PARP alleles within the linked chromosomal region are associated with systemic lupus erythematosus

Betty P. Tsao,[1] Rita M. Cantor,[2,3] Jennifer M. Grossman,[1] Nan Shen,[1] Nickolay T. Teophilov,[1] Daniel J. Wallace,[4] Frank C. Arnett,[5] Klaus Hartung,[6] Rose Goldstein,[7] Kenneth C. Kalunian,[1] Bevra H. Hahn,[1] and Jerome I. Rotter[2]

[1]Division of Rheumatology, Department of Medicine, and
[2]Departments of Pediatrics and Human Genetics, University of California–Los Angeles School of Medicine, Los Angeles, California 90095-1670, USA
[3]Division of Medical Genetics, Departments of Medicine and Pediatrics, Steven Spielberg Pediatric Research Center, and
[4]Rheumatology and Arthritic Disorders, Cedars-Sinai Research Institute, Los Angeles, California 90048, USA
[5]Division of Rheumatology, Department of Internal Medicine, University of Texas Health Sciences Center, Houston, Texas 77030, USA
[6]Zentralkrankenhaus Reinkenheide, Institut für Laboratoriums und Transfusionsmedizin, D-27574 Bremerhaven, Germany
[7]Division of Rheumatology, The Ottawa Hospital, University of Ottawa, Ottawa, Ontario K1H 8L6, Canada

Address correspondence to: Betty P. Tsao, Division of Rheumatology, Department of Medicine, University of California–Los Angeles School of Medicine, 32-59 Rehabilitation Center, Box 951670, Los Angeles, California 90095-1670, USA. Phone: (310) 825-8906; Fax: (310) 206-8606; E-mail: btsao@med1.medsch.ucla.edu

Received for publication December 4, 1998, and accepted in revised form March 2, 1999.

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by various autoantibodies that recognize autoantigens displayed on the surface of cells undergoing apoptosis. The genetic contribution to SLE susceptibility has been widely recognized. We previously reported evidence for linkage to SLE of the human chromosome 1q41–q42 region and have now narrowed it from 15 to 5 cM in an extended sample using multipoint linkage analysis. Candidate genes within this region include (a) *PARP*, poly(ADP-ribose) polymerase, encoding a zinc-finger DNA-binding protein that is involved in DNA repair and apoptosis; (b) *TGFB2*, encoding a transforming growth factor that regulates cellular interactions and responses; and (c) *HLX1,* encoding a homeobox protein that may regulate T-cell development. Using a multiallelic, transmission-disequilibrium test (TDT), we found overall skewing of transmission of *PARP* alleles to affected offspring in 124 families ($P = 0.00008$), preferential transmission of a *PARP* allele to affected offspring ($P = 0.0003$), and lack of transmission to unaffected offspring ($P = 0.004$). Similar TDT analyses of *TGFB2* and *HLX1* polymorphisms yielded no evidence for association with SLE. These results suggest that *PARP* may be (or is close to) the susceptibility gene within the chromosome 1q41–q42 region linked to SLE.

J. Clin. Invest. **103**:1135–1140 (1999).

Introduction

As is true for many multifactorial complex human diseases, cumulative studies suggest that interactions of multiple genes and environmental factors result in susceptibility to systemic lupus erythematosus (SLE) (1, 2). The prevalence of SLE in the US general population is approximately 15–50 per 100,000 (3). The relatively high incidence (10–16%) of more than one case in a family has suggested a genetic basis for SLE (3). The concordance rate of SLE in monozygotic twins (24–57%) is approximately 10 times higher than the rate in dizygotic twins (2–5%) (1, 3), again implicating genetic factors in this disorder. Based on these epidemiological studies, the relative risk for siblings of SLE patients, λs, defined as the risk for siblings of patients divided by the risk in the general population (4), is in the range of 20–40.

Case-control studies have shown that MHC class II genes (*HLA-DR* and/or *DQ* but not *DP*) and certain class III genes (*C2, C4, TNFα,* and *HSP70-2* alleles) confer susceptibility to SLE (1, 2). The overall relative risk associated with each MHC class II haplotype in various ethnic groups is estimated to be two- to threefold. Because of extensive linkage disequilibrium within the MHC region, the precise loci (and alleles) responsible for disease susceptibility have yet to be determined (1, 2). Other non-MHC genes have also been associated with SLE. Among them, evidence for homozygous deficiency of the first complement of the complement system, *C1q,* predisposing to SLE is particularly compelling; 90% of such individuals have SLE, and C1q knockout mice display an SLE-like phenotype (5, 6). In addition, polymorphisms in many genes encoding molecules with relevant immunological functions (including T-cell receptor α and β chains, immunoglobulin allotypes, FcγRIIa, FcγRIIIa, IL-6, IL-10, BCL-2, and mannose-binding protein), as well as deletions of specific variable gene segments of immunoglobulin genes, have been associated with SLE demonstrated mostly by the case-control studies (1, 2, 7–9). The contribution of these genes to the development of SLE is under investigation.

Linkage analysis for susceptibility loci of SLE has been more extensively studied in murine than in human SLE (reviewed in ref. 2). Using the identified murine susceptibility loci (the overlapping *Sle1/Nba2/Lbw7*) as a guide,

we examined seven markers located on a likely syntenic human chromosomal 1q31–q42 region in 52 affected sibpairs from Caucasian, Asian, and African-American families (2, 10–14). Five markers located at 1q41–q42 showed evidence for linkage (P = 0.0005–0.08) by the allele-sharing method using the model-free SIBPAL program of the S.A.G.E. package (ref. 14; Department of Biometry and Genetics, Louisiana State University Medical Center, New Orleans, USA). Subsequently, two independent reports supported linkage of this region; a lod score of 1.51 at 1q42 using a model-free linkage analysis of 105 affected sibpairs, and a lod score of 3.50 at 1q41 using a model-based linkage analysis in 31 African-American pedigrees (15, 16).

In this report, we describe results of fine mapping including a multipoint linkage analysis that narrows the linked region to 5 cM, and testing three positional candidate genes (*PARP, TGFB2,* and *HLX1*) for association with SLE. PARP is activated by DNA strand breaks introduced in the genome through environmental injuries (17). It catalyzes the attachment of ADP-ribose units from NAD to an array of nuclear acceptor proteins involved in cellular proliferation, differentiation and DNA repair (17). The potential involvement of PARP in SLE has been suggested by its lower-than-normal levels of activity and mRNA in patients with SLE and by its intermediate range of activity in unaffected family members of patients with SLE (18–20), making it an excellent positional candidate. Because of the well-established regulatory role of TGFβ1 (located on 19q13) in autoimmune diseases and the fact that same surface receptors bind to both TGFβ1 and TGFβ2 (21), we chose *TGFB2* as the second positional candidate gene. *HLX1* resembles a diverged *Drosophila* homeobox gene and is expressed in hematopoietic progenitors and activated lymphocytes. Functionally, this human homeodomain-containing protein has been shown to regulate the development of $CD4^+$ T cells in the transgenic mouse model (22). Because modulation of $CD4^+$ T cells by administration of a specific monoclonal antibody prevents and ameliorates murine SLE (23, 24), we chose *HLX1* as the third positional candidate gene. Our results of the family-based transmission-disequilibrium test (TDT) (25, 26) indicate that only one of these three candidate genes, *PARP,* is associated with SLE in this sample.

Methods

Subjects. This study was approved by the Human Subject Protection Committee of the University of California–Los Angeles (UCLA). Multiplex families were recruited by ascertaining nuclear families for two or more patients with SLE, which were extended to include parents and other siblings. These families were recruited at UCLA and at other collaborating sites, including the University of Texas–Houston (F.C. Arnett), the Zentralkrankenhaus Reinkenheide (K. Hartung), and The Ottawa Hospital (R. Goldstein). Simplex families were mainly recruited through UCLA rheumatologists to include patients with SLE, their parents, and other unaffected sibs if available. Medical records for patients with SLE were either reviewed by UCLA rheumatologists or were provided by the patients' rheumatologists in the form of completed checklists (27), and the checklist information was then entered into the UCLA database. This database was used for classification of patients as SLE (requiring at least four of the 11 American College of Rheumatology Classification criteria) (28, 29). Siblings of patients with SLE and who have no positive responses in the Connective Tissue Screening Questionnaire (30) are classified as unaffected sibs.

Genotyping. DNA was isolated from peripheral blood cells. Microsatellite markers at or near the specific candidate chromosomal region were selected based on a composite map (accessible at http://cedar.genetics.soton.ac.uk/pub/chrom1). The primers for these markers were purchased from Research Genetics Inc. (Huntsville, Alabama, USA). Microsatellite genotyping was determined by scoring the size of PCR products. PCR was performed in a 96-well plate using a thermocycler that was programmed for 95°C for one minute, 58°C for 30 s, and 72°C for one minute, with 25 cycles. The labeled PCR products were denatured and separated on a 5% sequencing gel.

The location of candidate genes were based on the GeneMap '98 (http://www.ncbi.nlm.nih.gov/genemap) and the Genome Database (http://gdbwww.gdb.org). The primer sequences used for the determination of genotypes of *PARP, HLX1,* and *TGFB2* were as described in the Genome Database. The PCR condition for *PARP* and *TGFB2* polymorphisms was 30 cycles of 93°C for one minute, 56°C for 30 s, and 72°C for one minute, plus a final extension of 72°C for five minutes. The *HLX1* PCR condition was 10 cycles of 94°C for 15 s, 55°C for 15 s, and 72°C for 30 s, followed by 20 cycles of 89°C for 15 s, 55°C for 15 s, and 72°C for 30 s, with a final extension at 72°C for 10 min. For these two candidate genes, the PCR mixture contained 40 ng genomic DNA, 20 mM Tris-HCl (pH 8.0), 2 mM $MgCl_2$, 10 mM KCl, 6 mM $(NH_4)_2SO_4$, 0.1% Triton X-100, 10 μg/ml BSA, 0.5 U native *Pfu* DNA polymerase (Stratagene, San Diego, California, USA), 200 μM dNTP, and 0.2 μM primers in a 5-μl reaction. The PCR mixture for *TGFB2* was similar to that described for *PARP* and *HLX1*, except for the buffer, which contained 10 mM Tris-HCl (pH 9.0), 1.5 mM $MgCl_2$, 50 mM KCl, and 0.1% Triton X-100. Aliquots of fluorescent PCR products of *PARP* and *HLX1* were electrophoresed using a 377 Prism ABI sequencer and analyzed by GeneScan and Genotyper programs (Applied Biosystems, Foster City, California, USA). The *TGFB2* genotyping was determined using radioactively labeled primers and autoradiography.

Data analysis. Multipoint linkage analysis of 14 microsatellite markers in the chromosome 1q31–42 region and the SLE phenotype (affected or unaffected) was conducted on nuclear families using the MAPMAKER/SIBS program (31) on a Sun Ultra 60 workstation (Sun Microsystems Inc., Palo Alto, California, USA), with a UNIX operating system. The program simultaneously incorporates allele-sharing information at multiple markers in a chromosomal region to estimate the expected proportions of affected sibpairs sharing zero, one, and two alleles identical by descent for points along the chromosomal region. Spouses of probands of multiplex families were used to estimate allele frequency. A lod score is generated by taking the log of the likelihood of the data at the maximum likelihood estimate of allele-sharing proportion, compared with the likelihood under the expectation of no linkage to the region.

The TDT can detect an allelic association of a marker with disease in the presence of its linkage to the disease (25). The method involves assessing the transmission of alleles from heterozygous parents to their affected offspring and testing whether there is evidence that transmission of alleles at a marker is different from 0.5 for each allele. To assess the deviation from 0.5 for all alleles simultaneously, the extended TDT (ETDT), which uses a logistic regression approach, has been conducted (software provided by the program authors) (26). If a significant deviation from 0.5 was detected, the same software was employed to examine each allele separately for a deviation from a 0.5 probability of transmission.

The relative risk for the 85-bp allele identified by the ETDT at

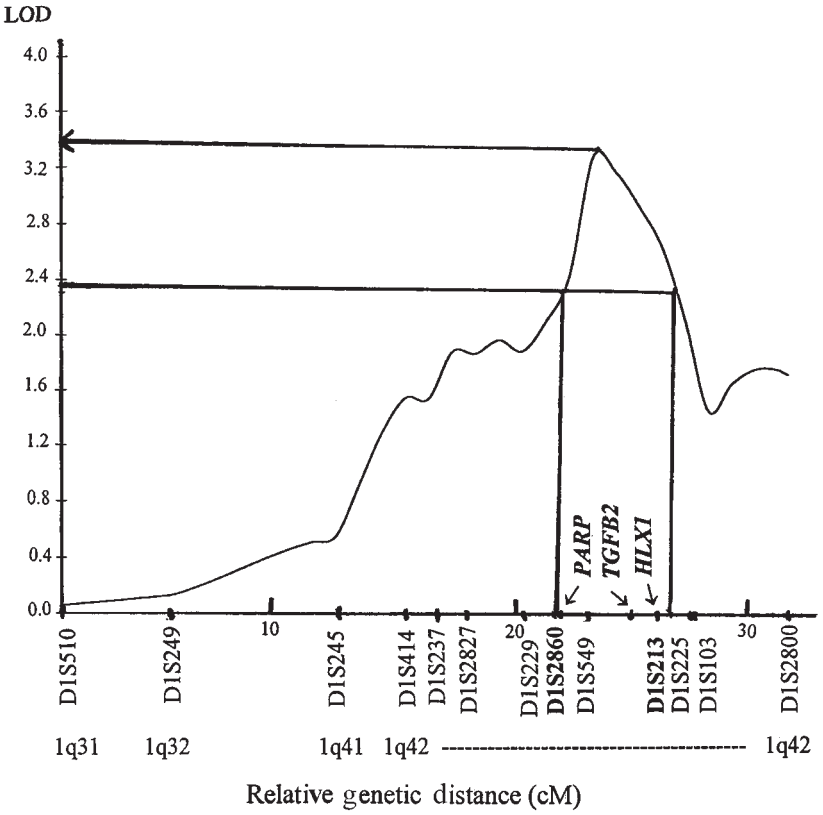

Figure 1
Multipoint linkage analysis of the chromosome 1q31–q42 region using the MAPMAKER/SIBS program. Positions of genetic markers relative to D1S510 are expressed in cM as the relative genetic distance shown on the *x* axis. All markers except *HLX1* and *TGFB2* were used in this analysis. The heuristic guideline of 1 lod below the peak value was used to identify a confidence interval of 5 cM (flanked by D1S2860 and D1S213) for the location of the putative SLE susceptibility gene.

the *PARP* locus has been assessed using a logistic regression method (32). Poisson regression analysis was applied to the numbers of parent-child triads observed among the oldest affected offspring with at least one parent heterozygous for the 85-bp allele. The risk of developing SLE with one or two 85-bp alleles was estimated by using the GENMOD procedure of the Statistical Analysis System (SAS) package computer program (33).

Results

We previously reported evidence for linkage of a 15-cM region on chromosome 1 to SLE using the affected sib-pair approach (14). To support and extend this finding, we have increased our sample size by 50%, from the initial 52 to 78 SLE-affected sibpairs from Caucasian, Asian, and African-American families. All participating members (77 parents; 134 affected and 89 unaffected siblings) were genotyped using 14 microsatellite markers within a 30-cM region (Figure 1) containing the previously identified 15-cM region. Previously, this linked region was established by model-free two-point linkage analyses using seven markers (D1S510, D1S249, D1S245, D1S229, D1S213, D1S225, and D1S103). As shown in Figure 1, multipoint linkage analysis on the current sample using the model-free MAPMAKER/SIBS program (31) identified a peak with a lod score of 3.3 and narrowed the region of interest to approximately 5 cM between markers D1S2860 and D1S213.

Because positive association results of candidate genes located within identified linked regions can narrow the search for susceptibility loci (e.g., apolipoprotein E type 4 allele in late-onset familial Alzheimer's disease and *CTLA4* in type 1 diabetes), we tested three candidate genes with known polymorphisms that are likely to localize within this narrowed region — specifically *PARP* (also known as *ADPRT), TGFB2,* and *HLX1* — using a multiallelic TDT approach (25, 26). The *PARP* polymorphism we tested is a CA repeat located 906 bp upstream of the transcription start site (36). There were nine *PARP* alleles detected in our cohort of 124 multiplex and simplex families consisting of 75 Caucasian, 25 Hispanic, 17 Asian, and seven African-American families, containing 223 parents, 289 patients, and 226 unaffected sibs. The oldest affected and unaffected offspring in each family were used for these analyses, as other sibs from the same families are not independent samples. Because racial origin is likely to contribute to genetic heterogeneity of SLE, we compared these candidate genes using one ethnic group, our largest one: the Caucasian sample. Table 1 summarizes data from all *PARP* alleles analyzed by the multiallelic TDT (25, 26) using our multiethnic cohort, and Table 2 summarizes data from the 75 Caucasian families. Analysis of the combined four frequent alleles (85, 93, 95, and 97 bp) showed the skewed transmission to affected offspring in the combined sample containing all ethnic groups ($P = 0.00008$; Table 1) as well as in the Caucasian sample ($P = 0.002$; Table 2). Further inclusion of the five infrequent *PARP* alleles (87, 89, 91, 99, and 101 bp) in the multiallelic TDT yielded an overall *P* value of 0.001, supporting significantly disproportional transmission to affected offspring when all nine alleles were analyzed as a group. Other ethnic groups were each too small in number to be analyzed separately.

Application of the TDT to these *PARP* alleles indicated that the 85-bp allele of *PARP* was preferentially transmitted to affected offspring (transmission/nontransmission = 66:33; $P = 0.0003$) and preferentially not

Table 1
Skewed transmission of PARP alleles in the multiethnic cohort

	All ethnic groups combined							
Alleles	Affected offspring				Unaffected offspring			
(bp)	t	nt	%t	*P* value	t	nt	%t	*P* value
85	66	33	67	0.0003	24	52	31	0.004
93	28	36	44	NS	24	21	53	NS
95	13	15	46	NS	13	5	72	NS
97	19	42	31	0.0009	31	20	61	NS
87	4	5			5	3		
89	0	3			2	0		
91	1	1			0	1		
99	4	0			2	0		
101	1	1			1	0		
Multialleles				0.00008				0.02

Data presented are derived from all PARP alleles transmitted (t) and not transmitted (nt) from heterozygous parents to offspring in 124 families. Data are shown as the percentage transmission of each allele (%t). The *P* value for each allele is the level of significance based on the deviation from the expected random (50%) allele transmission and was evaluated using a chi-squared statistic ($\chi^2 = [t - nt)^2/(t + nt)]$ with 1 degree of freedom). The multiallelic *P* value is the level of significance based on testing the four frequent alleles (85, 93, 95, and 97 bp) simultaneously for a deviation from the expected 50% transmission. NS, not significant ($P > 0.05$). Individual *P* values for the infrequent alleles (87, 89, 91, 99, and 101 bp) were not calculated.

Table 2
Skewed transmission of PARP alleles in Caucasians

	Caucasian							
Alleles	Affected offspring				Unaffected offspring			
(bp)	t	nt	%t	*P* value	t	nt	%t	*P* value
85	41	17	71	0.001	16	32	32	0.03
93	14	18	44	NS	12	9	57	NS
95	6	11	35	NS	10	2	83	0.03
97	10	23	30	0.02	16	12	57	NS
87	1	2			2	1		
89	0	0			0	0		
91	0	1			0	1		
99	0	0			0	0		
101	1	1			1	0		
multialleles				0.002				NS

Data presented are derived from 75 Caucasian families. See Table 1 for definitions of t, nt, %t, NS, and *P* value.

transmitted to unaffected offspring (transmission/nontransmission = 24:52; $P = 0.004$) in the 124 multiethnic families (Table 1). These data also indicated that the 97-bp allele of *PARP* might be protective, as it was preferentially not transmitted to affected offspring (transmission/nontransmission = 19:42; $P = 0.0009$) (Table 1). Consistently, in Caucasian families (Table 2), the 85-bp allele of *PARP* was significantly transmitted to offspring with SLE ($P = 0.001$), whereas the 97-bp allele was transmitted more frequently to unaffected offspring ($P = 0.02$). Compared with having no 85-bp alleles, the risk of developing SLE with one is 2.3 and is 4.0 for being homozygous (32).

Similar TDT analyses of a dinucleotide repeat within the *TGFB2* was performed using 81 Caucasian nuclear families. However, transmission data of seven *TGFB2* alleles from 149 parents to affected offspring demonstrated homozygosity in 83% of the analyzed parents. Among the 25 heterozygous parents studied, the most frequent *TGFB2* allele was transmitted 11 times, but not transmitted 14 times, to affected offspring, thus showing no obvious distortion from the expected random distribution ($\chi^2 = 0.36$; $P = 0.6$). TDT analyses of *HLX1* were also performed using an intronic dinucleotide repeat within this gene in an analogous fashion, but they yielded no evidence for association with SLE. Transmission data of the seven *HLX1* alleles to affected offspring from 129 heterozygous Caucasian parents are summarized in Table 3; no significant difference from the expected random distribution was detected (χ^2 for the genotype-wise TDT = 11.35; $P = 0.88$). In summary, TDT results of these three candidate genes yielded only evidence for association of the *PARP* polymorphism with SLE in our tested cohort. Thus, our data suggest that *PARP* may be the SLE susceptibility gene within the linked region previously identified, or at least is close to it.

Discussion

In this study, we have demonstrated that the 85-bp *PARP* allele is preferentially transmitted from heterozygous parents to offspring affected with SLE in a sample of 124 families comprising Caucasians, Hispanics, Asians, and African-Americans. Our rationale for performing the TDT analysis is to identify a genetic marker at or near the putative susceptibility gene within the large candidate region suggested by linkage analyses. Evidence for linkage of chromosome 1q41–q42 in human SLE is very strong, including (a) our previous report of a 15-cM region established with 52 affected sibpairs from multiple ethnic groups (14); (b) the narrowed 5-cM region with a maximum lod score of 3.3, as shown in Figure 1; (c) the lod score of 1.51 of a marker at 1q42 in an independent sample of 105 affected sibpairs (15); and (d) the lod score >2 at 1q41 in 31 African-American pedigrees (16). In the presence of established linkage to this region, we have tested three positional candidate genes for association by using the oldest affected offspring of each nuclear family in the TDT analysis. This family-based association test is considered to be a better study design than the population-based association test because the latter is more prone to spurious associations if cases and controls are

Table 3
HLX1 alleles are not associated with susceptibility to SLE

Alleles	Affected offspring		
(bp)	t	nt	%t
168	9	8	53%
166	10	9	53%
164	8	11	42%
162	17	18	49%
160	30	30	50%
158	39	32	55%
152	16	21	43%

The analyzed Caucasian families contained 129 heterozygous parents. The overall transmission of 7 tested alleles was not different from the expected random distribution with a *P* value of 0.88. See Table 1 for definitions of t, nt, %t, NS and *P* value.

drawn from unmatched populations (37). That none of these three candidate genes are located at the peak of the observed linkage (Figure 1) does not negate the possibility of their being the susceptibility gene. This may mainly reflect the imprecision of fine mapping using the linkage method due to the lack of one-to-one correlation between genotypes and phenotypes in complex diseases (37). These tested positional candidates have known polymorphisms of dinucleotide repeats with maximum heterozygosity indexes of 0.63, 0.52, and 0.82 for *PARP*, *TGFB2,* and *HLX1,* respectively (38). The low heterozygosity index of this polymorphism within *TGFB2* explains the low percentage of heterozygous parents we observed. Our studies of these three candidates showed that only *PARP* alleles were associated with SLE.

Our positive TDT results suggest that we are very close to the susceptibility gene based on linkage disequilibrium. The extent of linkage disequilibrium in the human genome appears quite variable; from 1–2 cM as observed in the MHC region to within 4.5 kb from studies of markers in the insulin gene (1, 39). In general, linkage disequilibrium may exist within a few hundred kilobase in most human genomic regions of nonisolated populations (40). Because map positions of *PARP, TGFB2, HLX1* and their flanking genes are all currently estimated to be within 6 megabase (38, 41), detailed physical mapping of the region surrounding *PARP* will be necessary to provide additional positional candidate genes.

An equally likely interpretation of our TDT results is that *PARP* is the SLE susceptibility gene within this linked region. The polymorphism of *PARP* we tested is located in the promoter region near the binding site of the transcription factor Ying Yang 1 (42) and, thus, may affect transcription. The functional role of PARP has been extensively studied by using a specific inhibitor (3-aminobenzamide or antisense RNA) and by studies of knockout mice (43). Cumulative data have shown that the absence of PARP activity results in elevated spontaneous genetic rearrangements and hypersensitive responses to DNA damage, suggesting a role for PARP in maintaining genomic stability (43). In addition, PARP is known to be involved in induced apoptosis. Specific cleavage of PARP occurs at an early stage of apoptosis, which may commit cells for self-destruction by eliminating protective mechanisms, including DNA repair (17). Both defective DNA repair and abnormal apoptosis have been implicated as causative factors for SLE (44–46). Although no gross defects in apoptosis are found in *PARP* knockout mice, splenocytes of these mice display a more rapid apoptotic response to an alkylating agent (47). Cell lines with disrupted PARP expression show insensitivity to apoptotic signals (48). Both experimental systems suggest a regulatory role of PARP in induced apoptosis, with impaired apoptosis less detectable in whole animals than in cell lines, probably because of other compensatory routes. Unlike C1q knockout mice, lupus-like phenotypes have not been reported in *PARP* knockout mice. It is worth noting that only 25% of C1q-deficient mice in the appropriate genetic background have histological evidence of glomerulonephritis at eight months of age (6). This observation has not been formally tested in PARP-deficient mice at the appropriate age. Our working hypothesis is that the 85-bp allele of *PARP* (or polymorphisms in linkage disequilibrium within *PARP*) confers defective DNA repair and abnormal apoptosis and thus predisposes to SLE. Previous studies of PARP activity and mRNA in patients with SLE and in family members further suggest a direct role of PARP in susceptibility for SLE (18–20). Our linkage and association suggest that *PARP* is an SLE susceptibility gene or, at the very least, is in physical proximity to an SLE susceptibility gene.

Acknowledgments

We are grateful to all patients and their family members who participated in this study and to the many physicians who referred families and verified diagnosis. We thank L. Ozaki, K. Salcedo, S. Pressman, C. McElree, and N. Biggs for their expert technical help. Supported in part by the Southern California Chapter of the Arthritis Foundation, National Institutes of Health grant RO1 AR-43814, funds from the Paxson family, the Cedars-Sinai Board of Governors' Chair in Medical Genetics, and the Arthritis Society (to R. Goldstein). Work was performed in the Dreyfuss Laboratory for Lupus Research at the University of California–Los Angeles, at the Bertram A. Maltz, M.D., Laboratory for Molecular Rheumatology, and at the Cedars-Sinai Research Institute in Los Angeles. George Ecker collected the Ottawa family material. R. Goldstein was a Research Scholar of the Arthritis Society. H. Deicher, J. Kalden, A. Lange, J. Lakomek, and H.H. Peter have contributed to the German family collection.

1. Arnett, F.C., Jr. 1997. The genetics of human lupus. In *Dubois' lupus erythematosus, 5th edition.* D.J. Wallace and B.H. Hahn, editors. Williams and Wilkins. Baltimore, MD. 77–117.
2. Vyse, T.J., and Kotzin, B.L. 1998. Genetic susceptibility to systemic lupus erythematosus. *Annu. Rev. Immunol.* **16**:261–292.
3. Hochberg, M.D. 1997. The epidemiology of systemic lupus erythematosus. In *Dubois' lupus erythematosus, 5th edition.* D.J. Wallace and B.H. Hahn, editors. Williams and Wilkins. Baltimore, MD. 49–65.
4. Risch, N. 1987. Assessing the role of HLA-linked and unlinked determinants of disease. *Am. J. Hum. Genet.* **40**:1–14 .
5. Bowness, P., *et al*. 1994. Hereditary C1q deficiency and systemic lupus erythematosus. *Q. J. Med*. **87**:455–464.
6. Botto, M., *et al*. 1998. Homozygous C1q deficiency causes glomerulonephritis associated with multiple apoptotic bodies. *Nat. Genet.* **19**:56–59.
7. Wu, J. *et al.* 1997. A novel polymorphism of FcγRIIIa (CD16) alters receptor function and predisposes to autoimmune disease. *J. Clin. Invest.* **100**:1059–1070.
8. Mehrian, R., *et al*. 1998. Synergistic effect between *IL-10* and *Bcl-2* genotypes in determining susceptibility to systemic lupus erythematosus. *Arthritis Rheum.* **41**:596–602.
9. Linker-Israeli M, *et al*. 1998. Novel alleles of the *IL-6* gene minisatellite are associated with systemic lupus erythematosus (SLE) and with differential IL-6 expression. *Arthritis Rheum.* **41**:S282. (Abstr.)
10. Kono, D.H., *et al*. 1994. Lupus susceptibility loci in New Zealand mice. *Proc. Natl. Acad. Sci. USA.* **91**:10168–10172.
11. Drake, C.G., *et al*. 1995. Analysis of the New Zealand black contribution to lupus-like renal disease: multiple genes that operate in a threshold manner. *J. Immunol.* **154**:2441–2447.
12. Morel, L., *et al*. 1994. Polygenic control of susceptibility to murine SLE. *Immunity.* **1**:219–229.
13. Mohan, C., *et al*. 1996. Genetic dissection of SLE pathogenesis: *Sle1* on murine chromosome 1 leads to a selective loss of tolerance to H2A/H2B/DNA subnucleosomes. *J. Clin. Invest.* **101**:1362–1372.
14. Tsao, B.P., *et al*. 1997. Evidence for linkage of a candidate chromosome 1 region to human systemic lupus erythematosus. *J. Clin. Invest.* **99**:725–731.
15. Gaffney, P.M., *et al*. 1998. A genome-wide search for susceptibility genes in human systemic lupus erythematosus sib-pair families. *Proc. Natl. Acad. Sci. USA.* **95**:14875–14879.
16. Moser K.L., *et al*. 1998. Genome scan of human systemic lupus erythematosus: evidence for linkage on chromosome 1q in African-American pedigrees. *Proc. Natl. Acad. Sci. USA*. **95**:14869–14874.
17. Lindahl, T., Satoh, M.S., Poirier, G.G., and Klungland, A. 1995. Post-translational modification of poly(ADP-ribose) polymerase induced by DNA strand breaks. *Trends Biochem. Sci.* **20**:405–411.

18. Sibley, J.T., Haug, B.L., and Lee, J.S. 1989. Altered metabolism of poly(ADP-ribose) in the peripheral blood lymphocytes of patients with systemic lupus erythematosus. *Arthritis Rheum.* **32**:1045–1049.
19. Haug, B.L., Lee, J.S., and Sibley, J.T. 1994. Altered poly(ADP-ribose) metabolism in family members of patients with systemic lupus erythematosus. *J. Rheum.* **21**:851–856.
20. Lee, J.S., Haug, B.L., and Sibley, J.T. 1994. Decreased mRNA levels coding for poly(ADP-ribose) polymerase in lymphocytes of patients with SLE. *Lupus.* **3**:113–116.
21. Letterio J.J., and Roberts A.B. Regulation of immune responses by TGF-β. *Annu. Rev. Immunol.* **16**:137–161.
22. Deguchi, Y., Agus, D., and Kehri, J.H. A human homeobox gene, HB24, inhibits development of CD4+ T cells and impairs thymic involution in transgenic mice. 1993. *J. Biol. Chem.* **268**:3646–3653.
23. Wofsy, D., and Seaman, W.E. 1985. Successful treatment of autoimmunity in NZB/NZW F1 mice with monoclonal antibody to L3T4. *J. Exp. Med.* **161**:378–391.
24. Wofsy, D., and Seaman, W.E. 1987. Reversal of advanced murine lupus in NZB/NZW F1 mice by treatment with monoclonal antibody to L3T4. *J. Immunol.* **138**:3247–3253.
25. Spielman, R.S., McGinnis, R.E., and Ewens, W.J. 1993. Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). *Am. J. Hum. Genet.* **52**:506–516.
26. Sham, P.C., and Curtis, D. 1995. An extended transmission/disequilibrium tests (TDT) for multi-allele marker loci. *Ann. Hum. Genet.* **59**:323–336.
27. Grossman, J.M., *et al*. Validation of a classification instrument for use in genetic studies of systemic lupus erythematosus. *Arthritis Rheum.* **41**:S330. (Abstr.)
28. Tan, E.M., *et al*. 1982. The 1982 revised criteria for the classification of SLE. *Arthritis Rheum.* **25**:1271–1277.
29. Hochberg, M.C. 1997. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum*. **40**:1725.
30. Karlson, W.E., *et al*. 1995. A connective tissue disease screening questionnaire for population studies. *Ann. Epidemiol.* **5**:297–302.
31. Kruglyak, L., and Lander, E.S. 1995. Complete multipoint sib-pair analysis of qualitative and quantitative traits. *Am. J. Hum. Genet.* **57**:439–454.
32. Weinberg, C.R., Wilcox, A.J., and Lie, R.T. 1998. A log-linear approach to case-parent-triad data: assessing effects of diseases genes that act either directly or through maternal effects and that may be subject to parental imprinting. *Am. J. Hum. Genet.* **62**:969–978.
33. SAS/STAT software changes and enhancement through release 6.11. 1996. SAS Institute Inc. Cary, NC.
34. Pericak-Vance, M.A., and Haines, J.I. 1995. Genetic susceptibility to Alzheimer disease. *Trends Genet.* **11**:504–508.
35. Nisticò, L., *et al*. 1996. The CTLA-4 gene region of chromosome 2q33 is linked to, and associated with, type 1 diabetes. Belgian Diabetes Registry. *Hum. Mol. Genet.* **5**:1075–1080.
36. Ogura, T., *et al*. 1990. Characterization of a putative promoter region of the human poly(ADP-ribose) polymerase gene: structural similarity to that of the DNA polymerase beta gene. *Biochem. Biophys. Res. Commun.* **167**:701–710.
37. Lander E.S., and Schork N.J. 1994. Genetic dissection of complex traits. *Science*. **265**:2037–2048.
38. http://gdbwww.gdb–org/.
39. Bennett, S.T., and Todd J.A. 1996. Human type 1 diabetes and the insulin gene: principles of mapping polygenes. *Annu. Rev. Genet.* **30**:343–370.
40. Jorde, L.B. 1995. Linkage disequilibrium as a gene-mapping tool. *Am. J. Hum. Genet.* **56**:11–14.
41. http://www.ncbi.nlm.nih.gov/genemap.
42. Oei, S.L., *et al*. 1997. Interaction of the transcription factor YY1 with human poly(ADP-ribosyl) transferase. *Biochem. Biophys. Res. Commun.* **240**:108–111.
43. Jeggo, P.A. 1998. DNA repair: PARP — another guardian angel? *Curr. Biol.* **8**:R49–R51.
44. Vaishnaw, A.K., McNally, J.C., and Elkon, K.B. 1997. Apoptosis in the rheumatic diseases. *Arthritis Rheum.* **40**:1917–1927.
45. Herrick, A.L., Rafferty, J.A., and Margison, G.P. 1995. DNA repair deficiency in systemic lupus erythematosus, cause or consequence of disease and implications for management [review]. *Lupus.* **4**:423–424.
46. McCurdy, D., Tai, L.-Q., Frias, S., and Wang, Z. 1997. Delayed repair of DNA damage by ionizing radiation in cells from patients with systemic lupus erythematosus and juvenile rheumatoid arthritis. *Radiat. Res.* **147**:48–54.
47. de Murcia, J.M., *et al*. 1997. Requirement of poly(ADP-ribose)polymerase in recovery from DNA damage in mice and in cells. *Proc. Natl. Acad. Sci. USA.* **94**:7303–7307.
48. Simbulan-Rosenthal, C.M., *et al*. 1998. Transient poly(ADP-ribosyl)ation of nuclear proteins and role of poly(ADP-ribose) polymerase in the early stages of apoptosis. *J. Biol. Chem.* **273**:13703–13712 .