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Poly(ADP-ribose) polymerase (PARP) polymorphisms associated with nephritis and arthritis in systemic lupus erythematosus

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***Objective*. The objective of this study was to confirm whether polymorphisms of the poly(ADP-ribose) polymerase gene (*PARP*) are associated with genetic susceptibility to systemic lupus erythematosus (SLE) and to investigate the possible association of nephritis and arthritis in SLE with *PARP* polymorphisms.**

***Methods*. Using direct DNA sequencing in 24 individuals, we identified 44 sequence variants within exons and their flanking regions, including the 1.5-kb promoter region of *PARP*. Six common polymorphic sites were selected for larger-scale genotyping (in 350 Korean SLE patients and 330 healthy controls), which identified six common haplotypes.**

***Results*. Although no statistically significant association with the risk of SLE was observed, we found that two single-nucleotide polymorphisms (SNPs −1963A → G and +28077G → A) were significantly associated with an increased risk of nephritis, and one non-synonymous variant [+40329T → C(V762A)] was also significantly associated with an increased risk of arthritis, while the −1963A → G SNP showed a protective effect on arthritis in Korean SLE patients.**

***Conclusion*. Our results demonstrate that *PARP* polymorphisms are not associated with SLE susceptibility, but that −1963A → G, +28077G → A and +40329T → C(V762A) are significantly associated with nephritis and arthritis in Korean SLE patients.**

KEY WORDS: Poly(ADP-ribose) polymerases, Single-nucleotide polymorphism, Systemic lupus erythematosus.

Systemic lupus erythematosus (SLE) is characterized by the accelerated apoptosis of lymphocytes and a failure in the clearance of apoptotic cells [1]. It has been postulated that several antinuclear antibodies (Abs) are present [2] and that DNA repair is impaired in patients with SLE [3, 4]. It is widely recognized that several genetic factors, including a gene linkage to human chromosome 1q41-42, contribute to SLE susceptibility [5]. The gene for poly(ADP-ribose) polymerase (PARP) is one of the candidate genes located in this region. The human *PARP* gene is 43 kb long and split into 23 exons [6].

PARP has been demonstrated to be a nuclear enzyme that catalyses the synthesis of poly(ADP-ribose) using NAD^+ as a substrate. This process is induced by DNA strand breaks that are generated directly or indirectly by genotoxic agents and enzymatic function, and it plays a pivotal role in DNA repair, proliferation, transcription regulation, apoptosis, genomic stability and malignancy [7, 8]. Several studies have suggested that levels of PARP activity and mRNA are subnormal in lupus patients (and intermediate in unaffected relatives of lupus patients), which consequently decreases poly(ADP-ribose) synthesis [9, 10]. These data implicate PARP in susceptibility to SLE.

It has been suggested that a microsatellite polymorphism—the CA dinucleotide repeat in the promoter region of *PARP*—affects transcription, because it is located close to the transcription factor Yin Yang 1 [11]. Family-based association studies have revealed that a polymorphic CA dinucleotide repeat in the promoter region of *PARP* is transmitted to affected offspring. An 85-bp *PARP* allele was found to be significantly associated with SLE, whereas a 97-bp *PARP* allele was protective in a multiethnic group comprising Caucasians, Hispanics, Asians and African Americans [5]. However, another study involving a similar cohort produced contradictory results [12], and case–control studies have shown no significant association in French and German Caucasians [13, 14].

Additionally, the human chromosome 1q31-42 region corresponds to the telomeric end of mouse chromosome 1, which is the region involving specific manifestations of murine lupus, including glomerulonephritis and IgG antichromatin Ab [15, 16]. Interestingly, it has been shown that *PARP* is overactivated in neighbouring cells by the massive synthesis of nitric oxide, which could lead to DNA breaks, and consequently necrosis triggered by depletion of NAD^+ cellular stores results in inflammatory injury [17]. Thus, *PARP* has been suggested to regulate the transcription of genes related to inflammation. The lineage of marker D1S235 to chromosome 1q41-43 has been demonstrated in genome-wide screening in multiplex rheumatoid arthritis families [18]. Furthermore, it was recently reported that one *PARP* haplotype including the 97-bp CA repeat was associated with rheumatoid arthritis in a Spanish population [19]. However, there is no reported evidence of an association of PARP with nephritis and arthritis in SLE.

In an effort to confirm whether *PARP* polymorphisms are associated with genetic susceptibility to SLE and investigate the possible association of nephritis and arthritis in SLE with *PARP*

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polymorphisms, we explored sequence variations of *PARP* in a Korean population, examined the genetic associations of single-nucleotide polymorphisms (SNPs) with SLE, and analysed the association of *PARP* polymorphisms with nephritis and arthritis among SLE patients. The study cohort consisted of 350 patients with SLE and 330 ethnically matched controls. Written informed consent was obtained from each subject. The study was approved by the Institutional Review Board of Hanyang University Medical Center.

Materials and methods

Patients and controls

A total of 350 Korean SLE patients who fulfilled the 1997 American College of Rheumatology (ACR) criteria of SLE [20] were consecutively enrolled between September 1998 and February 2002 from the Hospital for Rheumatic Diseases, Hanyang University, Seoul, Republic of Korea. The ethnicity of all patients and controls was Korean, and the following clinical and laboratory data were obtained: sex, age, ages at first symptom onset and clinical diagnosis, ACR diagnosis, and SLICC/ACR (Systemic Lupus International Collaborating Clinics/ACR) damage index [21]. Renal disorder was defined as persistent proteinuria greater than 0.5 g per day or greater than 3+ if quantitation was not performed or cellular casts were present. Arthritis was defined as non-erosive arthritis involving two or more peripheral joints, characterized by tenderness, swelling, or effusion according to ACR criteria [20]. As a control group, we included 330 healthy ethnically matched subjects in our examination of the genetic association of polymorphisms with susceptibility to SLE and related phenotypes. The mean age of the SLE patients was 35.6 yr (range 10.5–72.7 yr), and they comprised 15 males and 335 females. Antinuclear Abs were detected by indirect immunofluorescence using IT-1 cells and anti-double-stranded DNA (dsDNA) Abs in a *Crithidia luciliae* assay. Of these patients, 36.2% had renal disorders and 64.1% had non-erosive arthritis during any interval of observation.

PARP sequencing

We used the ABI Prism 3730 DNA analyser (Applied Biosystems, Foster City, CA, USA) to sequence exons and their flanking regions, including the promoter region (1.5 kb) of PARP in order to discover variants in 24 Korean unregulated individual DNA samples. Twenty-eight primer sets for the amplification and sequencing analysis were designed based on GenBank sequences (reference genome sequence for PARP: NT_004559.12, released in August 2004). The primer sets for amplification are listed in Supplementary Table 1. Sequence variants were verified in chromatograms.

PARP polymorphism genotyping

Genotyping was read blinded to clinical outcomes on the whole process. Polymorphic sites were genotyped using amplifying primers and probes designed for TaqMan [22]. Primer Express (Applied Biosystems) was used to design both the polymerase chain reaction (PCR) primers and the MGB TaqMan probes. One allelic probe was labelled with FAM™ dye and the other with the fluorescent VIC® dye. PCRs were run in the TaqMan Universal Master mix without UNG (Applied Biosystems), with PCR primer at a concentration of 900 nM and TaqMan MGB probe at a concentration of 200 nM. Reactions were performed in a 384-well format in a total reaction volume of 5 μl using 20 ng

Table 1. Logistic analysis of *PARP* polymorphisms with SLE using age and sex as covariates in a Korean population

Locus	Genotype	SLE	NC[a]	OR (95% confidence interval)	*P*
−1963A → G	A	164 (47.3%)	157 (47.6%)		
	AG	142 (40.9%)	146 (44.2%)	1.08 (0.86–1.37)	0.50
	G	41 (11.8%)	27 (8.2%)		
−759C → T	C	207 (61.4%)	194 (61.2%)		
	CT	114 (33.8%)	112 (35.3%)	1.04 (0.79–1.36)	0.79
	T	16 (4.8%)	11 (3.5%)		
+28077G → A	G	243 (69.8%)	224 (67.9%)		
	GA	93 (26.7%)	100 (30.3%)	1.02 (0.76–1.36)	0.92
	A	12 (3.5%)	6 (1.8%)		
+39794C → T	C	294 (84.5%)	278 (84.2%)		
	CT	53 (15.2%)	48 (14.6%)	0.89 (0.60–1.32)	0.57
	T	1 (0.3%)	4 (1.2%)		
+40329T → C(V762A)[b]	T	117 (33.5%)	97 (29.4%)		
	TC	171 (49.0%)	162 (49.1%)	0.86 (0.69–1.07)	0.17
	C	61 (17.5%)	71 (21.5%)		
+46133C → T	C	301 (86.7%)	288 (87.3%)		
	CT	44 (12.7%)	40 (12.1%)	1.04 (0.68–1.60)	0.85
	T	2 (0.6%)	2 (0.6%)		
ht1	–/–	148 (44.3%)	132 (41.6%)		
	–/ht1	149 (44.6%)	137 (43.2%)	0.89 (0.71–1.12)	0.31
	ht1/ht1	37 (11.1%)	48 (15.1%)		
ht3	–/–	240 (71.9%)	233 (73.5%)		
	–/ht3	87 (26.1%)	78 (24.6%)	1.08 (0.79–1.49)	0.61
	ht3/ht3	7 (2.1%)	6 (1.9%)		
ht4	–/–	241 (72.2%)	243 (76.7%)		
	–/ht4	82 (24.6%)	69 (21.8%)	1.22 (0.89–1.67)	0.22
	ht4/ht4	11 (3.3%)	5 (1.6%)		

Logistic regression models were used to calculate ORs (95% confidence intervals) and the corresponding *P* values for SNP sites and haplotypes, while controlling for age and sex as covariables. The common alleles were used as the reference genotype to the heterozygote and homozygote of the minor allele. Haplotypes with either equivalence with SNPs or with frequencies less than 5% were excluded from the statistical analyses.

[a]Normal control.

[b]Amino acid change.

of genomic DNA. The plates were then placed in a thermal cycler (PE 9700, Applied Biosystems) and heated at 50°C for 2 min and 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The TaqMan assay plates were transferred to a real-time PCR system (Prism 7900HT, Applied Biosystems) that read the fluorescence intensity in each well of the plate. Fluorescence data files from each plate were analysed using automated software (SDS 2.1; Applied Biosystems). The primers sequences are provided in Supplementary Table 2.

Statistical analysis

The χ^2 test was used to quantify the distribution of PARP alleles in SLE patients. We examined the linkage disequilibrium (LD), expressed as Lewontin's D′ (|D′|), where $D' = D/|D_{max}|$, and the LD coefficient r^2 between all pairs of biallelic loci [23, 24]. The haplotypes of each individual were inferred using the PHASE algorithm developed by Stephens *et al*. [25], which uses a Bayesian approach incorporating *a priori* expectations of haplotypic structure based on population genetics and coalescence theory. Genetic effects of inferred haplotypes were analysed in the same way as polymorphisms. The genotype distributions of PARP polymorphisms and haplotypes were compared between the SLE patients and healthy controls with multiple logistic regression models, while controlling for age (continuous variable), sex (male = 0, female = 1), and/or disease duration (continuous variable) as covariates. The common alleles were used as the reference genotype to the heterozygote and homozygote of the minor allele. *P* values of codominant, dominant and recessive models were also calculated.

Results

Polymorphisms and haplotypes

Direct sequencing identified 44 SNPs: five in the 5′ flanking region, six exonic SNPs (including four non-synonymous) and 33 intronic SNPs. Pairwise comparisons between SNPs revealed five sets of absolute LDs ($|D'| = 1$ and $r^2 = 1$) (Fig. 1A). Several complete LDs ($|D'| = 1$ and $r^2 \neq 1$) were also found (Fig. 1B). Six SNPs (−1963A → G, −759C → T, +28077G → A, +39794C → T, +40329T → C and +46133C → T) were selected for larger-scale ($n = 680$) genotyping based on LDs and frequencies (frequency > 0.05). The genotype distributions and frequencies of these six SNPs are listed in Supplementary Table 3. Three variants (+28077G → A, +39794C → T and +46133C → T) were newly identified in this study, and the others (−1963A → G, −759C → T and +40329T → C) have been reported previously [26, 27]. Six haplotypes (ht1 to ht6) inferred using the algorithm developed by Stephens *et al*. [25] were observed at frequencies higher than 5% (Fig. 1C). Because the three haplotypes ht2, ht5 and ht6 were almost equivalent to +28077G → A, +39794C → T and +46133C → T, respectively, they were excluded from further statistical analyses.

PARP polymorphisms and SLE susceptibility

These six SNPs and three haplotypes (ht1, ht3 and ht4) were used in the subsequent association study. A multiple logistic regression analysis controlling for age and sex as covariates revealed that none of the PARP polymorphisms was associated with SLE (Table 1).

Table 2. Logistic analysis of *PARP* polymorphisms with nephritis in SLE

		Renal disorder		Co-dominant		Dominant		Recessive	
Locus	Genotype	Positive	Negative	OR (95% confidence interval)	*P*	OR (95% confidence interval)	*P*	OR (95% confidence interval)	*P*
−1963A → G	A	47 (39.8%)	93 (50.5%)						
	AG	54 (45.8%)	70 (38.0%)	**1.47 (1.03–2.11)**	**0.03**	**1.76 (1.07–2.89)**	**0.03**	1.49 (0.72–3.09)	0.29
	G	17 (14.4%)	21 (11.4%)						
−759C → T	C	76 (66.7%)	106 (59.2%)						
	CT	34 (29.8%)	63 (35.2%)	0.81 (0.53–1.25)	0.34	0.80 (0.48–1.34)	0.40	0.65 (0.19–2.21)	0.49
	T	4 (3.5%)	10 (5.6%)						
+28077G → A	G	70 (58.8%)	140 (76.1%)						
	GA	43 (36.1%)	38 (20.7%)	**2.07 (1.33–3.21)**	**0.001**	**2.49 (1.46–4.24)**	**0.0008**	2.23 (0.68–7.34)	0.19
	A	6 (5.0%)	6 (3.3%)						
+39794C → T	C	103 (86.6%)	154 (83.7%)						
	CT	16 (13.5%)	29 (15.8%)	0.70 (0.36–1.38)	0.31	0.71 (0.36–1.43)	0.34		
	T	0 (0.0%)	1 (0.5%)						
+40329T → C(V762A)	T	42 (35.3%)	62 (33.5%)						
	TC	60 (50.4%)	89 (48.1%)	0.72 (0.50–1.04)	0.08	0.70 (0.41–1.19)	0.18	0.60 (0.30–1.17)	0.13
	C	17 (14.3%)	34 (18.4%)						
+46133C → T	C	103 (86.6%)	157 (85.8%)						
	CT	15 (12.6%)	25 (13.7%)	1.01 (0.53–1.95)	0.97	0.96 (0.48–1.94)	0.91	2.53 (0.15–41.68)	0.52
	T	1 (0.8%)	1 (0.6%)						
ht1	–/–	52 (46.4%)	79 (44.4%)						
	–/ht1	51 (45.5%)	76 (42.7%)	0.72 (0.49–1.06)	0.10	0.75 (0.45–1.24)	0.26	0.48 (0.20–1.13)	0.09
	ht1/ht1	9 (8.0%)	23 (12.9%)						
ht3	–/–	86 (76.8%)	126 (70.8%)						
	–/ht3	25 (22.3%)	47 (26.4%)	0.79 (0.48–1.31)	0.36	0.85 (0.48–1.50)	0.56	0.25 (0.03–2.17)	0.21
	ht3/ht3	1 (0.9%)	5 (2.8%)						
ht4	–/–	82 (73.2%)	127 (71.4%)						
	–/ht4	28 (25.0%)	42 (23.6%)	0.86 (0.54–1.37)	0.52	0.94 (0.54–1.64)	0.82	0.36 (0.07–1.79)	0.21
	ht4/ht4	2 (1.8%)	9 (5.1%)						

Logistic regression models were used to calculate ORs (95% confidence intervals) and the corresponding *P* values for SNP sites and haplotypes, while controlling for age, disease duration and sex as covariables. Genotype distributions and *P* values for logistic analyses of three alternative models (co-dominant, dominant, and recessive) are listed. Haplotypes with either equivalence with SNPs or with frequencies less than 5% were excluded from the statistical analyses. Significant associations are in bold type.

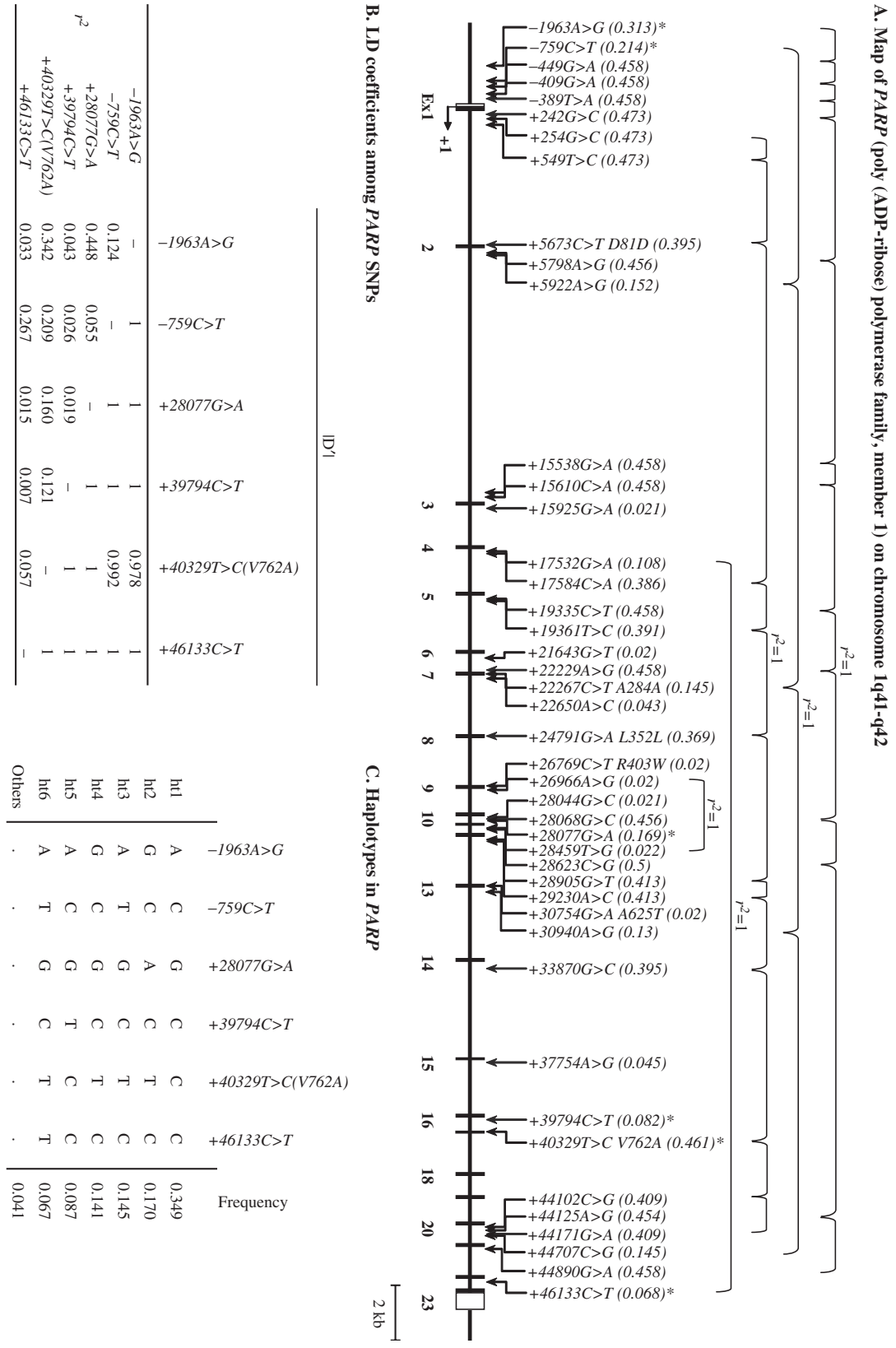

FIG. 1. Gene map and haplotypes of *PARP*. Coding exons are marked by black blocks, and 5′ and 3′ UTRs by white blocks. The first base of the translational start site is denoted as nucleotide +1. *indicate polymorphisms genotyped in a larger population ($N = 680$). The frequencies of polymorphisms not subjected to larger-scale genotyping were based on sequence data ($n = 24$). (A) Polymorphisms identified in *PARP* on chromosome 1q41-42 (reference genome sequence: NT_004559.12). (B) Linkage disequilibrium co-efficients (|D′| and r^2) among *PARP* polymorphisms. (C) Haplotypes of *PARP*. Only those with frequencies of at least 0.05 are shown. Others contain rare haplotypes: ACGCTC, GCGCCC and ATGCCC. Only two individuals (0.03%) showed haplotype probabilities lower than 95% in our study by PHASE.

TABLE 3. Logistic analysis of *PARP* polymorphisms with arthritis in SLE

		Arthritis		Co-dominant		Dominant		Recessive	
Locus	Genotype	Positive	Negative	OR (95% confidence interval)	*P*	OR (95% confidence interval)	*P*	OR (95% confidence interval)	*P*
−1963A → G	A	103 (50.7%)	37 (37.4%)						
	AG	83 (40.9%)	41 (41.4%)	**0.56 (0.39–0.80)**	**0.002**	**0.56 (0.34–0.93)**	**0.03**	**0.31 (0.15–0.64)**	**0.002**
	G	17 (8.4%)	21 (21.2%)						
−759C → T	C	121 (60.8%)	61 (64.9%)						
	CT	66 (33.2%)	31 (33.0%)	1.34 (0.85–2.11)	0.20	1.27 (0.75–2.16)	0.38	2.96 (0.64–13.70)	0.16
	T	12 (6.0%)	2 (2.1%)						
+28077G → A	G	147 (72.4%)	63 (63.0%)						
	GA	48 (23.7%)	33 (33.0%)	0.71 (0.46–1.10)	0.12	0.61 (0.36–1.03)	0.07	0.99 (0.28–3.47)	0.98
	A	8 (3.9%)	4 (4.0%)						
+39794C → T	C	173 (85.2%)	84 (84.0%)						
	CT	29 (14.3%)	16 (16.0%)	0.97 (0.50–1.88)	0.93	0.93 (0.47–1.84)	0.84	.	.
	T	1 (0.5%)	0 (0.0%)						
+40329T → C(V762A)	T	62 (30.4%)	42 (42.0%)						
	TC	103 (50.5%)	46 (46.0%)	**1.55 (1.06–2.26)**	**0.02**	**1.75 (1.03–2.96)**	**0.04**	1.77 (0.86–3.62)	0.12
	C	39 (19.1%)	12 (12.0%)						
+46133C → T	C	176 (87.1%)	84 (84.0%)						
	CT	24 (11.9%)	16 (16.0%)	0.95 (0.49–1.83)	0.88	0.87 (0.43–1.75)	0.69	.	.
	T	2 (1.0%)	0 (0.0%)						
ht1	–/–	80 (40.4%)	51 (55.4%)						
	–/ht1	91 (46.0%)	36 (39.1%)	**1.79 (1.18–2.73)**	**0.006**	**1.91 (1.13–3.23)**	**0.02**	**2.72 (0.98–7.53)**	**0.05**
	ht1/ht1	27 (13.6%)	5 (5.4%)						
ht3	–/–	142 (71.7%)	70 (76.1%)						
	–/ht3	50 (25.3%)	22 (23.9%)	1.39 (0.81–2.37)	0.23	1.26 (0.70–2.27)	0.45	.	.
	ht3/ht3	6 (3.0%)	0 (0.0%)						
ht4	–/–	152 (76.8%)	57 (62.0%)						
	–/ht4	42 (21.2%)	28 (30.4%)	**0.51 (0.32–0.81)**	**0.004**	**0.49 (0.29–0.86)**	**0.01**	**0.22 (0.06–0.83)**	**0.03**
	ht4/ht4	4 (2.0%)	7 (7.6%)						

Logistic regression models were used to calculate ORs (95% confidence intervals) and the corresponding *P* values for SNP sites and haplotypes, while controlling for age, disease duration and sex as covariables. Genotype distributions and *P* values for logistic analyses of three alternative models (co-dominant, dominant and recessive) are listed. Haplotypes with either equivalence with SNPs or with frequencies less than 5% were excluded from the statistical analyses. Significant associations are in bold type.

PARP polymorphisms and nephritis in SLE

We next tested the effect of PARP polymorphisms on renal manifestations using age, disease duration and sex as covariables in lupus patients. Two SNPs (−1963A → G and +28077G → A) were significantly associated with an increased risk of lupus glomerulonephritis among SLE patients (Table 2). The minor allele of –1963A → G and +28077G → A showed a strong susceptibility to lupus glomerulonephritis among SLE patients, in that the frequencies of the G-allele-containing genotypes of −1963A → G and the A allele of +28077G → A were significantly higher among SLE patients who were positive for renal disorder than among those who were negative [$P = 0.03$, odds ratio (OR) = 1.76 and $P = 0.0008$, OR = 2.49, respectively] (Table 2).

PARP polymorphisms and arthritis in SLE

We also investigated the genetic association with arthritis. Two SNPs (−1963A → G and +40329T → C[V762A]) and two common haplotypes (ht1 and ht3) were significantly associated with arthritis in SLE patients (Table 3). Variants −1963A → G and ht4 showed a strong protective effect in a gene-dose-dependent manner on arthritis in SLE patients ($P = 0.002$–0.03, OR = 0.56–0.31 and $P = 0.004$–0.01, OR = 0.51–0.22, respectively) (Table 3). On the other hand, +40329T → C(V762A) and ht1 had opposite effects on arthritis in SLE patients ($P = 0.02$–0.12, OR = 1.77–1.55 and $P = 0.006$–0.05, OR = 2.72–1.79, respectively) (Table 3). It was likely that the significant associations of ht1 (ACGCCC) depended on +40329T → C(V762A), because ht1 was almost tagged (frequency >0.90) by +40329T → C(V762A), which showed a positive association (Fig. 1B).

Discussion

Several studies have suggested that interactions of multiple genes and environmental factors result in susceptibility to SLE. Linkage analyses have shown that chromosome 1q41-42, which contains *PARP*, is one of the loci with a significant linkage to SLE [28, 29]. A case–control study involving Japanese subjects identified five *PARP* polymorphisms, including a CA-repeat polymorphism [30], and detected complete LD between C1362T and the $(CA)_{11}$ allele corresponding to the 85-bp allele [26]. Two SNPs identified in our study (−759C → T and −449G → A, which was linked with −1963A → G by absolute LD) correspond to C1362T and G1672A. Even though the −759C → T SNP is not considered to be linked to the $(CA)_{11}$ allele by absolute LD, our results show that −759C → T is not associated with an increased risk of SLE in Koreans and that its allele frequency (21.4%) is low, as in Japanese subjects (19.3–20.4%), compared with Caucasians (54%) [26].

PARP has been demonstrated to be a nuclear protein binding to DNA via two zinc-finger motifs, and to play a key role in DNA repair, which has led to the suggestion that PARP is crucial in the processing of DNA lesions by triggering the base-excision repair pathway; it has also been shown that PARP-deficient mice are hypersensitive to genotoxic agents [31, 32]. Furthermore, the significant delay in cell death in an experimental model with a non-cleavable variant of the enzyme during apoptosis led to the suggestion that loss of PARP function is required for the efficient completion of apoptosis [33]. Thus, it is likely that dysregulation of apoptosis by decreased PARP activity contributes to the development of SLE. However, a recent study implicated translocation of apoptosis-inducing factor (AIF) from the mitochondria to the nucleus in the initiation of apoptosis, and showed that PARP activation is necessary for

the translocation of AIF [34]. Therefore, further investigations are needed to clarify the role of PARP during apoptosis in SLE.

Tissue injury results from immune complex deposition, with a high level of autoantibodies directed to nuclear antigens in SLE. In particular, renal disorder with severe proteinuria and renal failure is a major complication of lupus. Although anti-dsDNA Abs have been shown to be strongly associated with renal involvement through deposition of these Abs directly on the glomerular basement membrane [35, 36], lupus glomerulonephritis can occur in the absence of anti-dsDNA Abs and breaking tolerance to nuclear antigens [37, 38]. Recently it was shown that breaking tolerance to dsDNA, nucleosomes and other nuclear antigens was not required for the development of lupus nephritis [39]. An immune response involving both T cells and B cells to kidney antigens is implicated in the pathogenesis of lupus nephritis [39]. On the other hand, increased IgG Abs reacting to PARP were observed in patients with SLE and autoimmune bowel diseases [40, 41]. These autoantibodies prevent caspase-3-mediated PARP cleavage during early apoptosis, which, if not regulated, can lead to prolonged autoimmune stimulation by increasing autoimmune cell survival [42]. These findings support the hypothesis that the *PARP* $-1963A \rightarrow G$ and $+28077G \rightarrow A$ SNPs identified in the present study can influence enzyme activity and subsequently result in an increased risk of lupus nephritis. Moreover, the common (frequency 0.313) promoter SNP, $-1963A \rightarrow G$, was analysed for a putative transcription-factor-binding site using TFSEARCH software (Searching Transcription Factor Binding Sites, V1.3; putative score >0.90), which revealed that it is located in the consensus sequence of ADR1 and NIT2 (zinc-finger transcription factor) binding sites. Thus, a nucleotide change from A to G could disrupt the binding site of these transcription factors. The present study also suggests that $+40329T \rightarrow C$(V762A) is consistently associated with an increased risk of arthritis, while the $-1963A \rightarrow G$ SNP is associated with protection from arthritis. The location of the associated non-synonymous $+40329T \rightarrow C$(V762A) SNP in exon 17 (frequency = 0.440) in the regulatory domain of *PARP* suggests its possible involvement in functional differences mediated by alternative amino acids.

Genetic associations with SLE manifestations have been reported, but only the association between polymorphism of low-binding Fcγ receptor genes and lupus nephritis has been replicated [43]. Therefore our results need to be replicated in larger studies because there may be a problem of replication validity in this genetic association study. In addition, future studies should investigate the relationship between amino acid substitutions and the activity of this gene.

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The authors have declared no conflicts of interest.

Supplementary data

Supplementary data are available at *Rheumatology* Online.

References

1. Emlen W, Niebur J, Kadera R. Accelerated *in vitro* apoptosis of lymphocytes from patients with systemic lupus erythematosus. J Immunol 1994;152:3685–92.
2. Tan EM. Antinuclear antibodies: diagnostic markers for autoimmune diseases and probes for cell biology. Adv Immunol 1989;44:93–151.
3. Golan TD, Foltyn V, Roueff A. Increased susceptibility to *in vitro* ultraviolet B radiation in fibroblasts and lymphocytes cultured from systemic lupus erythematosus patients. Clin Immunol Immunopathol 1991;58:289–304.
4. Rosenstein BS, Rosenstein RB, Zamansky GB. Repair of DNA damage induced in systemic lupus erythematosus skin fibroblasts by simulated sunlight. J Invest Dermatol 1992;98:469–74.
5. Tsao BP, Cantor RM, Grossman JM *et al.* PARP alleles within the linked chromosomal region are associated with systemic lupus erythematosus. J Clin Invest 1999;103:1135–40.
6. Auer B, Nagl U, Herzog H, Schneider R, Schweiger M. Human nuclear NAD+ ADP-ribosyltransferase (polymerizing): organization of the gene. DNA 1989;8:575–80.
7. Oliver FJ, Menissier-de Murcia J, de Murcia G. Poly(ADP-ribose) polymerase in the cellular response to DNA damage, apoptosis, and disease. Am J Hum Genet 1999;64:1282–8.
8. D'Amours D, Desnoyers S, D'Silva I, Poirier GG. Poly(ADP-ribosyl)ation reactions in the regulation of nuclear functions. Biochem J 1999;342:249–68.
9. Sibley JT, Haug BL, Lee JS. Altered metabolism of poly(ADP-ribose) in the peripheral blood lymphocytes of patients with systemic lupus erythematosus. Arthritis Rheum 1989;32:1045–9.
10. Haug BL, Lee JS, Sibley JT. Altered poly(ADP-ribose) metabolism in family members of patients with systemic lupus erythematosus. J Rheumatol 1994;21:851–6.
11. Oei SL, Shi Y. Transcription factor Yin Yang 1 stimulates poly(ADP-ribosyl)ation and DNA repair. Biochem Biophys Res Commun 2001;284:450–4.
12. Criswell LA, Moser KL, Gaffney PM *et al.* PARP alleles and SLE: failure to confirm association with disease susceptibility. J Clin Invest 2000;105:1501–2.
13. Delrieu O, Michel M, Frances C *et al.* Poly(ADP-ribose) polymerase alleles in French Caucasians are associated neither with lupus nor with primary antiphospholipid syndrome. GRAID Research Group. Group for Research on Auto-Immune Disorders. Arthritis Rheum 1999;42:2194–7.
14. Boorboor P, Drescher BE, Hartung K *et al.* Poly(ADP-ribose) polymerase polymorphisms are not a genetic risk factor for systemic lupus erythematosus in German Caucasians. J Rheumatol 2000;27:2061.
15. Kono DH, Burlingame RW, Owens DG *et al.* Lupus susceptibility loci in New Zealand mice. Proc Natl Acad Sci USA 1994;91:10168–72.
16. Drake CG, Rozzo SJ, Hirschfeld HF, Smarnworawong NP, Palmer E, Kotzin BL. Analysis of the New Zealand Black contribution to lupus-like renal disease. Multiple genes that operate in a threshold manner. J Immunol 1995;154:2441–7.
17. Szabo C, Dawson VL. Role of poly(ADP-ribose) synthetase in inflammation and ischaemia-reperfusion. Trends Pharmacol Sci 1998;19:287–98.
18. Jawaheer D, Seldin MF, Amos CI *et al.* A genomewide screen in multiplex rheumatoid arthritis families suggests genetic overlap with other autoimmune diseases. Am J Hum Genet 2001;68:927–36.
19. Pascual M, Lopez-Nevot MA, Caliz R *et al.* A poly(ADP-ribose) polymerase haplotype spanning the promoter region confers susceptibility to rheumatoid arthritis. Arthritis Rheum 2003;48:638–41.
20. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1997;40:1725.
21. Gladman D, Ginzler E, Goldsmith C *et al.* The development and initial validation of the Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index for systemic lupus erythematosus. Arthritis Rheum 1996;39:363–9.
22. Livak KJ. Allelic discrimination using fluorogenic probes and the 5′ nuclease assay. Genet Anal 1999;14:143–9.
23. Hedrick PW. Gametic disequilibrium measures: proceed with caution. Genetics 1987;117:331–41.

24. Hedrick P, Kumar S. Mutation and linkage disequilibrium in human mtDNA. Eur J Hum Genet 2001;9:969–72.
25. Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 2001;68:978–89.
26. Kato N, Morita H, Sugiyama T *et al*. Evaluation of the poly(ADP-ribose) polymerase gene in human stroke. Atherosclerosis 2000; 148:345–52.
27. Cottet F, Blanche H, Verasdonck P *et al*. New polymorphisms in the human poly(ADP-ribose) polymerase-1 coding sequence: lack of association with longevity or with increased cellular poly(ADP-ribosyl)ation capacity. J Mol Med 2000;78:431–40.
28. Gaffney PM, Kearns GM, Shark KB *et al*. A genome-wide search for susceptibility genes in human systemic lupus erythematosus sib-pair families. Proc Natl Acad Sci USA 1998;95:14875–9.
29. Moser KL, Neas BR, Salmon JE *et al*. Genome scan of human systemic lupus erythematosus: evidence for linkage on chromosome 1q in African-American pedigrees. Proc Natl Acad Sci USA 1998; 95:14869–74.
30. Fougerousse F, Meloni R, Roudaut C, Beckmann JS. Dinucleotide repeat polymorphism at the human poly (ADP-ribose) polymerase gene (PPOL). Nucleic Acids Res 1992;20:1166.
31. Wang ZQ, Stingl L, Morrison C *et al*. PARP is important for genomic stability but dispensable in apoptosis. Genes Dev 1997;11:2347–58.
32. Masutani M, Suzuki H, Kamada N *et al*. Poly(ADP-ribose) polymerase gene disruption conferred mice resistant to streptozotocin-induced diabetes. Proc Natl Acad Sci USA 1999;96:2301–4.
33. Oliver FJ, de la Rubia G, Rolli V, Ruiz-Ruiz MC, de Murcia G, Murcia JM. Importance of poly(ADP-ribose) polymerase and its cleavage in apoptosis. Lesson from an uncleavable mutant. J Biol Chem 1998;273:33533–9.
34. Yu SW, Wang H, Poitras MF *et al*. Mediation of poly(ADP-ribose) polymerase-1-dependent cell death by apoptosis-inducing factor. Science 2002;297:259–63.
35. Suzuki N, Mihara S, Sakane T. Development of pathogenic anti-DNA antibodies in patients with systemic lupus erythematosus. Faseb J 1997;11:1033–8.
36. Ravirajan CT, Rahman MA, Papadaki L *et al*. Genetic, structural and functional properties of an IgG DNA-binding monoclonal antibody from a lupus patient with nephritis. Eur J Immunol 1998;28:339–50.
37. Morel L, Rudofsky UH, Longmate JA, Schiffenbauer J, Wakeland EK. Polygenic control of susceptibility to murine systemic lupus erythematosus. Immunity 1994;1:219–29.
38. Mohan C, Alas E, Morel L, Yang P, Wakeland EK. 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 1998;101:1362–72.
39. Waters ST, McDuffie M, Bagavant H *et al*. Breaking tolerance to double stranded DNA, nucleosome, and other nuclear antigens is not required for the pathogenesis of lupus glomerulonephritis. J Exp Med 2004;199:255–64.
40. Decker P, Briand JP, de Murcia G, Pero RW, Isenberg DA, Muller S. Zinc is an essential cofactor for recognition of the DNA binding domain of poly(ADP-ribose) polymerase by antibodies in autoimmune rheumatic and bowel diseases. Arthritis Rheum 1998;41:918–26.
41. Reumaux D, Meziere C, Colombel JF, Duthilleul P, Mueller S. Distinct production of autoantibodies to nuclear components in ulcerative colitis and in Crohn's disease. Clin Immunol Immunopathol 1995;77:349–57.
42. Decker P, Isenberg D, Muller S. Inhibition of caspase-3-mediated poly(ADP-ribose) polymerase (PARP) apoptotic cleavage by human PARP autoantibodies and effect on cells undergoing apoptosis. J Biol Chem 2000;275:9043–6.
43. Karassa FB, Trikalinos TA, Ioannidis JP. The Fc gamma RIIIA-F158 allele is a risk factor for the development of lupus nephritis: a meta-analysis. Kidney Int 2003;63: 1475–82.