Fatty Liver in Hypertension

Identification of Mutated \textit{Srebf1} as a QTL Influencing Risk for Hepatic Steatosis in the Spontaneously Hypertensive Rat


Abstract—Approximately 30% of patients with hypertension have hepatic steatosis, and it has recently been proposed that fatty liver be considered a feature of the metabolic syndrome. Obesity, diet, and level of physical activity are likely factors modulating risk for hepatic steatosis, however genetic factors could also influence susceptibility or resistance to fatty liver in hypertensive or normotensive subjects. In genetic studies in spontaneously hypertensive rats (SHRs) and Brown Norway (BN) rats, we discovered that a variant form of sterol regulatory element binding transcription factor 1 (\textit{Srebf1} gene, SREBP-1 protein) underlies a quantitative trait locus (QTL) influencing hepatic cholesterol levels in response to a high cholesterol diet. Compared with the BN allele of \textit{Srebf1}, the SHR allele of \textit{Srebf1} includes variants in the promoter and coding regions that are linked to hepatic deficiency of SREBP-1 mRNA and protein, and reduced expression of the SREBP-1 target gene stearoyl-CoA desaturase 1, reduced promoter activity for SREBP-1c, and relative protection from dietary induced accumulation of liver cholesterol. Genetic correction of reduced SREBP-1 activity by derivation of congenic and transgenic strains of SHR increased hepatic cholesterol levels, thereby confirming \textit{Srebf1} as a QTL influencing hepatic lipid metabolism in the rat. The \textit{Srebf1} variant regulating hepatic cholesterol did not appear to affect blood pressure. These findings (1) are consistent with the results of association studies indicating that common polymorphisms affecting SREBP-1 may influence cholesterol synthesis in humans and (2) indicate that variation in \textit{Srebf1} may influence risk for hepatic steatosis. (\textit{Hypertension.} 2008;51:148-153.)

Key Words: fatty liver ■ quantitative trait loci ■ rats ■ inbred SHR ■ metabolic syndrome X ■ hypertension ■ sterol regulatory element binding protein 1

Genetic determinants involved in the pathogenesis of complex clinical disorders such as hypertension and related metabolic disturbances are often referred to as quantitative trait loci (QTL). In contrast to the identification of genes underlying mendelian disorders, molecular identification of QTL for complex traits has proven very difficult to accomplish even in animal models in which environmental and genetic factors can be carefully controlled. Although many QTL have been mapped to relatively broad chromosome regions, few QTL have been clearly isolated according to rigorous criteria proposed for establishing the identity of genes that underlie complex traits.\textsuperscript{1,2}

Hypertension is often accompanied by a variety of complex metabolic disturbances including fatty liver.\textsuperscript{3,4} Recently, Donati et al reported that 30% of nondiabetic subjects with arterial hypertension may have hepatic steatosis despite normal liver enzymes.\textsuperscript{4} Although obesity, level of physical activity, and diet are likely factors modulating susceptibility or resistance to fatty liver, it is also possible that genetic factors may influence whether an individual is at high or low risk for hepatic steatosis. Recently, in recombinant inbred (RI) strains derived from the spontaneously hypertensive rat (SHR), we mapped a QTL on Chromosome 10 that was associated with effects on hepatic cholesterol levels in animals fed a high cholesterol diet (designated HegI [Hepatic cholesterol level 1]).\textsuperscript{5} We subsequently found that the SHR harbors a rare allele of the gene encoding sterol regulatory element binding protein 1 (SREBP-1) that maps to a region of rat Chromosome 10 overlapping the QTL regulating hepatic cholesterol levels.\textsuperscript{6}

The gene for SREBP-1 (\textit{Srebf1}) has 2 alternate promoters and codes for 2 protein isoforms designated SREBP-1a and SREBP-1c, both of which are well known transcriptional regulators of hepatic cholesterol and fatty acid synthesis.\textsuperscript{7}
SREBP-2 is another SREBP isoform that is considered to play a greater role in the regulation of cholesterol biosynthesis than the SREBP-1 isoforms.7 However, the gene encoding SREBP-2 (Sreb2) is located on a different chromosome than Srebf1 and in our previous mapping studies, we did not detect any QTLs in the vicinity of Sreb2 on Chromosome 7 linked to the regulation of hepatic cholesterol levels. In addition, association studies in humans and animals have provided indirect evidence that Sreb1 might constitute a QTl regulating inherited variation in cholesterol and lipid metabolism.8,9 Given these observations, we focused on whether Sreb1 constitutes a bona fide QTl on Chromosome 10 that regulates inherited variation in hepatic cholesterol levels and risk for dietary induced hepatic steatosis in the SHR. Based on stringent criteria proposed by Glazier et al for QTl identification including linkage studies, fine genetic mapping, sequence analysis, and both in vitro and in vivo functional tests of the candidate gene,1 we now report that Sreb1 is a QTl regulating liver cholesterol levels and susceptibility to hepatic steatosis in SHRs fed a high cholesterol diet.

Methods

SHR Congenic Strain and Subline Derivation

Given our previous linkage results in RI strains,3 we performed additional mapping studies in a congenic strain and congenic subline to test whether Sreb1 might represent the QTl on Chromosome 10 linked to hepatic cholesterol levels in the SHR. We derived the congenic strain, SHR.BN-D10Mgh3/Sreb1 (hereafter referred to as the SHR-Chr.10 congenic strain), by a backcross breeding protocol in which we replaced a 53.7 megabase pair (Mbp) segment of Chromosome 10 including Sreb1 in the SHR/Ola strain (hereafter referred to as the SHR progenitor strain) with the corresponding chromosome segment from the Brown Norway (BN/Crl) strain (Figure 1). The SHR/Ola progenitor strain is a highly inbred strain referred to as the SHR progenitor strain) with the corresponding chromosome segment, the SHR-Chr.10 congenic strain and the SHR-Chr.10a congenic subline must be a consequence of genetic differences within the 2.5 Mbp differential chromosome segment that includes Sreb1.6 We then compared hepatic cholesterol levels in the SHR congenic strain and SHR progenitor strain fed a high-cholesterol diet as described further below. In these experiments, we studied male congenic rats that were the product of 10 generations of backcrossing to ensure a very high probability that the congenic and progenitor strains are homozygous identical at all loci outside the target chromosome segment. For any locus unlinked with the target chromosome segment, the probability of homozygosity after 10 generations of backcrossing is ~99.8%.14 After 10 backcross generations, the offspring were intercrossed to produce the homozygous congenic line for study.

We next refined the map location of the Hcl1 QTl to a 2.5 Mbp segment of Chromosome 10 by deriving a congenic subline, SHR.BN-D10Mgh3/D10Rat85 (hereafter referred to as the SHR-Chr.10a congenic subline) and comparing it to the SHR-Chr.10 congenic strain with respect to hepatic cholesterol levels. Specifically, we used backcross breeding with SSLP selection markers and the RFLP in Sreb1 to derive the SHR-Chr.10a congenic subline that is genetically identical to the SHR-Chr.10 congenic strain except for approximately 2.5 Mbp of Chromosome 10 in the region of Sreb1; the SHR-Chr.10 congenic strain carries the BN allele of Sreb1.

Figure 1. Schematic representation of the region of Chromosome 10 transferred from the BN strain (solid bar) onto the background of the SHR-Chr.10 congenic strain or SHR-Chr.10a congenic subline. The cross hatched shading denotes possible areas of residual heterozygosity within regions that immediately flank the transferred chromosome segment. The SHR-Chr.10 congenic strain and the SHR-Chr.10a congenic subline are genetically identical except for approximately 2.5 Mbp of Chromosome 10 that includes Sreb1. The SHR-Chr.10 congenic strain carries the BN allele for Sreb1 (solid bar), whereas the SHR-Chr.10a congenic subline carries the SHR allele for Sreb1 (open bar). The chromosome positions displayed across from each marker are taken from the version 3.4 assembly of the Rat Genome Sequencing Consortium.

SHR Transgenic Strain Derivation

In rats fed a high-cholesterol diet, we found that the SHR allele of Sreb1, compared with the wild-type BN allele of Sreb1, was associated with decreased hepatic levels of SREBP-1c mRNA and protein, and decreased hepatic levels of cholesterol (see results). Therefore, in SHR, we tested whether transgenic augmentation of hepatic SREBP-1c activity to a degree similar to that observed in the SHR-Chr.10 congenic strain harboring wild-type Sreb1 would restore hepatic cholesterol levels. We derived transgenic SHRs by microinjection of zygotes from the highly inbred SHR progenitor strain with a construct containing a truncated portion of the human cDNA encoding SREBP-1c (kindly provided by J. Horton and J. Goldstein, University of Texas Southwestern Medical Center, Dallas).15 The construct contained the rat PEPCk promoter fused to human SREBP-1c cDNA encoding amino acids 1 to 436 followed by 2 stop codons and human growth hormone (GH) poly A signal. This construct generates a dominant positive NH2-terminal fragment of SREBP-1c expressed in the liver that can enter the nucleus and be transcriptionally active without a requirement for proteolytic release from cell membranes.16 We intercrossed transgene positive rats to fix the transgene to be present in all rats in the transgenic colony and then compared the transgenic rats to the inbred SHR progenitor strain.
from which they were derived. We assessed the activity of SREBP-1c by measuring hepatic expression of stearoyl-coenzyme A (CoA) desaturase 1 (SCD-1), a key transcriptional target of SREBP-1 that plays a major role in the hepatic synthesis of cholesterol esters and triglycerides.15,16

**Experimental Protocol and Hepatic Lipid Measurements**

We fed all rats a standard diet until 7 weeks of age at which time they were switched to a diet containing 2% cholesterol (3018.03 diet, Hope Farms). We used a high-cholesterol diet in all experiments because this was the same diet used in the original QTL mapping studies.4 In pilot studies, we also found that administration of the high-cholesterol diet was necessary to elicit significant differences in hepatic lipid levels between the SHR progenitor and the SHR-Chr.10 congenic strain. After 4 weeks of feeding the high-cholesterol diet, we euthanized the animals and obtained liver samples for measurement of hepatic cholesterol and for preparation of mRNA to quantify gene expression levels as described further below. To measure hepatic cholesterol levels, frozen liver tissues were powdered under liquid N2 and extracted in chloroform-methanol (2:1 v/v). After adding 2% KH2PO4, the solution was centrifuged and the organic phase was removed and evaporated to dryness under N2. The residue was then dissolved in isopropyl alcohol, and cholesterol levels determined by enzymatic assay (Pliva-Lachema).

**Gene Expression and Protein Measurements**

We used real-time PCR to measure hepatic expression levels of mRNAs for SREBP-1a and SREBP-1c. As a measure of SREBP-1 activity, we also determined the expression of stearoyl-CoA desaturase 1 (SCD-1), a major target gene of SREBP-1. RNA was extracted from liver using Trizol reagent (Invitrogen), and cDNA was prepared and analyzed by real-time PCR testing using QuantiTect SYBR Green reagents (QIAGEN, Germany) on an Opticon continuous fluorescence detector (MJ Research) as previously described.17 Gene expression levels were normalized relative to the expression of cyclophilin, which served as the internal control, with results being determined in triplicate using the preferred method of Muller et al.18,19 We used Western blot analysis to measure hepatic levels of both precursor and mature forms of SREBP-1. SREBP-1 was detected using a rabbit polyclonal antibody (Santa Cruz Biotechnology Inc, catalog # sc-8984) developed against a recombinant protein corresponding to amino acids 41 to 200 of SREBP-1 of human origin and that cross-reacts with the mouse and rat SREBP-1 but not with SREBP-2. SREBP-1c is the predominant SREBP-1 isoform in liver and the immunofluorescence results are presumed to largely reflect detection of SREBP-1c.20

**Transfection Studies and DNA Sequence Analysis**

As described in the results, we found reduced hepatic expression of the mRNA encoding SREBP-1c in the SHR progenitor strain compared with the SHR-Chr.10 congenic strain. Therefore, we sequenced the SREBP-1c promoter regions of the SHR and BN strains and tested for strain differences in promoter function using in vitro transfection assays. Transfection studies were performed in HEK293 cells cultured in 10% fetal bovine serum. We cotransfected the cells with a firefly luciferase reporter vector pGL3 including the heparin-binding domain of the vitronectin receptor (Applied Biosystems). The 5' flanking region of the SREBP-1c gene (3500 bp) was amplified using PCR and subcloned into the pGL3 vector (Promega). HEK293 cells were transfected with Lipofectamine 2000 (Invitrogen) as described by the manufacturer. Luciferase activity was measured 48 hours post-transfection using the Dual Luciferase Reporter Assay System (Promega) and analyzed with a luminometer (Molecular devices). All results are expressed as means±SE of 3 replicate transfections.

**Results**

**Mapping the QTL for Hepatic Cholesterol Levels in Congenic Strains**

The SHR progenitor strain exhibited significantly lower hepatic cholesterol concentrations than the SHR-Chr.10 congenic strain that carried the BN variant of Srebf1 within a differential chromosome segment of approximately 53.7 Mbp (Figures 1 and 2). This finding confirmed the original linkage results in the RI strains in which the putative Hcl1 QTL of SHR origin was associated with lower hepatic cholesterol levels than the QTL of BN origin.5 Fine mapping of the Hcl1 QTL was achieved by comparison of the SHR-Chr.10 congenic strain to the SHR-Chr.10a congenic subline that is genetically identical except for approximately 2.5 Mbp of Chromosome 10 harboring Srebf1. All rats were allowed to recover for at least 5 to 7 days after surgical implantation of radiotelemetry transducers before the start of blood pressure recordings. Pulsatile pressures were recorded in 5-second bursts every 5 minutes throughout the day and night, and 24-hour averages for systolic arterial blood pressure were calculated for each rat for a 1-week period. The results from each rat in the same group were then averaged to obtain the group means.

**Statistical Analysis**

We studied 5 to 10 rats per group and performed the statistical analysis using t tests or rank sum tests for 2 group comparisons and ANOVA with Student-Newman-Keuls or Holm-Sidak testing for multiple group comparisons. All results are expressed as means±SEM unless otherwise specified.
variant of Srebf1 exhibited liver cholesterol concentrations lower than those in the SHR-Chr.10 congenic strain harboring the BN variant and similar to those found in the SHR progenitor strain (Figure 2). Thus, substitution mapping in the SHR-Chr.10 congenic strains confirmed genetic colocalization of the Hcl1 QTL and Srebf1 within a chromosome interval of approximately 2.5 Mb (Figure 1).

Hepatic triglyceride levels paralleled the hepatic cholesterol levels although the strain differences in triglycerides were not as great as the strain differences in cholesterol; liver triglyceride content in the SHR-Chr.10a subline carrying the SHR variant of Srebf1, 28.1±1.5 μmol/g, was similar to that in the SHR progenitor strain, 26.4±1.0 μmol/g, and significantly lower than that in the SHR-Chr.10 congenic strain carrying the BN variant of Srebf1, 37.9±2.8 μmol/g, (P<0.05 by Holm Sidak multiple comparison testing). We did not detect any differences in radiotelemetry measurements of 24-hour average systolic blood pressures between the SHR-Chr.10a subline harboring the SHR variant of Srebf1 and the SHR-Chr.10 congenic strain harboring the BN variant of Srebf1, 150±1.3 mm Hg versus 150±2.2 mm Hg, respectively. Thus, although the 2.5 Mb segment of Chromosome 10 including Srebf1 was linked to the regulation of hepatic lipid levels, it did not appear to affect blood pressure.

**Gene Expression and Protein Analysis**

Real-time PCR assays demonstrated lower amounts of mRNA for SREBP-1c in livers of the SHR progenitor strain compared with the SHR-Chr.10 congenic strain that carries the Srebf1 gene of the BN rat (Figure 3A). Hepatic expression of SREBP-1a mRNA was not different between the two strains (data not shown). The lower hepatic levels of SREBP-1c mRNA in the SHR progenitor strain compared with the SHR-Chr.10 congenic strain were also associated with lower hepatic levels of both the precursor and mature forms of SREBP-1 protein (Figure 3B). In addition, real-time PCR analysis showed that hepatic expression of the gene encoding stearoyl-CoA desaturase 1 (SCD-1), a major target regulated by SREBP-1, was significantly lower in the SHR progenitor strain compared with the SHR-Chr.10 congenic strain (Figure 4A). Thus, the SHR allele for Srebf1 is associated with deficient expression of both SREBP-1 mRNA and protein and reduced SREBP-1 target gene activity compared with the BN allele.

**Complementation Studies in the SREBP-1c Transgenic Strain**

We performed functional tests in transgenic rats to further test the causal relationship between reduced hepatic levels of SREBP-1 and reduced hepatic levels of cholesterol in SHRs fed a high-cholesterol diet. Hepatic activity of SREBP-1 in the SHR was augmented by transgenic expression of a dominant positive form of SREBP-1c in the liver under control of the PEPCK promoter. In mice, this construct has been previously shown to increase hepatic SREBP-1c activity and cause modest increases in hepatic levels of cholesterol. Transgenic augmentation of SREBP-1c in the SHR restored both SCD1 target gene expression (Figure 4A) and hepatic concentrations of cholesterol to levels similar to those observed in the SHR-Chr.10 congenic strain (Figure 2). Thus, augmentation of SREBP-1c activity by expressing either the wild-type form of SREBP-1c in congenic rats or a dominant positive form of SREBP-1c in transgenic rats complemented the low hepatic cholesterol levels linked to the SHR allele of Srebf1.

**Sequence Analysis and Transfection Studies**

Sequence analysis of Srebpf1 coding regions previously revealed that the SHR harbors a rare variant causing a valine to methionine substitution in the COOH terminal segment of both isoforms of SREBP-1c. However, in transfection studies with SHR and BN cDNAs and a sterol regulatory element-1
Discussion

Although the discovery of chromosome regions that contain QTL for complex cardiovascular and metabolic traits is now relatively straightforward, the task of moving beyond QTL mapping to pinpointing the identity of QTL at the molecular level has proven far more difficult.1,2 Despite major technical advances in genetics and molecular biology, relatively few studies have fulfilled rigorous criteria for QTL identification. In humans, it is not possible to individually manipulate candidate genes or to study the phenotypic effects of different allelic variants on fixed environmental and genetic backgrounds. Consequently, studies in humans have constrained ability to definitively establish proof of QTL identity at the molecular level. In contrast, the combined use of genetic studies with functional tests in animal models provides a means to unequivocally identify specific gene variants that mediate QTL effects on complex phenotypes.

In the current studies in the SHR-BN model, we have found that Srebf1 constitutes a QTL regulating hepatic cholesterol levels and susceptibility to dietary induced accumulation of liver cholesterol. The assertion that Srebf1 is a QTL influencing hepatic cholesterol levels in the SHR-BN model is based on the stringent criteria proposed by Glazier et al for QTL identification in studies of complex traits1: 1st criterion, Linkage. In RI strains fed a high cholesterol diet, a QTL associated with effects on hepatic cholesterol levels maps to rat Chromosome 10 near the Srebf1 candidate gene5,6; 2nd criterion, Fine mapping. In recombinant congenic sublines derived from the SHR and BN strains, the QTL regulating hepatic cholesterol levels and the Srebf1 positional candidate gene map to an interval of approximately 2.5 Mbp together with effects on mRNA and protein levels for SREBP-1; 3rd criterion, Sequence analysis. The SHR and BN strains carry different alleles for Srebf1 including variants in the promoter region for the SREBP-1c isoform; 4th criterion, Functional tests of candidate genes in vitro and in vivo. Compared with the BN allele for Srebf1, the SHR allele is associated with (1) reduced SREBP-1c promoter activity in vitro, (2) reduced SREBP-1c mRNA, protein, and target gene expression levels in vivo, and (3) reduced hepatic cholesterol levels in the environment of a high cholesterol diet. Finally, in congenic and transgenic strains of SHR, augmentation of SREBP-1c activity with wild-type or dominant positive forms of SREBP-1c increased liver cholesterol levels to a similar degree, thereby confirming that Srebf1 is a QTL regulating hepatic lipids in the SHR progenitors.

The finding that Srebf1 is a QTL influencing liver cholesterol levels in the SHR-BN model is consistent with the known biology of SREBP-1c as a transcriptional regulator of hepatic cholesterol metabolism.7 Our findings are also consistent with the results of association studies in humans suggesting that common polymorphisms affecting SREBP-1 may exert quantitative effects on cholesterol synthesis.8 In the current studies, the differential effect of the SHR versus BN allele on promoter function in vitro was less than the strain difference in mRNA expression observed in vivo. This could be due to the fact that the in vitro promoter studies are performed under artificial conditions that do not fully mimic the cellular milieu that can influence gene expression responses in vivo. It is also possible that additional promoter/enhancer polymorphisms might exist beyond those identified in this study that could further affect Srebf1 expression in vivo. Notwithstanding these limitations, the current findings together with the results of previous association studies indicate that genetic research in the SHR can be useful for
molecular isolation of QTL relevant to the regulation of complex metabolic traits in humans.

Although we have found that the SHR and BN variants have different effects on hepatic cholesterol metabolism, we have not found evidence suggesting differential effects of these alleles on blood pressure. The current results indicate that the Srebf1 allele of the SHR tends to decrease risk for dietary induced accumulation of liver cholesterol compared with the Srebf1 allele of the BN rat. Given that hypertension is often associated with multiple metabolic disturbances including fatty liver, the naïve expectation might have been for an opposite result, namely, that the Srebf1 allele of the SHR would be linked to greater accumulation of hepatic cholesterol than the Srebf1 allele of the normotensive BN strain. However, it is important to recognize that most nondiabetic patients with hypertension do not have fatty liver and 50% do not have the metabolic syndrome. The current findings suggest the possibility that some hypertensive patients, like the progenitor strain of the SHR, may harbor variants in Srebf1 that tend to protect against fatty liver whereas others, like the congenic strain of SHR carrying the BN allele for Srebf1, may harbor variants in Srebf1 that tend to increase the risk for fatty liver.

Perspectives
Based on stringent criteria for QTL identification in studies of genetically complex traits, we have found that Srebf1 constitutes a QTL regulating liver cholesterol levels in rats fed a high-cholesterol diet. These findings support the possibility that naturally occurring variants in Srebf1 might influence susceptibility to dietary induced accumulation of hepatic cholesterol in humans. Finally, given that hepatic steatosis is a common feature of the metabolic syndrome, future studies on the relationship between Srebf1 polymorphisms and risk for disordered glucose metabolism in the SHR-BN model should be of additional interest.

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Disclosures
None.

References
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Figure S1. DNA sequence of the SREBP1c promoter.

SHR
CTGGGCAGAAACGTGTCAAAAAAGGATGTTTTCTTTGGAACCTCCTGTGAGGATATAGA -1171
BN
CTGGGCAGAAACGTGTCAAAAAAGGATGTTTTCTTTGGAACCTCCTGTGAGGATATAGA -1164

SHR
AGGACCTAGAAAGGCCGGAGATGAAGTCGACTCTGTGAGGATATAGA -1111
BN
AGGACCTAGAAAGGCCGGAGATGAAGTCGACTCTGTGAGGATATAGA -1104

SHR
CAAGACTCTGAAAAGAGAGATGGGCCACAGGGATACAAGTCCCTGACACCCATGAGGAAA -1051
BN
CAAGACTCTGAAAAGAGAGATGGGCCACAGGGATACAAGTCCCTGACACCCATGAGGAAA -1044

SHR
CTTGGTTGCTGGTCCCCTCCCACTGATAGGCTGGCCAGCACTGGTGTGGTGGTGGTGGT  -991
BN
CTTGGTTGCTGGTCCCCTCCCACTGATAGGCTGGCCAGCACTGGTGTGGTGGTGGTGGT  -984

SHR
ACCTTGCTGGGCCACTGCCACATAAGCTAGGTCGCTGGCCAGCACTGGTGTGGTGGTGGT  -991
BN
ACCTTGCTGGGCCACTGCCACATAAGCTAGGTCGCTGGCCAGCACTGGTGTGGTGGTGGT  -924

SHR
GGTGATGATGATGGTGATGGCAGTGTGCTCGAGA -871
BN
GGTGATGATGATGGTGATGGCAGTGTGCTCGAGA ------ -870

SHR
GTGTGTGTGTGTGTGTGTGTGTACATGTATGAAAGAGCTTTTACCTTTGTCATCAAGGGGAC -811
BN
GTGTGTGTGTGTGTGTGTGTGTACATGTATGAAAGAGCTTTTACCTTTGTCATCAAGGGGAC -810

SHR
AACGACAGACTTGGCATACCCTTAATATCCACTGCCTTTCCCTTCTGTCCCAGAGACTGG -751
BN
AACGACAGACTTGGCATACCCTTAATATCCACTGCCTTTCCCTTCTGTCCCAGAGACTGG -750

SHR
GTTCCTGTCTGGTTTTTGCAAATTCTATAAGACAAGTGAGTCAGGCTTCACTTGTGGGGGC -691
BN
GTTCCTGTCTGGTTTTTGCAAATTCTATAAGACAAGTGAGTCAGGCTTCACTTGTGGGGGC -690

SHR
TGTGAGACCTTGAGGGAAGACACTGACCCAGTCAAGGGACAGAATATACTGGATGTGTGA -631
BN
TGTGAGACCTTGAGGGAAGACACTGACCCAGTCAAGGGACAGAATATACTGGATGTGTGA -630

SHR
TTTGGAGGTAGGATTTCCCTTGCTCACTCCTGAAGGCAGTTTCATGAGTGATCTAGGTCCG -571
BN
TTTGGAGGTAGGATTTCCCTTGCTCACTCCTGAAGGCAGTTTCATGAGTGATCTAGGTCCG -570

SHR
AGGCCATCAGCCCCTCCTTGAAACAAGTGTAAT CATCCTGGGGCTGCTCTGTAAGCTGGA -511
BN
AGGCCATCAGCCCCTCCTTGAAACAAGTGTAAT CATCCTGGGGCTGCTCTGTAAGCTGGA -510

Max ( E-box )
SHR
TTTCCCTGCACCAACTGCCACTATCCAAAGGCATCCATCCATTGGCTGTCCTCAGACTGT -451
BN
TTTCCCTGCACCAACTGCCACTATCCAAAGGCATCCATCCATTGGCTGTCCTCAGACTGT -450

SHR
AGCCTTATCATCTCTTGCTGCTGCCATTCAATGCGAAGGGACAGAAGTGGGTA AACTGAG -391
BN
AGCCTTATCATCTCTTGCTGCTGCCATTCAATGCGAAGGGACAGAAGTGGGTA AACTGAG -390

P300
SHR
GCTAAAATTGGCCATACTAGTTCTGGGTGTGTGCGAACCAGCGGTAGAAACACAGAGCTT -331
BN
GCTAAAATTGGCCATACTAGTTCTGGGTGTGTGCGAACCAGCGGTAGAAACACAGAGCTT -330

SHR
CCGGGATCCAGCCGCGCGCTGTCCAGATTCAATGGGACAGAAGTGGGTA AACTGAG -271
BN
CCGGGATCCAGCCGCGCGCTGTCCAGATTCAATGGGACAGAAGTGGGTA AACTGAG -270

Sp1
SHR
GGCTGCTGATTGG CCATGTG CGCTCACCCGAGGGGCGGGG CACGGGGGCGCTCAGCGGGC -31
BN
GGCTGCTGATTGG CCATGTG CGCTCACCCGAGGGGCGGGG CACGGGGGCGCTCAGCGGGC -30

LXRE
SHR
GCTGGCGCAGTTGCGGTTAAAGGCGGACGCCCGCTAGTAA CCCGGGGCTGCTCTCAGAC -151
BN
GCTGGCGCAGTTGCGGTTAAAGGCGGACGCCCGCTAGTAA CCCGGGGCTGCTCTCAGAC -150

LXRE
SHR
CCCGGTAAACCACGCGGCGCTGTCCAGATTCAATGGGACAGAAGTGGGTA AACTGAG -91
BN
CCCGGTAAACCACGCGGCGCTGTCCAGATTCAATGGGACAGAAGTGGGTA AACTGAG -90

Sp1
SHR
GGCTGCATTGGCGGCTGTCCAGATTCAATGGGACAGAAGTGGGTA AACTGAG -31
BN
GGCTGCATTGGCGGCTGTCCAGATTCAATGGGACAGAAGTGGGTA AACTGAG -30

NF-Y
SHR
TTTAAAAGCCTGGGGCGGGCTCATTACGGCGCTGTCCAGATTCAATGGGACAGAAGTGGGTA AACTGAG +30
BN
TTTAAAAGCCTGGGGCGGGCTCATTACGGCGCTGTCCAGATTCAATGGGACAGAAGTGGGTA AACTGAG +30

+1
SHR
GCACGCCCTAGGGGCGGGGCCGGACGAC +60
BN
GCACGCCCTAGGGGCGGGGCCGGACGAC +60