Identification of SMEK2 as a candidate gene for regulation of responsiveness to dietary cholesterol in rats[S]

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Abstract **We have previously mapped a diet-induced hypercholesterolemia locus (Dihc2) to chromosome 14 in the F2 generation cross of high-responsive exogenous hypercholesterolemia rats and low-responsive BN rats. To identify a causal gene within this locus, we constructed interval-specific congenic lines and carried out expression and sequencing analyses. Here we narrowed Dihc2 to a region including 33 genes and predicted transcripts and identified RGD1309450_predicted, a homologous gene of SMEK2, as a strong candidate for responsiveness to dietary cholesterol. Our finding provides new insights into the pathway underlying the individual responsiveness to dietary cholesterol in vivo.**—Asahina, M., W. Haruyama, Y. Ichida, M. Sakamoto, M. Sato, and K. Imaizumi. **Identification of SMEK2 as a candidate gene for regulation of responsiveness to dietary cholesterol in rats.** ***J. Lipid Res.*** **2009.** 50: **41–46.**

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Coronary heart disease is a leading cause of mortality in most industrialized countries. Epidemiological studies support that hypercholesterolemia is a major risk factor for coronary heart disease (1). The concentration of total cholesterol in serum is a quantitative and continuous trait that is controlled by complex systems involving environmental and polygenic factors and their interactions. Dietary cholesterol is an environmental factor that raises the total concentration of serum cholesterol in humans and animals (2, 3). However, individuals vary widely in the response to dietary cholesterol, implying individual genetic variability (4). Some genes whose polymorphisms influence the response to dietary cholesterol have already been reported in humans (5). In addition, novel quantitative trait loci (QTLs) for the response to dietary cholesterol have been identified. A genetic study of the stroke-prone spontaneously hypertensive rat, which showed an exaggerated response to a high-fat, high-cholesterol diet, showed QTLs for postdietary cholesterol levels on rat chromosomes 7, 15, and 16 (6). The genetic locus for diet-dependent hypercholesterolemia in the New Zealand obese mouse was also identified on distal mouse chromosome 5 (7). QTL analyses of an intercross of CAST/Ei and DBA/2J mice fed a high-cholesterol diet identified novel a QTL for total cholesterol on mouse chromosome 9 (8). However, these novel genetic loci have not been elucidated in the identification of causal genes.

Exogenously hypercholesterolemia (ExHC) rats generated from SD rats showed a 3-fold higher serum total cholesterol level than SD rats when fed a 1% cholesterol-containing diet for 2 weeks (9, 10). Thus, the ExHC rat is an appropriate model animal for evaluating the effects of dietary cholesterol on serum total cholesterol levels. To identify factors associated with the response to dietary cholesterol, we carried out QTL analyses using high-responsive ExHC rats, low-responsive BN rats, and (ExHC × BN)F2 progeny fed a diet containing 1% cholesterol (11). We mapped diet-induced hypercholesterolemia QTLs to rat chromosomes 5 and 14, and labeled them as diet-induced hypercholesterolemia1 (Dihc1) and Dihc2 (11).

In the present study, we first constructed an Ex.BN-Dihc2 congenic strain to confirm that Dihc2 is a QTL for diet-induced hypercholesterolemia. Second, we tried to identify more precisely a Dihc2 congenic fragment using interval-specific congenic lines (ISCLs). Third, we carried out expression analyses and sequencing of genes within the localized region for positional cloning or candidate gene cloning of a causal gene underlying Dihc2. Our findings suggest that a strong candidate gene in Dihc2 regulates the response to dietary cholesterol in rats.

MATERIALS AND METHODS

An expanded Materials and Methods section is in the online data supplement.

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[S] The online version of this article (available at http://www.jlr.org) contains supplementary Materials and Methods.

Inbred strains

BN/Sea (BN), Dahl S/Jr Sea (Dahl), and LEW/Sea (LEW) rats were obtained from KYUDO; ACI/N Slc (ACI), F344/N Slc (F344), and WKY/Izm (WKY/I) rats were from Japan SLC; and WKY/NCrlCrlj (WKY/N) rats were from Japan Charles River. ExHC/Sea (ExHC) rat colonies have been maintained in our institute with brother-sister matings.

The construction of congenic strains and a series of ISCLs

Ex.BN-Dihc2 congenic strains were constructed according to the speed congenic protocol (12). The congenic strains were backcrossed to ExHC rats for the production of rats with recombinant Dihc2 regions, and then recombinant rats were backcrossed to ExHC rats to construct a series of ISCLs.

Diets and phenotypes

Until they reached of 4 weeks of age, animals were maintained as described (11). Subsequently, rats were fed an AIN76-based purified diet containing 1% cholesterol and 10% olive oil for 2 weeks, and then blood, liver, and tail chips were obtained without fasting. Total cholesterol in the serum and triacylglycerol (TG) and cholesterol in the liver were measured as described (10, 11). The number of animals is shown in parentheses in each figure. These experiments were carried out under the guidelines for animal experiments of the Faculty of Agriculture and Graduate Course at Kyushu University and Law No. 105 and Notification No. 6 of the Government of Japan.

Genotyping

Individual DNA was extracted from tail tips and used for genotyping of simple sequence-length polymorphism markers as de scribed (11). The single nucleotide polymorphisms (SNPs) between the ExHC and BN alleles were determined by the direct-sequencing method, and some SNP markers were genotyped in ISCLs by the PCR-restriction fragment-length polymorphism method.

Expression analysis in liver

We utilized two databases, Ensembl and GeneBank, to find known genes and predicted transcripts located in the narrowed Dihc2 region. Then we carried out quantitative real-time PCR to compare mRNA expression between ExHC and BN rats. *Actb* was used as an internal control gene.

Sequencing

Two to three clones of the coding and upstream regions in ExHC and BN rats were sequenced. Nucleotide differences were confirmed in six inbred rat strains: ACI, Dahl, F344, LEW, WKY/I, and WKY/N.

URLs

Rat Genome Database is available at http://rgd.mcw.edu/, UCSC Genome Bioinformatics Genome Browser at http://genome.

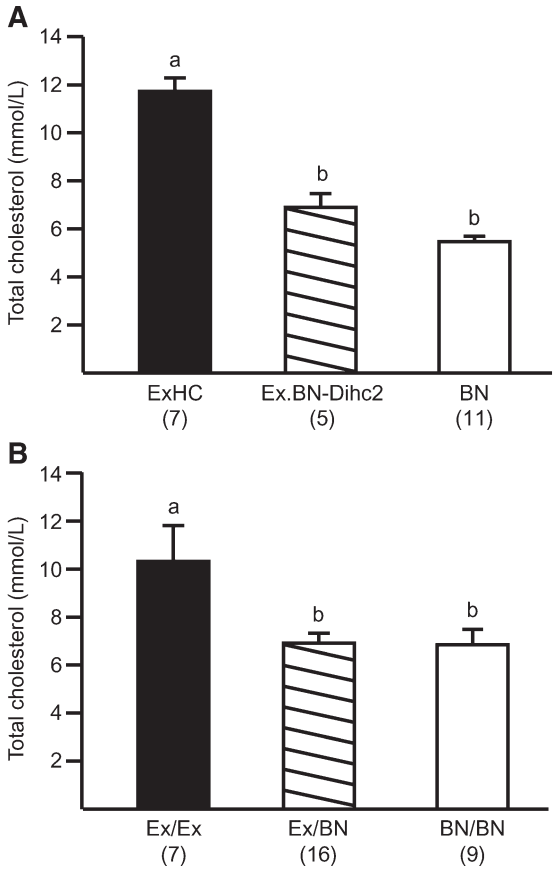

Fig. 1. Ex.BN-Dihc2 congenic rats are protected against diet-induced hypercholesterolemia. Serum cholesterol concentrations in male ExHC, BN, and Ex.BN-Dich2 rats (A) and Ex.BN-Dihc2 (BN/BN) rats and their littermates (Ex/BN and Ex/Ex) (B). The rats were fed a cholesterol-containing diet for 2 weeks. The number of animals is shown in parentheses. The values are means ± SEM. Different letters above the bars indicate a significant difference at $P < 0.01$.

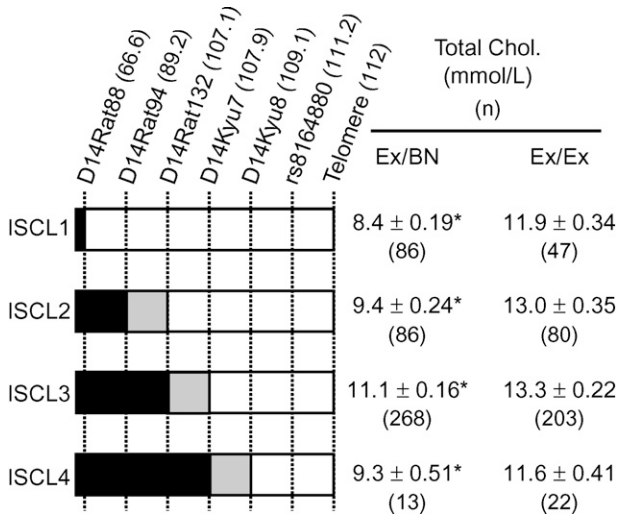

Fig. 2. Fine mapping of Dihc2 using interval-specific congenic lines (ISCLs). ISCLs were compared with their noncongenic littermates for postdietary serum total cholesterol levels. Left: A series of ISCLs, 1–4, were derived from recombinant rats of Ex.BN-Dihc2 congenic strains. White areas show the chromosomal segments homozygous for Ex/Ex alleles, black segments indicate the region heterozygous for Ex/BN alleles, and gray regions indicate the boundary regions where alleles are not determined. The markers and their positions on chromosome 14 are shown at the top of the figure. Right: Postdietary serum cholesterol levels (mean ± SEM) are given for the ISCL1–4 progeny (Ex/BN) and their noncongenic littermates (Ex/Ex). The rats were fed a cholesterol-containing diet for 2 weeks. Asterisks indicate significant differences at $P < 0.05$.

TABLE 1. Expression analyses of genes located within the refined 4.1 Mbp region

Gene ID	Gene Symbol	Expression Ratio		
		Expression	ExHC/BN	*P*
289855	RGD1311700_predicted	+	0.7 ± 0.09	0.04
305604	Efemp1	+	0.8 ± 0.04	0.13
364223	LOC364223	+	1.2 ± 0.08	0.27
360992	Pnpt1	+	0.8 ± 0.05	0.10
360993	RGD1309450_predicted	+	0.2 ± 0.01[a]	0.0000017
289859	RGD1311732	+	0.9 ± 0.08	0.52
679709	LOC679709	—		
679723	LOC679723	—		
679625	LOC679625	+	0.9 ± 0.08	0.60
305605	RGD1306694_predicted	+	1.0 ± 0.11	0.65
289864	RGD1308179_predicted	+	0.9 ± 0.14	0.70
305606	Mtif2	+	0.7 ± 0.02	0.03
81777	Rps27a	+	0.7 ± 0.08	0.03
289865	RGD1307937	+	0.8 ± 0.06	0.19
498428	Ndufs5a_predicted	+	1.2 ± 0.13	0.19
83765	Rtn4	+	1.2 ± 0.07	0.08
498429	RGD1561023_predicted	+	0.7 ± 0.03	0.003
305610	RGD1561672_predicted	+	0.7 ± 0.08	0.04
498430	RGD1565477_predicted	—		
679878	LOC679878	+	0.8 ± 0.21	0.51
679777	LOC679777	+	1.0 ± 0.14	0.86
305614	Spnb2	+	1.0 ± 0.10	0.74
498431	RGD1562229_predicted	—		
679981	LOC679981	+	1.7 ± 0.50	0.19
364224	LOC364224	+	1.3 ± 0.06	0.09
498432	LOC498432	—		
364225	Acyp2_predicted	—		
364226	LOC364226	+	1.1 ± 0.16	0.74
498433	Psme4	+	1.1 ± 0.06	0.11
498434	RGD1566385_predicted	+	1.0 ± 0.19	0.96
289874	RGD1306508_predicted	+	1.0 ± 0.05	0.60
360994	RGD1309120	+	1.0 ± 0.08	0.95
364227	Asb3_predicted	+	0.9 ± 0.07	0.50

The rats were fed a cholesterol-containing diet for 2 weeks (n = 6). The values are means ± SEM.
[a] Significant difference at $P < 0.001$.

ucsc.edu/cgi-bin/hgGateway/, and NCBI Rat Genome Resource at http://www.ncbi.nlm.nih.gov/genome/guide/rat/.

Statistical analysis

The statistical significance of differences between groups was determined using two-sided Student's *t*-test for comparisons between two groups or a one-way ANOVA with Sheffe's multiple comparisons post-test for comparisons among more than three groups.

RESULTS

Ex.BN-Dihc2 congenic rats are protected against diet-induced hypercholesterolemia

To confirm the effect of the Dihc2 locus on the responsiveness to dietary cholesterol, we constructed an Ex.BN-Dihc2 congenic strain that had the introgressed low-responsive BN allele of Dihc2 on the high-responsive ExHC background genome. Ex.BN-Dihc2 rats showed lower postdietary serum total cholesterol than the background strain (**Fig. 1A**). The congenic strain showed lower postdietary serum total cholesterol levels than its noncongenic Ex.Ex homozygous littermates (Fig. 1B). These results reveal that a gene influencing the response to dietary cholesterol is contained within the introgressed interval.

Fine mapping of Dihc2

To narrow the Dihc2 region, a series of ISCLs were constructed by continuous backcrosses of ExHC rats to Ex.BN-Dich2 congenic strains. The congenic strain with the Ex.BN heterozygous allele in Dihc2 had lower postdietary serum total cholesterol levels than did its noncongenic Ex.Ex homozygous littermates (Fig. 1B), indicating the dominance of the BN allele. Phenotypic comparisons between ISCLs with the Ex.BN heterozygous Dihc2 region and their Ex.Ex homozygous littermates in each ISCL were then conducted to determine which introgressed region affects the susceptibility to diet-induced hypercholesterolemia. ISCL1–4 rats showed significant decreases in the postdietary serum cholesterol level when compared with their Ex.Ex littermates, indicating that a causal gene is contained in the 4.1 Mb introgressed region spanning D14Kyu7 and the telomere (**Fig. 2**).

Expression analyses of genes within the localized Dihc2 region

Our previous study suggested that diet-induced hypercholesterolemia in ExHC rats could be attributed to increased secretion of slowly metabolized VLDL enriched in cholesteryl ester (CE) by the liver (10, 13). Therefore, we performed RT-PCR to evaluate the gene expression in

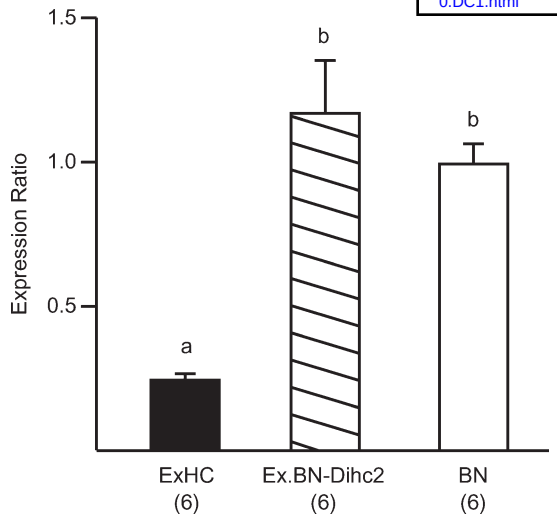

Fig. 3. Expression analyses of RGD1309450_predicted in ExHC, BN, and Ex.BN-Dihc2 rats. mRNA expression levels were determined by real-time quantitative PCR for each gene and standardized by *Actb* expression. Rats were fed a cholesterol-containing diet for 2 weeks. The number of animals is shown in parentheses. The values are means ± SEM. Different letters above the bars indicate a significant difference at $P < 0.01$.

the liver. Using public rat genome information, we initially found 33 genes and predicted transcripts located within the 4.1 Mbp region. Then mRNA expression in the liver was confirmed for 27 genes. We compared mRNA expression of these 27 genes between ExHC and BN strains using real-time quantitative PCR. Of these genes, RGD1309450_predicted showed a highly significant difference between the two strains ($P = 1.7 \times 10^{-6}$) (**Table 1**), with BN rats showing 5-fold higher mRNA expression than ExHC rats. To determine whether RGD1309450_predicted transcript levels were influenced by the Dihc2 locus, we examined mRNA expression in the Ex.BN-Dihc2 congenic strain. The congenic strain also exhibited significantly increased mRNA expression of RGD1309450_predicted when compared with the ExHC background strain (**Fig. 3**).

Sequencing analyses identified a 10 bp deletion leading to a nonsense mutation in ExHC rats

We sequenced the coding and upstream regulatory regions of RGD1309450_predicted in ExHC and BN rats. Two nucleotide differences in the upstream region and a 10 bp deletion in the coding region at codon 713 in ExHC rats were identified in RGD1309450_predicted (**Table 2, Fig. 4A**). This 10 bp deletion existed in ExHC rats but not in the other seven strains, which are not hyper-responders to dietary cholesterol (Table 2). Two SNPs in the upstream regions were not specific for ExHC rats (Table 2). A homology search showed that rat RGD1309450_predicted is highly conserved with mouse and human SMEK2, exhibiting 98% and 94% similarity, respectively (Fig. 4B). The10 bp deletion led to a nonsense mutation with the creation of a premature stop codon at the 758 position (Fig. 4B).

Liver lipid analyses in ExHC, BN, and Ex.BN-Dihc2 rats

Previously we showed that ExHC rats had lower liver TG levels than the original strain of ExHC rats, SD rats, which have a lower response to dietary cholesterol than do ExHC rats. We also showed increased formation of CE-rich VLDL in ExHC rats fed a cholesterol-containing diet (10, 13). These results raised the possibility that liver TG levels can be associated with the susceptibility to hypercholesterolemia in ExHC rats (10, 13). Therefore, we tested whether the Dihc2 locus is associated with the regulation of liver TG levels using the Ex.BN-Dich2 congenic strain. ExHC rats showed decreased liver TG levels when compared with BN and Ex.BN-Dihc2 rats (**Fig. 5A**). However, there was no difference in cholesterol levels between ExHC and Ex.BN-Dihc2 rats, and their cholesterol levels were lower than those of BN rats. These results indicate that Dihc2 is associated with the regulation of liver TG levels, but not cholesterol levels.

DISCUSSION

In this study, we identified RGD1309450_predicted as a candidate gene for the Dihc2 locus in rats using ISCLs and expression and sequencing analyses of genes. A homology search showed that rat RGD1309450_predicted is highly homologous to SMEK2 in humans and mice. It is therefore likely that SMEK2 is associated with the high responsiveness to dietary cholesterol in ExHC rats.

Our previous study showed that ExHC rats had decreased liver TG levels and secreted relatively CE-rich VLDL when compared with the SD original strain (10, 13). Liver lipid analyses in ExHC, BN, and Ex.BN-Dich2 rats indicated that the Dihc2 locus including SMEK2 is associated with liver TG levels, but not cholesterol levels (Fig. 5). Although the relative effects of CE versus TG in the regulation of VLDL

TABLE 2. Nucleotide differences in rat RGD1309450_predicted among eight inbred strains

		ExHC	BN	ACI	Dahl	F344	LEW	WKY/I	WKY/N
		(11.0)	(5.9)	(5.9)	(6.4)	(7.1)	(6.3)	(3.0)	(5.3)
−2235	SNP (T/C)	C	T	C	C	C	C	C	C
−1066	SNP (A/G)	G	A	G	G	G	G	G	G
2137	10 bp deletion	+	—	—	—	—	—	—	—

The total cholesterol levels are shown in parentheses. The values are means ± SEM (mmol/l). The rats were fed a cholesterol-containing diet for 2 weeks (n = 6).

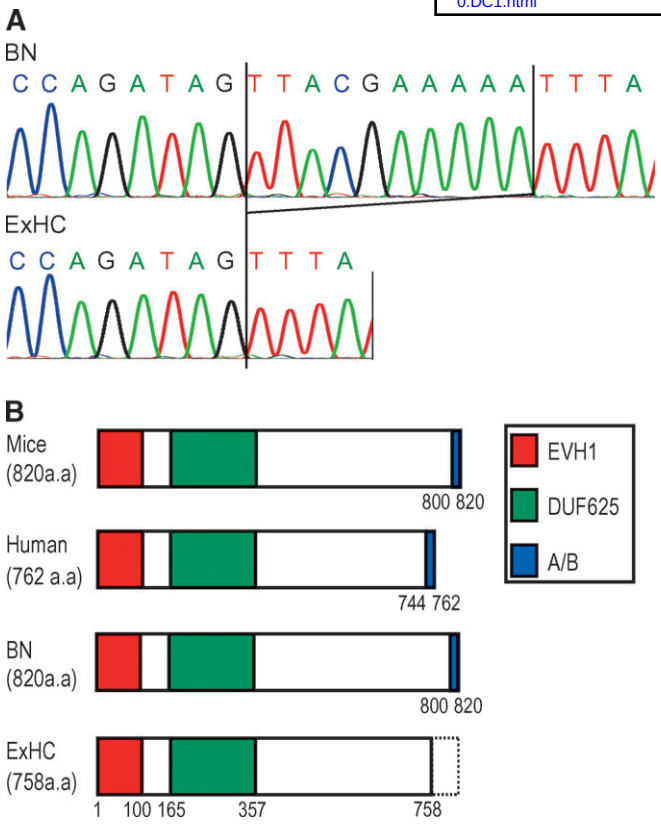

Fig. 4. Sequencing analyses identified a 10 bp deletion leading to a nonsense mutation in ExHC rats. A: Chromatograms showing the 10 bp deletion. B: The structure of the SMEK2 protein and the position of the stop codon arising from the nonsense mutation. Red areas show the EVH1 domain; green segments show the DUF-625 domain; and blue regions indicate the C-terminal conserved acidic/basic stretch (A/B).

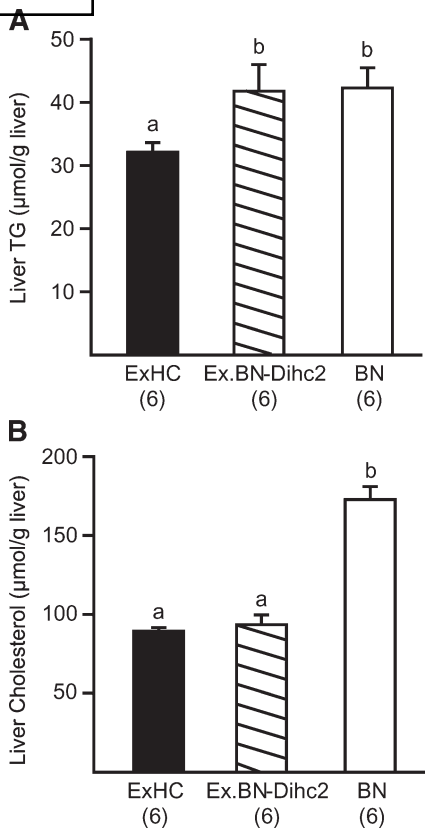

Fig. 5. Liver lipid analyses in ExHC, BN, and Ex.BN-Dihc2 rats. Liver triacylglycerol (A) and cholesterol (B) were measured. Rats were fed a cholesterol-containing diet for 2 weeks. The number of animals is shown in parentheses. The values are means ± SEM. Different letters above the bars indicate a significant difference at $P < 0.05$.

secretion remain controversial, the size of the intracellular triglyceride pool is known to determine VLDL secretion (14). There is evidence suggesting that 60–70% of VLDL-triglyceride is derived from lipolysis of cytoplasmic triglycerides (14). Thus, we suggest that ExHC rats secrete relatively CE-rich VLDL owing to their low levels of liver TG.

Tsai et al. (15) have shown that MEK1/2 inhibition significantly increases cellular and microsomal TG mass, but not cholesterol mass, in HepG2 cells and thereby induces the secretion of relatively-TG rich VLDL. This finding implies that MEK/ERK signal transduction is associated with TG levels in the liver and regulates VLDL components (15). SMEK2 was originally identified by Mendoza et al. (16) as a suppressor of MEK1 in *Dictyostelium* using a *mek1* mutant by using a second-site suppressor. They described that SMEK regulated some MEK1 effectors in a manner opposite to that of the MEK1/ERK1 pathway, and that SMEK signaling converged on a partially overlapping set of MEK1 effectors (16). These results suggest that SMEK is associated with the regulation of liver TG levels in a manner opposite to that of the MEK/ERK pathway.

In the present study, sequencing analysis detected an ExHC-specific 10 bp deletion that led to a nonsense mutation in SMEK2 in eight inbred rat strains. Premature mRNA may be degraded by nonsense-mediated decay, which is an mRNA surveillance mechanism that detects and selectively degrades mRNA transcripts containing premature termination codons (17). Thus, the ExHC-specific 10 bp deletion in SMEK2 is consistent with the highly decreased mRNA expression of SMEK2 in ExHC rats. In vegetative cells, SMEK localizes to the cell cortex through the EVH1 domain and translocates to the nucleus via a nuclear localization signal at its C terminus basic stretch in response to starvation signals (16). Because the SMEK2 protein lacks the conserved C-terminal basic stretch in ExHC rats (Fig. 4B), SMEK2 may not be translocated into the nucleus, and hence may not function as a mek1 suppressor in ExHC rats. Thus, we suggest that dysfunction and decreased mRNA expression of SMEK2 in ExHC rats led to the decreased TG levels in the liver. This speculation is consistent with the liver TG levels in ExHC, BN, and Ex.BN-Dihc2 rats (Fig. 5). SMEK2 is highly conserved from yeasts to humans, so its MEK suppressor function in *Dictyostelium* should parallel that in mammalian systems.

Although our approach has successfully identified SMEK2 as a strong candidate gene underlying Dihc2, further detailed functional studies are clearly required to confirm its candidacy reverse-genetically by gene-modified animals. In

conclusion, we suggest that SMEK2 is associated with diet-induced hypercholesterolemia in ExHC rats. Our finding provides new insight into the novel pathway underlying the responsiveness to dietary cholesterol in vivo.

REFERENCES

1. Criqui, M. H. 1986. Epidemiology of atherosclerosis: an updated overview. *Am. J. Cardiol.* **57:** 18C–23C.
2. Beynen, A. C., M. B. Katan, and L. F. Van Zutphen. 1987. Hypo and hyperresponders: individual differences in the response of serum cholesterol concentration to changes in diet. *Adv. Lipid Res.* **22:** 115–171.
3. Dietschy, J. M., S. D. Turley, and D. K. Spady. 1993. Role of liver in the maintenance of cholesterol and low density lipoprotein homeostasis in different animal species, including humans. *J. Lipid Res.* **34:** 1637–1659.
4. McNamara, D. J., R. Kolb, T. S. Parker, H. Batwin, P. Samuel, C. D. Brown, and E. H. Ahrens, Jr. 1987. Heterogeneity of cholesterol homeostasis in man. Response to changes in dietary fat quality and cholesterol quality. *J. Clin. Invest.* **79:** 1729–1739.
5. Ye, S. Q., and P. O. Kwiterovich, Jr. 2000. Influence of genetic polymorphisms on responsiveness to dietary fat and cholesterol. *Am. J. Clin. Nutr.* **72 (Suppl.):** 1275–1284.
6. Mashimo, T., H. Ogawa, Z. H. Cui, Y. Harada, K. Kawakami, J. Masuda, Y. Yamori, and T. Nabika. 2007. Comprehensive QTL analysis of serum cholesterol levels before and after a high-cholesterol diet in SHRSP. *Physiol. Genomics.* **30:** 95–101.
7. Giesen, K., L. Plum, R. Kluge, J. Ortlepp, and H.-G. Joost. 2003. Diet-dependent obesity and hypercholesterolemia in the New Zealand obese mouse: identification of a quantitative trait locus for elevated serum cholesterol on the distal mouse chromosome 5. *Biochem. Biophys. Res. Commun.* **304:** 812–817.
8. Lyons, MA. H. Wittenburg, R. Li, K. A. Walsh, G. A. Churchill, M. C. Carey, and B. Paigen. 2003. Quantitative trait loci that determine lipoprotein cholesterol levels in DBA/2J and CAST/Ei inbred mice. *J. Lipid Res.* **44:** 953–967.
9. Imai, Y., and H. Matsumura. 1973. Genetic studies on induced and spontaneous hypercholesterolemia in rats. *Atherosclerosis.* **18:** 59–64.
10. Imaizumi, K., A. Nagatomi, M. Sato, A. Tominaga, and M. Sugano. 1992. Cholesterol metabolism in ExHC (exogenous hypercholesterolemic) rats. *Biochim. Biophys. Acta.* **1123:** 101–109.
11. Asahina, M., M. Sato, and K. Imaizumi. 2005. Genetic analysis of diet-induced hypercholesterolemia in exogenously hypercholesterolemic rats. *J. Lipid Res.* **46:** 2289–2294.
12. Markel, P., P. Shu, C. Ebeling, G. A. Carlson, D. L. Nagle, J. S. Smutko, and K. J. Moore. 1997. Theoretical and empirical issues for marker-assisted breeding of congenic mouse strains. *Nat. Genet.* **17:** 280–284.
13. Sakono, M., H. Mori, K. Nagao, M. Sato, I. Ikeda, K. Yamamoto, and K. Imaizumi. 1996. Exogenous hypercholesterolemic rats, compared with their progenitor, Sprague-Dawley rats, promptly alter cholesterol metabolism in the liver and secrete cholesterol-rich particles in response to dietary cholesterol. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **113:** 803–808.
14. Lewis, G. F. 1997. Fatty acid regulation of very low density lipoprotein production. *Curr. Opin. Lipidol.* **8:** 146–153.
15. Tsai, J., W. Qiu, R. Kohen-Avramoglu, and K. Adeli. 2007. MEK-ERK inhibition corrects the defect in VLDL assembly in HepG2 cells: potential role of ERK in VLDL-apoB100 particle assembly. *Arterioscler. Thromb. Vasc. Biol.* **27:** 211–218.
16. Mendoza, M. C., F. Du, N. Iranfar, N. Tang, H. Ma, W. F. Loomis, and R. A. Firtel. 2005. Loss of SMEK, a novel conserved protein, suppresses *mek1* null cell polarity, chemotaxis, and gene expression defects. *Mol. Cell. Biol.* **25:** 7839–7853.
17. Baker, K. E., and R. Parker. 2004. Nonsense-mediated mRNA decay: terminating erroneous gene expression. *Curr. Opin. Cell Biol.* **16:** 293–299.