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Screening of genetic loci predisposing to herpes simplex virus infection on mouse chromosome 17

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ABSTRACT

Aim: The herpes simplex virus (HSV), one of the most common viruses infecting humans, is featured by a high infection rate and usually causes complex disorders difficult to diagnose and treat. Disease progression is always combined with the specific interaction between organism and environment, but genetic factors play a decisive role in most pathogenic processes. Like most human disorders, individual difference has also been involved in the pathogenesis of HSV infection. The present study aimed to screen the potential gene loci that regulates human predisposition to HSV infection. **Methods:** With reference to previous studies, inbred mouse lines with significantly distinct predisposition to HSV infection were chosen for gene loci screening. Gene sites on mouse chromosome 17 associated with susceptibility to HSV infection were then identified by correlation analysis and genome-wide scanning technique. **Results:** Genes affecting the vulnerability of mice to HSV infection were mapped to three regions on the 17th mouse chromosome, D17MIT51.1, D17MIT39.1 and the region between D17MIT180.1 and D17MIT184. **Conclusion:** The results suggest that the mouse genetic background plays an important role in its susceptibility to HSV-1 infection, which might be regulated by multiple predisposing quantitative trait loci.

INTRODUCTION

As an infection relapse could confer severe consequence in the pathogenicity of herpes simplex virus (HSV) infection, avoiding infection and preventing recurrence after treatment is of great importance. Individual differences involved in the pathogenesis of HSV infection in mice have been long studied. Several reports related to HSV infection susceptibility further pointed to the role of genetic background in the HSV infection process.[1-5] To further analyze the susceptibility to HSV infection in different inbred mouse lines, we scanned the 17th mouse chromosome for gene sites associated with it using the correlation analysis and

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genome-wide scanning technique.

METHODS

Genomic DNA extraction

Firstly, 50-100 frozen tissue samples were weighed, grinded into powder in liquid nitrogen using a grinding bowel and pestle, and then immediately mixed with 1 mL Tripure. Tissue sample powder was then further homogenized 10 times using a homogenizing drill on ice for 20 min until no tissue fluid particles was visible, centrifuged at the speed of 12,000 *g* at 4 ℃, for 10 min. The homogenate was then kept at room temperature for 5 min to make sure the nuclear protein was totally separated. Each milliliter homogenate was mixed with 0.3 mL chloroform and shaken vigorously at 4 ℃ for 15 s, kept at room temperature for 2-15 min, then Centrifuged at 12,000 *g* at 4 ℃, for 15 min. To get high quality DNA, the upper layer of colorless aqueous liquid after centrifugation was removed. Each milliliter homogenate was mixed with 0.2 mL 100% ethanol which was stored at 4 ℃, mixed completely by rotating the bottle upside down several times and kept at room temperature for 2-3 min for DNA precipitation. After centrifuging at 2,000 *g* for 5 min at 4 ℃, the upper layer liquid was removed with a pipette carefully. Each milliliter sample solution was then mixed with 1 mL of 0.1 mol/L sodium citrate dissolved in 10% ethanol, kept at room temperature for 30 min with frequent mixing, and centrifuged at 4 ℃ for 5 min at 2,000 *g* again. The upper layer liquid was collected, mixed with 75% ethanol, and kept at room temperature for 30 min with frequent mixing. Then, after centrifuging at 4 ℃ and 2,000 *g* for 5 min, the upper layer liquid was removed and the DNA sample was dried in the air or vacuum for 5-10 min. Finally, we dissolved the DNA with 50 uL TE solution, pipetted out 1 uL sample for color comparison and another 10 uL for electrophoresis, and the residual was stored at -20 ℃ for further analysis. DNA samples were diluted with 90 uL MQ water and analyzed with ultraviolet spectrophotometer. The OD260 value, OD280 value and OD260/280 value were used for calculating the concentration of DNA.

Primer design and synthesis

Primer design referenced information from the mouse genome program (detailed information can be viewed on the website of Jackson Laboratory), which was designed by Shanghai Jikang Biotechnology Limited Company [Table 1]. The detailed information about PCR reaction system and PCR reaction condition can be seen in Table 2.

Microsatellite loci detection

First, 1 mL Hi-Di Formamide was mixed with 50 uL GeneScan -500 LIZ Size Standard, then mixed with 9.5 uL polymerase chain reaction (PCR) product, which was diluted 20 times. Tubes containing the above solution were placed in the PCR instrument for degeneration at 95 ℃ for 5 min, kept on ice for more than 5 min. Using an ABI PRISMTM 310 genetic analyzer of the ABI Company for electrophoresis, the voltage was set to 15 KV and run at 60 ℃ for 28 min. The samples were then collected for further analysis.

Electrophoresis data processing

By using the software Genescan (311) and Genetyper (3.7), we could get the detected fragment size. The equipment was provided by ABI Company; the PCR amplification reagents by Baosheng Bioengineering Limited Company; and the fluorescent primers by Shanghai Jikang Biotechnology Limited Company.

RESULTS

Scanning 12 microsatellite spots on the 17th chromosome of two mouse lines

The differences of 12 microsatellite loci between two inbred mouse lines BALB/C and C57BL/6 were first scanned. Among the loci scanned, seven of them were significantly different: D17MIT245.1, D17MIT46, D17MIT51.1, D17MIT180.1, D17MIT20. 1, D17MIT184 and D17MIT39.1 [Table 3; Figure 1].

Scanning microsatellite loci on the 17th chromosome using three inbred mouse lines

To minimize false-positives among the above seven sites obtained using the two inbred mouse lines, we further searched the literature and found that another inbred mouse line, DBA-2, had similar susceptibility to HSV infection as BALB/C mice. Therefore, scanning these three inbred mouse lines for microsatellite loci led to the exclusion of two of the above seven loci, D17MIT245.1 and D17MIT46. Our updated scanning results showed that D17MIT51.1, D17MIT39.1 and the genomic region between D17MIT180.1 and D17MIT184 were mouse microsatellite regions affecting susceptibility to HSV infection [Table 4; Figure 2].

Bioinformatic analysis of genes in the HSV infection susceptibility regions on chromosome 17

For identifying potential genes involved in the susceptibility of mice to HSV infection, we used bioinformatics to analyze the genes localized in these regions. Based on the above results, bioinformatic analysis found approximately 140 genes in the positive sites D17MIT51.1, D17MIT39.1 and the region between D17MIT180.1 and D17MIT184 [Tables 5-7]. Among those genes, there were about 33 human

Table 1: Primer sequences for scanning microsatellite loci on the 17th chromosome of mouse genome

Loci name		Primer sequence	Genetic distance (cM)
D17MIT245.1	Forward	FAM-TGTGCTCTGGCTAGGGAGTT	3
	Reverse	CACATTCATATGTACACACACATGC	
D17MIT143.2	Forward	FAM-CTTACAAGCATCCTGTGGAACTC	5
	Reverse	GAGGACCAACAGTCAAACATAGC	
D17MIT46	Forward	FAM-TCCACCCCACTACCTGACTC	11.7
	Reverse	CCCTTCTGATGACCACAGGT	
D17MIT146.1	Forward	FAM-CTGTCAGCAGAACGTTCCTTAGT	17.1
	Reverse	CCAACTCAAGCCTTACATAGTGG	
D17MIT51.1	Forward	FAM-TCTGCCCTGTAACAGGAGCT	22.9
	Reverse	CTTCTGGAATCAGAGGATCCC	
D17MIT10.1	Forward	FAM-TGCACTTGCATAAGGAAAAC	24.5
	Reverse	GACTTTGGGGCCTACTTATG	
D17MIT180.1	Forward	FAM-AGACACTGTCTAAAAACACAAGATGG	29.4
	Reverse	TTGTGTTCATATGCATGTGTGC	
D17MIT20.1	Forward	FAM-AGAACAGGACACCGGACATC	34.3
	Reverse	TCATAAGTAGGCACACCAATGC	
D17MIT184	Forward	FAM-TGCACTACCCAAACATGCAT	38.5
	Reverse	ACTTCTGACAGGAAGCATCCA	
D17MIT93.1	Forward	FAM-TGTCCTTCGAGTGTTTGTGTG	44.5
	Reverse	TCCCCGGTGAATGAGTTATC	
D17MIT39.1	Forward	FAM-CCTCTGAGGAGTAACCAAGCC	45.3
	Reverse	CACAGAGTTCTACCTCCAACCC	
D17MIT122.1	Forward	FAM-TCTCTTCACTGCAATGGAACA	51.9
	Reverse	GAACCTATAGGCTCTTGAATAGATGG	

homologous genes that showed some of the following characteristics: (1) containing many quantitative trait loci, such as epididymal fat pad weight quantitative trait loci (QTL) 3, subcutaneous fat pad weight QTL 4, spleen weight QTL 9, *etc*.; (2) containing some genes related to the important physiological functions of the body such as Mut methylmalonyl-Coenzyme A mutase; (3) containing genes related to the developmental and physiological function such as early growth adjusted QTL 2, early growth QTL 5, *etc*.; (4) containing genes associated with some diseases such as the Down syndrome critical region gene 1-like 1, MSM lymphoma resistance 1, *etc*.; (5) containing mouse tissue associated antigen H-2.

Table 2: PCR reaction system and PCR reaction condition

PCR reaction system (total volume: 10 uL)	
Non-enzyme water	5.4 μL
10 × PCR buffer	1.0 uL
Mg^{2+} (25 mmol/L)	0.5 uL
dNTP (each 2.5 mmol/L)	1.0 μL
P1 (5 pM)	0.5 uL
P2 (5 pM)	0.5 uL
Template DNA (30-50 ng/uL)	1.0 uL
Ex-Taq enzyme (5 U/μL)	0.1 μL
PCR reaction condition	
95 ℃	5 min
94 ℃	30 s
Time	30 s
72 ℃	30 s
Repeat the 2nd to 4th steps for totally 38 cycles	
72 ℃	10 min
Store at 4 ℃	

PCR: polymerase chain reaction

DISCUSSION

It has been widely observed that different species or even individuals of the same species show differences in response to infection, but the explanation for this phenomenon is still rather controversial. There have

Table 3: Microsatellite loci scanning using BALB/C and C57BL/6 inbred mice

Microsatellite loci	BALB/C susceptible	C57BL/6 tolerant
D17MIT245.1	**194**	**202**
D17MIT143.2	112	112
D17MIT46	**218**	**236**
D17MIT146.1	166	166
D17MIT51.1	**152**	**154**
D17MIT10.1	155	155
D17MIT180.1	**139**	**137**
D17MIT20.1	**163**	**175**
D17MIT184	**126**	**128**
D17MIT93.1	155	155
D17MIT39.1	**86**	**104**
D17MIT122.1	141	141

Microsatellite loci with difference were marked in bold

Table 4: Microsatellite loci scanning using BALB/C, DBA-2 and C57BL/6 inbred mice

Microsatellite loci	Susceptible		Tolerant
	BALB/C	DBA-2	C57BL/6
D17MIT245.1	194	200	202
D17MIT143.2	112	112	112
D17MIT46	218	208	236
D17MIT146.1	166	166	166
D17MIT51.1	**152**	**152**	**154**
D17MIT10.1	155	149	155
D17MIT180.1	**139**	**139**	**137**
D17MIT20.1	**163**	**163**	**175**
D17MIT184	**126**	**126**	**128**
D17MIT93.1	155	169	155
D17MIT39.1	**86**	**86**	**104**
D17MIT122.1	141	123	141

Microsatellite loci with difference were marked in bold

been reports suggesting that the genetic background might play an important role.[1,6-8] The causative factors for different responses to infection, the possible ways of intervention, the revolutionary changes of infection prevention, and the control that resulted from those changes have aroused great interest among the scientific community. In this study, we analyzed the genetic background that contributes to the HSV infection susceptibility.

The Herpes virus genus (Herpesviridae) is among the enveloped, linear, double-stranded DNA viruses that widely exist in nature. Approximately 100 HSV species have already been identified or partially identified. Among them, two HSV species, SV-1 and HSV-2, that share 50% homology, have been closely associated with humans. According to statistics provided by the WHO, approximately 70% of the total population worldwide carries HSV antibodies and more than one-third suffers from recurrent HSV infection. Along with its high prevalence rate, a variety of human diseases are closely related with HSV infection, including human herpes labialis, herpes conjunctivitis, 20 herpes zoster encephalitis and other diseases causing great harm to human health. HSV encephalitis is the most common, sporadic, viral encephalitis, accounting for 10-20% of acute, viral, encephalitis and 60-80% of natural mortality. Understanding the complex and specific characteristics of HSV infection-related diseases has been a scientifically and socially pressing need that has led to overcoming the recent difficulties in diagnosis and treatment.

Table 5: Bioinformatics of genes in microsatellite loci D17MIT51.1 region

No.	Mouse genome	Corresponding human genes	Functions
1	Epididymal fat pad weight QTL 3		QTL
2	Subcutaneous fat pad weight QTL 4		QTL
3	Spleen weight QTL 9		QTL
4	Early growth adjusted QTL 2		QTL
5	Early growth QTL 5		QTL
6	Pulmonary adenoma susceptibility 12		QTL
7	Weight 6 weeks QTL 11		QTL
8	Weight 10 weeks QTL 12		QTL
9	DNA segment, Chr 17, Hunter 19		
10	DNA segment, Chr 17, Hunter 20		
11	DNA segment, Chr 17, Hunter 21		
12	Down syndrome critical region gene 1-like 1	*DSCR1L1*	
13	Fat pad 7		QTL
14	Mandibular morphogenesis 1		QTL
15	MSM lymphoma resistance 1		QTL
16	Bystin-like	*BYSL*	
17	DNA segment, Chr 17, ERATO Doi 191, expressed		
18	DNA segment, Chr 17, ERATO Doi 763, expressed		
17	Methylmalonyl-Coenzyme A mutase	*MUT*	
18	Neuroscience mutagenesis facility, 318		
19	Cysteine-rich secretory protein 2	*CRISP2*	
20	DNA segment, Chr 17, Tubingen 37		
21	DNA segment, Chr 17, Tubingen 16		
22	Ventral midbrain iron content 9		QTL
23	Soft tissue heal 11		QTL
24	DNA segment, Chr 17, Tubingen 37		
25	Gastritis type A susceptibility locus 4		QTL
26	H2 (histocompatibility-2, MHC)		Complex/cluster/region
27	Histocompatibility 2, Q region		Complex/cluster/region
28	Long bones 10		QTL
29	Lymphoma latency acceleration		QTL
30	Leishmaniasis resistance 1		QTL
31	Locomotor activity 2		QTL
32	T cell receptor beta variable 4, control 1		QTL
33	T-cell receptor induced activation 3		QTL
34	Modifier of Odc1		QTL
35	UVB induced immunosuppression 2		QTL
36	Cleidocranial dysplasia		Complex/cluster/region detail
37	Ectonucleotide Pyrophosphatase/phosphodiesterase 5	*ENPP5*	
38	T-complex-associated testis expressed 1	*TCTE1*	
39	Xenotropic murine leukemia virus 57		

QTL: quantitative trait loci

Table 6: Bioinformatics of genes in microsatellite loci D17MIT39.1 region on the 17 mouse chromosome

No.	Mouse genome	Corresponding human genes	Functions
1	Laminin receptor 9		
2	Proteoglycan induced arthritis 20		QTL detail
3	Ribosomal protein L19, related sequence 8		pseudogene
4	T-cell integration locus		
5	Xanthine dehydrogenase	*XDH*	xanthine dehydrogenase
6	Sine oculis-related homeobox 2 homolog (Drosophila)	*SIX2*	
7	Sine oculis-related homeobox 3 homolog (Drosophila)	*SIX3*	
8	MutS homolog 2 (E. coli)	*MSH2*	DNA mismatch repair protein, eukaryotic MSH2 type
9	Carcass protein in high growth mice 3		QTL
10	DNA segment, Chr 17, XREFdb 57		

QTL: quantitative trait loci

Table 7: Bioinformatics of genes in microsatellite loci from D17MIT180.1 to D17MIT184 region

No.	Mouse genome	Corresponding human genes	Functions
1	High mobility group nucleosomal binding domain 1, related sequence 8		
2	Cyclin D3	*CCND3*	
3	Ecotropic viral integration site 14		
4	High mobility group nucleosomal binding domain 2. related sequence 4		
5	DNA segment, Chr 17, Roswell Park 11, expressed		
6	Transplantation-specific integration cluster 1		
7	Body weight 2		
8	DNA segment, Chr 17, CEPH 9		
9	DNA segment, Chr 17, Le Roy 1		
10	DNA segment, Chr 17, Tubingen 40		
11	P300/CBP-associated factor	*PCAF*	
12	Progastricsin (pepsinogen C)	*PGC*	
13	Thymus specific insertion locus		
14	Meprin 1 alpha ;MGI:96963	*MEP1A*	
15	DNA segment, Chr 17, Seldin 7		
16	Macrophage migration inhibitory factor, pseudogene 8		Pseudogene
17	Cerebellar cAMP 8		QTL
18	DNA segment, Chr 17, John C. Schimenti 39		
19	DNA segment, Chr 17, National Cardiovascular Center, Shionogi 7		
20	DNA segment, Chr 17, National Cardiovascular Center, Shionogi 34		
21	DNA segment, Chr 17, XREFdb 556		
22	abdominal fat weight 3		QTL
23	DNA segment, Chr 17, Abbott 3		
24	DNA segment, Chr 17, Birkenmeier 8		
25	DNA segment, Chr 17, Tubingen 20		
26	Heligmosomoides polygyrus nematode resistance 7		QTL
27	Heligmosomoides polygyrus nematode resistance 7		QTL
28	Obesity and body weight QTL 4		QTL
29	RAB5A, member RAS oncogene family	*RAB5A*	
30	Skin tumor susceptibility 10		QTL
31	DNA segment, Chr 17, Brigham Young University 2		
32	High density lipoprotein (HDL) level 4		QTL
33	Vav 1 oncogene	*VAV1*	
34	Nrtn ;neurturin;	*NRTN*	
35	Creatine kinase, brain, related sequence 2		
36	Epstein-Barr virus induced gene 3	*EBI3*	
37	DNA segment, Chr 17, Hunter 24		
38	Ephrin A5	*EFNA5*	
39	RAS-like, family 2, locus 3		
40	Protein tyrosine phosphatase, receptor type, S	*PTPRS*	
41	Abdominal fat percentage 1		QTL
42	P. chabaudi malaria resistance QTL 7		
43	Caseinolytic peptidase, ATP-dependent, proteolytic subunit homolog (E. coli)	*CLPP*	
44	Plasmacytoma susceptibility 5		
45	Ribosomal protein L32, related sequence 7		Pseudogene
46	Sulfotransferase family, cytosolic, 1C, member 1		
47	DNA segment, Chr 17, Tubingen 23		
48	Complement component 3	*C3*	
49	CD86 expression in activated macrophages		QTL
50	DNA segment, Chr 17, Hunter 15		
51	DNA segment, Chr 17, University of California at Los Angeles 2		
52	EGF-like module containing, mucin-like, hormone receptor-like sequence 1	*EMR1*	
53	EGF-like module containing, mucin-like, hormone receptor-like sequence 4	*EMR4*	
54	Modifier of obesity 4		QTL
55	Fer (fms/fps related) protein kinase, testis specific 1		

Continued...

No.	Mouse genome	Corresponding human genes	Functions
56	tubulin, beta 4	*TUBB4*	
57	DNA segment, Chr 17, ERATO Doi 599, expressed		
58	DNA segment, Chr 17, Hunter 16		
59	SH3-domain GRB2-like 1	*SH3GL1*	
60	Thin fur		
61	Abdominal fat percent QTL 6		QTL
62	Early somite stage arrest 15a		
63	HDL QTL 29		QTL
64	KH-type splicing regulatory protein	*KHSRP*	
65	Regulatory factor X, 2 (influences HLA class II expression)	*RFX2*	
66	Skeletal muscle weight 5		QTL
67	DNA segment, Chr 17, Wayne State University 104, expressed	*C19orf10*	
68	DNA segment, Chr 17, XREFdb 173		
69	Immune response 5		
70	Feminization 1 homolog a (C. elegans)	*FEM1A*	
71	DNA segment, Chr 17, Indiana University Medical 1		
72	DNA segment, Chr 17, John C. Schimenti 20		
73	DNA segment, Chr 17, XREFdb 181		
74	Laminin, alpha 1	*LAMA1*	
75	RalA binding protein 1	*RALBP1*	
76	Twisted gastrulation homolog 1 (Drosophila)	*TWSG1*	
77	Protein tyrosine phosphatase, receptor type, M	*PTPRM*	
78	Age related hearing loss 3		QTL
79	Stathmin 1, related sequence 2		pseudogene
80	Abdominal fat weight QTL 7		QTL
81	DNA segment, Chr 17, Birkenmeier 9		
82	DNA segment, Chr 17, Brigham and Women's Genetics 1496 expressed		

QTL: quantitative trait loci

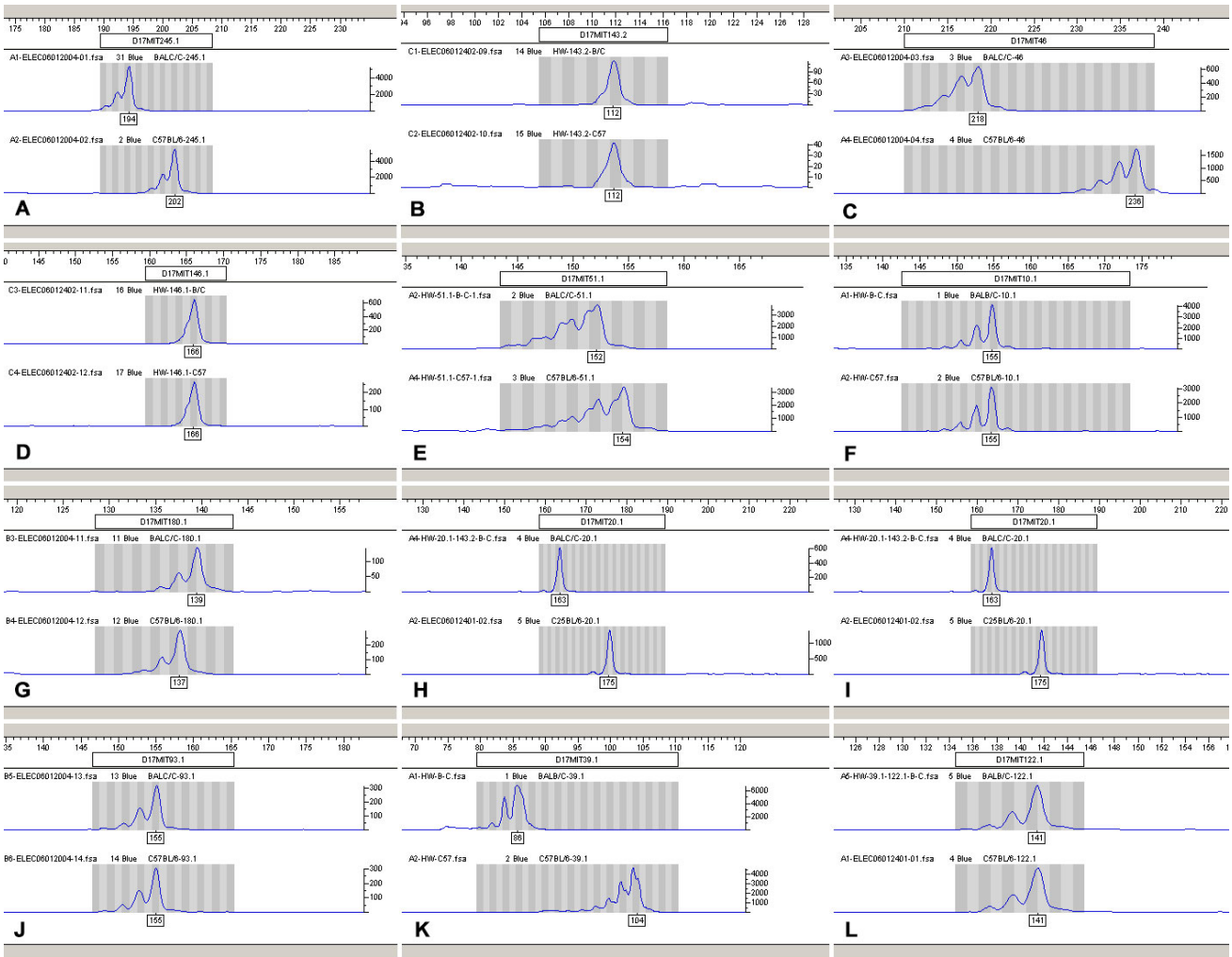

Figure 1: The microsatellite loci scanned using two mouse lines. A: D17MIT245.1; B: D17MIT143.2; C: D17MIT46; D: D17MIT46.1; E: D17MIT51.1; F: D17MIT10.1; G: D17MIT180.1; H: D17MIT20.1; I: D17MIT18; J: D17MIT93; K: D17MIT39.1; L: D17MIT122.1

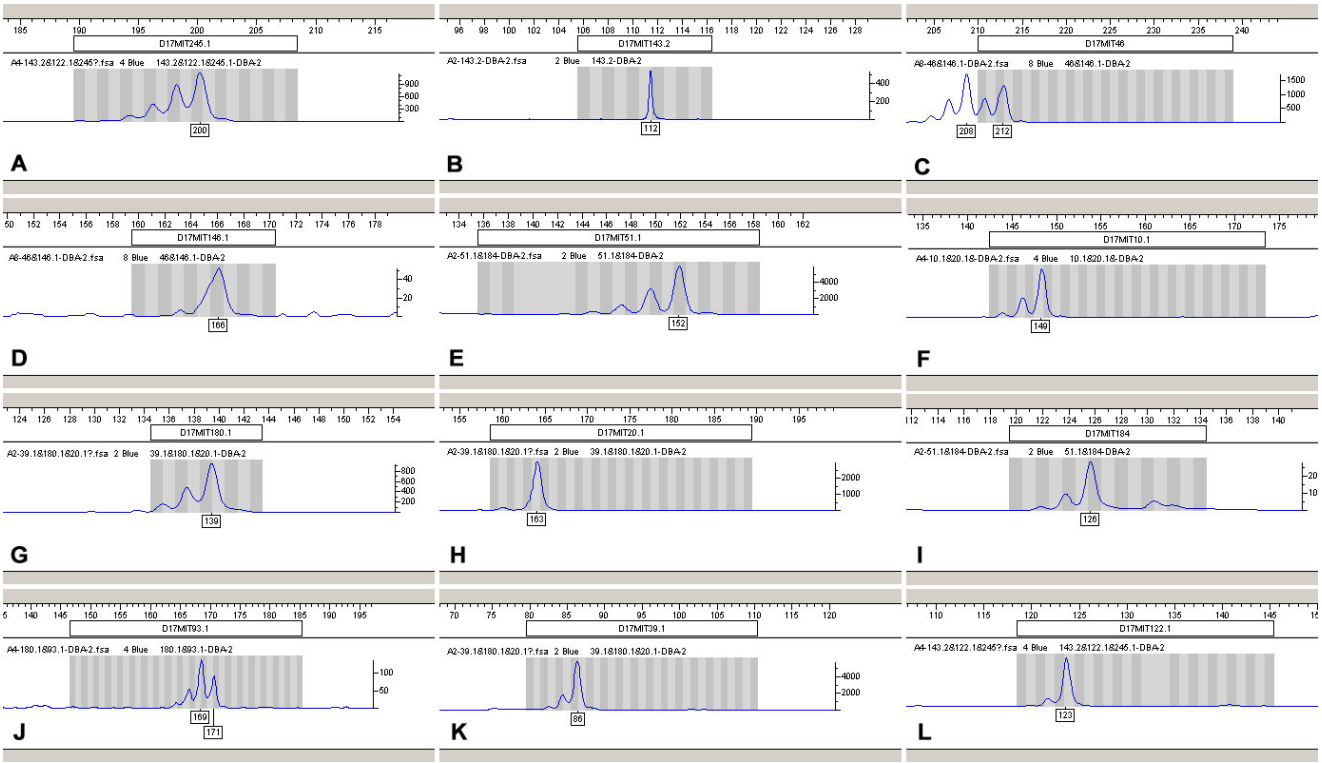

Figure 2: The microsatellite loci scan results of two DBA-2 mouse lines. A: D17MIT245.1; B: D17MIT143.2; C: D17MIT46; D: D17MIT46.1; E: D17MIT51.1; F: D17MIT10.1; G: D17MIT180.1; H: D17MIT20.1; I: D17MIT18; J: D17MIT93; K: D17MIT39.1; L: D17MIT122.1

As a viral disease seriously affecting human health with an increasing incidence in recent years, herpes simplex virus 1 (HSV-1) infection typically generates uncomfortable, watery blisters on the skin or mucous membranes of the mouth and lips,[9,10] potentially leads to encephalitis with remarkable sequelae, or vesicle eruption on genital organs.[11] More importantly, the eruption of these blisters and vesicles are frequently attributed to the long-term latent infection of HSV-1 in the nervous system.[12] Although the human HSV infection rate is very high, it is difficult to fully attract people's attention, so it's difficult for us to associate HSV infection with genetic background. Therefore, most of the previous HSV infection studies focused on the acquired immune response after infection, showing that T cell-mediated immune response plays an important role in resisting HSV-1 infection,[13,14] and immune suppressed or immune deficient individuals are vulnerable to opportunistic herpes virus infection.[15] Furthermore, recent studies[16,17] suggested that innate immune response plays a key role in limiting the spread of the virus. The development of innate immune germ line occurs earlier than acquired immune response system,[18] and these two mechanisms function differently. This is undoubtedly an important point that genetic backgrounds play an important role in HSV infection. Meanwhile, the clinical symptoms of acute infection, as well as the long-term pathologic processes induced by recurrent latent infection, have been shown to closely correlate with the complex viral genome structures and the molecular mechanism of viral gene transcriptional regulation and replication.[19,20]

In fact, as early as 1975, Lopez[1] reported that there are significant differences in the genetic backgrounds of inbred mice that had significantly different reactions to the same or similar HSV infection, which undoubtedly suggested that genetic background might be an important factor for susceptibility to infection. This mechanism revealed by Lopez has also been confirmed by other studies.[2,3] Also, there was a follow-up study on the relationship between the genetic background and susceptibility to HSV infection. Zawatzky *et al*.[21] showed that compared with susceptible DBA/2 mice, the relatively tolerant C57BL/6 mice could produce more interferons in the immune response when it comes to HSV-1 infection. But Brenner *et al*.[22] showed that there is no significant difference in the immune response to HSV infection among those two mouse lines. If the findings in mortality after infection phenotype, Simmons *et al*.[23] reported that only one gene loci functions in this process, while Kastrukoff *et al*.[24] reported that there were two loci separated in the role. We are inclined to believe that from the viewpoint of a gene associated with genetic background, it is undoubtedly that genetic background plays an important role in the phenotypic susceptibility to HSV infection. Since the genetic

background is polygenic, and HSV has no apparent genetic background, the susceptibility of HSV infection itself is very likely regulated by more than one gene controlling quantitative traits.

By bioinformatics analysis, our results suggest that approximately 140 genes were found in the area of D17MIT51.1, D17MIT39.1 and the genomic region between D17MIT180.1 and D17MIT184, and functions of the majority of those genes are not fully revealed. There is a possibility that the above sites are related to the susceptibility to HSV infection, especially the growth-related genes which are highly suggestive of the importance of genetic background. Among these 140 genes, there were about 33 genes homologous to human genes. Their main functions include binding with other partners, regulating a variety of physiological processes, and the modulation of the phosphorylation process of various enzymes and coenzymes.

Based on our experimental results and bioinformatics analysis, the genetic background might play an important role in susceptibility to HSV infection, which is also consistent with most previous studies. It is worth noting that our finding is likely to be a quantitative trait locus, and may not be a particular system or population-specific mechanism. It is just a hint of this phenomenon in a particular system or population which has a relative higher or lower incidence in another race or ethnic groups. This is not consistent with some previous research.

In summary, the biological information and related data analysis suggest that these genes have important physiological and pathological functions. However, up to now, their associations with HSV susceptibility infection have not been reported, suggesting that they could be potential candidate genes that contributed to the different susceptibility to HSV infection.

Because HSV infection phenotypes have not been clearly defined yet, some issues are far from a consensus. For instance, whether the differences in response to HSV infection really exist and whether the genetic background plays a role in it. All these issues will undoubtedly limit the objectivity of this research. It is also necessary to validate the results in the present study by expanding the sample size, further investigating the role of the regulatory regions in regulating susceptibility to HSV infection. Furthermore, the functions of genes near these microsatellite loci, as well as their functions in regulating susceptibility, deserve further investigation.

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Conflicts of interest
There are no conflicts of interest.

Patient consent
There is no patient involved.

Ethics approval
Ethics approval was obtained prior to the commencement of the study.

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