Mapping and Congenic Dissection of Genetic Loci Contributing to Hyperglycemia and Dyslipidemia in Mice

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Abstract

Background
Patients with dyslipidemia have an increased risk of developing type 2 diabetes, and diabetic patients often have dyslipidemia. Potential genetic connections of fasting plasma glucose with plasma lipid profile were evaluated using hyperlipidemic mice.

Methods
225 male F2 mice were generated from BALB/cJ (BALB) and SM/J(SM) Apoe-deficient (Apoe−/−) mice and fed a Western diet for 5 weeks. Fasting plasma glucose and lipid levels of F2 mice were measured before and after 5 weeks of Western diet and quantitative trait locus (QTL) analysis was performed using data collected from these two time points. 144 SNP (single nucleotide polymorphism) markers across the entire genome were typed.

Results
One major QTL (logarithm of odds ratio (LOD): 6.46) peaked at 12.7 cM on chromosome 9, Bglu16, and 3 suggestive QTLs on chromosomes 15, 18 and X were identified for fasting glucose, and over 10 loci identified for lipid traits. Bglu16 was adjacent to a major QTL, Hdlq17, for high-density lipoprotein (HDL) cholesterol (LOD: 6.31, peak: 19.1 cM). A congenic strain with a donor chromosomal region harboring Bglu16 and Hdlq17 on the Apoe−/− background showed elevations in plasma glucose and HDL levels. Fasting glucose levels were significantly correlated with non-HDL cholesterol and triglyceride levels, especially on the Western diet, but only marginally correlated with HDL levels in F2 mice.

Conclusions
We have demonstrated a correlative relationship between fasting glucose and plasma lipids in a segregating F2 population under hyperlipidemic conditions, and this correlation is partially due to genetic linkage between the two disorders.
Introduction
Dyslipidemia, characterized by elevations in plasma triglyceride and LDL cholesterol levels and reductions in HDL cholesterol levels, frequently occurs with hyperglycemia as part of the metabolic syndrome, which also includes abdominal obesity, insulin resistance, and hypertension [1]. Although the nature for the close association between dyslipidemia and hyperglycemia is not well understood, pleiotropic effects of genetic mutants or variants affecting both traits appear to play a role. Indeed, a few rare genetic mutations involving ABCA1 [2], LIPE [3], LPL [4], or LRP6 [5] cause both dyslipidemia and hyperglycemia. Genome-wide association studies (GWAS) have identified a number of common variants associated with variations in plasma lipids [6] [7] and fasting plasma glucose [8] [9] [10]. Over a dozen of them are associated with both traits at the genome-wide significance level (http://www.genome.gov/GWAStudies/). Unexpectedly, half of them, including CETP, MLXIPL, PLTP, GCKR, APOB, APOE-C1-C2, CYP7A1, and TIMD4, have exhibited opposite allelic effect on plasma lipid and glucose levels [11], a finding that is in contrast to the positive correlations observed in the clinical situation. Furthermore, it is quite challenging to establish causality between a common variant and a complex trait in humans due to small gene effect, complex genetic structure, and environmental influences.

One approach to the problems encountered in human genetic studies is to use inbred strains of mice differing in glucose and lipid profile. Apolipoprotein E-deficient (Apoe−/−) mice develop spontaneous dyslipidemia on a low fat chow diet, with elevated non-HDL cholesterol levels and reduced HDL levels [12] [13]. Feeding a high fat diet aggravates dyslipidemia. Moreover, these mice develop all phases of atherosclerotic lesions seen in humans [14] [15] [16] [17] [18]. We have found that Apoe−/− mice with the C57BL/6J, C3H/HeH, SM/J (SM) or SWR/J genetic background develop significant hyperglycemia when fed a Western diet but become resistant when transferred on to the BALB/cJ (BALB) background [19] [20] [16]. In the present study, we performed quantitative trait locus (QTL) analysis using a male cohort derived from BALB-Apoe−/− and SM-Apoe−/− mice to find potential genetic connections between plasma glucose and lipid traits.

Methods
All procedures were carried out in accordance with current National Institutes of Health guidelines and approved by the University of Virginia Animal Care and Use Committee (Assurance #A3245-01, Animal Protocol #3109).

Mice
BALB and SM Apoe−/− mice were created using the classic congenic breeding strategy, as described [16]. BALB-Apoe−/− mice were crossed with SM-Apoe−/− mice to generate F1s, which were intercrossed by brother-sister mating to generate a cohort of F2 mice. Mice were weaned at 3 weeks of age onto a rodent chow diet. At 8 weeks of age, male F2 mice were started on a Western diet containing 21% fat, 34.1% sucrose, 0.15% cholesterol, and 19.5% casein by weight (Harlan Laboratories, TD 88137) and maintained on the diet for 5 weeks.

Measurements of plasma glucose and lipid levels
Mice were bled twice: once before the start of the Western diet and once at the time of euthanasia. Mice were fasted overnight before blood was drawn from the retro-orbital venous plexus with the animals under isoflurane anesthesia. Plasma glucose was measured with a Sigma glucose (HK) assay kit, as reported [21]. Total cholesterol, HDL cholesterol, and triglyceride...
were measured using Thermo DMA (Louisville, CO) assay kits [13]. Non-HDL cholesterol was calculated as the difference between total and HDL cholesterol.

Genotyping
Genomic DNA was isolated from the tails of mice by using the phenol/chloroform extraction and ethanol precipitation method. F2 mice were genotyped by the Jackson Laboratory Genotyping Services using mouse strain-specific SNP arrays. DNA samples from the two parental strains and their F1s served as controls. 144 SNPs and 225 F2 mice were included for QTL analysis.

Studies with congenic mice
Construction of a congenic strain in which a chromosome 9 segment from 5 to 61 cM was transferred from C3H/HeJ- Apoe−/− mice onto the C57BL/6J- Apoe−/− background was previously reported [22]. Male congenic and C57BL/6J- Apoe−/− control mice were started with the Western diet at 6 weeks of age and maintained on the diet for 12 weeks. Blood samples were collected from overnight fasted mice before and after 12 weeks of Western diet.

Statistical analysis
QTL analysis was performed using the standard analysis software J/qtl and Map Manager QTX as we previously reported [19] [23] [24]. One thousand permutations of trait values were run to define the genome-wide LOD (logarithm of odds) score threshold for significant or suggestive linkage of each trait. Loci that exceeded the 95th percentile of the permutation distribution were considered significant (P<0.05) and those exceeding the 37th percentile were suggestive (P<0.63). Student's unpaired t test was used to determine statistical significance between congenic and control mice in trait values.

Results
Trait value distributions
Values of fasting plasma glucose and triglyceride levels of F2 mice on both chow and Western diets and of HDL and non-HDL cholesterol levels on the chow diet are normally or approximately normally distributed (Fig 1). Values of Ln (natural logarithm)-transformed HDL and non-HDL cholesterol levels on the Western diet approach the normal distribution. These data were analyzed using J/qtl software to search for QTLs affecting the traits. Loci with a genome-wide suggestive or significant P value are presented in Table 1.

Fasting glucose levels
A genome-wide scan for main effect QTL revealed a highly significant QTL in the proximal region of Chr9 for fasting glucose levels when mice were fed the chow diet (12.7cM, LOD:6.463) (Fig 2 and Table 1) (original genotype and phenotype data used for QTL analysis are provided in Table A in S1 text). This locus is overlapping in position with Bglu16, recently mapped in female F2 mice derived from BALB and SM Apoe−/− mice. Two suggestive loci, located on Chr15 and Chr18, for fasting glucose were also detected when the cross were on the chow diet. The Chr15 locus replicates Bglu8 and the Chr18 locus replicates Bglu10, initially mapped in a NZB/B1NJ x NZW/LacJ intercross [25]. When F2 mice were fed the Western diet, a suggestive locus for fasting glucose was detected in the distal region of ChrX (68.38 cM, LOD: 3.069). This locus was novel. Inheritance of BALB alleles conferred an increased glucose level.
for the Chr9 and Chr15 QTLs while inheritance of SM alleles conferred increased glucose levels for Chr18 and ChrX QTLs (Table 2).

**Fasting lipid levels**

Genome-wide scans for main effect QTLs detected multiple loci for HDL, non-HDL cholesterol, and triglyceride levels (Figs 3, 4 and 5, Table 1). For HDL, 3 suggestive QTLs, located on Chr1, Chr12 and Chr20, were found on the chow diet and 5 QTLs, located on Chr1, 9, 13, 15
and 17, were found on the Western diet. The Chr9 QTL peaked at 19.13 cM and had a highly significant LOD score of 6.31 (Table 1). This QTL is overlapping in position with Hdlq17, mapped in female B6x129S1/SvImJ F2 mice [26]. Though partially overlapping, the position of this QTL was noticeably different from that of Bglu16 (Fig 4). The Chr1 locus replicated Cq1 and Hdlq5, which have been mapped in numerous crosses[27]. The Chr13 QTL replicated Hdlcl2, initially mapped in (PERA/EiJ x B6-Ldlr) x B6-Ldlr backcross [28]. The Chr15 QTL replicated Hdlq45, previously mapped in a B6 x A/J intercross[29]. The Chr17 locus replicated Hdlq56, mapped in a B6 x 129 intercross [30].

For non-HDL, 2 QTLs on Chr1 and Chr15 were detected when mice were fed the Western diet. The Chr1 QTL peaked at 60.14 cM and had a LOD score of 3.94 (Fig 5 and Table 1). This QTL replicated Nhdlq13, mapped in a B6 x C3H Apoe-/- intercross [31]. The QTL on Chr15 peaked at 41.66 cM and had a LOD score of 3.52. It replicated Nhdlq9, mapped previously in PERA/EiJ X DBA/2J and B6-Apoe-/- X C3H-Apoe-/- intercrosses[32][33].

For triglyceride, 3 suggestive QTLs, located on Chr1 and ChrX, were detected when mice were fed the Western diet (Fig 6). The QTL on ChrX had a LOD score of 3.25 and peaked at 66.4 cM. This QTL was replicated on the chow diet and thus named Tgq35. The 2 suggestive QTLs on Chr1, peaked at 41.8 and 80.1 cM, replicated Tgq9 and Tglq1, respectively [34].
Correlations between plasma glucose and lipid levels

Correlations of fasting plasma glucose levels with plasma levels of HDL, non-HDL cholesterol or triglyceride were evaluated in the F2 population fed either chow or Western diet (Fig 7). Significant correlations of fasting glucose with non-HDL cholesterol and triglyceride were observed when mice were fed either chow ($R^2 = 0.1498$ and $P = 5.3E-4$ for non-HDL; $R^2 = 0.1193$ and $P = 6.56E-7$ for triglyceride) or Western diet ($R^2 = 0.3899$ and $P = 4.48E-25$ for non-HDL; $R^2 = 5782$ and $P = 2.61E-43$ for triglyceride). F2 mice with higher non-HDL cholesterol or triglyceride levels also had higher fasting glucose levels, especially on the Western diet. In contrast, HDL cholesterol levels were only marginally correlated with fasting glucose levels on either chow ($R^2 = 0.0724$ and $P = 6.3E-6$) or Western diet ($R^2 = 0.0199$ and $P = 0.035$).
Confirmation of chromosome 9 QTLs

C3H/HeJ and BALB strains share essentially identical haplotype blocks for the chromosome 9 region harboring Bglu16 and Hdlq17 (10–30 cM), and also QTLs for fasting glucose and HDL have been mapped in this region using intercrosses derived from C3H/HeJ[19][35]. Thus, we used a congenic strain carrying a chromosomal region harboring Bglu16 and Hdlq17 from the C3H/HeJ donor strain to test QTL effects on fasting glucose and lipid profile. Male congenics had significantly higher fasting plasma glucose levels than C57BL/6 Apoe−/− mice on either chow (189.1 ± 8.8 vs. 142.0 ± 15.2 mg/dl; P = 0.017) or Western diet (348.8 ± 19.0 vs. 215.9 ± 20.6 mg/dl; P = 0.00017) (Fig 8 and Table B in S1 text). HDL cholesterol levels were nearly 2-fold higher in congenics than in C57BL/6 Apoe−/− mice on the chow diet (133.0 ± 12.0 vs. 88.4 ± 6.4 mg/dl; P = 0.0039). On the Western diet, HDL cholesterol levels were also higher in congenics (71.1 ± 12.5 vs. 55.6 ± 9.7 mg/dl), although the difference did not reach statistical significance (P = 0.339). In contrast, congenics were comparable with C57BL/6 Apoe−/− mice in non-HDL cholesterol levels (chow: 191.5 ± 15.2 vs. 160.1 ± 16.5 mg/dl, P = 0.177; Western: 809.5 ± 40.7 vs. 784.2 ± 46.8 mg/dl, P = 0.689) and triglyceride levels (chow: 73.1 ± 3.8 vs. 70.4 ± 3.9 mg/dl, P = 0.626; Western: 70.0 ± 4.5 vs. 73.7 ± 3.8 mg/dl, P = 0.543).

Discussion

BALB Apoe−/− mice have much higher HDL and lower non-HDL cholesterol levels and are more resistant to development of type 2 diabetes compared to SMapoemice[16][20]. In this study, we performed QTL analysis using a male F2 cohort derived from the two Apoe−/− mouse strains to investigate genetic connections between glucose and lipid-related traits. One major QTL for
fasting glucose, \textit{Bglu16}, is immediately adjacent but not coincident with a major QTL for HDL cholesterol, \textit{Hdlq17}, on proximal chromosome 9. The presence of these two QTLs was confirmed with a congenic strain. Moreover, strong correlations of fasting glucose with non-HDL and triglyceride levels were observed in F2 mice when fed the Western diet.

In this study, one significant QTL and three suggestive QTLs have been identified to influence glucose homeostasis under fasting conditions. The significant QTL on proximal chromosome 9 is coincident with \textit{Bglu16}, recently mapped in a female intercross between BALB and SM Apoe\textsuperscript{−/−} mice. A suggestive QTL for fasting glucose has also been mapped to this position in a C57BL/6 x BALB Apoe\textsuperscript{−/−} intercross\cite{21}. For all three intercrosses, inheritance of BALB alleles at the locus contributed to increased fasting glucose levels. A locus for glucose-stimulated insulin secretion has been mapped to this position in a C57BL/6J x C3H/HeJ intercross \cite{36}. The suggestive QTL on chromosome 15 is close to \textit{Bglu8}, mapped in a NZB x NZW intercross \cite{25}. Linkages close to this locus have been detected in two other crosses involving BALB mice but inheritance of BALC alleles was associated with reduced fasting glucose levels \cite{21}\cite{37}. The QTL on chromosome 18 is coincident with \textit{Bglu10}, mapped in a NZB x NZW intercross \cite{25}.
All QTLs identified for plasma lipids confirm those mapped in previous studies except for the one on X chromosome for triglyceride that is new and named $Tgq30$. This QTL occurred with a suggestive LOD score under both chow and Western diet feeding conditions. It is considered appropriate to name a suggestive QTL if it has been repeatedly observed [38]. The major QTL for HDL is coincident with $Hdlq17$, mapped in a C57BL/6 x 129S1/SvImJ female intercross [26]. This QTL has been replicated in multiple crosses, including B6 x 129, B6 x CAST/EiJ, B6-Apo$e^{-/-}$ x C3H-Apo$e^{-/-}$, and B6-Apo$e^{-/-}$ x BALB-Apo$e^{-/-}$ intercrosses [33][39][40][41][30][31]. We conducted haplotype analysis for this QTL and narrowed candidates down to two dozen genes (Table C in S1 Text). These candidate genes contain one or more non-synonymous SNPs in the coding regions or SNPs in the upstream regulatory region that are shared by the high allele strains but are different from the low allele strains at the QTL. Among them, $Ubash3b$, $Phldb1$, $Sorl1$, $Sik3$, and $Apoa1$ have been shown to be associated with variations in total, HDL cholesterol or triglyceride levels in humans (http://www.ebi.ac.uk/gwas/home). Linkage close to this locus has also been detected in a female intercross derived from BALB and SM Apo$e^{-/-}$ mice but BALB alleles were associated with reduced HDL levels [42]. The opposite allelic effect on HDL in the male vs. female crosses suggests that two or more genes in this region contributed to the trait.

**Fig 4. Interval mapping graphs for fasting glucose (left panel) and HDL (right panel) on chromosome 9.** The histogram in the plot estimates the confidence interval for a QTL. Note the difference in position between the two QTLs. Two green vertical lines represent genome-wide significance thresholds for suggestive or significant linkage ($P = 0.63$ and $P = 0.05$, respectively). Black plots reflect the LOD score calculated at 1-cM intervals, the red plot represents the effect of BALB alleles, and the blue plot represents the effect of SM alleles.

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As BALB and C3H/HeJ strains share essentially identical haplotype blocks for the chromosomal region harboring \textit{Bglu16} and \textit{Hdlq17} and as QTLs for fasting glucose and HDL have been mapped to this region in crosses derived from C3H/HeJ mice \cite{19}\cite{35}, we used a congenic strain carrying the C3H/HeJ chromosome 9 donor alleles to confirm the presence of the two QTLs. However, as the congenic strain carries a chromosomal segment much longer than the confidence interval of \textit{Bglu16} and \textit{Hdlq17}, other QTLs in the congenic region might also contribute to the QTL effects observed in the congenic mice.

We have observed positive correlations of fasting glucose levels with non-HDL cholesterol and triglyceride levels in the F2 population under either feeding condition. The correlations were extremely high when mice developed significant dyslipidemia on the Western diet. Similar findings have been observed in other crosses derived from Apoe\textsuperscript{-/-} mouse strains\cite{21} and humans \cite{11}\cite{43}. Emerging human studies have also shown associations of non-HDL cholesterol and ApoB with incident type 2 diabetes \cite{44},\cite{45},\cite{46}. Despite the strong correlations, only one suggestive QTL for fasting glucose was coincident with one QTL for triglyceride on chromosome X and there was no coincident QTL between glucose and non-HDL traits.

\textbf{Fig 5. Genome-wide scans to search for loci influencing non-HDL cholesterol levels.} (A) F\textsubscript{2} mice were fed a chow diet. (B) Mice were fed a Western diet. Two loci on chromosomes 1 and 15 were identified for non-HDL cholesterol levels under the Western diet.

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We observed a slight but positive correlation between HDL cholesterol and fasting glucose levels in male F2 mice on either chow or Western diet. This finding is in contrast with the negative correlation between the two traits in a female F2 cohort derived from the same parental strains[42]. Prospective human studies have shown that HDL is inversely correlated with the risk of type 2 diabetes [47][48]. HDL can increase insulin secretion from \( \beta \)-cells, improve insulin sensitivity of the target tissues, and accelerate glucose uptake by muscle due to its diverse functions, including cholesterol efflux and reverse cholesterol transport, anti-oxidation, anti-inflammation and activation of the AMP-activated protein kinase[49][50]. The positive correlation between HDL and fasting glucose observed in this study may suggest that HDL has lost its anti-diabetic function. Indeed, under pathological conditions such as the acute phase response and chronic inflammatory diseases, HDL undergoes qualitative changes in both

Fig 6. Genome-wide scans to search for loci influencing triglyceride levels. (A) F2 mice were fed a chow diet. (B) Mice were fed a Western diet. Three suggestive loci on chromosomes 1 and X were identified for triglyceride levels.

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components and structure and can lose protective function[51]. The observed correlation between HDL and fasting glucose could also be derived from the genetic effect of two closely linked QTLs with each affecting one trait, like \textit{Bglu16} and \textit{Hdlq17}. The reasons for the discrepancy between male and female F2 mice in the correlations are unknown. Multiple factors could contribute: First, female mice were fed the western diet for 12 weeks starting at 6 weeks of age while males were fed the diet for 5 weeks starting at 8 weeks of age. Second, male F2s had higher glucose levels (chow: 110 vs 99, Western: 191 vs 147 mg/dl) than their female counterparts, suggesting that males are more susceptible to diet-induced type 2 diabetes. Finally, sex differences in metabolic traits have been observed in humans and mice [52][53].

Dyslipidemia and hyperglycemia are integral components of metabolic syndrome, a group of risk factors that increase risk for cardiovascular disease and type 2 diabetes. We have identified multiple loci contributing to dyslipidemia and hyperglycemia from a male F2 cohort. One major QTL for fasting glucose, \textit{Bglu16}, is adjacent to \textit{Hdlq17}, a QTL for HDL on chromosome 9. The strong correlations of fasting glucose with non-HDL cholesterol and triglyceride support the hypothesis that dyslipidemia plays a causative role in the development of type 2 diabetes [54].
Fig 8. Comparison of male congenic and background control mice in fasting plasma glucose, HDL, non-HDL cholesterol, and triglyceride levels when fed a chow or Western diet. Congenic mice contained a donor C3H/HeJ chromosome 9 segment harboring Bglu16 and Hdlq17 on the C57BL/6J Apoe−/− background. Results are means ± SE of 9 to 14 mice. * P<0.05.

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Supporting Information
S1 Text. Supporting tables: genotypic and phenotypic data used for quantitative trait locus (QTL) analysis, characterization of congenic strains, and haplotype analysis. (XLSX)

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Author Contributions
Conceived and designed the experiments: WS. Performed the experiments: WS QW WC JL. Analyzed the data: WS. Contributed reagents/materials/analysis tools: WS. Wrote the paper: WS. Constructed the congenic strain and Apoe−/− mouse strains: WS.

References
1. Kaur J. A comprehensive review on metabolic syndrome. Cardiol Res Pract. 2014; 2014: 943162.
2. Saleheen D, Nazir A, Khanum S, Haider SR, Frossard PM. R1615P: a novel mutation in ABCA1 associated with low levels of HDL and type II diabetes mellitus. Int J Cardiol. 2006; 110: 259–260. PMID: 16055210

3. Albert JS, Yerges-Armstrong LM, Horenstein RB, Pollin TI, Sreenivasan UT, Chai S, et al. Null mutation in hormone-sensitive lipase gene and risk of type 2 diabetes. N Engl J Med. 2014; 370: 2307–2315. doi: 10.1056/NEJMoa1315496 PMID: 24848981

4. Hu Y, Ren Y, Luo RZ, Mao X, Li X, Cao X, et al. Novel mutations of the lipoprotein lipase gene associated with hypertriglyceridemia in members of type 2 diabetic pedigrees. J Lipid Res. 2007; 48: 1681–1688. PMID: 17476032

5. Mani A, Radhakrishnan J, Wang H, Mani A, Mani MA, Nelson-Williams C, et al. LRP6 mutation in a family with early coronary disease and metabolic risk factors. Science. 2007; 315: 1278–1282. PMID: 17332414

6. Teslovich TM, Musunuru K, Smith AV, Edmondson AC, Stylianou IM, Koseki M, et al. Biological, clinical and population relevance of 95 loci for blood lipids. Nature. 2010; 466: 707–713. doi:10.1038/nature09270 PMID: 20686565

7. Global Lipids Genetics Consortium, Willer CJ, Schmidt EM, Sengupta S, Peloso GM, Gustafsson S, et al. Discovery and refinement of loci associated with lipid levels. Nat Genet. 2013; 45: 1274–1283. doi: 10.1038/ng.2797 PMID: 24097068

8. Dupuis J, Langenberg C, Prokopenko I, Saxena R, Soranzo N, Jackson AU, et al. New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. Nat Genet. 2010; 42: 105–116. doi: 10.1038/ng.250 PMID: 20081858

9. Soranzo N, Sanna S, Wheeler E, Gieger C, Radke D, Dupuis J, et al. Common variants at 10 genomic loci influence hemoglobin A1c levels via glycemic and nonglycemic pathways. Diabetes. 2010; 59: 3229–3239. doi: 10.2337/db10-0502 PMID: 20858683

10. Manning AK, Hivert MF, Scott RA, Grimsby JL, Bouatia-Naji N, Chen H, et al. A genome-wide approach accounting for body mass index identifies genetic variants influencing fasting glycemic traits and insulin resistance. Nat Genet. 2012; 44: 659–669. doi: 10.1038/ng.2274 PMID: 22581228

11. Li N, van der Sijde MR, Bakker SJ, Dullaart RP, van der Harst P, et al. Pleiotropic effects of lipid genes on plasma glucose, HbA1c, and HOMA-IR levels. Diabetes. 2014; 63: 3149–3158. doi: 10.2337/db13-1800 PMID: 24722249

12. Shi W, Wang NJ, Shihi 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. PMID: 10827138

13. Tian J, Pei H, James JC, Li Y, Matsumoto AH, Helm GA, et al. Circulating adhesion molecules in apoE-deficient mouse strains with different atherosclerosis susceptibility. Biochem Biophys Res Commun. 2005; 329: 1102–1107. PMID: 15752767

14. Nakashima Y, Plump AS, Raines EW, Breslow JL, Ross R. ApoE-deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree. Arterioscler Thromb. 1994; 14: 133–140. PMID: 8274468

15. Shi W, Zhang Z, Chen MH, Angle JF, Matsumoto AH. Genes within the MHC region have a dramatic influence on radiation-enhanced atherosclerosis in mice. Circ Cardiovasc Genet. 2010; 3: 409–413. doi:10.1161/CIRCGENETICS.10.957449 PMID: 20729504

16. Liu S, Li J, Chen MH, Liu Z, Shi W. Variation in Type 2 Diabetes-Related Phenotypes among Apolipoprotein E-Deficient Mouse Strains. PLoS One. 2015; 10: e0120935. doi: 10.1371/journal.pone.0120935 PMID: 25946019

17. Zhang Y, Kundu B, Zhong M, Huang T, Li J, Chordia MD, et al. PET imaging detection of macrophages with a formyl peptide receptor antagonist. Nucl Med Biol. 2015; 42: 381–386. doi: 10.1016/j.nucmedbio.2014.12.001 PMID: 25532700

18. Breslow JL. Genetic differences in endothelial cells may determine atherosclerosis susceptibility. Circulation. 2000; 102: 5–6. PMID: 10880406

19. Su Z, Li Y, James JC, Matsumoto AH, Helm GA, Lusis AJ, et al. Genetic linkage of hyperglycemia, body weight and serum amyloid-P in an intercross between C57BL/6 and C3H apolipoprotein E-deficient mice. Hum Mol Genet. 2006; 15: 1650–1658. PMID: 16595606

20. Li J, Wang Q, Chai W, Chen MH, Liu Z, Shi W. Hyperglycemia in apolipoprotein E-deficient mouse strains with different atherosclerosis susceptibility. Cardiovasc Diabetol. 2011; 10: 117. doi: 10.1186/1475-2840-10-117 PMID: 22204493

21. Zhang Z, Rowian JS, Wang Q, Shi W. Genetic analysis of atherosclerosis and glucose homeostasis in an intercross between C57BL/6 and BALB/cJ apolipoprotein E-deficient mice. Circ Cardiovasc Genet. 2012; 5: 190–201. doi: 10.1161/CIRCGENETICS.111.961649 PMID: 22294616
22. Manichaikul A, Wang Q, Shi YL, Zhang Z, Leitinger N, Shi W. Characterization of Ath29, a major mouse atherosclerosis susceptibility locus, and identification of Rcn2 as a novel regulator of cytokine expression. Am J Physiol Heart Circ Physiol. 2011; 301: H1056–61. doi: 10.1152/ajpheart.00366.2011 PMID: 21666121

23. Yuan Z, Pei H, Roberts DJ, Zhang Z, Rowlan JS, Matsumoto AH, et al. Quantitative trait locus analysis of neointimal formation in an intercross between C57BL/6 and C3H/HeJ apolipoprotein E-deficient mice. Circ Cardiovasc Genet. 2009; 2; 220–228. doi: 10.1161/CIRCGENETICS.108.792499 PMID: 19718279

24. Su Z, Li Y, James JC, McDuffie M, Matsumoto AH, Helm GA, et al. 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. PMID: 16387874

25. Su Z, Tsaih SW, Szatkiewicz J, Shen Y, Paigen B. Candidate genes for plasma triglyceride, FFA, and glucose revealed from an intercross between inbred mouse strains NZB/B1NJ and NZW/LacJ. J Lipid Res. 2008; 49: 1500–1510. doi: 10.1194/jlr.M800053-JLR200 PMID: 18362393

26. Ishimori N, Li R, Kelmenson PM, Konstanje R, Walsh KA, Churchill GA, et al. Quantitative trait loci analysis for plasma HDL-cholesterol concentrations and atherosclerosis susceptibility between inbred mouse strains C57BL/6J and 129S1/SvImJ. Atheroscler Thromb Vasc Biol. 2004; 24: 161–166. PMID: 14928247

27. Ackert-Bicknell C, Paigen B, Konstanje R. Recalculation of 23 mouse HDL QTL datasets improves accuracy and allows for better candidate gene analysis. J Lipid Res. 2013; 54: 984–994. doi: 10.1194/ jlr.M033035 PMID: 23933935

28. Seidelmann SB, De Luca C, Leibel RL, Breslow JL, Tall AR, Welch CL. Quantitative trait locus mapping of genetic modifiers of metabolic syndrome and atherosclerosis in low-density lipoprotein receptor-deficient mice: identification of a locus for metabolic syndrome and increased atherosclerosis on chromosome 4. Atheroscler Thromb Vasc Biol. 2005; 25: 204–210. PMID: 15514201

29. Stylianou IM, Tsaih SW, DiPetrillo K, Ishimori N, Li R, Paigen B, et al. Complex genetic architecture revealed by analysis of high-density lipoprotein cholesterol in chromosome substitution strains and F2 crosses. Genetics. 2006; 174: 999–1007. PMID: 16951076

30. Su Z, Wang X, Tsaih SW, Zhang A, Cox A, Sheehan S, et al. Genetic basis of HDL variation in 129/SvImJ and C57BL/6J mice: importance of testing candidate genes in targeted mutant mice. J Lipid Res. 2009; 50: 116–125. doi: 10.1194/jlr.M090305 PMID: 18772481

31. Rowlan JS, Zhang Z, Wang Q, Fang Y, Shi W. New quantitative trait loci for carotid atherosclerosis identified in an intercross derived from apolipoprotein E-deficient mouse strains. Physiol Genomics. 2013; 45: 332–342. doi: 10.1152/physiogenomics.00099.2012 PMID: 23463770

32. Wittenburg H, Lyons MA, Li R, Kurtz U, Wang X, Mossner J, et al. QTL mapping for genetic determinants of lipoprotein cholesterol levels in combined crosses of inbred mouse strains and F2 crosses. J Lipid Res. 2006; 47: 1780–1790. PMID: 16685081

33. Rowlan JS, Li Q, Manichaikul A, Wang Q, Matsumoto AH, Shi W. Atherosclerosis susceptibility loci identified in an extremely atherosclerosis-resistant mouse strain. J Am Heart Assoc. 2013; 2: e000260. doi: 10.1161/JAHA.113.000260 PMID: 23938286

34. Stylianou IM, Langley SR, Walsh K, Chen Y, Revenu C, Paigen B. Differences in DBA/1J and DBA/2J reveal lipid QTL genes. J Lipid Res. 2008; 49: 2402–2413. doi: 10.1194/jlr.M800244-JLR200 PMID: 18503028

35. Rowlan JS, Zhang Z, Wang Q, Fang Y, Shi W. New quantitative trait loci for carotid atherosclerosis identified in an intercross derived from apolipoprotein E-deficient mouse strains. Physiol Genomics. 2013.

36. Toye AA, Lippiat JD, Proks P, Shimomura K, Bentley L, Hugill A, et al. A genetic and physiological study of impaired glucose homeostasis control in C57BL/6J mice. Diabetologia. 2005; 48: 675–686. PMID: 15729571

37. Shike T, Hirose S, Kobayashi M, Funabiki K, Shirai T, Tomino Y. Susceptibility and negative epistatic interaction of quantitative trait loci contributing to type 2 diabetes and related phenotypes in a KK/Ta mouse model. Diabetes. 2001; 50: 1943–1948. PMID: 11473059

38. Abiola O, Angel JM, Avner P, Bachmanov AA, Belknap JK, Bennett B, et al. The nature and identification of quantitative trait loci: a community's view. Nat Rev Genet. 2003; 4: 911–916. PMID: 14634638

39. Seahayek E, Duncan EM, Yu HJ, Petukhova L, Breslow JL. Loci controlling plasma non-HDL and HDL cholesterol levels in a C57BL/6J x CASA/Rk intercross. J Lipid Res. 2003; 44: 1744–1750. PMID: 12810823

40. Li Q, Li Y, Zhang Z, Gilbert TR, Matsumoto AH, Dobrin SE, et al. Quantitative trait locus analysis of carotid atherosclerosis in an intercross between C57BL/6 and C3H apolipoprotein E-deficient mice. Stroke. 2008; 39: 166–173. PMID: 18048852
41. Lyons MA, Korstanje R, Li R, Walsh KA, Churchill GA, Carey MC, et al. Genetic contributors to lipoprotein cholesterol levels in an intercross of 129S1/SvImJ and RIIIS/J inbred mice. Physiol Genomics. 2004; 17: 114–121. PMID:14872007

42. Wang Q, Grainger AT, Manichaikul A, Farber E, Onengut-Gumuscu S, Shi W. Genetic linkage of hyperglycemia and dyslipidemia in an intercross between BALB/cJ and SM/J Apoe-deficient mouse strains. BMC Genet. 2015; 16: 133. doi: 10.1186/s12863-015-0292-y PMID: 26555648

43. Bayram F, Kocer D, Gundogan K, Kaya A, Demir O, Coskun R, et al. Prevalence of dyslipidemia and associated risk factors in Turkish adults. J Clin Lipidol. 2014; 8: 206–216. doi: 10.1016/j.jacl.2013.12.011 PMID: 24636181

44. Hwang YC, Ahn HY, Yu SH, Park SW, Park CY. Atherogenic dyslipidaemic profiles associated with the development of Type 2 diabetes: a 3.1-year longitudinal study. Diabet Med. 2014; 31: 24–30. doi: 10.1111/dme.12278 PMID: 24636181

45. Hwang YC, Ahn HY, Park SW, Park CY. Apolipoprotein B and non-HDL cholesterol are more powerful predictors for incident type 2 diabetes than fasting glucose or glycated hemoglobin in subjects with normal glucose tolerance: a 3.3-year retrospective longitudinal study. Acta Diabetol. 2014; 51: 941–946. doi: 10.1007/s00592-014-0587-x PMID: 24816996

46. Ley SH, Harris SB, Connelly PW, Mamakeesick M, Gittelsohn J, Wolever TM, et al. Association of apolipoprotein B with incident type 2 diabetes in an aboriginal Canadian population. Clin Chem. 2010; 56: 666–670. doi: 10.1373/clinchem.2009.136994 PMID: 21010448

47. Wilson PW, Kannel WB, Anderson KM. Lipids, glucose intolerance and vascular disease: the Framingham Study. Monogr Atheroscler. 1985; 13: 1–11.

48. Wilson PW, Meigs JB, Sullivan L, Fox CS, Nathan DM, D'Agostino RB S. Prediction of incident diabetes mellitus in middle-aged adults: the Framingham Offspring Study. Arch Intern Med. 2007; 167: 1068–1074. PMID: 17533210

49. Drew BG, Rye KA, Duffy SJ, Barter P, Kingwell BA. The emerging role of HDL in glucose metabolism. Nat Rev Endocrinol. 2012; 8: 237–245. doi: 10.1038/nrendo.2011.235 PMID: 22271188

50. Farbstein D, Levy AP. HDL dysfunction in diabetes: causes and possible treatments. Expert Rev Cardiovasc Ther. 2012; 10: 353–361. doi: 10.1586/erc.11.182 PMID: 22390807

51. Navab M, Reddy ST, Van Lenten BJ, Fogelman AM. HDL and cardiovascular disease: atherogenic and atheroprotective mechanisms. Nat Rev Cardiol. 2011; 8: 222–232. doi: 10.1038/nrcardio.2010.222 PMID: 21304474

52. Weng C, Yuan H, Yang K, Tang X, Huang Z, Huang L, et al. Gender-specific association between the metabolic syndrome and arterial stiffness in 8,300 subjects. Am J Med Sci. 2013; 346: 289–294. doi: 10.1097/MAJ.0b013e3182732e97 PMID: 23503333

53. Mbikay M, Siros F, Gyamera-Achampong C, Wang GS, Rippstein P, Chen A, et al. Variable effects of gender and Western diet on lipid and glucose homeostasis in aged PCSK9-deficient C57BL/6 mice CSK9PC57BL/6. J Diabetes. 2015; 7: 74–84. doi: 10.1111/1753-0407.12139 PMID: 24548670

54. Li N, Fu J, Koonen DP, Kuivenhoven JA, Snieder H, Hofker MH. Are hypertriglyceridemia and low HDL causal factors in the development of insulin resistance? Atherosclerosis. 2014; 233: 130–138. doi: 10.1016/j.atherosclerosis.2013.12.013 PMID: 24529133