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Genetic analysis of a mouse cross implicates an anti-inflammatory gene in control of atherosclerosis susceptibility

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Abstract

Nearly all genetic crosses generated from *Apoe*^{-/-} or *Ldlr*^{-/-} mice for genetic analysis of atherosclerosis have used C57BL/6J (B6) mice as one parental strain; thus limiting their mapping power and coverage of allelic diversity. SM/J-*Apoe*^{-/-} and BALB/cJ-*Apoe*^{-/-} mice differ significantly in atherosclerosis susceptibility. 224 male F₂ mice were generated from the two *Apoe*^{-/-} strains to perform quantitative trait locus (QTL) analysis of atherosclerosis. F₂ mice were fed 5 weeks of Western diet and analyzed for atherosclerotic lesions in the aortic root. Genome-wide scans with 144 informative SNP markers identified a significant locus near 20.2 Mb on chromosome 10 (LOD score: 6.03), named *Ath48*, and a suggestive locus near 49.5 Mb on chromosome 9 (LOD: 2.29; *Ath29*) affecting atherosclerotic lesion sizes. Using bioinformatics tools, we prioritized 12 candidate genes for *Ath48*. Of them, *Tnfaip3*, an anti-inflammatory gene, is located precisely underneath the linkage peak and contains two nonsynonymous SNPs leading to conservative amino acid substitutions. Thus, this study demonstrates the power of forward genetics involving the use of a different susceptible strain and bioinformatics tools in finding atherosclerosis susceptibility genes.

Keywords

Atherosclerosis; quantitative trait locus; TNF alpha induced protein 3; mouse

Introduction

Atherosclerosis is the underlying cause of heart attack, ischemic stroke and peripheral arterial disease and claims more lives than any other disease in the United States

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Author contributions

ENG sectioned samples and quantitated atherosclerotic lesions. ATG analyzed the data. JL analyzed candidate gene expression. MHC supervised morphometric analysis of atherosclerotic lesions. WS constructed the cross, collected samples and supervised the study. All authors read and edited the manuscript.

Conflict of interest

The authors declare no conflicting interests.

(Mozaffarian et al. 2015). Prospective studies of twins, families or cohorts have demonstrated a strong genetic component in coronary heart disease (Marenberg et al. 1994), (Lloyd-Jones et al. 2004), (Bachmann et al. 2012). Meta-analysis of genome-wide association studies (GWAS) have identified over 50 loci harboring common variants significantly associated with coronary heart disease (Nikpay et al. 2015), (CARDIoGRAMplusC4D Consortium et al. 2013). However, the collective effect of all these loci explains only 26% of the total heritability of coronary heart disease (Nikpay et al. 2015), suggesting that many more loci remain to be discovered. Moreover, all the loci detected have small effect sizes with odds ratios (OR) <1.25 (Nikpay et al. 2015), and thus may have little value for being used to diagnose the disease or develop targeted therapy.

Studies of animal models have contributed greatly to the identification of genes and pathways involved in many complex human traits or diseases, including atherosclerosis. Apolipoprotein E-null (*Apoe*^{-/-}) and LDL receptor-null (*Ldlr*^{-/-}) mice develop all phases of atherosclerotic lesions seen in humans (Nakashima et al. 1994), (Ishibashi et al. 1994). To date, we and others have generated 12 intercrosses or backcrosses from *Apoe*^{-/-} or *Ldlr*^{-/-} mouse strains for conducting quantitative trait locus (QTL) analysis of atherosclerotic lesions in the aortic root and identified ~50 loci for the trait (<http://www.informatics.jax.org/allele>). Of the 12 crosses generated, C57BL/6 (B6) mice have been used as one susceptible progenitor strain for 10; thus limiting the power of QTL analysis and the coverage of allelic diversity. Generation of new crosses using a different atherosclerosis-susceptible strain may discover new loci and also empower bioinformatics analysis for prioritizing candidate genes. We recently constructed SM/J (SM) and SWR/J *Apoe*^{-/-} mouse strains, and found them susceptible to atherosclerosis compared with C3H/HeJ (C3H) or BALB/cJ (BALB) *Apoe*^{-/-} mice (Liu et al. 2015). In the present study, we performed QTL analysis using a male F₂ cohort derived from BALB-*Apoe*^{-/-} and SM-*Apoe*^{-/-} mice and identified a major QTL (*Ath48*) on chromosome 10 influencing atherosclerotic lesion size. We further used bioinformatics tools to prioritize *Tnfrsf25* as a likely candidate gene for the QTL.

Materials and Methods

Ethics statement

All procedures were conducted under the current National Institutes of Health guidelines and approved by the Institutional Animal Care and Use Committee (Assurance #A3245-01, Animal Protocol #3109).

Generation and treatment of the cross

BALB-*Apoe*^{-/-} and SM-*Apoe*^{-/-} mice were generated in our laboratory using the traditional breeding strategy as reported (Liu et al. 2015). The creation of a male F₂ cohort from the two *Apoe*^{-/-} strains was recently described (Shi et al. 2016). Briefly, BALB-*Apoe*^{-/-} mice were crossed with SM-*Apoe*^{-/-} mice to generate F₁s, which were intercrossed to generate 224 male F₂ mice. At 8 weeks of age, these mice were started with a Western diet containing 21% fat, 34.1% sucrose, 0.15% cholesterol and 19.5% casein, and fed the diet for 5 weeks.

Aortic lesion measurement

Atherosclerotic lesion areas in the aortic root of mice were measured as reported (Rowlan et al. 2013)(Grainger et al. 2016). The vasculature of the animals was perfusion-fixed with 10% formalin through the heart. The aortic root and adjacent portion of the heart were harvested, cross-sectioned in 10- μ m thickness, and stained with oil red O and hematoxylin. Atherosclerotic lesion areas were measured under a microscope using Zeiss AxioVision 4.8 software. Lesion areas on five sections with the largest readings were averaged for each mouse, and this number was used for statistical analysis.

Plasma lipid and glucose analyses

Blood was drawn under isoflurane anesthesia from the retro-orbital venous sinus after mice were fasted overnight. EDTA was used as an anticoagulant. Plasma levels of total cholesterol, HDL cholesterol, triglyceride and glucose were measured using commercial kits as reported (Shi et al. 2016)(Su et al. 2006a). Non-HDL cholesterol was the difference between total and HDL cholesterol.

Genotyping

Genomic DNA was isolated from the tail of mice using the phenol/chloroform extraction and ethanol precipitation method. F₂ mice were genotyped through the Jackson Laboratory genotyping services using mouse strain-specific SNP arrays (Shi et al. 2016). DNA from the two parental strains and their F1s served as controls. 144 informative SNPs across the whole genome were typed.

Statistical analysis

QTL analysis was performed using J/qtl and Map Manager QTX software as previously reported (Grainger et al. 2016)(Shi et al. 2016),(Wang et al. 2015). LOD threshold values for significant and suggestive linkage were determined through 1000 permutations of the achieved data, which are provided in supplementary materials.

Prioritization of candidate genes

Positional candidate genes for the significant QTL obtained were prioritized using bioinformatics tools. Probable candidate genes were defined as those that contained a non-synonymous SNP in a coding region or a SNP in upstream regulatory region, and this SNP was shared by the parental strains carrying the high allele but was different from the one shared by the parental strains carrying the low allele at a QTL, as reported (Rowlan et al. 2013)(Grainger et al. 2016). Analysis was performed using a combination of SNP databases, including the Sanger Mouse Genomes Project, Mouse Phenome Database, and Ensembl. Online software RaptorX was used to predict the potential effect of an amino acid substitution on the 3D structure of protein product (<http://raptorx.uchicago.edu/StructurePrediction/predict/>).

Tnfaip3 mRNA expression

mRNA expression levels of *Tnfaip3* and *Gapdh* in the liver of mice was determined by real-time PCR. RNA extraction, cDNA synthesis and PCR amplification were performed as

reported (Manichaikul et al. 2011). Primers for *Tnfrsf25* were: 5'-GGTGACCCTGAAGGACAGTG-3'/5'-CTTCAAACCTACCCCGGTCT-3', and for *Gapdh* were: 5'-GAGGCCGGTGCTGAGTATGT-3'/5'-AAGGGTGGAGCCAAAAGGGTCATC-3').

Results

Trait value distribution

Values in atherosclerotic lesion areas of F₂ mice showed a Pareto distribution in which the number of animals with a lesion area of 70,000 μm^2 was the largest and then gradually decreased as lesion areas increased (Fig. 1). After being squared root-transformed, the values of lesion areas approached a normal distribution. Genome-wide scans were performed using both transformed and untransformed lesion data to identify QTLs for atherosclerotic lesions.

QTL analysis

A genome-wide scan using the untransformed lesion data through the non-parametric mode of J/qtl revealed a significant QTL on chromosome 10 and a suggestive QTL on chromosome 9 influencing atherosclerotic lesion sizes (Fig. 2). Details of these two QTLs, including locus name, LOD score, peak location, effect size, 95% CI (confidence interval), *P* value, high allele, and mode of inheritance, are presented in Table 1. The chromosome 10 QTL had a significant LOD score of 5.05 and peaked at 20.2 Mb. This QTL exerted effect in an additive mode of inheritance with the BALB allele conferring larger lesion size (Fig. 3). This locus overlapped in the confidence interval with *Ath11*, mapped in a *B6-Apoe*^{-/-} × *FVB-Apoe*^{-/-} intercross (Dansky et al. 2002), and *Ath20*, mapped in a B6 × 129 intercross (Ishimori et al. 2004). However, because this QTL was mapped in a cross derived from progenitor strains different from previous ones, it was designated *Ath48* to represent a new locus for atherosclerosis. The chromosome 9 QTL had a suggestive LOD score of 2.12 and peaked at 49.5 Mb. This QTL affected atherosclerosis in a heterotic mode of inheritance. A genome-wide scan using the square-root-transformed lesion data through the parametric mode of J/qtl yielded the same two QTLs as the last scan, but their respective LOD scores were larger: the chromosome 10 QTL had a LOD score of 6.03 and the chromosome 9 QTL had a LOD score of 2.29 (Table 1).

Candidate genes for *Ath48*

Ath48 overlaps in the confidence interval with *Ath11* mapped in a *B6-Apoe*^{-/-} × *FVB-Apoe*^{-/-} intercross and *Ath20* mapped in a B6 × 129 intercross (Dansky et al. 2002), (Ishimori et al. 2004). Among the 3 crosses that have yielded the chromosome 10 QTLs, B6 and SM strains carry the low allele conferring reduced atherosclerosis, while BALB, FVB and 129 strains carry the high allele conferring increased atherosclerosis. We searched the Sanger Mouse Genomes database for genes containing nonsynonymous SNP(s) or SNP(s) in upstream regulatory regions that were shared by the high allele strains (BALB, FVB and 129) but were different from ones shared by the low allele strains (B6) within the confidence interval of *Ath48*. 12 candidate genes were identified, including *Mthfd11*, *Zbtb2*, *Esr1*, *Mtrf11*, *Pcmt1*, *Katna1*, *Ppil4*, *Ust*, *Sash1*, *Tnfrsf25*, *Fam54a*, and *Raet1e*. For these genes, we

further searched other publicly available databases, including the Mouse Phenome Database (<http://phenome.jax.org/db/q?rtn=snp/home>) and Ensembl (<http://www.ensembl.org/index.html>), and compiled the results in Table 2. Of them, *Tnfaip3* (19.0 Mb) and *Fam54a* (20.36 Mb) are located precisely underneath the linkage peak of *Ath48*. Two nonsynonymous SNPs in the *Tnfaip3* gene led to substitutions of glutamic acid (Glu) to alanine (Ala) at residue 627 and proline (Pro) to leucine (Leu) at residue 699 (Fig. 4). Comparison of *Tnfaip3* protein sequences among man, rat, and mouse suggested that 627Glu and 699Pro are conserved in humans, rats, and B6 mice (Fig. 4). Thus, 627Ala and 699Leu are mutations in the high allele BALB, FVB and 129 strains. Online software RaptorX was then used to predict the potential impact of these substitutions on the 3D structure of *Tnfaip3* protein (<http://raptorx.uchicago.edu/StructurePrediction/>). The predicted 3D structure of this protein was noticeably different between B6 and BALB (Fig. 5).

Real-time PCR was performed to assess the expression of *Tnfaip3* mRNA in the liver of B6 and BALB *Apoe*^{-/-} mice. BALB expressed ~3-fold as much *Tnfaip3* mRNA as B6 based on the relative ratio to *Gapdh* determined by real-time PCR (*Tnfaip3*/*Gapdh* ratio: 0.00172±0.00011 vs. 0.000673±0.000092; *P*=0.00037) (Fig. 6).

Correlations between plasma glucose and lipid levels

Correlations of atherosclerotic lesion areas with fasting plasma lipid and glucose levels were evaluated in the F₂ population (Fig. 7). Significant correlations with non-HDL cholesterol (*R*=0.185; *P*=0.0079) and triglyceride (*R*=0.143; *P*=0.038) were observed. F₂ mice with higher non-HDL cholesterol or triglyceride levels tended to develop larger atherosclerotic lesions than those with lower levels. There was a trend toward an inverse correlation with HDL cholesterol levels (*R*=-0.106; *P*=0.128) and a positive correlation with glucose levels (*R*=0.120; *P*=0.084).

Discussion

BALB and SM mouse strains differ significantly in their susceptibility to atherosclerosis when fed an atherogenic diet or deficient in *Apoe* (Nishina et al. 1993), (Liu et al. 2015). Using a male F₂ cohort derived from the two *Apoe*^{-/-} strains, we identified a significant QTL, *Ath48*, and one suggestive QTL that influenced atherosclerotic lesion sizes. Further bioinformatics analysis prioritized 12 candidate genes for *Ath48* with *Tnfaip3* being the most likely candidate gene. We also observed significant correlations of atherosclerotic lesion areas with non-HDL cholesterol and triglyceride levels and suggestive correlations with HDL and glucose levels in the F₂ mice.

Ath11 is an atherosclerosis susceptibility QTL mapped in two separate intercrosses between B6-*Apoe*^{-/-} and FVB-*Apoe*^{-/-} mice (Dansky et al. 2002). It is located on mouse chromosome 10 peaked at 10 cM (~20 Mb) with the FVB allele conferring increased atherosclerosis and the B6 allele conferring decreased atherosclerosis. An epistatic QTL for atherosclerosis, *Ath20*, has been mapped to this location in a B6 × 129 female intercross with the 129 allele increasing and the B6 allele decreasing atherosclerosis (Ishimori et al. 2004). The present study mapped a major atherosclerosis susceptibility QTL to this location. As this QTL was identified in a cross derived from different progenitor strains, it was given

a new designation, *Ath48*, in accordance to the guideline provided by the International Committee on Standardized Genetic Nomenclature for Mice (<http://www.informatics.jax.org/mgihome/nomen/gene.shtml>). The high allele of *Ath48* was derived from a progenitor strain resistant to atherosclerosis in that F₂ mice homozygous for the BALB allele had a larger atherosclerotic lesion than those homozygous for the SM allele. However, it is somewhat surprising that for all of the 3 crosses that have mapped the chromosome 10 QTLs, the high allele is derived from an atherosclerosis-resistant strain and the low allele derived from a susceptible strain.

The reason why the effect of *Ath11* or *Ath48* on atherosclerosis has not shown up in the high allele progenitor strains is unknown. The finding from our studies of C3H/HeJ mice suggests that the action of some QTLs is dependent on the presence of another QTL. Despite carrying multiple atherosclerosis susceptibility QTLs, including *Ath28* on chromosome 2 and *Ath33* on chromosome 15 (Rowlan et al. 2013), C3H/HeJ mice are extremely resistant to atherosclerosis. However, after their *H2^k* haplotype (murine major histocompatibility complex) is replaced with the *H2^b* haplotype and bone marrow is reconstituted with syngeneic marrow, C3H/HeJ mice developed 4-fold larger atherosclerotic lesions than atherosclerosis-susceptible B6 mice (Shi et al. 2010).

By examining genes containing SNPs that are shared among the high allele strains (BALB, FVB, 129) but different from those shared by the low allele strains (C57BL/6J, SM/J), we have reduced the number of candidate genes for *Ath48* down to 12. This analysis is based on the assumption that most genetic variations between mouse strains are ancestral and inherited primarily from their progenitors *M. m. musculus* and *M. m. domesticus* (Wade et al. 2002). All strains carrying an allele that results in a high value for a given trait often share the same ancestral allele harboring the underlying causal gene, while strains carrying an allele that produces a low value for the trait share a different ancestral allele (Peters et al. 2007). Because a QTL is derived from changes in the function or the amount of a gene product, we have focused on genes that carry a nonsynonymous SNP or a SNP in upstream regulatory region segregating between the high allele and the low allele strains of the QTL crosses. *Tnfaip3* is located precisely underneath the linkage curve peak and contains two nonsynonymous SNPs and one SNP in the upstream regulatory region segregating between the high allele and low allele progenitor strains. The two nonsynonymous SNPs occur in the conserved coding regions and have led to changes in the 3D structure of *Tnfaip3* protein in the high-allele strains. *Tnfaip3* encodes the TNF alpha induced protein 3, which exerts a potent anti-inflammatory function in endothelial cells and smooth muscle cells by inhibiting NF-κB activation (Cooper et al. 1996)(Patel et al. 2006). Haploinsufficiency of *Tnfaip3* increased atherosclerosis while overexpression of *Tnfaip3* suppressed atherosclerosis in *Apoe*^{-/-} mice (Wolfrum et al. 2007). We found that *Tnfaip3* was more abundantly expressed in the liver of the high allele BALB strain than the low allele B6 strain, which might reflect a compensatory change in response to the insufficiency of protein function in the high allele strain.

Raet1e is also a likely candidate gene for *Ath48*. This gene is highly polymorphic between the high allele and low allele strains, with multiple SNPs leading to amino acid substitutions.

Increased expression of *Raet1e* in the aorta suppresses atherosclerosis in either subcongenic or transgenic mice (Rodriguez et al. 2013).

The chromosome 9 QTL for atherosclerosis overlaps with *Ath29*, initially mapped in a B6 x C3H *Apoe*^{-/-} intercross and then replicated in several mouse crosses (Su et al. 2006b) (Tomita et al. 2010)(Wang et al. 2007a)(Wang et al. 2007b). This QTL overlaps with a HDL QTL we recently identified using the same mouse cross (Shi et al. 2016). There was also a trend toward a significant correlation between HDL cholesterol levels and atherosclerotic lesion areas in this cross. In this regard, *Sor11*, *Sik3*, and *Apoa1* that affect lipid metabolism are strong candidate genes for *Ath29*. These genes each contain one or more SNPs segregating between the high allele strains and the low allele strains of the QTL cross (data not shown). Another promising candidate gene is *Rcn2*, which plays a key role in regulation of both basal and oxidized lipid-induced proinflammatory gene expression in endothelial cells (Manichaikul et al. 2011).

Recent human genetic studies suggest that LDL and triglycerides are causally related to risk of coronary heart disease (CHD) (Global Lipids Genetics Consortium et al. 2013),(Holmes et al. 2015). Genetic variants that increase LDL cholesterol or triglyceride levels result in an increased risk for CHD. The significant correlations of atherosclerotic lesion areas with non-HDL cholesterol and triglyceride levels observed in this cross suggest that the increased CHD risk is partially due to increased atherosclerotic lesion formation. For HDL, no association has been found between genetic variants and CHD risk once adjusted for triglycerides or LDL cholesterol in humans (Holmes et al. 2015). Accordingly, no significant correlation between HDL cholesterol levels and atherosclerotic lesion areas was observed in this cross or a previous cross (Su et al. 2006b).

We observed a marginal correlation between atherosclerotic lesion areas and plasma glucose levels in this cross. When fed a Western diet, both BALB-*Apoe*^{-/-} and SM-*Apoe*^{-/-} mice show significant increases in plasma glucose levels and atherosclerotic lesion areas (Liu et al. 2015). The small correlation suggests that other factors than plasma glucose contributed to aggravated atherosclerosis in the *Apoe*^{-/-} mice.

In summary, we have identified a major QTL for atherosclerosis using a segregating F₂ population, and further applied bioinformatics resources to prioritize *Tnfrsf25* as the most likely causal gene. As *Tnfrsf25* exerts effect through inhibition of the NF-κB pathway, our findings highlight the significance of anti-inflammatory action in atherogenesis and its potential for developing anti-atherosclerotic therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Frequency distributions of atherosclerotic lesion areas in male F_2 mice derived from BALB- $Apoe^{-/-}$ and SM- $Apoe^{-/-}$ mice. Left panel: the distribution of untransformed atherosclerotic lesion areas; right panel: the distribution of square-root transformed atherosclerotic lesion areas. Graphs were created using a plotting function of J/qtl software.

Figure 2.

Genome-wide scans to search for main effect QTLs influencing atherosclerotic lesion areas in male F_2 mice. Chromosomes 1 through 20 are represented numerically on the X-axis. Each minor tick on the X axis represents one informative SNP. The Y-axis represents the LOD score. Two horizontal dashed lines denote genome-wide thresholds for suggestive ($P=0.63$) and significant ($P=0.05$) linkage. The upper panel (A) shows a genome-wide scan for atherosclerotic lesion areas using the non-parametric mode, and the lower panel (B) shows a genome-wide scan for atherosclerotic lesion areas using the parametric mode. Graphs were created by J/qtl

Figure 3.

Interval mapping graphs of chromosomes 10 (left panel) and 9 (right panel) for atherosclerotic lesion areas created by MapManager QTX. The black plot represents LOD scores calculated at 1-cM intervals, the red plot denotes the effect of BALB alleles, and the blue plot represents the effect of SM alleles. The histogram in the plot estimates the confidence interval for a QTL. Two green vertical lines denote genome-wide significance thresholds for suggestive or significant linkage ($P=0.63$ and $P=0.05$, respectively).

B6: cqkcfieaqnqr ... hrge**p**tpeeppk
BALB: cqkcfi**a**aqnqr ... hrge**L**tpeeppk
Rat: cqkcfieaqnqr ... hrgeptpeeppk
Human: cqkcfieaqnqr ... hrgepapedppk

↑
627

↑
699

Figure 4.

Comparison of selected *Tnfaip3* protein sequences in three mammal species, including rat, human, B6 and BALB mice. Amino acid residues that are different among the species are highlighted.

Figure 5.
3D structure of *Tnfaip3* protein for B6 (left panel) and BALB (right panel) mice predicted by RaptorX software. The predicted 3D structure is noticeably different between the two mouse strains.

Figure 6.

Real-time analyses of *Tnfaip3* and *Gapdh* mRNA expression in the liver of B6 and BALB *Apoe*^{-/-} mice fed a Western diet. Results were expressed as a ratio of *Tnfaip3* to *Gapdh* in real-time PCR cycle threshold (Ct) values of 4 individual mice per strain. * $P < 0.05$.

Figure 7.

Correlations of atherosclerotic lesion sizes with plasma levels of HDL, non-HDL cholesterol, triglyceride, and glucose in the F_2 mice. Each point represents values of an individual F_2 mouse. The correlation coefficient (R) and significance (P) are shown.

Table 1

Significant and suggestive QTLs for atherosclerotic lesion areas in male F₂ mice derived from BALB-*Apoe*^{-/-} and SM-*Apoe*^{-/-} mice.

Locus name	Chr	Mode of analysis	LOD ^a	Peak (Mb)	Closest marker	Effect size (%) ^b	95%CI ^c	High allele	Mode of inheritance ^d
<i>Ath29</i>	9	Nonparametric	2.12	49.5	rs3688870	5	9.14–59.14	H	Heterosis
<i>Ath48</i>	10	Nonparametric	5.05	20.2	rs3667084	13	9.16–23.16	B	Additive
<i>Ath29</i>	9	Parametric	2.29	49.5	rs3688870	5	9.14–49.14	H	Heterosis
<i>Ath48</i>	10	Parametric	6.03	20.2	rs3667084	13	9.16–23.16	B	Additive

^aLOD scores were obtained from genome-wide QTL analysis using J/qtl. The significant LOD scores were highlighted in bold. The suggestive and significant LOD score thresholds were determined by 1,000 permutation tests. Suggestive and significant LOD scores were 2.092 and 3.468, respectively, for untransformed atherosclerotic lesion data (nonparametric), and 2.174 and 3.572, respectively, for square root-transformed lesion areas (parametric).

^bEffect size (%) of the marker closest to the QTL peak was determined by Map Manager.

^c95% Confidence interval in cM defined by a whole genome QTL scan.

^dMode of inheritance was defined according to allelic effect at the nearest marker of a QTL.

Table 2

Haplotype analysis for *Ath48* on chromosome 10 (0 to 50 Mb).

Position	Gene	dbSNP	Low allele			High allele			Csq	SIFT
			C57BL/6	129	BALB	FVB				
3978708	Mthfd1l	rs29320259	T	C	C	C			missense_variant	1
4007800	Mthfd1l	rs29378605	T	C	C	C			missense_variant	0.18
4369441	Zbb2	rs254179209	T	G	G	G			missense_variant	0.71
4966154	Esr1	rs29315913	G	T*	T*	T*			missense_variant nc_transcript_variant	0.16
5823831	Mtrf1l	rs29343310	T	C	C	C			5_prime_utr_variant	-
7638968	Pcm1l	rs47720906	T	C*	C*	C*			missense_variant nmd_transcript_variant	0.04
7741513	Katna1	rs13470645	T	C*	C*	C*			missense_variant nmd_transcript_variant	0.67
7821178	Ppil4	rs29359974	C	A	A	A			missense_variant	1
8518788	Ust	rs29378325	T	C	C	C			5_prime_utr_variant	-
8725513	Sash1	rs29375710	C	T	T	T			missense_variant	0.31
19003739	Tnfaip3	rs29345620	T	G*	G*	G*			missense_variant nc_transcript_variant	1
19002963	Tnfaip3	rs29353577	A	G	G	G			missense_variant	0.29
19015224	Tnfaip3	rs29315296	C	G*	G*	G*			5_prime_utr_variant nc_transcript_variant	-
20357285	Fam54a	rs48866598	C	T	T	T			missense_variant	0.21
22180662	Raet1e	rs49018430	T	C	C	C			missense_variant	0.33
22180672	Raet1e	rs230540838	T	C	C	C			missense_variant	0.23
22180675	Raet1e	rs250225184	C	T	T	T			missense_variant	0.67
22180681	Raet1e	rs49817652	G	A	A	A			missense_variant	1
22180687	Raet1e	rs47146068	T	C	C	C			missense_variant	1
22180696	Raet1e	rs49126056	C	T	T	T			missense_variant	0.08
22180710	Raet1e	rs49637041	A	C	C	C			missense_variant	1
22180726	Raet1e	rs50722116	A	G	G	G			missense_variant	0.65
22180821	Raet1e	rs51079123	G	A	A	A			missense_variant	0.11

Analysis was performed based on the Mouse Genomes Project and Mouse Phenome Databases. Functional candidate genes are denoted in bold. Csq: consequences;

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most likely SNP. SIFT (Sorting Intolerant From Tolerant): A parameter for predicting the effect of an amino acid substitution on protein function.
*