Am. J. Hum. Genet. 52:183–191, 1993

The Mapping of the Human 52-kD Ro/SSA Autoantigen Gene to Human Chromosome 11, and Its Polymorphisms

Mark Barton Frank,* Kazuko Itoh,*,[1] Atsushi Fujisaku,‡ Pierre Pontarotti,§ Marie-Genevieve Mattei,‖ and Barbara R. Neas*,†

*Arthritis and Immunology Program, Oklahoma Medical Research Foundation; and †Department of Biostatistics and Epidemiology, University of Oklahoma Health Sciences Center, Oklahoma City; ‡Second Department of Internal Medicine, Hokkaido University School of Medicine, Sapporo; §Centre de Recherches sur le Polymorphisme Génétique des Populations Humaines, Toulouse; and ‖INSERM U 242, Centre de Génétique Médicale, Marseille

Summary

Autoantibodies to Ro/SSA occur in nearly half of the patients with systemic lupus erythematosus and are associated with lymphopenia, photosensitive dermatitis, and pulmonary and renal disease, which suggests that they have an immunopathologic role. The majority of Ro/SSA precipitin-positive patients produce serum antibodies that bind to the 60-kD and 52-kD Ro/SSA proteins. We previously isolated and determined the nucleotide sequence of a cDNA clone that encodes the 52-kD form of the human Ro/SSA protein. In the present study, we have determined the chromosomal location of the gene by in situ hybridization to the end of the short arm of chromosome 11. Hybridization of portions of the cDNA probe to restriction enzyme–digested DNA indicated the gene is composed of at least three exons. The exon encoding the putative zinc fingers of this protein was found to be distinct from that which encodes the leucine zipper. An RFLP of this gene was identified and is associated with the presence of lupus, primarily in black Americans.

Introduction

Systemic lupus erythematosus (SLE) is a rheumatic, multisystemic autoimmune disease that affects approximately 1/1,000 females and occurs in approximately nine times as many females as males. Racial variations in the incidence of this disease have been reported (Rothfield 1985; Fessel 1988). Patients with this disease can have combinations of arthritis, skin rashes, serositis, and hematological, neuropsychiatric, and immunological abnormalities (Tan et al. 1982). While SLE is not inherited as a simple Mendelian trait, evidence for a genetic basis for lupus is strong. The incidence of the disease among first-degree relatives of lupus patients is approximately 100 times higher than that which is found in the general population (Leonhardt 1964; Arnett and Shulman 1976). The concordance rate in MZ twins varies between 24% and 69%, as opposed to 2%–5% in DZ twins (Block et al. 1975, 1976; Winchester and Lahita 1987; Deapen et al. 1992). On the basis of these estimates, the number of genes that contribute to susceptibility to SLE has been calculated to be between two and four, depending on the assumed dominance relationships between alleles at these loci (Winchester and Lahita 1987). It has been suggested that one of these may be a dominant susceptibility locus for autoimmune disorders (Bias et al. 1986).

High concentrations of serum autoantibodies directed against particular intracellular components are a common feature of lupus and have allowed the subclassification of patients into more homogeneous subsets for study (Wasicek and Reichlin 1982; Smolen et al. 1985; Mond et al. 1989). Autoantibodies that bind to the ribonucleoprotein Ro/SSA occur in 35%–50% of SLE patients and in up to 97% of patients with Sjogren syndrome (Harley et al. 1986; Reichlin 1986). This Ro/SSA particle consists of a single 60-kD immu-

Received February 10, 1992; final revision received September 4, 1992.

Address for correspondence and reprints: Dr. Bart Frank, Arthritis and Immunology Program, Oklahoma Medical Research Foundation, 825 N.E. 13th Street, Oklahoma City, OK 73104.

1. Present address: Department of Pediatrics, Nippon Medical School Tamanagayama Hospital, Tokyo.

0002-9297/93/5201-0021$02.00

noreactive protein noncovalently bound with one of four small (83–112 nucleotides) RNA molecules (Clark et al. 1969; Alspaugh and Tan 1975; Lerner et al. 1981; Wolin and Steitz 1984; Yamagata et al. 1984). The majority of anti-Ro/SSA–positive sera have antibodies that bind to the 60-kD Ro/SSA protein in western blots, as well as to a 52-kD Ro/SSA protein (Ben-Chetrit et al. 1988; Rader et al. 1989; Itoh et al. 1990). Antibodies eluted from the 52-kD band bind back to the 52-kD protein but not to the 60-kD protein. Similarly, antibodies eluted from the 60-kD Ro/SSA protein do not bind to the 52-kD protein, which suggests significant differences in these proteins (Itoh et al. 1990). The functions of these Ro/SSA proteins have not been determined.

Autoantibodies to Ro/SSA have been isolated from acid eluates of nephritic kidneys of lupus patients (Maddison and Reichlin 1979), and strong associations with clinical aspects of the disease have been reported, which suggests that they have pathophysiologic importance (Maddison et al. 1979; Wasicek and Reichlin 1982; Smolen et al. 1985; Harley et al. 1989; Hedgpeth and Boulware 1989; Mond et al. 1989). Anti–60-kD Ro/SSA antibodies can cross the placenta and have been implicated in the dermatitis and congenital heart block of infants with neonatal lupus that were born to anti–60-kD Ro/SSA–positive mothers (Watson et al. 1984; Ramsey-Goldman et al. 1986; Silverman et al. 1991). However, virtually all of these studies either were performed before the 52-kD Ro/SSA antigen was appreciated or failed to examine associations with the anti–52-kD Ro/SSA autoantibodies by using purified 60-kD Ro/SSA protein in their assays. It is only recently, with the advent of electrophoretic techniques to separate the 52-kD Ro/SSA species from La/SSB (Buyon et al. 1990) and the recent availability of a recombinant 52-kD protein, that a clear distinction between the coordinately expressed anti–60-kD and –52-kD Ro/SSA autoimmune responses can be made. By such methods, data from Buyon et al. (1989) indicate that anti-La/SSB and anti–52-kD Ro/SSA autoantibodies, but not anti–60-kD Ro/SSA antibodies, are associated with clinical features of neonatal lupus.

To better appreciate such distinctions and to further our analysis of immunogenetic parameters affecting anti-Ro/SSA autoantibody production (Frank et al. 1990; Fujisaku et al. 1990), we have cloned and sequenced the cDNA that encodes the 52-kD Ro/SSA protein (Itoh et al. 1991). A similar cDNA sequence, which differs in a single replacement substitution in the coding region, was simultaneously reported by Chan et al. (1991). No relationship between the 60-kD and 52-kD cDNA or their predicted protein sequences was observed, which is consistent with the absence of immunological cross-reactivity (Deutscher et al. 1988; Ben-Chetrit et al. 1989). The 52-kD Ro/SSA protein appears to be novel in that it contains motifs both for zinc fingers and for a leucine zipper. This suggests that it may function in nucleic acid binding. In the present study, we have further characterized the gene that encodes this protein. We report the mapping of this gene to chromosome 11, and we discuss findings of the exon structure and polymorphisms of this locus.

Material, Subjects, and Methods

Chromosome-Spread Preparation

In situ hybridization was carried out on chromosome preparations obtained from phytohemagglutinin-stimulated human lymphocytes cultured for 72 h. 5-Bromodeoxyuridine was added for the final 7 h of culture (60 μg/ml of medium) to ensure good-quality posthybridization chromosomal banding.

Probe Preparation and In Situ Hybridization

The cDNA encoding the 52-kD Ro/SSA protein containing a 1,843-bp insert in pUC18 (Itoh et al. 1991) was tritium labeled by nick-translation to a specific activity of 1.3×10^8 dpm/μg. The radiolabeled probe was hybridized to metaphase spreads at a final concentration of 6 ng/ml of hybridization solution, according to a method described elsewhere (Mattei et al. 1985). After being coated with nuclear-track emulsion (Kodak NTB2), slides were exposed for 25 d at 4°C and then were developed. To avoid any slipping of silver grains during banding procedures, chromosome spreads were first stained with buffered Giemsa solution and the metaphase spreads were photographed. R-banding was then performed using the fluorochrome-photolysis-Giemsa (FPG) method, and metaphase spreads were rephotographed before analysis (Perry and Wolff 1974).

Patients and Controls

Peripheral blood was collected from 141 SLE patients undergoing treatment at clinics of either the University of Oklahoma Health Sciences Center (Oklahoma City) or Hokkaido University (Sapporo). Patients in both countries met the revised diagnostic criteria

for SLE (Tan et al. 1982). Of the 65 patients who lived in the Oklahoma City area, 26 were black and 39 were Caucasian; the remaining patients were Japanese. The overall proportion of female patients was 87%, which is in agreement with the sexual distribution of this disease. Patient sera were tested for their anti-Ro/SSA activity by Ouchterlony double immunodiffusion against calf thymus extract and were confirmed by counterimmunoelectrophoresis against human tissue extract. Levels of anti–52-kD Ro/SSA autoantibodies were determined by ELISA using a human recombinant 52-kD Ro/SSA protein. Peripheral blood was also collected from 76 healthy controls. Because the incidence of SLE is much higher among first-degree relatives of patients than in the general population (Leonhardt 1964; Arnett and Shulman 1976), only healthy individuals who lacked SLE in their immediate family were solicited from the general population. Twenty-nine of the 60 Oklahoma City–based controls were black, and 31 were Caucasian. Seventy percent of all controls were female, although the distribution of the 52-kD Ro/SSA *Bgl*II RFLP did not differ between females and males.

cDNA Probes

The 1,843-bp cDNA containing the coding region for the 52-kD Ro/SSA protein (Itoh et al. 1991) was separately digested with the restriction enzymes *Bam*HI, *Rsa*I, and *Pst*I. Three subregions of this cDNA were cloned: a 5′ 440-bp *Eco*RI-*Rsa*I fragment that includes the coding region for the putative zinc fingers, an internal 141-bp *Pst*I fragment that contains the coding region of the leucine zipper, and a 539-bp *Bam*HI fragment that contains the coding region for the COOH-terminal third of the protein. The full-length cDNA and each subregion were individually radioactively labeled (Feinberg and Vogelstein 1983, 1984) and were used as probes for restriction mapping and RFLP analysis. Restriction endonucleases, the Klenow fragment of *Escherichia coli* DNA polymerase, and T4 DNA ligase were purchased from Promega Biotech (Madison, WI) and Bethesda Research Laboratories (Gaithersburg, MD). [α-^{32}P]dCTP was purchased from ICN Pharmaceuticals (Costa Mesa, CA).

Determination of the Exon Structure and RFLPs of the 52-kD Ro/SSA Gene

DNA was extracted from peripheral blood leukocytes with proteinase K and phenol (Frank et al. 1990). Ten-microgram samples of genomic DNA were digested with restriction enzymes and were subjected to agarose gel electrophoresis. DNA was transferred to nylon membranes by the method of Southern (1978). After hybridization to nucleic acid probes, membranes were washed to high stringency with 0.2 × SSPE (30 mM NaCl, 2 mM NaH_2PO_4, 0.2 mM EDTA, pH 7.0) and 0.1% SDS at 65°C and were subjected to autoradiography.

Results

The 52-kD Ro/SSA Gene Maps to Chromosome 11 by In Situ Hybridization

In order to determine the chromosomal location of the gene encoding the 52-kD Ro/SSA protein, metaphase chromosome spreads from phytohemagglutinin-stimulated peripheral blood mononuclear cells were hybridized to the full-length radiolabeled 52-kD cDNA probe (Itoh et al. 1991). Of the 100 spreads that were examined after in situ hybridization, 334 silver grains were associated with the chromosomes. Seventy (20.9%) of these were located on chromosome 11 (fig. 1, *left*), which comprises approximately 4% of the human genome (Morton et al. 1982). The remaining silver grains were equally distributed among the other chromosomes. The distribution of grains on chromosome 11 was not random. Fifty-eight (82.8%) of 70 mapped to the p15.3-p15.5 region of the chromosome 11, with the majority in the p15.5 band (fig. 1, *right*). These results allowed us to map the gene encoding the 52-kD Ro/SSA protein to the terminal portion of the short arm of this chromosome in the human genome.

The Zinc Finger and Leucine Zipper Motifs Are Encoded by Separate Exons

A restriction-enzyme map of the gene encoding the 52-kD Ro/SSA protein was determined from genomic DNA from three healthy Caucasian donors, after digestion with the restriction enzymes *Eco*RI, *Eco*RV, *Bgl*II, *Kpn*I, and *Hin*dIII. DNA was transferred to nylon membranes (Southern 1978) and hybridized with a radiolabeled full-length cDNA probe that encodes the 52-kD Ro/SSA protein. To explore the exon structure of this gene, fragments of the whole cDNA were isolated and used as probes. Probe A consisted of the 5′ 440 bp of the cDNA; probe B contained nucleotides 632–772; while probe C contained nucleotides 912–1449, which encode approximately the COOH-terminal third of the protein. All restriction-enzyme fragments that hybridized to the full-length cDNA

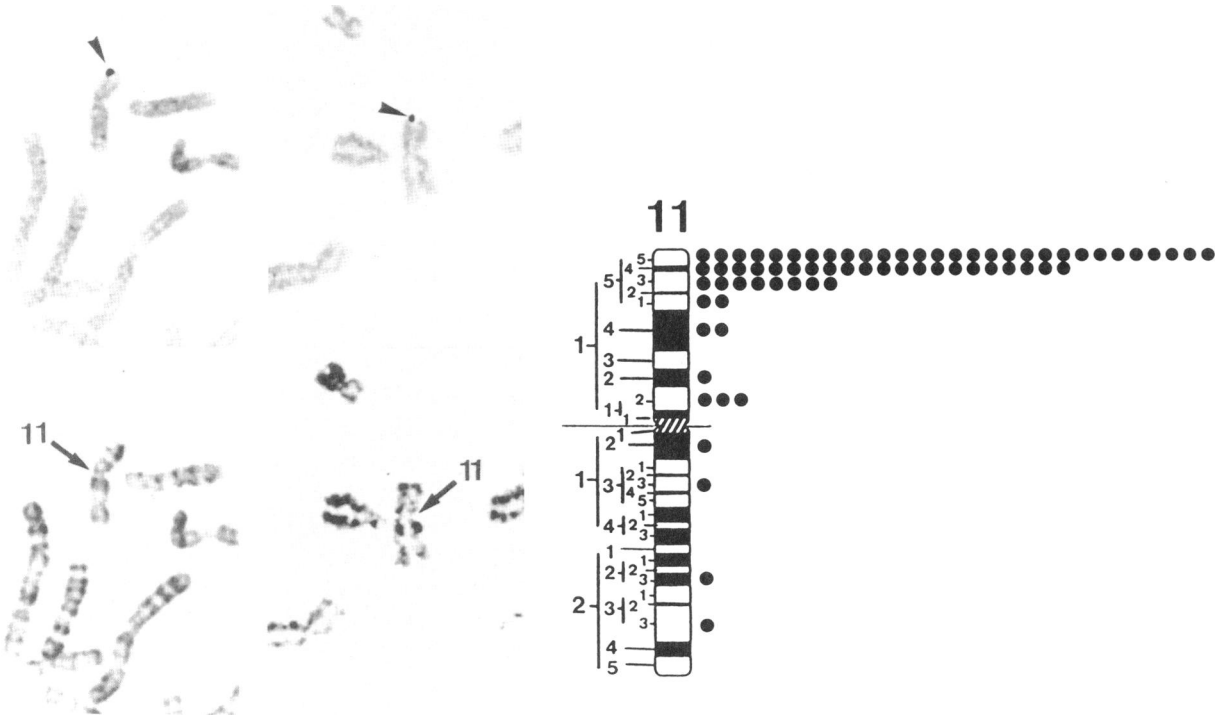

Figure 1 Chromosomal location of the 52-kD Ro/SSA gene. *Left,* Two partial human metaphase chromosome spreads showing the specific site of hybridization to chromosome 11. In the top photographs, arrowheads indicate the positions of silver grains on Giemsa-stained chromosomes after autoradiography. In the bottom photographs, the same chromosomes are identified by R-banding (FPG technique). *Right,* Ideogram of the human G-banded chromosome 11 illustrating the distribution of labeled sites for the 52-kD Ro/SSA gene probe. Dots indicate silver grains counted in the adjacent regions of the chromosome.

probe were accounted for with these subregion probes (table 1). A restriction-enzyme map of this gene was determined using these probes, after digestion with combinations of these restriction enzymes (fig. 2). Probe A hybridized between an *Eco*RV site and a *Bgl*II site (fig. 2, *left shaded region*) upstream of a *Hin*dIII-*Eco*RI region, which hybridized to probe B (fig. 2, *right shaded region*). We were able to position the COOH-terminal exon by using the internal *Bgl*II site of probe C. Analysis of the predicted amino acid se-

Table 1

Restriction-Enzyme Fragments of the 52-kD Ro/SSA Locus

	Size of Hybridizing Fragment[a] (kb)			
Restriction Enzyme	Whole cDNA	A	B	C
*Eco*RI	13.5 and 4.1	13.5	13.5	4.1
*Eco*RV	9.5	9.5	9.5	9.5
*Hin*dIII	6.0 and 3.95	6.0	3.95	3.95
*Kpn*I	6.0 and 3.8	3.8	6.0	6.0
*Bgl*II	3.75 and 1.5/1.3[b]	1.5/1.3[b]	3.75	3.75

[a] Fragments of the full-length cDNA designated A, B, and C are described in the Material, Subjects, and Methods section. The four individual probes are listed above each column.

[b] The polymorphic *Bgl*II 1.5- and 1.3-kb RFLPs were identified with probe A.

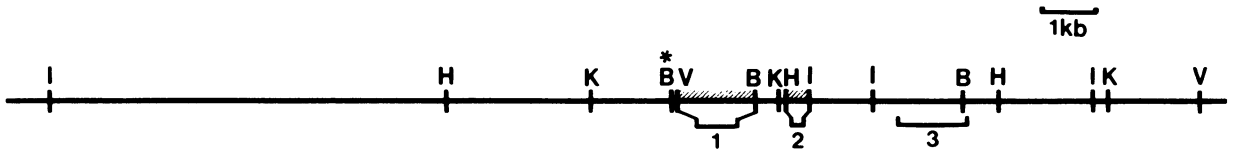

Figure 2 Restriction-enzyme map of the 52-kD Ro/SSA locus. Three exons were identified using probes from different areas of the coding region of the 52-kD cDNA. Probe A hybridized to exon 1, probe B to exon 2, and probe C to exon 3. I = *Eco*RI; H = *Hin*dIII; K = *Kpn*I; B = *Bgl*II; and V = *Eco*RV. An asterisk (*) indicates the polymorphic *Bgl*II site.

quence of the 52-kD Ro/SSA protein suggested the presence of zinc fingers (encoded by nucleotides 84–407) and a leucine zipper (nucleotides 669–734) on this protein (Itoh et al. 1991). These data indicate that these motifs are encoded by separate exons. Both of these structures have been identified on other proteins that are known to bind to nucleic acids (Hanas et al. 1983; Berg 1986; Evans and Hollenberg 1988; Landschulz et al. 1988; Johnson and McKnight 1989).

Polymorphisms and Racial Variations of the 52-kD Ro/SSA Gene

DNA was digested from 20 SLE patients by using the restriction enzymes *Eco*RI, *Eco*RV, *Bgl*II, *Kpn*I, and *Hin*dIII. When the full-length 52-kD cDNA was used as a probe, a *Bgl*II 3.75-kb band was detected in all individuals. Polymorphic bands of 1.5 and 1.3 kb were also observed (fig. 3). The inheritance of these RFLPs was studied in three simplex families and was consistent with that of a single-locus, autosomal codominant trait (data not shown). No RFLPs were observed with the other four enzymes. By using the three nonoverlapping probes that span the coding region (described in the previous section), the polymorphic *Bgl*II RFLP bands were detected only after hybridization with the 5′ 440-bp region probe. (The other two subregion probes hybridized with the invariant band.) We have mapped the polymorphic *Bgl*II site at or immediately adjacent to the initiation codon of this gene. Since the chromosomal sequence of this gene is unknown, the exact location has not been determined. However, analysis of the nucleotide sequences of the cDNA in this region indicates the presence of five of the six bases of a *Bgl*II recognition site 36 bp upstream of the initiation codon (Chan et al. 1991).

In order to determine the frequency of this RFLP and to analyze its disease significance, DNA was initially digested with *Bgl*II from 65 SLE patients and from 44 healthy controls. The 1.3-kb RFLP band was absent in 42% of patients but in none of the controls (χ^2 = 6.18, P = .01), which suggests a difference in the proportion of 1.5-kb RFLP homozygotes between these groups. To further appreciate these differences, we have determined the RFLP genotype from 141 patients and 76 controls. Our initial association between

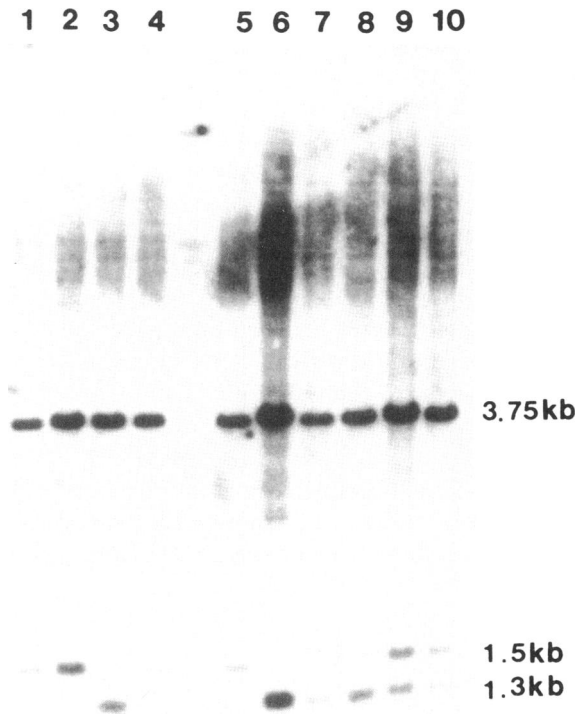

Figure 3 Restriction-enzyme-length polymorphisms of the 52-kD Ro/SSA gene. DNA isolated from 10 SLE patients was digested with *Bgl*II and hybridized to the full-length cDNA for the 52-kD Ro/SSA gene. Lanes 1, 2, and 5, Homozygous pattern for the 1.5-kb RFLP. Lanes 3 and 6–8, Homozygous pattern for the 1.3-kb RFLP. Lanes 4, 9, and 10, Heterozygous pattern. The sizes of the hybridizing fragments are shown to the right of the figure.

Table 2

Distribution of RFLPs among Patients and Controls

Group	No. of Subjects			
	1.5-kb RFLP Band[a]		1.3-kb RFLP Band[b]	
	+	−	+	−
SLE	100	41	98	43
Control	49	27	66	10

Note. — A plus sign (+) denotes presence of the RFLP band, and a minus sign (−) denotes absence of the RFLP band.

[a] $\chi^2 = 0.68$; $P < .41$.

[b] $\chi^2 = 7.13$; $P < .008$.

SLE and homozygosity for the *Bgl*II 1.5-kb RFLP was maintained (table 2).

Because of the apparent association of this RFLP with the disease and because of the reported variation in the incidence of SLE in different races (Rothfield 1985), we have investigated the effect of race and this RFLP on lupus. The number and proportion of individuals with each RFLP genotype are listed in table 3. Within each race, the proportion of the *Bgl*II 1.5-kb homozygote controls was always less than that in patients. In fact, there were no 1.5-kb homozygotes among healthy Japanese. However, differences in the genotypic distribution between races were apparent. A highly significant statistical association between this RFLP and lupus is limited to the black Americans in this study ($\chi^2 = 15.04$, $P < .0005$). No significant difference in the distribution of the genotypes between patients and healthy controls was found in the Caucasian or Japanese cohorts, although the proportion of 1.5-kb RFLP homozygous controls was lower, particularly among Japanese. No associations between these RFLPs and either (*a*) the presence or titers of anti–52-kD autoantibodies or (*b*) particular clinical manifestations of this disease have been detected. These data indicate that this newly identified polymorphism may serve as a general disease marker within particular racial groups.

Discussion

Previously our laboratory reported the sequence of a cDNA that encodes the 52-kD human Ro/SSA protein (Itoh et al. 1991). This cDNA contained a single open reading frame of 1,425 nucleotides, which encodes a predicted 475-amino-acid polypeptide with a predicted molecular mass of 54,108 daltons. A similar predicted protein sequence was simultaneously reported by Chan et al. (1991). This 52-kD protein appears to be unique in that it contains both zinc fingers and a leucine zipper motif. Similarities between the zinc finger motif of 52-kD Ro/SSA protein and those of two other proteins, rfp and rpt-1, have been found. Each of these proteins contains multiple zinc fingers in the NH_2-terminal 145-amino-acid residues, which are conserved in amino acid sequence and spacing but are quite different from zinc fingers of many other proteins, which suggests that these may represent a novel family of proteins. The function of rfp is unknown; however, a portion of this gene has been shown to undergo translocation in human thyroid papillary carcinomas and lymphomas (Takahashi and Cooper 1987; Takahashi et al. 1988; Greico et al. 1990). Within the short arm of chromosome 11, a number of fragile sites have been identified (11p13-11p15.1), and gene rearrangements have been associated with particular tumors (Sutherland 1990). Analy-

Table 3

Number and Percentage of Subjects for the *Bgl*II RFLP Genotypes

Group and Race	No. (%) of Subjects with Genotypes			Total
	1.5/1.5	1.5/1.3	1.3/1.3	
SLE:				
Black	18 (69)	7 (27)	1 (4)	26 (100)
Caucasian	9 (23)	18 (46)	12 (31)	39 (100)
Japanese	16 (21)	32 (42)	28 (37)	76 (100)
Control:				
Black	6 (21)	13 (45)	10 (34)	29 (100)
Caucasian	4 (13)	18 (58)	9 (29)	31 (100)
Japanese	0 (0)	8 (50)	8 (50)	16 (100)

ses of genes that are altered in the Wilms tumor 11p13 chromosomal rearrangement suggest a zinc finger–containing protein (Rauscher et al. 1990). However, the zinc fingers encoded by this putative Wilms tumor locus and by the 52-kD Ro/SSA protein are clearly different, and lupus erythematosus has not been associated with tumor development. We have found no evidence of translocation of the zinc-finger exon of this Ro/SSA protein in peripheral blood cells of lupus patients or controls by using restriction enzyme–digest analyses.

It is of interest that the *Bgl*II polymorphism that occurs in a gene encoding the 52-kD Ro/SSA autoantigen is not associated with anti–52-kD Ro/SSA antibodies but, rather, with the disease as a whole. Experiments that focus on the location and nature of this nucleic acid difference, as well as on the function of 52-kD Ro/SSA protein, will be critical in the understanding of the disease association reported here. Other reports of allelic variants associated with lupus in particular ethnic groups, which are not associated with autoantibodies, have been reported for antigen-recognition molecules, such as a T cell receptor α RFLP (Tebib et al. 1989), and Gm and Km allotypic markers of immunoglobulins (Fedrick et al. 1983; Whittingham et al. 1983; Schur et al. 1985; Stenszky et al. 1986; Hoffman et al. 1991). We have identified gene markers at the T cell receptor β locus and HLA-DQ, both of which are associated with the presence of anti–60-kD Ro/SSA antibodies (Frank et al. 1990; Fujisaku et al. 1990).

Our analysis of this RFLP indicates a strong association with the presence of lupus in black Americans. While it is possible that this RFLP is a marker for an important linked locus in SLE, the presence of high-titer autoantibodies that bind to this gene's product in a high proportion of these patients argues against this possibility. Determination of the nucleotide sequence of this gene from blacks of different genotypes will be necessary to understand the physiologic significance of this association. The paucity of the 1.5-kb homozygotes in Caucasian and Japanese controls may be suggestive that this association extends to other races. Family studies will be particularly valuable in determining the extent to which the disease cosegregates with the RFLP in multiplex SLE families. Race-related genetic heterogeneity in the transmission of lupus might be demonstrated by such means. The analysis of other disease-associated gene markers and the *Bgl*II RFLP will be needed to test this hypothesis. Future studies using this 52-kD Ro/SSA gene marker and polymorphisms at other loci whose products are active in the immune system should also be important in furthering our understanding of both the genetic basis and the immunopathology underlying this disease.

Acknowledgments

The authors thank Dr. Morris Reichlin for helpful discussions, Ms. Bonnie Wilds for exceptional secretarial support, and Mrs. Yumiko Shinohe for technical assistance. This work was supported by grants AI21568 and AI24717 from the National Institutes of Health.

References

Alspaugh MA, Tan EM (1975) Antibodies to cellular antigens in Sjogren's syndrome. J Clin Invest 55:1067–1073

Arnett FC, Shulman LE (1976) Studies in familial systemic lupus erythematosus. Medicine 55:313–322

Ben-Chetrit E, Chan EKL, Sullivan KF, Tan EM (1988) A 52-kD protein is a novel component of the SS-A/Ro antigenic particle. J Exp Med 167:1560–1571

Ben-Chetrit E, Gandy BJ, Tan EM, Sullivan KF (1989) Isolation and characterization of a cDNA clone encoding the 60-kD component of the human SS-A/Ro ribonucleoprotein autoantigen. J Clin Invest 83:1284–1292

Berg JM (1986) Potential metal binding domains in nucleic acid binding proteins. Science 232:485–487

Bias WB, Reveille JD, Beaty TH, Meyers DA, Arnett FC (1986) Evidence that autoimmunity in man is a Mendelian dominant trait. Am J Hum Genet 39:584–602

Block SR, Lockshin MD, Winfield JB, Weksler ME, Masakatsu I, Winchester RJ, Mellors RC, et al (1976) Immunologic observations on 9 sets of twins either concordant or discordant for SLE. Arthritis Rheum 19:545–554

Block SR, Winfield JB, Lockshin MD, D'Angelo WA, Christian CL (1975) Studies of twins with systemic lupus erythematosus: a review of the literature and presentation of 12 additional sets. Am J Med 59:533–552

Buyon JP, Ben-Chetrit E, Karp S, Roubey RA, Pompeo L, Reeves WH, Tan EM, et al (1989) Acquired congenital heart block: pattern of maternal antibody response to biochemically defined antigens of the SSA/Ro-SSB/La system in neonatal lupus. J Clin Invest 84:627–634

Buyon JP, Slade SG, Chan EKL, Tan EM, Winchester R (1990) Effective separation of the 52-kDa SSA/Ro polypeptide from the 48 kDa SSB/La polypeptide by altering conditions of polyacrylamide gel electrophoresis. J Immunol Methods 129:207–210

Chan EKL, Hamel JC, Buyon JP, Tan EM (1991) Molecular definition and sequence motifs of the 52-kD component of human SS-A/Ro autoantigen. J Clin Invest 87:68–76

Clark G, Reichlin M, Tomasi TB Jr (1969) Characterization of a soluble cytoplasmic antigen reactive with sera from

patients with systemic lupus erythematosus. J Immunol 102:117–122

Deapen D, Escalante A, Weinrib L, Horwitz D, Bachman B, Roy-Burman P, Walker A, et al (1992) A revised estimate of twin concordance in systemic lupus erythematosus. Arthritis Rheum 35:311–318

Deutscher SL, Harley JB, Keene JD (1988) Molecular analysis of the 60-kDa human Ro ribonucleoprotein. Proc Natl Acad Sci USA 85:9479–9483

Evans RM, Hollenberg SM (1988) Zinc finger: gilt by association. Cell 52:1–3

Fedrick JA, Pandey JP, Chen Z, Fudenberg HH, Ainsworth SK, Dobson RL (1983) Gm allotypes in blacks with systemic lupus erythematosus. Hum Immunol 8:177–181

Feinberg AP, Vogelstein B (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13

——— (1984) Addendum: a technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 137:266–267

Fessel WJ (1988) Epidemiology of systemic lupus erythematosus. In: Klippel JH (ed) Systemic lupus erythematosus: rheumatic clinics of North America. WB Saunders, Philadelphia, pp 15–23

Frank MB, McArthur R, Harley JB, Fujisaku A (1990) Anti-Ro(SSA) autoantibodies are associated with T cell receptor beta genes in systemic lupus erythematosus. J Clin Invest 85:33–39

Fujisaku A, Frank MB, Neas B, Reichlin M, Harley JB (1990) HLA-DQ gene complementation and other histocompatibility relationships in man with the anti-Ro/SSA autoantibody response of systemic lupus erythematosus. J Clin Invest 86:606–611

Greico M, Santoro M, Berlingieri MT, Melillo RM, Donghi R, Bongarzone I, Pierotti MA, et al (1990) PTC is a novel rearranged form of the ret proto-oncogene and is frequently detected in vivo in human thyroid papillary carcinomas. Cell 60:557–563

Hanas JS, Hazuda DJ, Bogenhagen DF, Wu FY-H, Wu C-W (1983) *Xenopus* transcription factor A requires zinc for binding to the 5S RNA gene. J Biol Chem 258:14120–14125

Harley JB, Alexander EL, Bias WB, Fox OF, Provost TT, Reichlin M, Yamagata H, et al (1986) Anti-Ro/SSA and anti-La/SSB in patients with Sjogren's syndrome. Arthritis Rheum 29:196–206

Harley JB, Sestak AL, Willis LG, Fu SM, Hansen JA, Reichlin MR (1989) A model for disease heterogeneity in systemic lupus erythematosus: relationships between histocompatibility antigens, autoantibodies, lymphopenia and proteinurea. Arthritis Rheum 32:826–836

Hedgpeth MT, Boulware DW (1989) Interstitial pneumonitis in antinuclear antibody negative lupus erythematosus: a new clinical manifestation and possible association with anti-Ro(SS-A) antibodies. Arthritis Rheum 31:545–548

Hoffman RW, Sharp GC, Irvin WS, Anderson SK, Hewett JE, Pandey JP (1991) Association of immunoglobulin Km and Gm allotypes with specific antinuclear antibodies and disease susceptibility among connective tissue disease patients. Arthritis Rheum 34:453–458

Itoh K, Itoh Y, Frank MB (1991) Protein heterogeneity in the human Ro/SSA ribonucleoproteins: the 52- and 60-kD autoantigens are encoded by separate genes. J Clin Invest 87:177–186

Itoh Y, Rader MD, Reichlin M (1990) Heterogeneity of the Ro/SSA antigen and autoanti-Ro/SSA response: evidence for 4 antigenically distinct forms. Clin Exp Immunol 81: 45–51

Johnson PF, McKnight SL (1989) Eukaryotic transcriptional regulatory proteins. Annu Rev Biochem 58:799–839

Landschulz WH, Johnson PF, McKnight SL (1988) The leucine zipper: a hypothetical structure common to a new class of DNA binding proteins. Science 240:1759–1764

Leonhardt T (1964) Family studies in systemic lupus erythematosus. Acta Med Scand 176, Suppl 416:1–156

Lerner MR, Boyle JA, Hardin JA, Steitz JA (1981) Two normal classes of small ribonucleoproteins detected by antibodies associated with lupus erythematosus. Science 211:400–402

Maddison PJ, Mogavero H, Provost TT, Reichlin M (1979) The clinical significance of antibodies to a soluble cytoplasmic antigen in SLE and other connective tissue diseases. J Rheumatol 6:189–195

Maddison PJ, Reichlin M (1979) Deposition of antibodies to a soluble cytoplasmic antigen in the kidneys of patients with systemic lupus erythematosus. Arthritis Rheum 22: 858–863

Mattei JF, Philip N, Passage E, Moisan JP, Mandel JL (1985) DNA probe localization at 18p11.3 band by in situ hybridization and identification of a small supernumerary chromosome. Hum Genet 69:268–271

Mond CB, Peterson MGE, Rothfield NF (1989) Correlation of anti-Ro antibody with photosensitivity skin rash in systemic lupus erythematosus. Arthritis Rheum 32:202–204

Morton NE, Lindsten J, Iselius L, Yee S (1982) Data and theory for a revised chiasma map of man. Hum Genet 62: 266–270

Perry P, Wolff S (1974) New giemsa method for the differential staining of sister chromatids. Nature 251:156–158

Rader MD, Codding C, Reichlin M (1989) Differences in the fine specificity of anti-Ro (SS-A) in relation to the presence of other precipitating autoantibodies. Arthritis Rheum 32:1563–1571

Ramsey-Goldman R, Hom D, Deng JS, Ziegler GC, Kahl LE, Steen VD, LaPorte RE, et al (1986) Anti-SS-A antibodies and fetal outcome in maternal systemic lupus erythematosus. Arthritis Rheum 29:1269–1273

Rauscher FJ III, Morris JF, Tournay OE, Cook DM, Curran

T (1990) Binding of the Wilms' tumor locus zinc finger protein to the EGR-1 consensus sequence. Science 250: 1259–1262

Reichlin M (1986) Significance of the Ro antigen system. J Clin Immunol 6:339–348

Rothfield NF (1985) Systemic lupus erythematosus: clinical aspects and treatment. In: McCarty DJ (ed) Arthritis and allied conditions, 10th ed. Lea & Febiger, Philadelphia, pp 911–935

Schur PH, Pandey JP, Fedrick JA (1985) Gm allotypes in white patients with systemic lupus erythematosus. Arthritis Rheum 28:828–830

Silverman E, Mamula M, Hardin JA, Laxer R (1991) Importance of the immune response to the Ro/La particle in the development of congenital heart block and neonatal lupus erythematosus. J Rheumatol 18:120–124

Smolen JS, Morimoto C, Steinberg AD, Wolf A, Schlossman SF, Steinberg RT, Penner E, et al (1985) Systemic lupus erythematosus: delineation of subpopulations by clinical, serologic and T cell subset analysis. Am J Med Sci 289: 139–147

Southern EM (1978) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503–517

Stenszky V, Kozma L, Szegedi G, Farid NR (1986) Interplay of immunoglobulin G heavy chain markers (Gm) and HLA in predisposing to systemic lupus nephritis. J Immunogenet 13:11–17

Sutherland GR (1990) Human fragile sites. In: O'Brien SJ (ed) Genetic maps: locus maps of complex genomes. Book 5: Human maps. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, pp 5.193–5.195

Takahashi M, Cooper GM (1987) Ret transforming gene encodes a fusion protein homologous to tyrosine kinase. Mol Cell Biol 7:1378–1385

Takahashi M, Inaguma Y, Hiai H, Hirose F (1988) Developmentally regulated expression of a human "finger"-containing gene encoded by the 5′ half of the ret transforming gene. Mol Cell Biol 8:1853–1856

Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, Schaller JG, et al (1982) The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 25:1271–1277

Tebib J, Alarcon-Segovia D, Alcocer-Varela J, Schur PH (1989) Association of a T cell receptor alpha chain RFLP and systemic lupus erythematosus (SLE). Arthritis Rheum Suppl 31:S121

Wasicek CA, Reichlin M (1982) Clinical and serological differences between systemic lupus erythematosus patients with antibodies to Ro vs patients with antibodies to Ro and La. J Clin Invest 69:835–843

Watson RM, Lane AT, Barnett NK, Bias WB, Arnett FC, Provost TT (1984) Neonatal lupus erythematosus: a clinical, serological and immunogenetic study with review of the literature. Medicine (Baltimore) 63:362–378

Whittingham S, Mathews JD, Schanfield MS, Tait BD, Mackay IR (1983) HLA and Gm genes in systemic lupus erythematosis. Tissue Antigens 21:50–57

Winchester RJ, Lahita RG (1987) Genetic susceptibility to systemic lupus erythematosus. In: Lahita RG (ed) Systemic lupus erythematosus. John Wiley & Sons, New York, pp 81–118

Wolin SL, Steitz JA (1984) The Ro small cytoplasmic ribonucleoproteins: identification of the antigenic protein and its binding on the Ro RNAs. Proc Natl Acad Sci USA 81: 1996–2000

Yamagata H, Harley JB, Reichlin M (1984) Molecular properties of the Ro/SSA antigen and enzyme-linked immunosorbent assay for quantitation of antibody. J Clin Invest 74:625–633