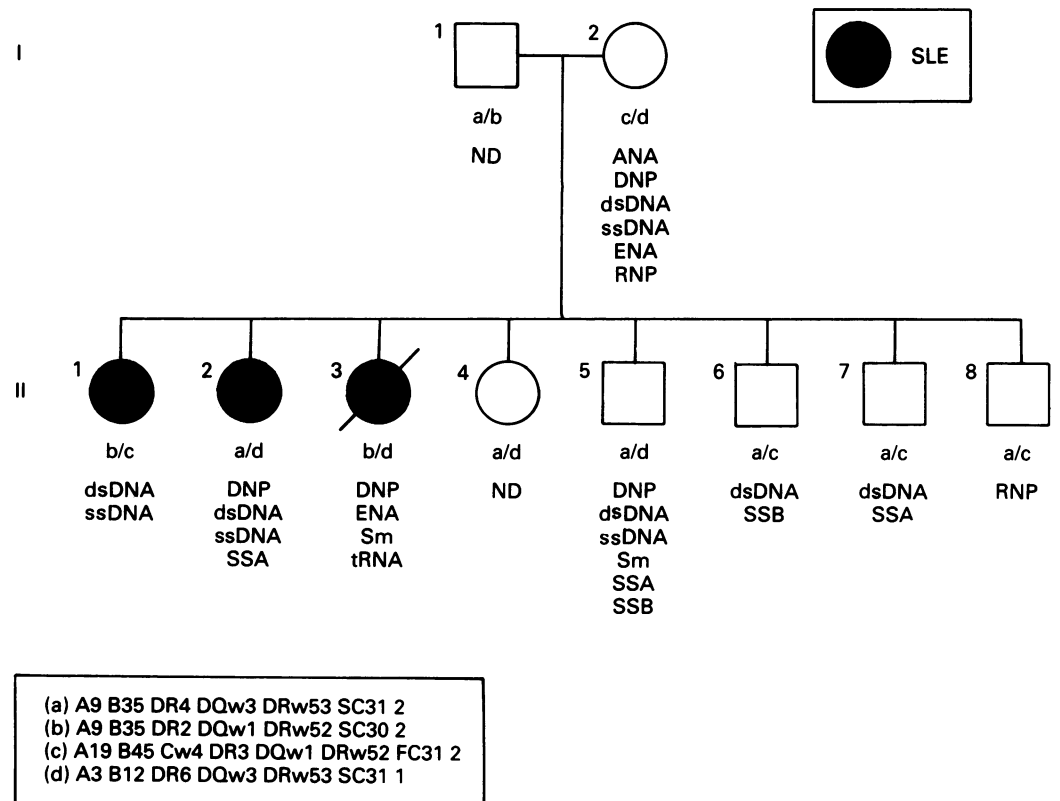
I

1 □——2 ○
a/b   c/d
ND   ANA
         DNP
         dsDNA
         ssDNA
         ENA
         RNP

● SLE

II

1 ●   2 ●   3 ●⁄   4 ○   5 □   6 □   7 □   8 □
b/c   a/d   b/d   a/d   a/d   a/c   a/c   a/c
dsDNA   DNP   DNP   ND   DNP   dsDNA   dsDNA   RNP
ssDNA   dsDNA   ENA   dsDNA   SSB   SSA
         ssDNA   Sm   ssDNA
         SSA   tRNA   Sm
                                      SSA
                                      SSB

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(a) A9 B35 DR4 DQw3 DRw53 SC31 2
(b) A9 B35 DR2 DQw1 DRw52 SC30 2
(c) A19 B45 Cw4 DR3 DQw1 DRw52 FC31 2
(d) A3 B12 DR6 DQw3 DRw53 SC31 1
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Figure 4   Family No 4. Patient No II.3 died at the age of 18 from pulmonary haemorrhage. For abbreviations see fig 1.
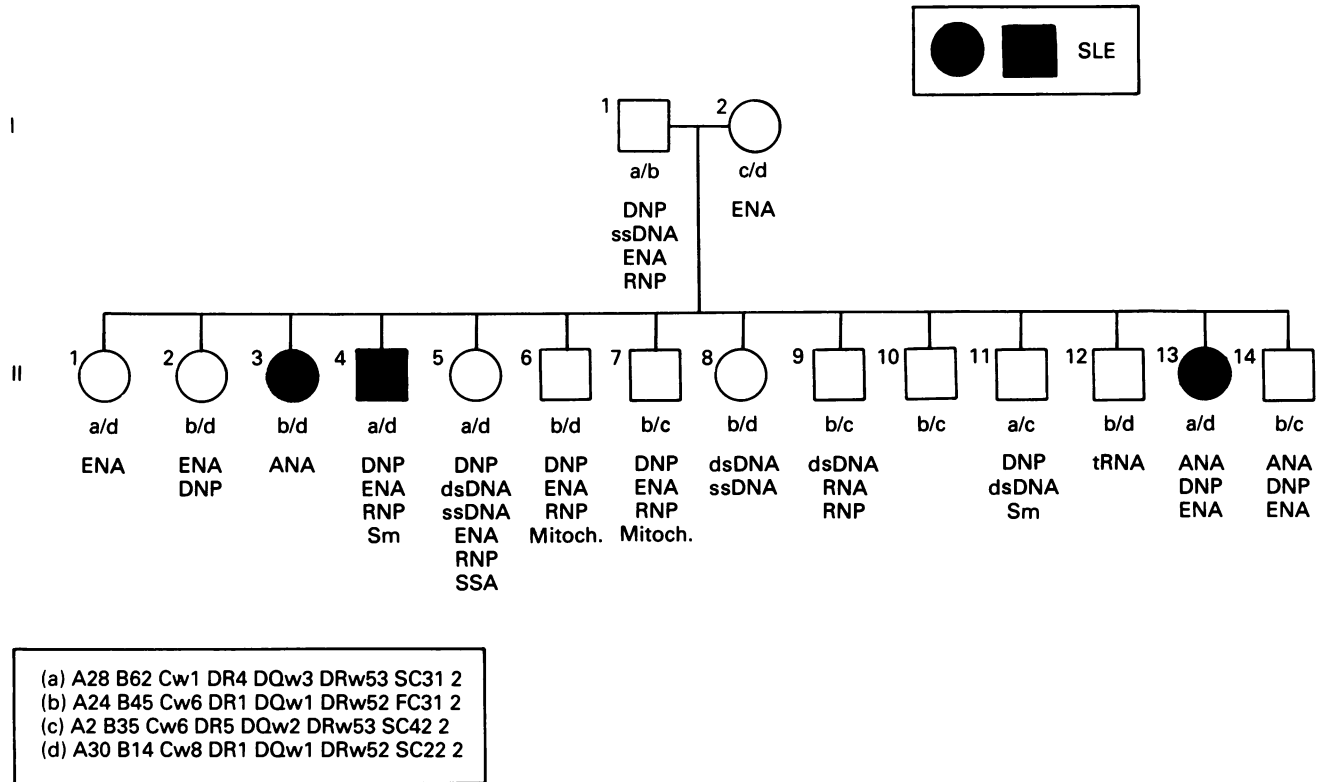
Figure 5   Family No 5. Note that one (II.10) out of 14 siblings was negative for all autoantibodies tested. For abbreviations see fig 1.

from antibodies (except for a borderline positive autoantibody for DNP). Subjects II.1 and II.3 in this family also might have developed these antibodies by sharing the household with their sick relatives.

Figure 3 (family 3) depicts an additional family in which the parents seemed to be completely healthy, yet four sisters (II.4–II.7) developed overt SLE; one (II.7) died before we studied her. It might be that the haplotype inherited from the father conferred suceptibility to SLE. The father with this haplotype was weakly positive for antibodies to dsDNA, SSA, and SSB (data not shown). We cannot discount the possible influence of the haplotype inherited from the mother as she was not screened for autoantibodies.

Figure 4 (family 4) represents another family with multiple cases of SLE with three affected sisters (II.1–II.3) having different HLA phenotypes, again with diverse patterns of auto-antibodies. In this family the mother had many antinuclear autoantibodies and all the children tested, including four sons, had autoantibodies.

Figure 5 (family 5) shows another complex family with two parents having antinuclear autoantibodies. Two daughters (II.3, II.13) and one son (II.4) were affected by overt SLE, but not all shared the same HLA phenotype. It is clear that a special pattern of autoantibodies is not necessarily related to overt SLE. The different patterns of autoantibodies in patients with SLE, even in those having the same HLA phenotype, might reflect differences in treatment or disease activity, or both, at the time of the study.

Figure 6 (family 6) details three generations of a family. Remarkably, it seems that overt

disease is limited to the second generation, while both the first and third generations, despite the presence of large numbers of auto-antibodies in all members, have not developed SLE. The different pattern of autoantibodies is displayed in the figure by two identical twins. It is noticeable that the two twins with identical HLA phenotype have similar levels of all auto-antibodies. The twin sister, however, had slightly higher levels than her twin brother.

Figure 7 (family 7) presents a family of five members. Two of three daughters developed SLE; with identical HLA. The apparently healthy sister presents a wide variety of auto-antibodies, and interestingly, her HLA phenotypes are different from those of her two sisters with SLE. This autoantibody presentation might be due to environmental factors. It should be noted that the sisters with SLE possess the 'c' haplotype which contains DR4, an antigen known to give suceptibility to the development of SLE; as well as a null allele of complement, also associated with the disease.

Figure 8 (family 8) depicts another family with multiple cases of SLE, in which all the healthy relatives tested have developed a wide variety of autoantibodies.

Although in these families there were some members with null alleles of C4A or C4B, no cosegregation was found between the presence of any antibody and either of these null alleles in this group of families.

16/6 DETERMINATION

The serological variability was also expressed in 16/6 levels. Members of families 1, 3, 4, and 5 were all negative for the idiotype. In family No
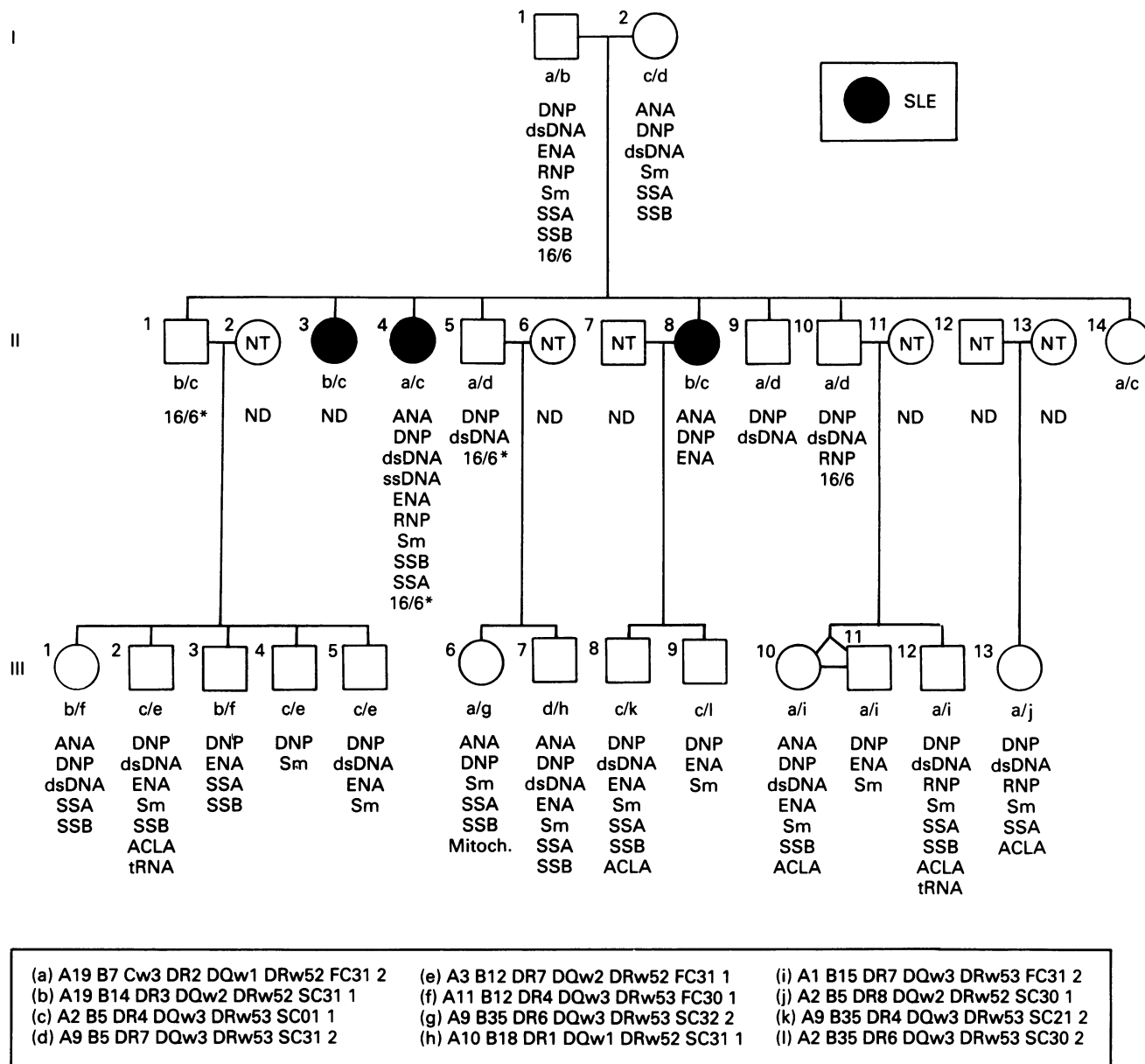
I

● SLE

1 a/b

DNP
dsDNA
ENA
RNP
Sm
SSA
SSB
16/6

2 c/d

ANA
DNP
dsDNA
Sm
SSA
SSB

II

1 b/c
16/6*

2 NT
ND

3 b/c
ND

4 a/c
ANA
DNP
dsDNA
ssDNA
ENA
RNP
Sm
SSB
SSA
16/6*

5 a/d
DNP
dsDNA
16/6*

6 NT
ND

7 NT
ND

8 b/c
ANA
DNP
ENA

9 a/d
DNP
dsDNA

10 a/d
DNP
dsDNA
RNP
16/6

11 NT
ND

12 NT
ND

13 NT
ND

14 a/c

III

1 b/f
ANA
DNP
dsDNA
SSA
SSB

2 c/e
DNP
dsDNA
ENA
Sm
SSB
ACLA
tRNA

3 b/f
DNP
ENA
SSA
SSB

4 c/e
DNP
Sm

5 c/e
DNP
dsDNA
ENA
Sm

6 a/g
ANA
DNP
Sm
SSA
SSB
Mitoch.

7 d/h
ANA
DNP
dsDNA
ENA
Sm
SSA
SSB

8 c/k
DNP
dsDNA
ENA
Sm
SSA
SSB
ACLA

9 c/l
DNP
ENA
Sm

10 a/i
ANA
DNP
dsDNA
ENA
Sm
SSB
ACLA

11

12 a/i
DNP
ENA
Sm

a/i
DNP
dsDNA
RNP
Sm
SSA
SSB
ACLA
tRNA

13 a/j
DNP
dsDNA
RNP
Sm
SSA
ACLA

(a) A19 B7 Cw3 DR2 DQw1 DRw52 FC31 2   (e) A3 B12 DR7 DQw2 DRw52 FC31 1   (i) A1 B15 DR7 DQw3 DRw53 FC31 2
(b) A19 B14 DR3 DQw2 DRw52 SC31 1   (f) A11 B12 DR4 DQw3 DRw53 FC30 1   (j) A2 B5 DR8 DQw2 DRw52 SC30 1
(c) A2 B5 DR4 DQw3 DRw53 SC01 1   (g) A9 B35 DR6 DQw3 DRw53 SC32 2   (k) A9 B35 DR4 DQw3 DRw53 SC21 2
(d) A9 B5 DR7 DQw3 DRw53 SC31 2   (h) A10 B18 DR1 DQw1 DRw52 SC31 1   (l) A2 B35 DR6 DQw3 DRw53 SC30 2

Figure 6 Family No 6. 16/6* indicates that serum samples from those subjecs had borderline values (that is, between two and three standard deviations above the mean level of 38 controls) for this idiotype. Ages of the third generation range from 7 to 20 years, and at the present time none of them has developed SLE. Note that all members belonging to the third generation are positive for a great variety of autoantibodies. Members of the first and second generations were also positive to some autoantibodies except for subjects II.1 and II.14. For abbreviations see fig 1.

Percentage of positive autoantibody presentation in families with multiple cases of systemic lupus erythematosus (SLE)*

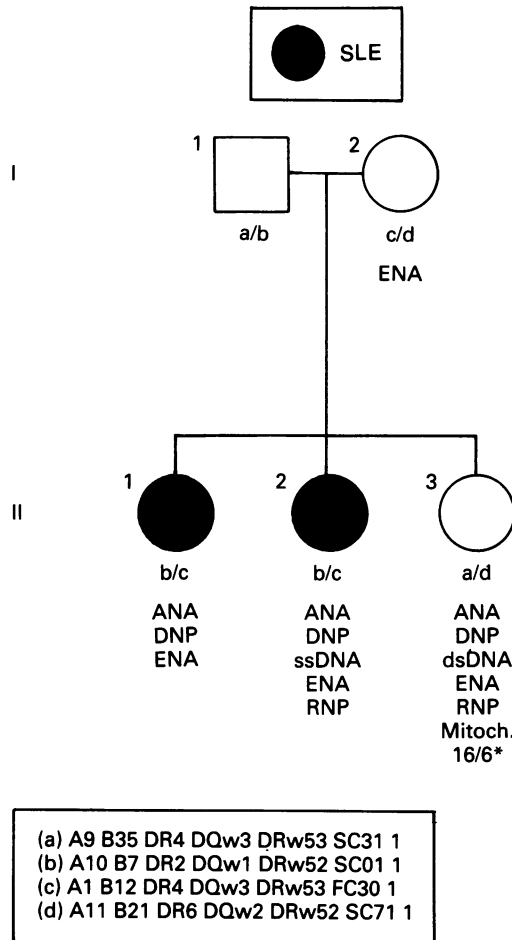| Autoantibody†	SLE (n=17)	First degree relatives (n=43)	Second degree relatives‡ (n=11)	Total relatives without SLE	Controls§ (n=38)
ANA	81·3	17·6	57·0	24·4	0·0
DNP	88·2	61·0	100·0	69·2	4·0
dsDNA	52·9	48·8	63·6	51·8	0·0
ssDNA	41·2	11·6	0·0	9·3	0·0
ENA	87·5	42·5	63·6	47·1	3·6
RNP	40·0	31·0	18·2	28·3	0·0
Sm	59·0	14·0	81·8	27·8	0·0
SSA	35·3	14·0	54·5	22·2	0·0
SSB	31·3	12·5	63·6	23·5	0·0
ACLA	23·5	4·7	36·4	11·1	3·3
tRNA	23·5	7·0	18·2	9·3	0·0
Mitoch	14·3	10·0	9·1	9·8	3·4
16/6 Idiotype	5·6	6·8	0·0	5·5	3·0

*Percentage positive subjects are given as those with antibody levels equal to or higher than three SD above mean level of normal human serum.
†ANA=antinuclear antibodies; DNP=dinitrophenyl; dsDNA=antibodies to double stranded DNA; ssDNA=antibodies single stranded DNA; ENA=antibodies to extractable nuclear antigens; ACLA=anticardiolipin antibodies; Mitoch=antibodies to mitochondria
‡Second degree relatives belong only to one family.
§Mexican families selected for that specific purpose.

Figure 7   Family No 7.
For abbreviations see fig 1.

(a) A9 B35 DR4 DQw3 DRw53 SC31 1
(b) A10 B7 DR2 DQw1 DRw52 SC01 1
(c) A1 B12 DR4 DQw3 DRw53 FC30 1
(d) A11 B21 DR6 DQw2 DRw52 SC71 1

(a) A19 B17 DR1 DQw1 DRw52 SC31 2
(b) A9 B35 DR8 DQw2 DRw52 SC01 2
(c) A3 B12 DR7 DQw3 DRw53 FC30 2
(d) A2 B7 DR6 DQw1 DRw52 SC31 2
(e) Ax Bx DRx DQwx DRwx SC02 1

Figure 8   Family No 8. For abbreviations see fig 1.

2 only one of the propositae was highly positive (patient I.2). Family 6 was remarkable in having four healthy members (I.1, II.1, II.5, and II.10) positive for the idiotype, none of them having overt SLE. Although subjects I.1, II.5, and II.10 had many autoantibodies, no autoantibody was detected in the serum of subject II.1. This idiotype was also present in five members of family 8 (one patient with SLE (I.4) and four subjects healthy (I.3, II.2–II.4) at the moment they were studied) and in one member (II.3) of family 7.

The table summarises the incidence of auto-antibodies found among the patients with SLE and their healthy relatives.

Discussion

The aim of the study was to analyse the pattern of inheritance of a panel of autoantibodies in families with multiple cases of SLE and their first degree relatives. The inheritance seemed to be related to some unknown genetic factors but not clearly related to HLA in those 'multiple case' families where the genetic predisposition often came from both parents as judged by the phenotypes shared or the presence of autoantibodies in them. The existence of even a great variety of antinuclear autoantibodies in the serum of a subject was not necessarily associated with overt disease. Only prolonged follow up will point to the pathogenicity of the autoantibodies in first degree relatives of patients with SLE.

Surprisingly, the incidence of the 16/6 idiotype among patients and their relatives was low. The 16/6 idiotype was reported by us to be a pathogenic idiotype.[27] [30-32] Its titres correlated with disease activity and it was found to be deposited in tissue afflicted by SLE. Recently, immunisation of naive mice with the 16/6 idiotype has been followed by induction of an SLE-like disease.[29] [31] [33] Therefore, we expected increased titres of the 16/6 idiotype to be found in higher numbers in patients and first degree relatives in families with multiple cases of SLE in comparison with the incidences reported previously (40% and 25% respectively).[32]

Conceivably, in the families reported here other pathogenic idiotypes might play a significant part. We have reported previously a pathogenetic role for the 16/6 idiotype in at least one family, but the members of that family had an additional risk factor for SLE—namely, C4 complement component deficiencies either at C4A or C4B.[34]

It would be interesting to see whether the autoantibody burden is higher in families with multiple cases of SLE than in families with only single case, particularly as our data suggest that 'multiple case' families may be genetically different from single case ones. On the other hand, the presence of this idiotype in healthy relatives supports the suggestion that it is a germline encoded natural autoantibody. The presence or absence of this phenotype in healthy relatives reflects B cell activation (for example, by Epstein-Barr virus) or the presence of antibodies to the idiotype which impair its detec-
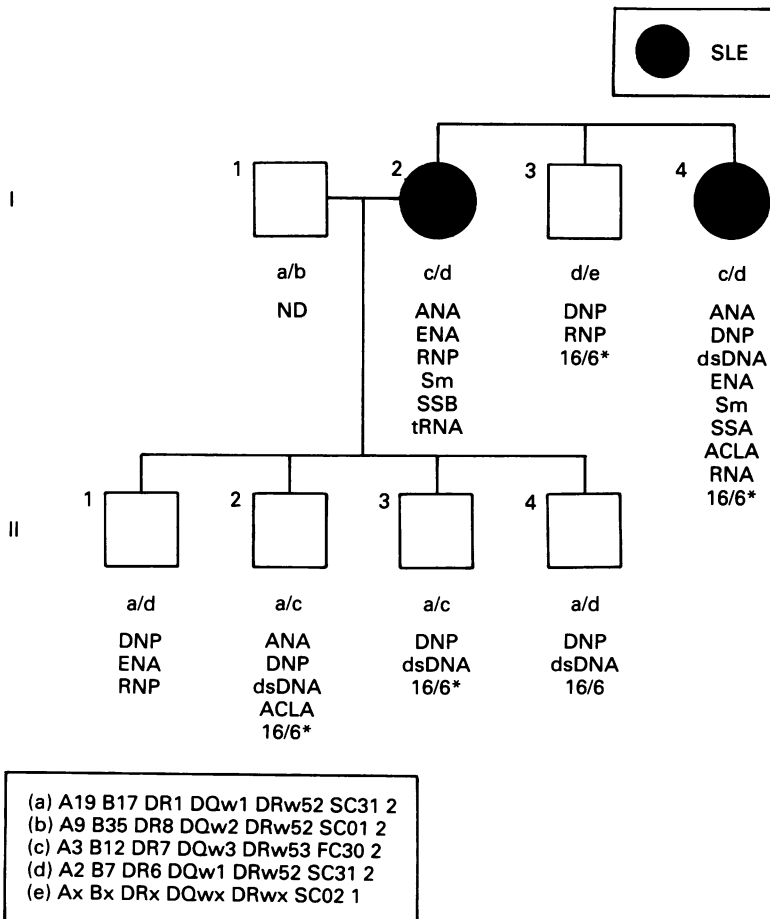
tion. This might also occur in patients with SLE at the time of disease remission—hence the correlation of the 16/6 idiotype with disease activity.

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