Published in final edited form as:

Atherosclerosis. 2008 January ; 196(1): 234–239.

Association of a *Vcam1* mutation with atherosclerosis susceptibility in diet-induced models of atherosclerosis

Toru Miyoshi^{*}, Zuobiao Yuan^{*}, and Weibin Shi

Departments of Radiology and the Cardiovascular Research Center, University of Virginia, Charlottesville, VA 22908

Abstract

We previously identified a G>A single nucleotide polymorphism (SNP) between C57BL/6J (B6) and C3H/HeJ (C3H) mouse strains at position 2077 in the coding region of *Vcam1* that leads to substitution of an amino acid from aspartic acid (D) to asparagine (N) in the protein product. In the present study, we investigated the association of this SNP with atherosclerosis susceptibility using a panel of inbred mouse strains, a set of recombinant inbred (RI) strains derived from B6 and C3H mice, and a cohort of F2 mice derived from B6 and C3H apolipoprotein E-deficient (apoE^{-/-}) mice. Inbred strain analysis revealed that mouse strains with the B6 *Vcam1* genotype developed significantly larger atherosclerotic lesions than strains with the C3H genotype (4622 ± 2816 vs. $362 \pm 697 \mu\text{m}^2/\text{section}$; $P=0.029$). BXH RI strains with the B6 *Vcam1* genotype also developed larger atherosclerotic lesions than those with the C3H genotype (8305 ± 9031 vs. $2139 \pm 2931 \mu\text{m}^2/\text{section}$) although the difference was not statistically significant ($P=0.13$). In contrast, no association was detected between *Vcam1* and atherosclerotic lesion size in F2 mice. The present data indicate that the G>A mutation of *Vcam1* is associated with atherosclerotic lesion formation in the dietary but not apoE^{-/-} models of atherosclerosis and this association suggests a role for the *Vcam1* gene in influencing atherosclerosis susceptibility.

Keywords

Vcam1; single nucleotide polymorphism; atherosclerosis; mouse

INTRODUCTION

Atherosclerotic vascular disease frequently clusters in families. Although environmental factors such as diet, exercise, and smoking play a role, genetic factors are a major determinant for development of the disease [1]. Twin and adoption studies provide evidence that genetic factors account for approximately 50% of the variability in the manifestation of the disease in human populations [2–5]. Identifying the genes that influence risk for atherosclerosis has not readily been achieved in humans.

Studies using inbred mouse strains demonstrate significant inter-strain differences in susceptibility to atherosclerosis [6–8]. Animal model studies also show continuous

Correspondence: Weibin Shi, University of Virginia, Department of Radiology, Box 801339, MR4 Room 1171, 409 Lane Road, Charlottesville, VA 22908, Phone: (434) 243-9420, Fax: (434) 982-5680, Email: ws4v@virginia.edu.

^{*} Authors contributed equally.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

distributions of atherosclerotic lesion sizes, indicating the polygenic nature of atherosclerotic lesions. C57BL/6 (B6) and C3H/HeJ (C3H) mice are two commonly used inbred strains that differ strikingly in the development of atherosclerosis when fed an atherogenic diet or when deficient in apoE (apoE^{-/-}) [8,9]. B6 mice readily develop atherosclerosis whereas C3H mice are highly resistant to it. We have recently identified a single nucleotide polymorphism (SNP) between strains B6 and C3H in the coding region of the *Vcam1* gene that leads to substitution of an amino acid in the protein product [10]. We have also observed marked differences between the two strains in the expression of VCAM-1 by endothelial cells at either baseline or after stimulation with oxidized LDL [10,11]. However, it is unclear whether *Vcam1* is one of the genes that influence atherosclerosis susceptibility in mice. Co-segregation of intragenic recombinant genotypes in a candidate gene with a phenotype constitutes a genetic proof that the candidate gene or a closely linked gene contributes to the phenotype. In the present study, we examined whether allelic variation at the *Vcam1* gene locus was associated with atherosclerotic lesion size in a panel of inbred mouse strains, in a set of recombinant inbred (RI) strains derived from B6 and C3H mice, and in an intercross between B6 and C3H apolipoprotein E-deficient (apoE^{-/-}) mice.

MATERIALS AND METHODS

Sequence analysis

Genomic DNA of inbred mouse strains and BXH RI strains was purchased from the Jackson Laboratory. The coding region, spanning the G2077A polymorphism, of the *Vcam1* gene was PCR-amplified with a primer pair 5'-ATTTCTGTGCCTCCACCAGACTGT-3'/5'-GCAACCATGAGCTGCTTATTTGGG-3' and the resulting PCR product was sequenced for the aforementioned mouse strains. Two independent PCR products for each strain were gel fractionated, purified with a QIAquick PCR purification kit, and then sequenced on an ABI Prism Cycle Sequencer 310 (Applied Biosystems). The coding regions of *Mtp* and *Nfkb1* that are polymorphic between B6 and C3H strains were also sequenced for mouse strains whose atherosclerosis phenotype is available from previous studies. The primer pair used for PCR amplification of *Mtp* was 5'-GGTGCGTGGAATGCATTGTACCTT-3'/5'-TCACACAGGGCACACAGACATACA-3' and for *Nfkb1*, it was 5'-GACAGCATCAGCTTCAGCCCTTTA-3'/5'-GCTTGAATACGCCCGTTTGCAGAT-3'.

Vcam1 genotypic analysis

The *Vcam1* genotypes of F2 mice derived from B6.apoE^{-/-} and C3H.apoE^{-/-} mice were determined by PCR-restriction fragment length polymorphism (PCR-RFLP)-based method. An A/G polymorphism (rs3722079) in intron 7 of the *Vcam-1* gene altered a restriction sequence so that the *Vcam1* B6 allele contained an *MspAII* restriction enzyme site that was absent in the C3H allele. A primer pair was designed to amplify a 559-bp PCR product, spanning the A/G polymorphism, from genomic DNA (5'-GGGCGTTTGACCAATTCCCAGT AA-3'/5'-ACAGAAACCAAGCTGGAAGCTCAG-3'). *MspAII* cut the B6 PCR product into fragments of 249 bp and 310 bp, whereas the C3H product was not cut. Thus, the BB alleles would exhibit two bands, the CC alleles one band, and the BC alleles three bands on an agarose gel. The accuracy of this assay was evaluated by comparing the strain distribution pattern of *Vcam1* genotypes with that of the flanking marker *D3Mit102*.

Genetic correlation

Correlations of *Vcam1*, *Mtp* or *Nfkb1* genotypes with atherosclerotic lesion size were examined using a panel of inbred mouse strains, a set of BXH RI strains, and a cohort of BXH F2s. A mouse homozygous for the B6 allele at a specific gene locus was assigned a genotypic score of 1, a mouse homozygous for the C3H allele assigned a genotypic score of 0, and a

mouse heterozygous for the B6 and C3H alleles assigned a genotypic score of 0.5. Genetic correlations between genotypes and atherosclerotic lesion phenotypes were determined by linear regression analysis.

Statistical analysis

All data were expressed as Means $\pm$ SD. ANOVA was used for determining if the mean phenotypic values of F2 mice with different genotypes at the *Vcam1* locus were significantly different. The Student *t* test was used when only two means were compared. Differences were considered statistically significant at $P < 0.05$.

RESULTS

Our previous studies identified a nonsynonymous A>G SNP between strains B6 and C3H at position 2077 within the coding region of the *Vcam1* gene that changes the sequence of the predicted protein at amino acid residue 693 from aspartic acid (D) to asparagine (N) [10]. Sequence analysis of the SNP for additional 44 inbred strains identified 12 strains that shared the B6 allele (129, C57L, SM, BTBR, C57BL/10, C57BR, KK/H1, LP, MA, RIII, SEA, WSB) and 32 strains that shared the C3H allele (A/J, AKR, BALB/cByJ, C58, CAST, CZECHII, DBA, FVB, MOLF, NOD, NZB, PERA, PL, SJL, SPRET, SWR, BUB, C57BLKS, CBA, CE, DDK, I, JF1, LG, MA, MSM, NON, NZW, PWD, SEG, ST, ZALLENDE) (Table 1).

By comparing the VCAM-1 peptide sequences of eight mammal species, including the mouse, we found that aspartic acid (D) at residue 693 is conserved in humans, chimpanzees, monkeys, cattle, pigs, dogs, rats, and C3H mice (Figure 1), suggesting that Asn⁶⁹³ (N) is a mutation in B6 mice.

We then determined whether this amino acid change was associated with variation in susceptibility to atherosclerosis in 15 female inbred mouse strains, whose average atherosclerotic lesion size is available from a previous study of Paigen et al [7]. These mice were fed an atherogenic diet containing 15% fat, 1.25% cholesterol, and 0.5% cholate (the Paigen diet) for 14 weeks. A significant correlation was detected between the *Vcam1* genotypes and atherosclerotic lesion size ($R=0.79$; $P=0.00044$) (Figure 2). Mouse strains with the B6 *Vcam1* genotype developed significantly larger atherosclerotic lesions than strains with the C3H genotype (4622 ± 2816 vs. $362 \pm 697 \mu\text{m}^2/\text{section}$; $P=0.029$). *Mtpp* (66.2 cM from the centromere), encoding microsomal triglyceride transfer protein, and *Nfkb1* (68.9 cM from the centromere), encoding nuclear factor of kappa light polypeptide gene enhancer in B-cells 1, are two genes adjacent to *Vcam1* (50.8 cM from the centromere) that may influence atherosclerosis. Thus, they were also tested for associations with atherosclerotic lesion size in these inbred strains. No significant correlations with atherosclerotic lesion size were detected for either *Mtpp* ($R=0.42$; $P=0.117$) or *Nfkb1* ($R=0.10$; $P=0.723$) (Figure 3).

We also assessed the associations of *Vcam1* genotypes with atherosclerotic lesion size in a set of BXH RI strains. Mean atherosclerotic lesion size for 10 female BXH RI strains and the two progenitor strains is available from a previously published study of Liao et al [12]. These mice were fed the atherogenic diet for 15 weeks. The strains possessing the B6 *Vcam1* genotype tended to develop larger atherosclerotic lesions than strains with the C3H genotype (8305 ± 9031 vs. $2139 \pm 2931 \mu\text{m}^2/\text{section}$), but this correlation was not statistically significant ($R=0.42$; $P=0.176$), probably due to the limited number of RI strains (Figure 4). Compared to *Vcam1*, *Mtpp* and *Nfkb1* showed weaker correlations with atherosclerotic lesion size ($R=0.30$; $P=0.34$ for both genes) (Figure 5).

Genetic correlation analysis was finally performed in 215 female F2 mice derived from B6.apoE^{-/-} and C3H.apoE^{-/-} mice. Mice were fed a Western diet containing 42% fat, 0.15%

cholesterol and 19.5% casein for 12 weeks. The phenotypic measurements, including atherosclerotic lesion size, of F2 mice were previously reported [13]. *Vcam1* genotypes of F2 mice were determined by the PCR-RFLP method. No significant correlation was detected between *Vcam1* genotypes and atherosclerotic lesion size ($R=0.04$; $P=0.56$) (Figure 6).

DISCUSSION

In this study, we evaluated the significance of the A>G mutation in the coding region of the *Vcam1* gene in the development of atherosclerosis in mice. We detected a significant association of the *Vcam1* genotypes with atherosclerotic lesion size in a panel of inbred mouse strains and an apparent trend of association in a set of BXH RI strains that were fed an atherogenic diet, while in BXH F2 mice that were deficient in apoE and fed a Western diet for 12 weeks the association was not significant. These findings suggest that *Vcam1* or a closely linked gene influence atherosclerosis susceptibility in diet-induced but not apoE^{-/-} models of atherosclerosis.

Our present association analysis using 15 inbred mouse strains suggested *Vcam1* as a candidate gene to influence atherosclerosis susceptibility. *Vcam1*, encoding vascular cell adhesion molecule-1 (VCAM-1) expressed by endothelial cells, mediates adhesion to and rolling of monocytes along the endothelial border [14]. All phases of atherosclerosis involve the recruitment of monocytes from circulation and their transendothelial migration. This process is predominantly mediated by cellular adhesion molecules expressed on the endothelium. Expression of VCAM-1 is upregulated in atherosclerotic lesions [15]. Blockade of the VCAM-1/VLA-4 pathway with antibodies reduces monocyte adhesion and infiltration induced by injury [16]. VCAM-1 domain 4-deficiency markedly reduces arterial VCAM-1 expression, monocyte adherence in the aortic root, and fatty streak formation in apolipoprotein E-deficient mice [17]. There is suggestive evidence supporting the involvement of VCAM-1 in regulation of atherosclerosis susceptibility in standard mouse strains. C3H and BALB/c mice, which have lower VCAM-1 levels in plasma and arterial walls, develop smaller atherosclerotic lesions than B6 mice, which display higher VCAM-1 expression on the apoE^{-/-} background [10,18]. A significant atherosclerosis susceptibility locus on chromosome 3 near the *Vcam1* gene was recently identified using a F2 cross between B6 and FVB strains deficient in the LDL receptor [19].

Association studies using 15 inbred mouse strains may lead to false positive findings. Thus, confirmation analyses were performed in two independent populations. In BXH RI strains, a trend of association between *Vcam1* genotypes and atherosclerotic lesion size was apparent, supporting the probability that *Vcam1*, or a closely linked gene, influences atherosclerotic lesion formation. Because the association analysis uses data from ten BXH RI strains and the two progenitor strains, the small number of mouse strains may have influenced the power of association analysis. In contrast, in the F2 population derived from B6.apoE^{-/-} and C3H.apoE^{-/-} mice, the association between *Vcam1* genotypes and atherosclerotic lesion size was not detected. The confounding influence of atherosclerosis susceptibility loci on chromosomes 9 and 11 [13] may have diminished the power to detect associations between *Vcam1* and atherosclerotic lesions using the cross. Another explanation is that VCAM-1 contributed less significantly to the development of atherosclerosis in the apoE^{-/-} model than in the diet-induced model. Both inbred strains and BXH RI strains used for the present association analyses were fed the cholate-containing diet while the F2 mice were fed a Western diet without cholate. The cholate diet has been shown to cause a chronic inflammatory state, with the expression of inflammatory and oxidative stress genes, in the liver and probably also in the vessel walls [12]. It is plausible to speculate that VCAM-1, as a proinflammatory gene, is more highly expressed in the arterial wall and consequently contributes more significantly to the initiation and progression of atherosclerosis in the diet-induced models than in the

apoE^{-/-} model. Other studies have also shown that different models may lead to different atherosclerosis susceptibility loci or genes detected. For example, an atherosclerosis susceptibility locus on chromosome 3 was detected in a B6 X FVB F2 cross deficient in the LDL receptor but not in a B6 X FVB F2 cross deficient in apoE [19,20].

Significant association of a polymorphism with a disease trait may not ensure that the polymorphism is a primary factor for the disease susceptibility. There is a possibility that the observed association is due to linkage disequilibrium and that a closely-linked gene that is polymorphic between the B6 and C3H strains may actually determine the disease susceptibility. *Mtp* and *Nfkb1* are two genes adjacent to *Vcam1* on chromosome 3 that are polymorphic between the two strains and are known to influence atherosclerosis. However, both genes showed weaker associations than *Vcam1* with atherosclerotic lesion size for both the inbred mouse strains and the BXH RI strains.

In summary, this study provides evidence supporting *Vcam1* to be a candidate gene that influences atherosclerosis susceptibility in the dietary model of atherosclerosis. With the completion of human and mouse genome sequencing and the availability of sophisticated statistical tools, screening of positional candidate genes within a certain chromosomal region may lead to accelerated identification of causal genes. However, there is a possibility that a different linked gene may contribute to the phenotype and that the observed association might be a secondary one resulting from linkage disequilibrium. Thus, traditional approaches, including construction and analysis of congenic, knockout or transgenic rodents, may still be needed to validate the phenotypic association.

Acknowledgements

This work was supported by National Institutes of Health grants HL71844 and HL75433. The authors thank Jessica Rowland for critically reviewing the manuscript.

References

1. Lusis AJ, Mar R, Pajukanta P. Genetics of atherosclerosis. *Annu Rev Genomics Hum Genet* 2004;5:189–218. [PubMed: 15485348]
2. Bak S, Gaist D, Sindrup SH, Skytthe A, Christensen K. Genetic liability in stroke: a long-term follow-up study of Danish twins. *Stroke* 2002;33:769–774. [PubMed: 11872902]
3. Berg K. Genetic epidemiological approaches to the prevention of coronary heart disease—summary report of WHO consultations. *Prog Clin Biol Res* 1984;147:85–87. [PubMed: 6739499]
4. Marenberg ME, Risch N, Berkman LF, Floderus B, de FU. Genetic susceptibility to death from coronary heart disease in a study of twins. *N Engl J Med* 1994;330:1041–1046. [PubMed: 8127331]
5. Zdravkovic S, Wienke A, Pedersen NL, Marenberg ME, Yashin AI, De FU. Heritability of death from coronary heart disease: a 36-year follow-up of 20 966 Swedish twins. *J Intern Med* 2002;252:247–254. [PubMed: 12270005]
6. Dansky HM, Charlton SA, Sikes JL, Heath SC, Simantov R, Levin LF, Shu P, Moore KJ, Breslow JL, Smith JD. Genetic background determines the extent of atherosclerosis in ApoE-deficient mice. *Arterioscler Thromb Vasc Biol* 1999;19:1960–1968. [PubMed: 10446078]
7. Paigen B, Ishida BY, Verstuyft J, Winters RB, Albee D. Atherosclerosis susceptibility differences among progenitors of recombinant inbred strains of mice. *Arteriosclerosis* 1990;10:316–323. [PubMed: 2317166]
8. Shi W, Wang NJ, Shih DM, Sun VZ, Wang X, Lusis AJ. Determinants of atherosclerosis susceptibility in the C3H and C57BL/6 mouse model: evidence for involvement of endothelial cells but not blood cells or cholesterol metabolism. *Circ Res* 2000;86:1078–1084. [PubMed: 10827138]
9. Paigen B, Albee D, Holmes PA, Mitchell D. Genetic analysis of murine strains C57BL/6J and C3H/HeJ to confirm the map position of *Ath-1*, a gene determining atherosclerosis susceptibility. *Biochem Genet* 1987;25:501–511. [PubMed: 3128974]

10. Pei H, Wang Y, Miyoshi T, Zhang Z, Matsumoto AH, Helm GA, Tellides G, Shi W. Direct evidence for a crucial role of the arterial wall in control of atherosclerosis susceptibility. *Circulation* 2006;114:2382–2389. [PubMed: 17101850]
11. Shi W, Haberland ME, Jien ML, Shih DM, Lusis AJ. Endothelial responses to oxidized lipoproteins determine genetic susceptibility to atherosclerosis in mice. *Circulation* 2000;102:75–81. [PubMed: 10880418]
12. Liao F, Andalibi A, Qiao JH, Allayee H, Fogelman AM, Lusis AJ. Genetic evidence for a common pathway mediating oxidative stress, inflammatory gene induction, and aortic fatty streak formation in mice. *J Clin Invest* 1994;94:877–884. [PubMed: 8040344]
13. Su Z, Li Y, James JC, McDuffie M, Matsumoto AH, Helm GA, Weber JL, Lusis AJ, Shi W. Quantitative trait locus analysis of atherosclerosis in an intercross between C57BL/6 and C3H mice carrying the mutant apolipoprotein E gene. *Genetics* 2006;172:1799–1807. [PubMed: 16387874]
14. Huo Y, Ley K. Adhesion molecules and atherogenesis. *Acta Physiol Scand* 2001;173:35–43. [PubMed: 11678724]
15. O'Brien KD, Allen MD, McDonald TO, Chait A, Harlan JM, Fishbein D, McCarty J, Ferguson M, Hudkins K, Benjamin CD. Vascular cell adhesion molecule-1 is expressed in human coronary atherosclerotic plaques. Implications for the mode of progression of advanced coronary atherosclerosis. *J Clin Invest* 1993;92:945–951. [PubMed: 7688768]
16. Manka DR, Wiegman P, Din S, Sanders JM, Green SA, Gimple LW, Ragosta M, Powers ER, Ley K, Sarembock IJ. Arterial injury increases expression of inflammatory adhesion molecules in the carotid arteries of apolipoprotein-E-deficient mice. *J Vasc Res* 1999;36:372–378. [PubMed: 10559677]
17. Dansky HM, Barlow CB, Lominska C, Sikes JL, Kao C, Weinsaft J, Cybulsky MI, Smith JD. Adhesion of monocytes to arterial endothelium and initiation of atherosclerosis are critically dependent on vascular cell adhesion molecule-1 gene dosage. *Arterioscler Thromb Vasc Biol* 2001;21:1662–1667. [PubMed: 11597942]
18. Tian J, Pei H, James JC, Li Y, Matsumoto AH, Helm GA, Shi W. Circulating adhesion molecules in apoE-deficient mouse strains with different atherosclerosis susceptibility. *Biochem Biophys Res Commun* 2005;329:1102–1107. [PubMed: 15752767]
19. Teupser D, Tan M, Persky AD, Breslow JL. Atherosclerosis quantitative trait loci are sex- and lineage-dependent in an intercross of C57BL/6 and FVB/N low-density lipoprotein receptor $-/-$ mice. *Proc Natl Acad Sci USA* 2006;103:123–128. [PubMed: 16380418]
20. Dansky HM, Shu P, Donavan M, Montagno J, Nagle DL, Smutko JS, Roy N, Whiteing S, Barrios J, McBride TJ, Smith JD, Duyk G, Breslow JL, Moore KJ. A phenotype-sensitizing Apoe-deficient genetic background reveals novel atherosclerosis predisposition loci in the mouse. *Genetics* 2002;160:1599–1608. [PubMed: 11973313]

B6		L T L D V K G K E H N K N Y F S P E L L
C3H	NP_035823	L T L D V K G K E H N K D Y F S P E L L
Rat	NP_037021	L T L D V K G K E N N K D Y F S P E L L
Dog	NP_001003298	L T L D V K G R E N N K D Y F S P E L L
Pig	NP_999056	I T L D V K G R E S N K D Y F S S E L L
Cattle	NP_776909	L T L D V K G R E S N K D Y F S P E L L
Monkey	XP_001107924	L T L D V Q G R E N N K D Y F S P E L L
Chimpanzee	XP_001135527	L T L D V Q G R E N S K D Y F S P E L L
Human	NP_001069	L T L D V Q G R E N N K D Y F S P E L L

693 in mouse

Figure 1.

Comparison of partial VCAM-1 protein sequences in eight mammal species. The amino acid residue differing between B6 and C3H mice at position 693 is highlighted. GenBank accession No. are shown following the names of the species.

Figure 2.

Association between atherosclerotic lesion size and *Vcam1* genotypes in 15 female inbred mouse strains analyzed by linear regression analysis. Mouse strains were grouped by their *Vcam1* genotypes. Phenotypic data were from a previously published study by Paigen et al (7). Mice were fed an atherogenic diet for 14 weeks. Inbred mouse strains with the B6 *Vcam1* genotype developed significantly larger atherosclerotic lesions than strains with the C3H *Vcam1* genotype.

Figure 3.

Association between atherosclerotic lesion size and *Mtp* or *Nfkb1* genotypes in 15 female inbred mouse strains described above. *Mtp* (66.2 cM from the centromere) and *Nfkb1* (68.9 cM from the centromere) are two genes linked to *Vcam1* (50.8 cM from the centromere) on mouse chromosome 3 and are polymorphic between the B6 and C3H strains. Correlation coefficient, R, and P value are shown on the figure.

Figure 4.

Association between atherosclerotic lesion size and *Vcam1* genotypes in BXH recombinant inbred (RI) strains derived from B6 and C3H mice. BXH RI strains were grouped by their *Vcam1* genotypes. Phenotypic data were from a previously published study by Liao et al (12). Mice were fed an atherogenic diet for 15 weeks. RI strains with the B6 *Vcam1* genotype tended to develop larger atherosclerotic lesions than strains with the C3H *Vcam1* genotype.

Figure 5. Association between atherosclerotic lesion size and *Mtp* or *Nfkb1* genotypes in BXH RI strains derived from B6 and C3H mice. RI strains were grouped by their *Mtp* or *Nfkb1* genotypes. Correlation coefficient, R, and P value are shown on the figure.

Figure 6.

Association between atherosclerotic lesion size and *Vcam1* genotypes in 215 female F2 mice derived from B6.apoE^{-/-} and C3H.apoE^{-/-} strains. Mice were fed a Western diet for 12 weeks. BB, CC and BC designate F2 mice homozygous for the B6 allele, C3H allele and heterozygous at the *Vcam1* locus, respectively. Correlation coefficient, R, and P value are shown on the figure.

Table 1

Inbred strains were separated into two groups according to the G2077A polymorphism in the coding region of the *Vcam1* gene

Allele Amino acid residue	G/G Asp ⁶⁹³	A/A Asn ⁶⁹³
Strains	A/J, AKR, BALB/cByJ, C3H, C58, CAST, CZECHII, DBA, FVB, MOLF, NOD, NZB, PERA, PL, SJL, SPRET, SWR, WSB BUB, C57BLKS, CBA, CE, DDK, I, JF1, LG, MA, MSM, NON, NZW, PWD, SEG, ST, ZALENDE	1291, C57BL/6, BTBR, C57L, SM, C57BL/10, C57BR, KK/H1, LP, MA, RIII, SEA,

Asp: aspartic acid and Asn: asparagine.