Supporting Methods

Histology analyses

For DCC phenotypic characterizations, starting at 6-8 weeks of age, age-matched mice were placed on HP diet containing calcium (0.4%), high phosphate (0.85%), and low (0.04%) magnesium (HP diet; Teklad TD00442, Harlan-Teklad, Madison, WI). After 4 weeks, the heart tissue was collected as described (1). Briefly, freshly isolated heart was washed with phosphate-buffered saline, embedded in optimal cutting temperature (OCT) reagent (Tissue-Tek, Inc.), and snap-frozen on dry ice. Serial 10-μm cryosections of the ventricle were collected on superfrost/plus microscope slides (Fisher). Every fifth section was used for histochemical staining. Generally 60-80 sections for each heart were stained for hematoxylin and/or Von Kossa and then examined by light microscopy for identification of calcification deposits. Mice with > 20% of tissue sections containing calcification were defined as DCC susceptible.

Methods for determining arterial calcification, aortic aneurysms, and atherosclerotic lesions, were described previously (2, 3). Briefly, serial 10-μm cryosections were collected from the ventricle to the aortic arch. Every other section was collected beginning from the aortic valves. Sections were stained with Oil red-O and hematoxylin, and counter-stained with Fast Green. Calcification score and lesion

size were quantified by light microscopy. To determine the degree of severity of aneurysms in aortic sections, a semi-quantitative method was used, which evaluated the extent of medial destruction as well as the number of sections in which aneurysms could be observed. A scoring system was developed with values ranging from zero to six, with zero being no sign of aneurysm and no disruption of the media, and six being complete destruction of the media with the elastin layer disrupted for >2 μm and the aortic lesion being protruding into the adventitia. For each mouse, the summarized aneurysm score for all aortic sections was used.

GFP reporter assay

The full length 3'-UTR of *Abcc6* from either the B6 or the C3H allele was cloned into the reading frame of the expression vector pEGFP-c1 under the control of the CMV promoter (Clonetech). The chimeric GFP reporter constructs containing *Abcc6* 3'-UTR were transiently transfected into cultured Hela cells for 48 hrs. Expression of the GFP gene was determined by quantitative PCR relative to the endogenous kanamycin resistance gene. Sequence of primer used for GFP gene quantitative PCR: Forward: ACGGCAAGCTGACCCTGAAGTTCATC; Reverse: GCATGGCGGACTTGAAGAAGTCGTG. Sequence of primer used for kanamycin resistance gene quantitative PCR: Forward: CTGAAGAGCTTGGCGGCGAATG; Reverse: GCGTCGCTTGGTCGGTCATTTC.

Microarray data set statistical analyses

Detailed information about the BxD and BxH intercrosses were reported previously (4, 5). Briefly, the original data from the BxH intercross was generated in the apoE null genetic background and these mice were fed high fat diet to stimulate hyperlipidemia. The cross was made to study atherosclerosis, diabetes, and metabolic disease-related traits. To generate the microarray database, we collected multiple tissues, including liver, muscle, adipose, and brain. For the BxD cross where an atherogenic diet was used to induce hyperlipidemia, only liver tissue was arrayed. Therefore, for the liver pathway analyses, both of the BxD and BxH databases were used, but for the muscle pathway analyses, only BxH database was available.

In the BxD and the BxH microarray databases, the expression of each gene transcript was quantified as the mean of the two color-channel-$\log_{10}$(expression ratio)-values, where the expression ratio (mean log ratio or "mlratio") is the expression intensity normalized to the overall pooled signal, and adjusted for background noise. The expression of each gene was treated as a quantitative trait to be studied for linkage in a segregating F_2 population. Lod scores obtained from the two F_2 intercrosses (4, 5) using the MapMaker QTL and QTL Cartographer programs were used to study the positional candidate genes in the 840Kb *Dyscalc1* region. A *cis*-eQTL was defined as an eQTL mapped to the gene itself, which means in practice, within 10cM or 20Mb of the structural gene locus (6). A lod score > 4.3 represents a threshold of

significance in an F_2 intercross with a genome-wide *P* value < 0.05. We studied liver microarray data for both the BxD and BxH crosses, and muscle, brain and adipose microarray databases from the BxH cross. Given on our hypothesis that C3H and DBA share a common disease-causing allele, we first examined the liver array data from the two crosses. Liver expression data from BxH revealed that, 15 of the 34 genes represented on the array exhibited a significant ($P < 0.05$) difference in transcript levels between the two groups of mice with different parental genotypes at the *Dyscalc1* locus (supporting Table 2). Using the Wilcoxon nonparametric test, 12 of the 15 genes remained significant ($P < 0.05$). After Bonferroni correction, 10 genes remained statistically significant in both tests. Of the 34 genes from the 840kb *Dyscalc1* region represented on the microarray, eight had significant *cis*-eQTL (lod score > 4.3) in the BxH cross and two in the BxD cross using the liver array database. Only the ATP binding cassette transporter, *Abcc6*, exhibited significant *cis*-eQTL lod scores in both crosses (supporting Table 2). We also found *Plekha4* and *Kcnj14* demonstrating significant *cis*-eQTL in adipose and brain respectively (lod scores = 7.68 and 11.09). Interestingly, no significant *cis*-eQTL was identified in skeletal muscle for the *Dyscalc1* region. Comparing *Abcc6*, *Plekha4* and *Kcnj14*, only liver expression of *Abcc6* correlated significantly with both myocardial calcification ($P < 0.0001$) and aortic medial calcification ($P = 0.0007$). Thus, our results suggest *Abcc6* expression variations from the liver contribute to the DCC phenotype in the heart and skeletal muscle, indicating a systemic disease mechanism for DCC.

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