An epistatic effect of the female-specific loci on the development of autoimmune vasculitis and anti-nuclear autoantibody in murine lupus

Extended report

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Abstract

Objective. To identify the genetic loci regulating incidence and severity of renal autoimmune vasculitis developed in murine lupus.

Methods. Vasculitis of renal arteries was histopathologically evaluated in MRL/Mp-*Fas*^{lpr} (MRL/lpr), C57BL/6-*Fas*^{lpr} (B6/lpr), (MRL/lpr × B6/lpr) F_1 and MRL/lpr × (MRL/lpr × B6/lpr) F_1 backcross mice. Using genomic DNA samples of the backcross mice, genome-wide scan, association study and linkage analysis were carried out based on genotypes of polymorphic microsatellite markers. Correlations of vasculitis grade and levels of various autoantibodies were also evaluated.

Results. we identified two recessive susceptibility loci of MRL allele on chromosomes 4 and 1, which previously had been defined as the autoimmune-related loci termed *Arvm1* and *Sle-1/Nba2*, respectively. The former was epistatic to the latter in a female-specific manner. The titer of anti-nuclear autoantibody (ANA) in IgG class, but not ANA in IgM class or anti-dsDNA in both IgG and IgM, significantly correlated to vasculitis grade.

Conclusion. The present loci have been reported in previous studies using the different set of strains of mice, suggesting a significance of these loci in developing autoimmune vasculitis in murine models. Coincidence of the development of autoimmune vasculitis and ANA in IgG class suggests a shared genetic factor regulatory to these traits.

Introduction

A number of studies have demonstrated a significance of genetic predisposition in developing common autoimmune diseases including systemic lupus erythematosus (SLE). Taking advantage of inbred genetic composition and high genetic penetrance to disease onset, murine autoimmune models have provided not only evidences to support the notion on genetic predisposition, but also valuable clues to understand generalized genetic interpretations for human diseases, i.e., polygenic modes of inheritance, candidate genes and genetic interaction.

The MRL/lpr mice spontaneously develop a spectrum of autoimmunity resembling human SLE. These mice stably develop glomerulonephritis, systemic vasculitis, arthritis and sialadenitis coincidentally with serum autoimmune traits and remarkable lymphoproliferation [1]. MRL/lpr is the mutant strain defective in expression of the *cd95* gene [2]. As *cd95* encodes the receptor CD95 that mediates apoptosis signal in lymphocytes [3], its defective mutation (*lpr*) may explain lymphoproliferation and a defect in activation-induced cell death of T cells [2][4][5]. The *cd95* sufficient mice MRL/+ are known to develop glomerulonephritis and pancreatitis with serum autoimmune traits very late in life [6], but the severity of glomerulonephritis and the levels of autoantibodies are much less than those in MRL/lpr, indicating that the *lpr* is primarily associated with the accelerated pathogenesis of autoimmunity in MRL/lpr mice. Importantly, the *lpr* congenic mice B6/lpr barely develop autoimmune diseases [1]. This fact further indicates that MRL-specific genetic factors other than the *lpr* are prerequisite to the development of autoimmune diseases in MRL/lpr mice [7][8]. Serum autoimmune traits such as ANA and anti-dsDNA antibody are also known to depend on a genetic background other than the *lpr* [8]. To date, the question has been posed as to strain-specific genetic factors critically involved in developing autoimmune traits.

We previously reported that renal vasculitis and glomerulonephritis were genetically segregated in the MRL/lpr×(MRL/lpr×B6/lpr) F_1 backcross mice [9]. The results suggested a differential genetic control for the development of each disease. However, substantial information in this concern remains to be known. The present study primarily aimed to solve problems on genetic loci associated with vasculitis. To achieve this aim, we here prepared a large number of MRL/lpr×(MRL/lpr×B6/lpr) F_1 backcross mice (MBN_2). A total of 458 MBN_2 mice were subjected to genetic study to define susceptibility loci to renal vasculitis. The quantitative trait locus (QTL) analysis finally demonstrated two significant loci to interpret the pathogenesis in the female mice. The two loci were located on chromosomes 4 and 1 in a recessive susceptibility mode of inheritance, and the former locus had an epistatic effect on the latter. As the latter locus overlapped the common interval previously represented as *Sle-1* or *Nba2*, which was known to be crucially associated with ANA production [10], we examined if there was correlation between vasculitis and autoantibody emergence in MBN_2 female mice. We will present a new evidence for epistatic interaction of the known loci and a serum autoimmune trait indicative of autoimmune vasculitis.

Methods

Mice. MRL/lpr and B6/lpr mice were originally purchased from The Jackson Laboratory (Bar Harbor, ME) and bred under specific-pathogen-free condition in the Integrated Center for Sciences (INCS) in Ehime University. Using the (MRL/lpr×

B6/lpr) F_1 (MBF_1) and the MRL/lpr×(MRL/lpr×B6/lpr) F_1 backcross mice (MBN_2) were prepared and housed in the same center and in the laboratory animal center of Kawashima Co. Ltd., Gifu, Japan. For all animal experiments, we observed the Ehime University guidelines for animal experimentation.

Histological examination and grading criteria of vasculitis lesion. Mice were killed under ether anesthesia at 19-20 weeks of age. Serum samples were collected and stored at -80℃ until use. The kidneys were fixed with 10% formalin in 0.01 M phosphate buffer (pH 7.2), and embedded in paraffin. Tissue sections were stained with hematoxylin and eosin (H&E) or elastica-Masson (EM) method for histological examination by light microscopy. Histopathological grading for renal vasculitis lesion was performed as described elsewhere [11]. In brief, severity of vasculitis was graded as follows: 0, normal to minimal perivacular lymphocyte infiltration; 1, moderate perivascular cell infiltration associated with destruction of external elastic lamina; 2, intimal thickening with the destruction of internal elastic lamina in addition to the findings of grade 1. We observed more than 20 arteries in 6 sections prepared from bilateral kidneys, and defined vasculitis grade of animal with the maximal grade in those observations. An animal with one or more vasculitis lesions diagnosed as grade 1 or 2 was considered to have vasculitis.

Measurements of autoantibodies. The titer of ANA was measured with immunoreactivity to murine normal hepatocytes as described previously [12]. Nuclear samples were prepared by stamping minced liver tissue on glass slide, dried completely and fixed in ice-cold acetone for 10 min. Prior to co-incubation of serum samples and nuclear samples, serum samples were incubated with 5 μg/ml dsDNA for 2 h at 4℃ to adsorb possible cross-reactivity to dsDNA. After masking nuclear samples with 10% normal goat serum, serial dilutions of serum sample were incubated on nuclear samples for 2 hr at 4℃. Washed with PBS, those were further incubated with secondary antibody of FITC-conjugated anti-mouse IgG, γ chain specific (Zymed, South San Francisco, CA) or anti-mouse IgM, μ chain specific (Zymed).

Titer index of ANA was determined with maximal dilution point to visualize nuclear signal; 0, no staining with 1/100 dilution; 1, faintly stained with 1/100 dilution; 2, strongly stained with 1:100 dilution; 3, stained with $1:10^3$ dilution; 4, stained with $1:10^4$ dilution; 5, stained with $1:3\times10^4$ dilution; 6, stained with $1:9\times10^4$ dilution. Anti-dsDNA titers were measured by ELISA as described previously [13]. A unit for anti-dsDNA titer was defined as an equivalent titer in the serum of female MRL/lpr mice.

Mapping of vasculitis susceptibility loci. Genotypes of the MBN_2 mice were determined by microsatellite analyses. We first performed the selective mapping using 48 samples consisted of 24 non-affected (grade 0) and 24 affected mice (grade 2). A total of 126 microsatellite markers were used, which provided a full coverage of mouse autosomes with an average of 12.9 cM and with a maximum distance of 23 cM. The candidate chromosomes were determined with a statistical index ($p<0.05$) given by the chi-square test. Second, QTL analysis was performed on candidate chromosomes using whole MBN_2 samples. We determined the polymorphism of the *cd72* gene (forward 5'-CAGCGGCTTAGAGTTGC-3' and reverse 5'-GCAGGGCTTATGGAAGTAAT-3') and the *fcgr2b* gene, for which primers was kindly provided by Dr. S. Hirose (Juntendo University School of Medicine, Tokyo, Japan). The genetic positions of microsatellite

markers and genes were based on information from the Mouse Genome Informatics (MGI), The Jackson laboratory.

Statistical analysis. In association study, $p<0.0034$ ($\chi^2>8.58$, 1df) and $p<0.0001$ ($\chi^2>15.13$, 1df) were accepted as suggestive and significant linkage, respectively [14]. The QTL analysis was performed by using Windows QTL Cartographer (V2.5) software developed by Zeng et al with histopathological grades of vasculitis as the indicator for phenotype [15]. In detail, composite interval mapping of Model 6 was adopted, and the control marker number and window size was 5 and 10 cM, respectively. The walk speed was 2 cM, and the forward regression method was selected. The threshold level of statistical significance for QTL was determined by the permutation test (1000 times permutation at $p=0.05$) developed by Churchill and Doerge [16]. The Kruskal-Wallis test was used to determine the association of ANA titer to vasculitis. We regarded $p<0.05$ as significance in the Kruskal-Wallis test and the chi-square test, and $p<0.01$ in paired comparison in three groups.

Results

Incidence of renal vasculitis. In MBF_1, MBN_2 and MRL/lpr, renal vasculitis was characterized by a granulomatous arterial lesion associated with mononuclear cell infiltration in perivascular region and the destruction of external elastic lamina. In addition, some mice displayed intimal thickening accompanied by the destruction of internal elastic lamina. In an affected mouse with vasculitis, we could identify a few or mostly one lesion in 20 arteries observed, suggesting a sporadic incidence of vasculitis in the kidney. Irrespective of the severity, vasculitis generally affected a larger artery such as interlobar or arcuate artery in the kidney. These pathologic characters were very similar to those observed in MRL/lpr mice and intercrosses of MRL/lpr and C3H/HeJ-*Fas*^{lpr} (C3H/lpr) strains [11]. The histological evaluation on the severity of vasculitis in B6/lpr, MBF_1 and MBN_2 groups of mice are summarized in Table 1. There were no incidence of vasculitis observed in B6/lpr group. On the other hand, MBF_1 and MBN_2 groups showed the incidence of vasculitis by the rates of 20.4% and 39.1%, respectively. These rates were much lower than that of parental MRL/lpr gourp (82.9%; $p<0.01$ for MBF_1 and MBN_2). Vasculitis seemed inherited from MRL/lpr in an incompletely recessive manner. Sex difference in vasculitis incidence was not evident in all affected groups.

Table 1. Incidence of vasculitis in MRL/lpr, B6/lpr MBF_1 and MBN_2 mice.

	Vasculitis grade			incidence‡	
Strain, sex	0	1	2	No.	(%)
MRL/lpr					
Female	10	26	20	46/50	(82.1)
Male	4	9	13	22/26	(84.6)
Total	14	35	33	68/82	(82.9)
B6/lpr					
Female	10	0	0	0/10	(0)
Male	10	0	0	0/10	(0)
Total	10	0	0	0/10	(0)
MBF_1*					
Female	25	7	1	8/33	(24.2)
Male	18	3	0	3/21	(14.3)
Total	43	10	1	11/54	(20.4)
MBN_2†					
Female	124	49	37	86/210	(41.0)
Male	155	64	29	93/248	(37.5)
Total	279	113	66	179/458	(39.1)

* (MRL/lpr x B6/lpr)F_1.

† MRL/lpr x (MRL/lpr x B6/lpr)F_1.

‡ Grades 1 and 2 were regarded as positive individuals for vasculitis.

Mapping of vasculitis susceptibility loci. We first determined four candidate chromosomes in a genome-wide search with 126 informative microsatellite markers, then in an association study with all samples, defined two markers, *D4Mit271* (20.8 cM) and *cd72* (22.5 cM), on chromosome 4 with statistically suggestive linkage ($p < 0.0034$) (Table 2A). Of particular importance, the significant linkage to these markers was shown only in the female group ($p<0.0001$). These results were supported by QTL analysis, in which the highest LOD score (2.083) was given at *cd72* only by the female group (Figure 1A).

Table 2. Association of microsatellite genotype and incidence of renal vasculitis in MBN $_2$ mice.

Chromosome/Sex	Position	Vasculitis					
		Negative*		Positive*			
Marker	cM	*MM* †	*MB*	*MM*	*MB*	χ^2, 1 df	*p* ‡
A.							
Chr.4/Female							
D4Mit319	12.1	47	77	54	32	12.6	0.00039
D4Mit271	20.8	47	77	58	28	17.7	< 0.0001§§
cd72	22.5	46	78	56	30	16.0	< 0.0001§§
D4Mit17	31.4	52	72	58	28	13.2	0.00027
Chr.4/Male							
D4Mit319	12.1	70	85	48	45	0.97	0.32
D4Mit271	20.8	74	81	49	44	0.57	0.45
cd72	22.5	71	84	49	44	1.10	0.29
D4Mit17	31.4	65	90	44	49	0.68	0.41
Chr.4/Total							
D4Mit319	12.1	116	163	102	77	10.4	0.0013
D4Mit271	20.8	120	159	107	72	12.3	0.0005§
cd72	22.5	116	163	105	74	12.7	0.0004§
D4Mit17	31.4	116	163	102	77	10.4	0.0013
B.							
Chr.1/Female							
D1Mit268	83.4	56	68	47	39	1.83	0.18
fcgr2b	92.3	54	70	50	36	4.32	0.04
D1Mit356	95.8	56	68	51	35	4.06	0.04
D1Mit291	101.5	60	64	44	42	0.16	0.69
Chr.1/Male							
D1Mit268	83.4	68	87	48	45	1.40	0.24
fcgr2b	92.3	69	86	48	45	1.17	0.28
D1Mit356	95.8	69	86	49	44	1.56	0.21
D1Mit291	101.5	69	86	49	44	1.56	0.21
Chr.1/Total							
D1Mit268	83.4	124	155	95	84	3.25	0.07
fcgr2b	92.3	123	156	98	81	4.96	0.03
D1Mit356	95.8	125	154	100	79	5.34	0.02
D1Mit291	101.5	129	150	93	86	1.43	0.23

* Grades 1 and 2 were regarded as positive individuals for vasculitis.

†Genotypes of *MM* and *MB* indicate MRL/MRL homozygote and MRL/B6 heterozygote, respectively.

‡ The chi-square test.

§ Significant linkage.

§§ Suggestive linkage.

Epistasis of the two loci associated with incidence and severity of vasculitis.

The distal region of mouse chromosome 1 has been frequently reported as a susceptibility locus to autoimmune traits, including glomerulonephritis and autoantibody emergence [10][17][18][19]. The present results showed a trace linkage of this region to vasculitis, though it was not supported by statistics (Table 2B). We then sorted a large panel of mice into two groups by the *cd72* genotype, thereafter termed MM-group (MRL homozygote) and MB-group (MRL/B6 heterozygote), and each group was analyzed in the same way as above. Notably, we could identify a suggestive linkage of *fcgr2b* (92.3 cM) and *D1Mit356* (95.8 cM) in the MM-group (p<0.0034), whereas no linkage was shown in the MB-group (Table 3). In QTL analysis, the MM-group, but not the MB-group, again demonstrated a significant linkage (LOD = 3.408) at *D1Mit356*

Table 3. Association of microsatellite genotype and incidence of renal vasculitis in *CD72MM* -group* of MBN_2 mice.

		Vasculitis					
Chromosome/Se›	Position	Negative†		Positive†			
Marker	cM	*MM* ‡	*MB*	*MM*	*MB*	χ^2, 1 df	*p*
Chr.1/Female							
D1Mit268	83.4	17	28	32	24	3.75	0.05
fcgr2b	92.3	13	32	32	24	8.06	0.005
D1Mit356	95.8	13	32	32	24	8.06	0.005
D1Mit291	101.5	15	30	28	28	2.83	0.09
Chr.1/Male							
D1Mit268	83.4	25	46	24	25	2.27	0.13
fcgr2b	92.3	24	47	24	25	2.78	0.10
D1Mit356	95.8	24	47	25	24	3.56	0.06
D1Mit291	101.5	24	47	24	25	2.78	0.10
Chr.1/Total							
D1Mit268	83.4	42	74	56	49	6.55	0.01
fcgr2b	92.3	37	79	56	49	10.4	0.0013§
D1Mit356	95.8	37	79	57	48	11.3	0.0008§
D1Mit291	101.5	39	77	52	53	5.75	0.02

* *CD72MM* -group includes MRL/MRL homozygotes at the *cd72* locus.

† Grades 1 and 2 were regarded as positive individuals for vasculitis.

‡ Genotypes of MM and MB indicate MRL/MRL homozygote and MRL/B6 heterozygote, respectively.

§ Suggestive linkage, the chi-square test

(Figure 1B).

Next, we evaluated a combined effect of the two susceptibility loci, *D1Mit356* and *cd72*, on the incidence or the severity of vasculitis (Table 4). The two susceptibility loci showed an additive effect on the incidence and the severity of vasculitis in a

female-specific manner. On the other hand, the additive effect was not proven statistically in the male group. Importantly, a susceptible contribution of the *D1Mit356* locus to the incidence and the severity of vasculitis was highly influenced by the *cd72* locus. These findings indicate an epistasis of the *cd72* locus to the *D1Mit356* locus.

Table 4. Epistatic interaction of the *Arvm1* locus (*cd72*) and *D1Mit356*.

	genotype		incidence*			
Sex	*cd72*	*D1Mit356*	*(%)*		mean of grade	
Female						
	MM †	*MM*	71.1	‡	1.04	§
	MM	*MB*	42.9		0.57	
	MB	*MM*	30.6		0.47	
	MB	*MB*	23.4		0.32	
Male						
	MM	*MM*	51.0		0.65	
	MM	*MB*	33.8		0.42	
	MB	*MM*	34.8		0.48	
	MB	*MB*	33.9		0.46	
Total						
	MM	*MM*	60.6	‡	0.84	§
	MM	*MB*	37.8		0.49	
	MB	*MM*	32.8		0.47	
	MB	*MB*	29.2		0.40	

* Percentage of positive individuals for vasculitis (grades 1and 2).

†Genotypes of *MM* and *MB* indicate MRL/MRL homozygote and MRL/B6 heterozygote, respectively.

‡ $p<0.01$, The chi-square test.

§ $p<0.01$, The Mann-Whitney's test.

Autoantibody correlated to vasculitis. It has been frequently reported that an autoantibody emergence is linked to a genetic interval in the distal region of chromosome 1 in murine lupus models [10][17][18][19]. Because the present results proved a genetic linkage of this region to vasculitis in the female group, we examined a quantitative correlation between vasculitis and autoantibody in the female group. Titers of ANA and anti-dsDNA were measured in the serum samples collected from the 181 MBN_2 female mice. The results indicated a significant correlation between a titer of ANA in IgG class and vasculitis in the MM-group of the *cd72* locus ($p=0.00064$), while no correlation was proven in the MC-group ($p=0.31$). Titers of ANA in IgM class and anti-dsDNA in both classes were not significantly associated with vasculitis (Table 5).

Table 5. Correlation of autoantibody titer and vasculitis grade in MBN_2 female mice.

Genotype of *cd72*,	vasculitis grade			
Autoantibody	0	1	2	*p*
CD72-MM *	N=37	N=31	N=19	
ANA-IgM	1.81 (0.97)†	1.77 (1.15)	2.11 (0.88)	0.46
ANA-IgG	3.27 (0.84)	4.06 (1.09)	4.31 (1.11)	0.00064§§
dsDNA-IgM	0.47 (0.53)	0.44 (0.38)	0.51 (0.48)	0.41
dsDNA-IgG	0.19 (0.26)	0.23 (0.24)	0.14 (0.09)	0.59
CD72-MB *	N=70	N=13	N=11	
ANA-IgM	1.91 (1.11)	2.08 (1.19)	2.00 (0.77)	0.74
ANA-IgG	3.78 (1.23)	4.08 (1.32)	4.45 (1.37)	0.31
dsDNA-IgM	0.56 (0.54)	0.55 (0.49)	0.67 (0.48)	0.66
dsDNA-IgG	0.37 (0.56)	0.18 (0.19)	0.68 (1.15)	0.29

* Genotypes of *CD72-MM* and *CD72-MB* indicate MRL/MRL homozygote and MRL/B6 heterozygote at the *cd72* locus, respectively.

† Mean titer value (S.D.) of autoantibody.

§§ Significant differece in autoantibody titers by the 3 vasculitis grades, the Kruskal-Wallis test

Discussion

The two loci identified in this study remind previous studies indicating similar autoimmune loci to ours. The chromosome 4 locus in this study was found to overlap the *Arvm1* locus identified in the previous study [11]. The *Arvm1* locus was demonstrated as a significant susceptibility locus to renal vasculitis in analyzing differently backcrossed mice MRL/lpr×(MRL/lpr×C3H/lpr) F_1 (MCN_2). That study further suggested that allelic variant of *cd72* gene was a candidate of genetic factors for vasculitis in the *Arvm1* locus. Indeed, primary structure of CD72 is highly polymorphic between MRL/lpr and C3H/lpr [11]. CD72 is an inhibitory receptor to influence on B cell antigen receptor signaling [20][21][22]. It may be possible that such allelic difference of CD72 affects B cell antigen receptor signaling, and somehow develops autoimmunity. Since primary structure of CD72 of B6/lpr is totally same as that of C3H/lpr, the *cd72* gene is highly polymorphic between MRL/lpr and B6/lpr [11][23]. Again, this study supports the consideration on candidacy of the *Arvm1* locus. We would like to emphasize a significance of the *Arvm1* locus in developing autoimmune vasculitis in MRL/lpr genetic background.

The present findings demonstrated a female-specific efficacy of the *Arvm1* locus in MBN_2 mice. This is not true of the previous conclusion with MCN_2 mice. Three possible reasons may be considered for this discrepancy. The first reason is an suppressive effect of minor loci in B6/lpr on the susceptibility of the *Arvm1* locus in a male specific manner. We might look over such minor loci in this study. The second reason is an epistatic effect of B6/lpr Y chromosome on the *Arvm1* locus. The Y chromosome of MCN_2 male mouse was originated from C3H/lpr, while that of MBN_2 male mouse was originated from B6/lpr. To prove this point, we need to study further

with reciprocal breeding to generate MBN_2 mice. The third reason is a difference of the gene(s) truly involved in vasculitis proven in MCN_2 and MBN_2 cases. In this case, the function of susceptibility gene could be controlled in a sex-sensitive manner. Further studies are needed to validate these possible reasons.

The distal region of chromosome 1 defined in this study overlaps the loci previously defined as autoimmune susceptibility ones. Several lines of studies with different combinations of mouse strains and the congenic strains harboring this region have emphasized the significance of this region in the development of autoimmunity, including glomerulonephritis and emergence of ANA [10][24][25]. It is a new finding that this region is a potent susceptibility locus to renal vasculitis in a sensitive mode to the *Arvm1* locus. This region is known to consist of many genes of immunological importance. For examples, there can be listed the inhibitory Fc receptor gene (*fcgr2b*) [26][27][28], the signaling lymphocyte-activation molecule (SLAM) family genes [29][30][31][32][33][34] and interferon-inducible protein genes (*Ifi*) [35][36]. Recently accumulating evidence has delineated genetic polymorphism of this region in relation to mouse autoimmunity. According to the latest evidence, B6/lpr belongs to the group III of *fcgr2b* gene and the haplotype 1 of SLAM family genes, typical of non-autoimmune prone haplotypes. On the other hand, MRL/lpr and C3H/lpr can be defined as the group I or *autoimmune fcgr2b prompter haplotype* (AIH) of *fcgr2b* gene, and the haplotype 2 of SLAM family genes, typical of autoimmune prone haplotypes [37][38][39]. It may be reasonable that this haplotypic difference between B6/lpr and MRL/lpr is also involved in the development of vasculitis in association to ANA production. It is of particular interest to identify the gene(s) in this region commonly involved in a broad spectrum of autoimmunity. In the previous study using MCN_2 mice, this region was not shown to be susceptible to vasculitis. This may be due to an overall correspondence of this region between C3H/lpr and MRL/lpr.

Our present data demonstrated an epistatic effect of the *Arvm1* locus on susceptibility of the chromosome 1 locus. Further studies should be achieved to know the mechanism of epistatic interaction of the two loci. Interestingly, recent study on human SLE has shown that *cd72* polymorphisms associated with alternative splicing modify susceptibility to human SLE through epistatic interaction with *fcgr2b* [40]. The human loci including *cd72* and *fcgr2b* genes are syntenic of the *Arvm1* locus and the distal region of chromosome 1, respectively. Although it is unclear whether the epistatic effect in human SLE is related to vasculitis or not, the notion of an epistatic mode of complex loci may be important to understand a genetic basis for pathogenesis of SLE.

SLE usually develops a variety of diseases, including glomerulonephritis, vasculitis, dermatitis, interstitial pneumonia and so forth. Currently increasing evidence from genetic studies on mouse SLE model has been demonstrated that the development of each disease in SLE is controlled by a different set of genetic factors [9]. This may provide a possible interpretation for the pathogenic mechanism by which human SLE appears in varied spectrums of diseases. Increased serum level of autoantibody was a universal feature of SLE both in human and in murine models. Many kinds of autoantibody are detectable there. Little is known of a genetic basis for coincidence of autoantibodies and diseases, and specific correlation of each class of autoantibody and an elemental disease in SLE. Although the present study was not conducted to fully address this issue, our findings indicate correlation between ANA in IgG and vasculitis. Moreover, the difference in the contribution of each class of autoantibody to vasculitis

suggests differential genetic regulation for the development of those antibodies as suggested previously [8][41]. ANA and anti-dsDNA are known to correlate to the onset of glomerulonephritis, thus a single specificity of ANA to vasculitis is not highly evaluated. Combinatory evaluations of two or more autoantibodies may be useful for specific diagnosis of SLE variants.

In conclusion, we defined a female-specific vasculitis locus on chromosome 4 using MRL/lpr×(MRL/lpr×B6/lpr) F_1 backcross mice. Epistasis of this locus was demonstrated to the major autoimmune-related locus on chromosome 1. Taking advantage of murine autoimmune model, we should make more efforts to uncover genetic interaction modes of the complex loci associated with polygenic diseases. Further understanding in this concern will provide a new insight into the pathogenic mechanism for human SLE.

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Competing interests

The authors have no competing interests to disclose.

Figure legend

Figure 1. Plots of the logarithmic odds (LOD) scores of QTL for renal vasculitis on (A) chromosome 4 and (B) chromosome 1. We adopted composite interval mapping of Model 6 in the Windows QTL Cartographer (V2.5) software. The control marker number and window size were 5 and 10 cM, respectively. Horizontal lines indicate the threshold levels of statistic significance, which were determined by the permutation test (1000 times permutations, p=0.05). Genetic positions with MIT markers are indicated on horizontal axis.

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Figure 1, Zhang, et al.